AMERICAN THYROID ASSOCIATION

Optimal Thyroid Health for All

1923 – 2023

AMERICAN
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(*continued*)

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Welcome to the American Thyroid Association 2023 Annual Meeting

Dear ATA® Annual Meeting Attendees,

On behalf of the American Thyroid Association® Board of Directors, we are thrilled to welcome you to Washington, DC. This year's meeting is particularly special because it is the culmination of planning to celebrate the ATA's Centennial. Our organization has grown and supported members since its initial inception on December 9, 1923, when the Illinois Clinical Club met in Bloomington, IL and created The American Association for the Study of Goiter. This growth and advancement are chronicled online in our Clark T. Sawin History Resource Center, specifically in three timelines created by our History & Archives Committee. We'd particularly like to thank Valerie Anne Galton, PhD, for her leadership of this initiative over the years. The fascinating biographies and interesting photographs you see represent the culmination of many hours of devoted research by the key committee members.

This celebration would not be possible without acknowledging the many contributing members. We would like to thank the co-chairs of our Centennial Task Force, Peter A. Kopp, MD, and Elizabeth N. Pearce, MD, and staff liaison Sharleene Cano, CAE, for their tireless efforts to ensure that our celebration is enduring. You will see the results of this work in this printed piece and online with the different manuscripts and videos that were created to capture the spirit of the ATA and highlight the many accomplishments and advancements of our members over the past 100 years. Thank you to, Anna M. Sawka, MD, PhD, Angela M. Leung, MD, and Catherine F. Sinclair, MD, the Editors-in-Chief for *Thyroid*®, *Clinical Thyroidology*® and *VideoEndocrinology*™, respectively, for coordinating these efforts with the Centennial Task Force. Also, a special ''thank you'' to David S. Cooper, MD, for his hours of research exploring the Sawin Library resources and working with Dr. Kopp on the historical manuscript for *Thyroid* and this year's Sawin Lecture.

Special thanks to this year's Annual Meeting Program Co-Chairs, Alexandria M. Dumitrescu, MD, PhD, and Elizabeth Gardner Grubbs, MD, for creating an exceptional program that highlights science and innovation from bench to bedside and features two new pre-meeting courses designed to meet our attendees' evolving needs.

It goes without saying that we would also like to thank you, the ATA members and supporters, for being part of our community, being the source of our energy and providing the enthusiasm, talent, and expertise that makes our organization unique. We hope that this keepsake brings you joy as you celebrate the centennial onsite with us and look ahead to the next 100 years with pride and excitement as part of the ATA community. We've left some pages at the back of the book for you to gather signatures and notes from other attendees – consider it your ''ATA Centennial Yearbook'' of sorts.

With gratitude and thanks,
Julie Ann Sosa, MD, President
Jacqueline Jonklaas, MD, PhD, Secretary
Amanda Perl, Executive Director

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The American Thyroid Association 1923–2023: Honoring Our Past, Embracing Our Future

David S. Cooper[1] and Peter A. Kopp[2,3]

The year 2023 marks the centennial year of the American Thyroid Association (ATA). Hence, it seems fitting to briefly review the history of our organization.* The American Association for the Study of Goiter (AASG) had its beginnings in the early 1920s when a group of surgeons began to meet informally in Bloomington, IL, and other medical centers in the United States as the ''Illinois Clinical Club'' under the direction of Edwin Plummer Sloan, a thyroid surgeon.[1] The purpose of these meetings was to update the participants on the latest advances in thyroid surgery and anesthesia.

Thyroid disease, predominantly manifested as goiter, was a very common problem throughout the United States. This was not limited to the ''goiter belt'' (Upper Midwest, Great Lakes regions, Appalachians, and Pacific Northwest) with endemic iodine deficiency, where the rates of goiter in some communities were >60%.[2] In addition, World War I had created a demand for surgeons, who were then trained in large medical centers, often in areas of the country where goiter was prevalent. However, on their return from the war, there were often operative complications due to the surgeons' inexperience with thyroid surgery.

It was in this environment that Dr. Sloan proposed an organization of ''medical men'' from across the country that would include not only surgeons, but also experts from all medical disciplines, to stimulate research into the cause of goiter (it was not clear that much of it was due to iodine deficiency at that time), and to advocate for improved treatments and possible prevention. This was to be accomplished through meetings and subsequent publication of information about goiter that would be available to all physicians interested in caring for patients with goiter.

On December 6, 1923, in Bloomington, IL, Dr. Sloan's vision was realized in the formation of the AASG (Fig. 1).[3] Dr. Sloan was inaugurated as the first president for the years 1923–1924 (Fig. 2).† According to a recollection of the early history of the society, there were 26 ''Charter Members,'' including a Denver surgeon, Seymour D. Van Meter (who initiated the Van Meter award in 1930 during his presidency), as well as his daughter, Dr. Virginia Van Meter, who was also surgeon. Of note, Dr. Henry S. Plummer, an internist, was also one of the founding members of the society.

The first regular meeting occurred at the Unitarian Church during January 23–25, 1924, in Bloomington, IL, with >200 physicians in attendance (Fig. 3).[4] Lectures included ''An Analysis of the Types of Goiter with Indications for Treatment,'' ''Radium Treatment of Goiter,'' and ''A Review of Another Years Work with Thyroid Disease'' by Dr. Frank Lahey (who could not attend the meeting), and, interestingly, ''Use of Seaweed in the Prevention and Treatment of Goiter,'' by Dr. J.W. Turrentine from the U.S. Department of Agriculture. There were also debates on the causes and

*Much of the history of the ATA to be recounted in this review is contained in society meeting minutes that are available in the ATA archives, funded in part by the Clark T. Sawin, MD, History and Resource Center at the ATA offices. The Transactions of the American Association for the Study of Goiter, also included in the ATA archives, and that were published by various journals over the years, including the *Journal of Clinical Endocrinology*, served as an additional source of information. Finally, the Daily Pantagraph, the Bloomington, IL, newspaper (provided by the McLean County [IL] Museum of History), provided documentation about the initial meeting of the AASG in January 1924.

†According to a document by J. Rudolph Yung dated October 2, 1950, entitled ''American Association for the Study of Goiter: Early History; International Conference on Goiter'', the founding members of the American Association for the Study of Goiter were Elmer R. Arn*, Joseph L. DeCourcy, Frank Deneen, Frank B. Dorsey*, J.W. Dreyer, William Englebach, Gordon S. Fahrni*, L.W. Frank, N.W. Gilette, Arthur E. Hertzler, J. W. Hoschelle, A.S. Jackson, H.M. Joy, R.A. McGillicuddy, G.W. Newell, A.J. Paulson, H.S. Plummer*, A.F. Renneker, Marcus O. Shivers*, R.G. Stevens, Edwin P. Sloan, A.C. Scott, Seymour Van Meter*, Virginia Van Meter (?), F.S. Wetheraell, and J. Rudolph Yung*.
*Indicates individuals who became presidents of the AASG. This document is stored in the ATA Archives in a volume entitled ''American Goiter Association History and Minutes 1923–1949.'' It remains unknown whether this manuscript has ever been published.

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[3]Division of Endocrinology, Metabolism and Molecular Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA.

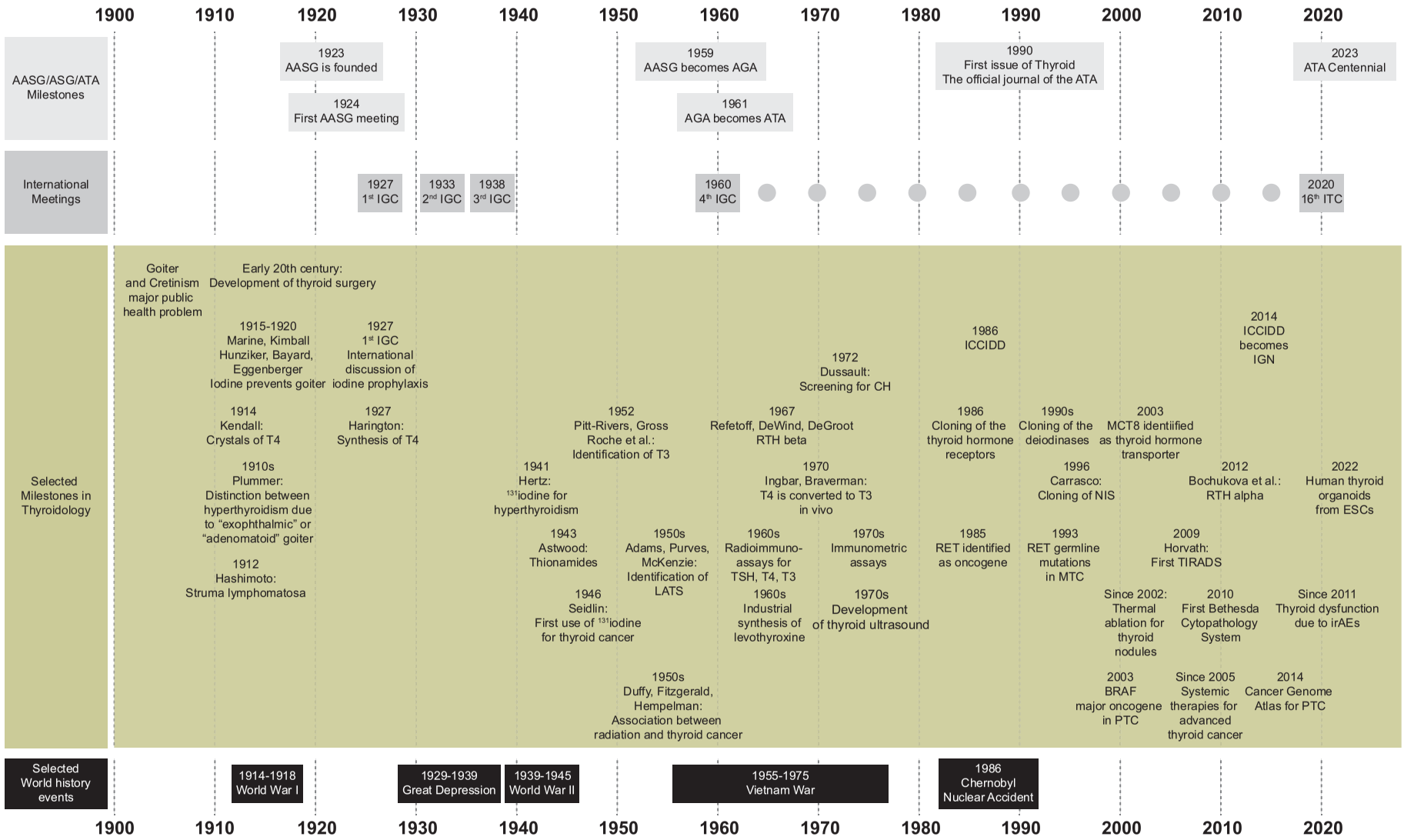
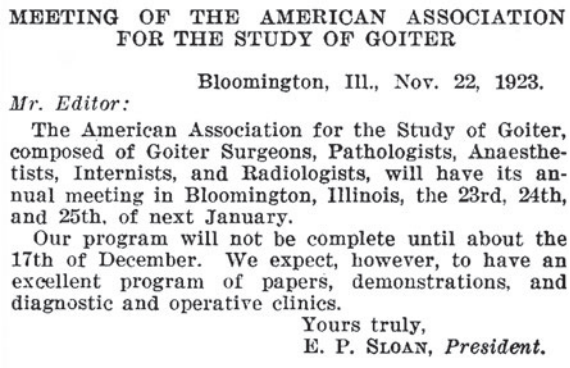


FIG. 1. Selected milestones illustrating the history of the ATA, the International Thyroid Meetings, and thyroidology. For more details see references.[3,5,6] AASG, American Association for the Study of Goiter; AGA, American Goiter Association; ATA, American Thyroid Association; BRAF, proto-oncogene B-RAF; CH, congenital hypothyroidism; ESCs, embryonic stem cells; ICCIDD, International Council for the Control of Iodine Deficiency Disorders Global Network; IGC, International Goiter Conference; IGN, Iodine Global Network; irAEs, immune-related adverse events; ITC, International Thyroid Congress; LATS, long-acting thyroid stimulator; MCT8, monocarboxylate transporter 8; MTC, medullary thyroid cancer; NIS, sodium iodide symporter; PTC, papillary thyroid cancer; RET, rearranged during transfection; RTH, resistance to thyroid hormone; T3, triiodothyronine; T4, thyroxine; TIRADS, Thyroid Imaging Reporting and Data System; ●, ITCs.

FIG. 2. Edwin Plummer Sloan, first president of the American Society for the Study of Goiter. Dr. Sloan was born in Missouri and graduated from medical school in 1898. After a surgical residency, he received additional training at clinics in Berlin, Germany, and with Theodor Kocher in Berne, Switzerland. Further information can be found in the Clark T. Sawin History Resource Center of the ATA that includes biographical notes and pictures of all past presidents.[9]



MEETING OF THE AMERICAN ASSOCIATION FOR THE STUDY OF GOITER

Bloomington, Ill., Nov. 22, 1923.

Mr. Editor:

The American Association for the Study of Goiter, composed of Goiter Surgeons, Pathologists, Anaesthetists, Internists, and Radiologists, will have its annual meeting in Bloomington, Illinois, the 23rd, 24th, and 25th, of next January.

Our program will not be complete until about the 17th of December. We expect, however, to have an excellent program of papers, demonstrations, and diagnostic and operative clinics.

Yours truly,
E. P. SLOAN, *President.*

FIG. 3. Call for the first meeting of the American Association for the Study of Goiter. From N Engl J Med 189: 961, 1923.

classification of goiter, with emphasis on the benefit of appropriate classification,[‡] which might, importantly, lead to nonoperative treatment.

In addition to >50 lectures during the 3-day meeting, operative clinics were held each day at 2 local hospitals where surgeons demonstrated the latest surgical and anesthetic techniques (Fig. 4). Interestingly, the names of the patients who were to have surgery were published in the local newspaper (*The Daily Pantagraph*, Bloomington, IL) each morning.

The first meeting of the AASG concluded with the announcement that the officers would continue their role in the following year, and that the site of the following years' meeting was yet to be decided (the meeting, in fact, took place in Bloomington, IL, in conjunction with the annual meeting of the American Medical Association in Chicago). In 1959, the AASG was incorporated in the New York State as the American Goiter Association, and in 1961 the name was changed to the American Thyroid Association (Fig. 1).

Programmatic Themes 1923–2023

The founding members of the AASG were focused on the improvement of thyroid surgery and outcomes, still a significant challenge in the 1920s. Gordon S. Fahrni, a founding member of the AASG, wrote in his recollections in 1973: ''Its birth was a response to a need across the country to increase our knowledge of the whole subject and improve the care of people so afflicted.''[4]

The subsequent developments are reflected in the topics of the meeting programs and abstracts, in publications, and the membership composition (see Composition of the ATA by Medical Specialty and Sex). Accompanied by numerous scientific, medical, technological, and societal developments, there has been a constant and dynamic development, which has impacted the focus and the composition of the ATA. A detailed summary of these milestones is not possible due to space constraints, but the timelines in the Clark T. Sawin History Resource Center illustrate the landmarks in the history of the ATA,[3] the International Goiter and Thyroid Meetings,[5] and the history of thyroidology (Fig. 1).[6]

Composition of the ATA by Medical Specialty and Sex

In the beginning, the AASG was composed almost exclusively of male surgeons.[4] Despite a focus on surgical aspects,[§] the group had an interest in nonsurgical topics related to the thyroid already at the inaugural meeting in January 2024. For example, Dr. Henry Plummer spoke, although the topic of his address is not known. Presumably, it related to the use of potassium iodide to prepare patients with Graves' disease for thyroidectomy, an approach that significantly improved the outcomes of hyperthyroid patients undergoing surgery.[4,7]

Dr. Plummer would go on to become the first nonsurgeon to be elected president of the society in 1933. At the January 1927 meeting in Philadelphia, James Howard Means spoke on ''The Development of Our Knowledge of the Thyroid Gland.'' Dr. Means would later become the second nonsurgeon to become president of the society in 1948. From the early days, the AASG was committed to integrate other disciplines interested in thyroid pathophysiology.[**] However,

[‡]In 1924, a committee of the AASG was formed to better understand and classify goiter, and, working with other American and European experts, proposed, after five years of work, a definition of goiter that was ''a disease of unknown cause and numerous characteristics of which are disturbance of functions and enlargements of the thyroid gland.'' In addition, a classification of goiter was proposed, which, in its simplest form, divided goiter into four categories: diffuse nontoxic goiter, diffuse toxic goiter, nodular nontoxic goiter, and nodular toxic goiter.

[§]Recollections of Gordon S. Fahrni: ''The first decade in the life of our Association typified somewhat, that of a Surgical Club, the members of which assembled each year to compare experience and to work toward a broader perspective.''[2]

[**]Recollections of Gordon S. Fahrni: ''Basic in this plan was to bring into membership the other branches of our profession interested in thyroid problems. In this we were successful and some of our first recruits were internists and research workers in this field, then followed endocrinologists and biochemists and as the years went by our membership increased and broadened to include all phases of effort in thyroid problems.''[2]

GOITER SURGEONS HAVE A BUSY DAY

Fifty Persons Presented Themselves at Diagnostic Clinic Conducted Here Thurs.

MANY ADRESSES HEARD

That the advancement that has been made and is being made will result within ten years, of a decrease in the prevalence of goiter, equally as great as the study and methods of treatment of tuberculosis, have decreased the latter disease, was the prediction of Dr. William Peck of Freeport, president of the Tri-State Medical Society, made yesterday before the American Association for the Study of Goiter at the session in the Unitarian church. The various methods of treatment, either medical, X-ray, non-operative, or operative, have taken such a notable advance and the results have become so satisfactory, that their continuation and the researches, experimentation and studies, that are yet to be made, will have wonderful effect, he predicted in lessening goiter, and its gradual elimination as one of the afflictions of the human race.

Other outstanding features of the discussion of yesterday related to the advance that had been in relation to classification of cases. It was the consensus of opinion that an accurate diagnosis according to the classification of a case, vital to the success of treatment, and this preliminary study and the kind of treatment after a case has been properly diagnosed and correctly classified is apt to have beneficial results. It sometimes develops that if this classification is accurately made that a cure can be effected without the necessity for an operation. For this reason, the wisdom of such classification and the decision in determining in which class the various cases belong is now generally conceded by the medical fraternity, and that the success of the treatment largely depends upon this course.

Fifty persons presented themselves for the diagnostic clinic yesterday, conducted by Dr. Andre Crotti of Columbus, and the number was so great that it was impossible to take care of them all.

Cause of goiter remains a mystery to the medical profession, but Dr. Crotti gave some information that he had procured as the result of a series of experiments and investigations and which in time, might solve this problem to the satisfaction of all. The doctor gave some interesting information concerning goiter, where it is found most numerously, and some theories that have been advanced concerning it by authorities. Some have argued that goiter was produced by a deficiency of iodine in the system. Another maintains that it is due to infection, while others assert that it is due to germs in drinking water.

Dr. E. R. Arn, of Dayton, O., and Dr. J. L. DeCourcey of Cincinnati, led the discussion in relation to the use of iodine preparation in the schools and as a preventive following operations. It was the general opinion, however, that the indiscriminate use of iodine was dangerous, at least more so in some cases, than others.

Deleterious effects upon the heart of goiter poison secretions, was discussed by Dr. Frank N. Wilson of Ann Arbor, Mich. He described a number of cases of cardiac disturbances in association with diseases of the thyroid gland and the successful handling of them, and the relief experienced by the removal of goiters.

Dr. Wayne Babcock of Philadelphia referred to a number of extreme cases and which are extremely poisonous, telling of the preliminary methods to be used and the advisability of secondary operation and treatment when such a type of cases are found.

Dr. William Englebach of St. Louis discussed the functions of ductless glands and their relation to other glands and also told of the physical effect in diseases, producing many deformities, such as obesity, gianism, dwarfism, goiter and the like.

Dr. Horace M. Brown of Milwaukee discussed the endocrine system and the relation of the thyroid and other ductless glands to, and the effect upon, the moral, mental and physical being.

Dr. E. G. Blair, of Kansas City, dwelt upon the advisability of secondary operations in extreme cases when the full operation would not be advisable at the outset. He referred to a number of such cases where the secondary method of treatment had proven successful.

A banquet last evening was one of the enjoyable features of the day. There was a large attendance and the menu was an elaborate one.

Today's Program.

There will be operative clinics again today at St. Joseph and Mennonite hospitals. The program today will have many interesting speakers. One address by Dr. F. M. Hagans of Lincoln will relate to the use of radium in the treatment of goiter.

The order of today's program is as follows:

General Session—7:00 A. M.

Commander Wm. Seaman Bainbridge, New York.—"Goiter in the Navy, and in Europe."

Dr. Frank H. Lahey, Boston, Mass.—"A Review of Another Year's Work with Thyroid Disease."

Dr. E. R. Arn, Dayton, O.—"An Analysis of Types of Goiter with Indications for Treatment."

Dr. Roswell Petitt, Ottawa, Ill.—"Incipient Goiter vs. Incipient Tuberculosis."

Dr. E. W. Rowe, Lincoln, Neb.—"The Roentgen Treatment of Thyrotoxicosis."

Dr. F. M. Hagans, lincoln, Jll.—"Radium Treatment of Goiter."

Dr. J. W. Turrentine, Ph. D., bureau of soils, U. S. Department of Agriculture, Washington, D. C.—"Use of Seaweed in the Prevention and Treatment of Goiter."

FIG. 4. Goiter surgeons have a busy day. Excerpt of the *The Daily Pantagraph*, Bloomington, IL, January 25, 1924 (page 3), describing educational events presented at the first meeting of the American Association for the Study of Goiter.

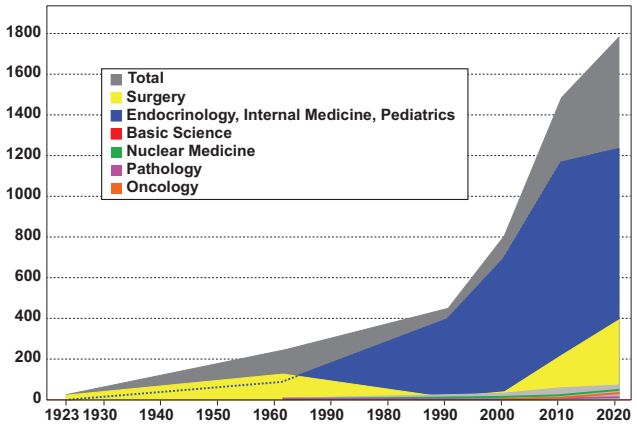
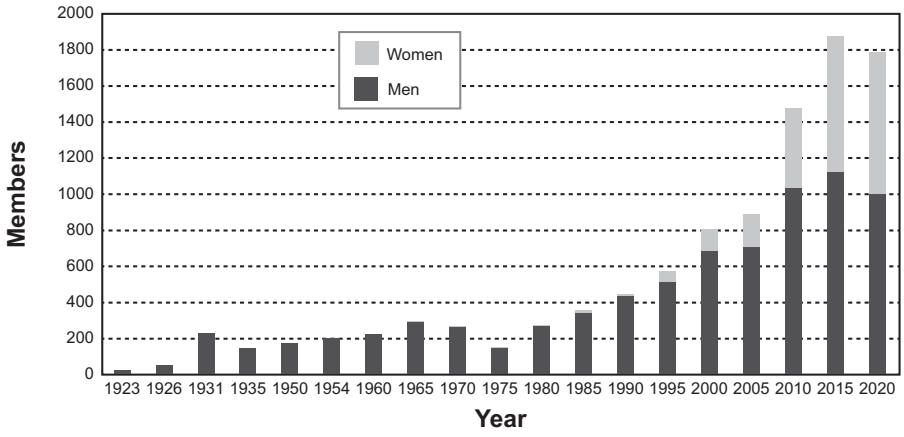


FIG. 5. Demographic development by specialty groups within the ATA.

unfortunately, there did not appear to be a formal breakdown of medical specialty within the organization until 1961 (Fig. 5).

At that time, ~50% of active members were surgeons and the other half were composed mostly of endocrinologists and internists in equal proportion. Over the subsequent three decades, the number of surgeons in the organization decreased dramatically to only 3% in 1990. The reasons for this decline are uncertain, but possibly relate to a decrease in nontoxic goiter prevalence due to iodine sufficiency in the population, as well as a decrease in surgical therapy for many thyroid conditions, especially Graves' disease and toxic nodular goiter. Beginning in 2000, there has been a welcomed increase in the proportion of surgeons, both endocrine surgeons and head and neck surgeons. This is likely reflective of the increase in the prevalence of thyroid nodules and thyroid cancer, as well as important advances in thyroid cancer care. Over the years,

FIG. 6. Virginia Kneeland Frantz, first female president of the ATA in 1962. Dr. Frantz pursued medical studies at Columbia College of Physicians and Surgeons, graduating in 1922, and she became the first woman surgery intern at New York Presbyterian Hospital. In 1935, she described insulin-secreting tumors of the pancreas in collaboration with Dr. Allen Whipple. In 1942, she was involved in the pioneering study demonstrating radioiodine uptake in a metastatic focus of well-differentiated thyroid cancer but not in coexisting poorly differentiated metastases.[9,10]



Women	0	0	0	0	0	0	0	1	1	3	3	18	8	57	121	179	444	749	781
Men	26	53	231	150	175	205	230	291	270	150	272	342	440	515	687	714	1035	1124	1005

FIG. 7. Membership by Sex in the ATA. (For the period until the early 1960s, it is difficult to extract information about the makeup of the organization by sex. The listings utilize first name initials and the sex of individual participants can usually not be identified).

there have always been society members who were pathologists, basic scientists, radiologists, and pediatricians, and these proportions have remained relatively stable over time. Paralleled by the development of systemic therapies for advanced thyroid cancer, the ATA membership now also includes a group of dedicated oncologists.

Initially, the AASG was composed entirely of men (with the exception of Dr. Virginia Van Meter), reflecting the paucity of women physicians in the United States. Dr. Virginia Van Meter did address the group at the January 1925 meeting in Bloomington, IL, on the topic of ''Goiter Survey in the Public Schools of Denver.'' In reviewing minutes of society meetings and member participation lists at annual meetings is difficult to extract information about the makeup of the organization by sex, since the listings utilize first name initials, so the sex of individual participants cannot be identified.

Dr. Virginia Frantz, an esteemed thyroid pathologist at Columbia University became the first woman president of the ATA in 1962 (Fig. 6). The number of women members in the society has continued to increase progressively over the past several decades, reflecting increasing number of women in medicine in general, and in endocrinology and surgical specialties in particular. By the year 2020, 44% of ATA members were women (Fig. 7). There are currently no reliable longitudinal or cross-sectional data on ethnic/racial background, minorities, and diversity.

Mission and Perspectives

The ATA's vision is committed to achieving the goal of *Optimal Thyroid Health for All*, and its mission statement is *Transforming thyroid care through clinical excellence, education, scientific discovery and advocacy in a collaborative community.*

Efforts toward clinical excellence and education are strongly supported by the ATA's dedication to developing high-quality guidelines, the educational meetings, and its publication portfolio. *Thyroid*, the official journal of the ATA, was launched in 1990 with Dr. Jerome Hershman as founding editor.[8] It is complemented by *Clinical Thyroidology* (year of inception 1988), *Clinical Thyroidology for the Public* (2008), and *VideoEndocrinology* (2014).

Scientific discovery is, in part, dependent on appropriate funding, sources that must be defended. Much work needs to be done toward the elimination of national and global disparities and inequities in terms of access to health care and education. The ATA is committed to valuing diversity and integration, and the organization is embedded in a global context. Worldwide collaborative efforts and connections are key to respond to the challenges of the future with constructive and sustainable solutions.

All these goals can only be achieved by ongoing engagement and citizenship of the ATA membership and, as illustrated by our history, openness to dynamic change and reorientation. To quote Gordon Fahrni: ''… our Thyroid Association, … kept pace with and helped to stimulate and develop the sequence of changes over the years of which we are all so proud.''[4]

Let us enter into the ATA's new century with a renewed commitment to the prevention and innovative treatment of thyroid disorders, with dedication to excellence in clinical care and research, and with a collaborative approach and an inclusive membership.

Acknowledgments

We thank Sharleene Cano, Director, Membership and Publications, American Thyroid Association, for the information on membership statistics, and Bill Kemp, McLean County Museum of History, Bloomington, IL, for providing materials from *The Pantagraph*.

Authors' Contribution

The article was conceived and written by David S. Cooper and Peter A. Kopp.

Author Disclosure Statement

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References

1. Yung J. American Association for the Study of Goiter. Origin—History—Accomplishment. Presidential Address. Transactions of the American Association for the Study of Goiter. West J Surg Obstet Gynecol 1936: 44(Suppl):24–32.
2. Leung AM, Braverman LE, Pearce EN. History of U.S. iodine fortification and supplementation. Nutrients 2012; 4(11):1740–1746.
3. Clark T. Sawin History Resource Center: American Thyroid Association Timeline. Available from: https://www.thyroid.org/about-american-thyroid-association/history/clark-t-sawin-history-resource-center/american-association-timeline/ [Last accessed: August 1, 2023].
4. Fahrni G. Recollections of the Organization and Growth of the American Thyroid Association. 1973. Available from: https://thyroid.org/wp-content/uploads/timeline/Fahrni-Recollections-1912-1950.pdf [Last accessed: August 1, 2023].
5. Clark T. Sawin History Resource Center: International Thyroid Congress Timeline. Available from: https://www.thyroid.org/about-american-thyroid-association/history/clark-t-sawin-history-resource-center/international-congress-timeline/ [Last accessed: August 1, 2023].
6. Clark T. Sawin History Resource Center: Thyroid History Timeline. Available from: https://www.thyroid.org/about-american-thyroid-association/history/clark-t-sawin-history-resource-center/thyroid-history-timeline/ [Last accessed: August 1, 2023].
7. Kopp PA. Iodine in the therapy of Graves' disease: A century after Henry S. Plummer. Thyroid 2023;33(3):273–275.
8. Kopp P. Welcome to a new year in Thyroid. Thyroid 2013; 23:5–6.
9. Clark T. Sawin History Resource Center: Past Presidents. Available from: https://www.thyroid.org/about-american-thyroid-association/history/past-presidents/ [Last accessed: August 1, 2023].
10. Keston AS, Ball RP, Frantz VK, et al. Storage of radioactive iodine in a metastasis from thyroid carcinoma. Science 1942;95(2466):362–363.

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ASSOCIATION

Optimal Thyroid Health for All

1923 – 2023

ATA Presidents

1923–1925 **E.P. Sloan**
1926 **E.G. Blair**
1927 **Emil Goetsch**
1928 **Gordon S. Fahrni**
1929 **S.D. Van Meter**
1930 **A.R. Arn**
1931 **Kerwin Kinard**
1932 **M.O. Shivers**
1933 **Henry S. Plummer**
1934 **R.M. Howard**
1935 **Allen Graham**
1936 **J.R. Yung**
1937 **Nelson M. Percy**
1938 **Frank H. Lahey**
1939 **F.B. Dorsey, Jr.**
1940 **J.K. McGregor**
1941 **Frank E. Rogers**
1942–1946 **J. deJ. Pemberton**
1947 **W.B. Mosser**
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1949 **Arnold S. Jackson**
1950 **Samuel F. Haines**
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1953 **Claude J. Hunt**
1954 **Merrill N. Foote**
1955 **Richard B. Cattell**
1956 **Rulon W. Rawson**
1957 **Brown M. Dobyns**
1958 **Elmer C. Bartels**
1959 **Warren H. Cole**
1960 **Howard Mahorner**
1960 **Edwin G. Ramsdell**
1961 **Alexander Albert**
1962 **V. Kneeland Frantz (Virginia)**
1963 **John C. McClintock**
1964 **J.E. Rall**
1965 **F.R. Keating, Jr.**
1966 **Lawrence W. Sloan**
1967 **G. H. Klinck**
1968 **Lindon Seed**
1969 **John B. Stanbury**
1970 **Theodore Winship**
1971 **Samuel B. Barker**
1972 **Robert L. Kroc**
1973 **Sidney C. Werner**
1974 **David H. Solomon**
1975 **Jacob Robbins**
1976 **William M. McConahey**
1977 **Sidney H. Ingbar**
1978 **Farahe Maloof**
1979 **Alvin B. Hayles**
1980 **Monte A. Greer**
1981 **Robert Volpe**
1982 **Leslie J. DeGroot**
1983 **David V. Becker**
1984 **J. Maxwell McKenzie**
1985 **Lewis E. Braverman**
1986 **Jack H. Oppenheimer**
1987 **Gerard N. Burrow**
1988 **John T. Nicoloff**
1989 **Delbert A. Fisher**

1990 **John F. Wilber**
1991 **Constance S. Pittman**
1992 **Ralph R. Cavalieri**
1993 **Jerome M. Hershman**
1994 **P. Reed Larsen**
1995 **Leonard Wartofsky**
1996 **Colum A. Gorman**
1997 **E. Chester Ridgway**
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Thyroid Hormone Metabolism: A Historical Perspective

Valerie Anne Galton[1] and Arturo Hernandez[2]

In this article, starting with the recognition that iodine is essential for normal thyroid function and is a component of thyroid hormone (TH) molecules, we discuss the many seminal observations and discoveries that have led to identification of various pathways of TH metabolism and their potential roles in TH economy and action. We then recount evidence that TH metabolism participates in maintaining the appropriate content of active hormone in a TH-responsive tissue or cell. Thus, metabolism of the TH is not merely a means by which it is degraded and eliminated from the body, but an essential component of an intricate system by which the thyroid exerts its multiple regulatory effects on almost all organs and tissues. The article ends with a summary of the current concepts and some outstanding questions that are awaiting answers.

Keywords: deiodinases, thyroxine, thyroxine glucuronide, thyroxine sulfate, triiodothyronine

Introduction

In 1820, Coindet reported that the newly discovered element, iodine, could be used successfully to treat endemic goiter, suggesting that it is important for normal functioning of the thyroid gland (1). In 1874, Gull published the symptoms of a disease that he described as a cretinoid state supervening in adult life in women (2), and in 1878, Ord proposed the term myxedema for this disease and noted that it was associated with atrophy of the thyroid (3).

Five years later, J and A Reverdin described the clinical picture following 21 thyroidectomies and called it ''myxoedème opératoire'' (4,5), and Kocher reported the results of 200 thyroidectomies performed by himself and other surgeons in Switzerland and Germany. He termed the resulting condition, which resembled that of myxedema, cachexia strumipriva (6).

In 1888, a committee that had been established to consider these conditions reported their conclusion that myxedema, *cretinism*, and the conditions that resulted from total thyroidectomy resulted from impaired thyroid function (7). In 1890, Bettencourt R and Serrano J-A reported rapid improvement in a patient with myxedema who had received a graft of sheep thyroid (8), and a year later, Murray showed that myxedema could be successfully treated with daily injections of sheep thyroid extract (9).

Then in 1896, Baumann reported that the thyroid gland contained significant amounts of iodine, contained predominantly in a protein fraction that on hydrolysis yielded a substance that ameliorated symptoms of myxedema in women and thyroidectomy in animals (10,11). Chemical purification of an active principle from the thyroid, which contained 65% iodine, was achieved by Kendall in 1914 and he named it thyroxin (12). Harington determined that the compound is a p-hydroxyphenyl ether of tyrosine, with iodine atoms located in the 3,5,3′ and 5′ positions, and he renamed it thyroxine (T4) (13) (Fig. 1).

Although it was then evident that most of the biological activity of the thyroid was due to T4, it was not clear whether it was also the circulating hormone. In fact, it was not until 1948 that Taurog and Chaikoff, using [^{131}I]T4, found that in normal animals, most (if not all) of the organic iodine in the circulation consisted of T4 reversibly bound to plasma proteins (14). This was unequivocally confirmed by Laidlaw, using the newly developed technique of paper chromatographic analysis (15).

Studies of T4 Metabolism *In Vivo*

In the 1940s, the availability of radioactive iodine and paper chromatography opened up a new era of thyroid hormone (TH) research, including studies of T4 metabolism.

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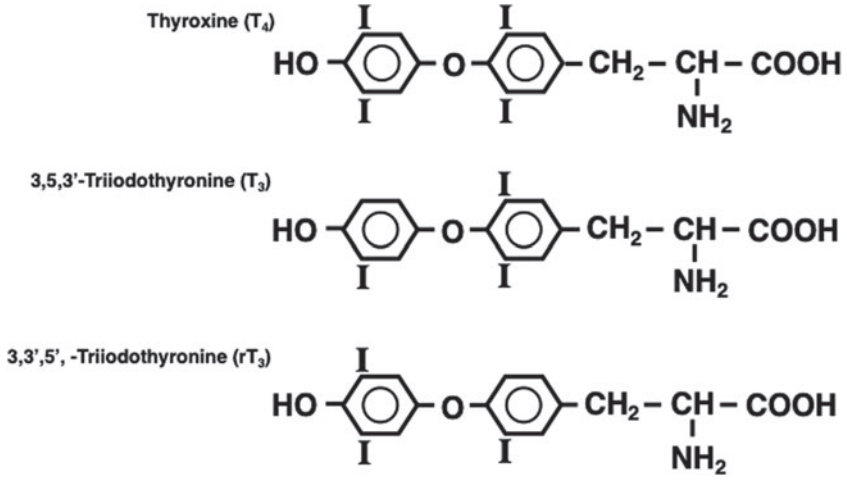

FIG. 1. Chemical structures of Thyroxine (T4), 3,5,3′-triiodothyronine (T3) and 3,3′,5′-triiodothyronine (reverseT3, rT3).

Gross and Leblond reported that 2 hours following injection of [^{131}I]T4 into rats, up to 50% of the injected radioactivity was located in the liver, bile, and gastrointestinal tract, and after 24 hours, as much as 80% was located in the feces as [^{131}I]T4, while 10% appeared in the urine as [^{131}I]iodide (16).

Taurog *et al.* demonstrated that majority of the organic iodine in bile was T4-glucuronide (T4G), a compound formed by conjugation of glucuronic acid (GA) with the phenolic hydroxyl group of T4 (17). T4 was also present in bile in the form of a sulfate ester (T4S) (18). The conjugated forms were shown to be readily hydrolyzed in the intestine and thus T4 was excreted in feces in the unconjugated form (17,18). Initially it was felt that this metabolic pathway was solely a means by which the liver eliminated excess T4 from the body. However, Albert and Keating, using a physiological level of [^{131}I]T4, demonstrated the presence of enterohepatic circulation of T4, as indicated by the finding that the rate of secretion of radioactivity into the bile was much higher than its rate of excretion in the feces (19). The proportion of hormone resorbed from the intestine and the relative fractions of hormonal iodine excreted in urine and feces were found to vary widely among species (20).

Many derivatives of T4, including 3,5,3′-triiodothyronine (T3) and 3,3′5′-triiodothyronine (rT3) (Fig. 1), and the acetic acid derivatives of both T4 and T3 (tetrac, TA4, and triac, TA3), can also undergo conjugation with GA and esterification with sulfate. Several studies indicate that whereas T4 appears to conjugate more readily with GA in the liver, T3 is esterified primarily with sulfate (21).

In rats given exogenous T4, the clearance rate of T4 through the hepatic/fecal route steadily increases as the dose of T4 is raised, suggesting that the process participates in regulation of the serum T4 level (20). T4G is also formed and sequestered in the kidney, and it has been suggested that this organ provides an additional mechanism for regulating the circulating T4 level (20).

TH also can undergo oxidative deamination and decarboxylation. In 1954, Jouan *et al.* reported the presence of triac in the kidneys of rats given [^{131}I]T3 (22), and Albright *et al.* demonstrated the formation of both Tetrac and Triac from the parent hormones in rat kidney slices (23). Both analogs are formed from endogenous T4 and T3 in the liver and kidney (24).

However, although the acetic acid analogs have significant thyromimetic activity, the importance of this metabolic pathway and the physiological role of endogenous acetic acid analogs were unknown at the time (21). The pathways of T4 metabolism recognized by the end of the 1970s are shown in Figure 2.

The Role of T4 in TH Action

Although by the 1950s it was recognized that T4 is quantitatively the major iodinated compound secreted by the

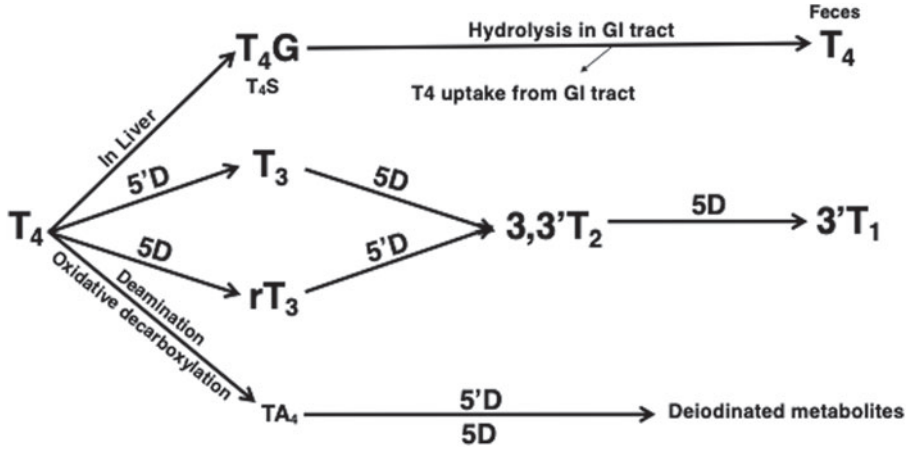

FIG. 2. Pathways of metabolism and excretion of T4 identified by 1980. 5′D, 5′-deiodination; 5D, 5-deiodination; NB, significant amounts of T4G are also formed and stored in the kidneys; T4G, T4 glucuronide; T4S, T4 sulfate; TA4, tetrac tetraiodothyroacetic acid.

thyroid and present in circulation, there was some doubt that it was the active form of the TH. The observations that T4 had a long latent period of action when administered *in vivo* and had no significant effect when studied in tissues *in vitro* suggested that it had to be metabolized in peripheral tissues to an active form.

This concept was substantiated when T3 was identified in the thyroid and circulation by Gross and Pitt-Rivers. They showed that T3 was more potent, and its action more rapid, than T4 and hypothesized that T3 was the active form of the hormone and T4 was its precursor (25). However, the necessary proof was elusive. As early as 1955, Pitt-Rivers pointed out that although rats responded more rapidly to T3 than to T4, the response was still measured in hours and injected [^{131}I]T3 disappeared more rapidly from the body than did [^{131}I]T4. Furthermore, T3 also appeared to be inactive when added to tissue preparations *in vitro*.

Arguably, the more significant issue was that, despite several attempts, the presence of [^{131}I]T3 following injection of [^{131}I]T4 into athyreotic humans could not be unequivocally demonstrated. Although in 1955 Pitt-Rivers and Stanbury reported the presence of [^{131}I]T3 in circulation of patients given [^{131}I]T4 (26), Stanbury became concerned that the reported data yielded curves for the appearance of labeled T3 and failed to conform to the theory that T3 was formed from T4 by a simple precursor–product relationship. There was also considerable overlap of T4 and T3 on the chromatograms. He repeated the study in six patients using a solvent system that provided excellent separation of the two hormones and found no evidence of any T4 to T3 conversion (27).

Attempts to detect T4 to T3 conversion *in vitro* were also largely unsuccessful. Although conversion of [^{131}I]T4 to [^{131}I]T3 in rat kidney slices was reported (28,29), the findings could not be confirmed (23), and many other groups were unable to demonstrate [^{131}I]T3 generation from [^{131}I]T4 in a variety of tissues despite the fact that deiodination of T4, as evidenced by the release of [^{131}I]iodide, clearly occurred (21).

At this point, concern was growing regarding the physiological significance of studies of T4 deiodination *in vitro*. There were many reports describing the presence in tissues, particularly in broken cell preparations, of T4 deiodinating systems that were heat stable and thus unlikely to be mediated by an enzyme. Furthermore, several compounds were not only found to stimulate deiodination, including hydrogen peroxide, flavin compounds, ferrous ions, and ascorbic acid, but most of the effects were also obtained in heat-treated tissue preparations.

In all these studies, the reaction product was invariably inorganic iodide, but no T3 was generated. These diverse studies, the possible mechanisms involved, and their physiological significance are considered in a 1963 review (21).

By the mid-1960s, it was felt that the results obtained in studies of T4 deiodination *in vitro* were of questionable physiological importance, and investigators focused on studies *in vivo*, in particular the isotopic equilibrium technique developed by Van Middlesworth (30). This involved giving rats a daily injection of [^{131}I]T4. Once the daily output of radioactivity in urine and feces became constant, the model could be used to test the effects of conditions or substances on the urinary output of [^{131}I]iodide as a measure of T4 deiodination. For example, Van Middlesworth used this technique to demonstrate that 6-n-propyl-2-thiouracil (PTU) partially inhibited the deiodination of T4. However, it was not possible to determine from these studies whether deiodination is a process essential for TH action or merely one by which it is degraded, and no evidence of [^{131}I]T3 generation was obtained.

The answer finally came in the early 1970s as a result of two critical findings. First, in 1970, Braverman *et al.* demonstrated unequivocally that T4 to T3 conversion occurs in athyreotic humans (31), and Sterling *et al.* confirmed this by demonstrating that [^{14}C]T3 was present in euthyroid human subjects injected with [^{14}C]T4. In humans, up to a third of the T4 that was metabolized was converted to T3 (32), and in rats, at least 20% of the total body extrathyroidal T3 was derived from T4 (33). These convincing findings laid to rest a major inconsistency in the theory that T3 was the active form of the hormone, namely, the previous failures to demonstrate T4 to T3 conversion.

The second finding was the discovery in 1972 of TH receptors (TRs) by Oppenheimer *et al.*, who demonstrated that they are located in the nucleus and have much higher affinity for T3 than for T4 (34). Furthermore, the majority of iodothyronine bound to TRs in rat liver and kidney was T3 (35).

Together, these findings provided convincing evidence that the majority of TH action is initiated by T3 rather than T4. Thus, 5′-deiodination (5′D) is an activating process and therefore an essential component of TH action.

Identification and Characterization of Deiodinases

Now that the importance of 5′D for production of T3 in peripheral tissues was established, interest turned to the identification and characterization of the specific enzyme(s) involved. Three reviews documenting the progress made in this area by Leonard and Visser (1986), Bianco *et al.* (2002), and St Germain *et al.* (2009) are available (36–38).

As discussed above, earlier attempts to study these enzymes *in vitro* had been repeatedly unsuccessful. However, this problem was resolved in 1976 when Visser *et al.* reported that thiol groups were essential cofactors for deiodination *in vitro*, and in the presence of dithiothreitol, subcellular fractions of rat liver readily converted T4 to T3 (39).

It was soon established that there are two enzymes that catalyze the conversion of T4 to T3, the type 1 and 2 deiodinases (D1 and D2). D1 and D2 were distinguished initially because D1, in contrast to D2, was inhibited by PTU (40). D1 is a dual-purpose enzyme. In addition to its ability to activate T4 by converting it to T3 by 5′D, it can also inactivate iodothyronines by inner-ring or 5-deiodination (5D), in particular after they have undergone esterification with sulfate. Thus, whereas D1 is capable of deiodinating unconjugated T4 by 5′D or 5D with comparable efficiency, after sulfation of T4 to T4S, 5′D by D1 is essentially blocked and T4S is subjected to 5D by D1 to generate the inactive metabolite, rT3S (41). It is notable that the preferred substrate of D1 for 5′D is not T4, but rT3. D1 activity is increased in hyperthyroidism and decreased in hypothyroidism. Its expression is most abundant in the kidney, liver, and thyroid but it is also expressed at a low level in other tissues (36–38).

D2 catalyzes only 5′D and its K_d for T4 is in the nanomolar range, approximately three orders of magnitude lower than that of D1 for T4. In contrast to D1, the substrate preference of D2 for T4 is greater than that for rT3 and its expression is increased in hypothyroidism. D2 activity is expressed at low levels in many tissues, but most abundantly in the pituitary, brain, and brown fat (BAT) (36–38).

A third deiodinase (type 3 deiodinase [D3]), first demonstrated in cultured monkey hepatocarcinoma cells (42), catalyzes only 5D and inactivates both T4 and T3. D3 activity is expressed at high levels in the placenta and pregnant uterus and at lower levels in fetal and neonatal tissues, in particular the brain. In nonpregnant adults, it is found predominantly in the brain and skin (36–38).

Evidence concerning the role of 5′D in peripheral tissues was also accumulating from studies *in vivo*. In 1979, Larsen *et al.* demonstrated that inhibition of intrapituitary 5′D activity prevented the acute suppression of thyrotropin release by T4 in hypothyroid rats, indicating that conversion to T3 is essential for the action of T4 in this tissue (43). The following year it was shown that T3 produced from T4 by 5′D supplies much of the endogenous T3 in rat brain, including that it was located on the nuclear TRs (44,45).

It was also found that T3 derived from T4 by 5′D is exchanged with the circulating T3 (46). [^{131}]rT3 was also found in brain tissue following injection of [^{131}I]T4, suggesting that D3 may play a role in TH economy in this organ (47). Further evidence for this concept was provided by Kaplan and Yaskoski (48) who measured 5′D and 5D in areas of rat brain, starting at birth. It was notable that in each area studied, 5D was highest at, or soon after, birth and then decreased, whereas 5′D was relatively low at birth and then increased markedly over the neonatal period, a time when considerable maturation of the brain occurs.

Additional evidence for the role of 5′D came from studies in BAT. In 1985, Silva and Larsen demonstrated that in rats exposed to cold, 5′D activity in BAT was enhanced and this was associated with an increase in both local and plasma T3 content (49). Local conversion of T4 to T3 by 5′D activity was found to be critical for thermogenesis in BAT (50). It was now clearly evident that in addition to the control of TH secretion by the thyroid gland, TH economy and presumably action are also regulated, at least in part, by both 5′D and 5D in peripheral tissues.

By the mid-1980s, it was widely accepted that a major role of D1 is to generate T3 for circulation, whereas that of D2 is to generate T3 from T4 for local use within the same cell or tissue. T3 generated by D2 can also contribute to the circulating T3 (49). However, the precise roles of each of the three deiodinases *in vivo*, particularly in tissues that express more than one deiodinase, remained to be established.

One approach to defining the role of a deiodinase is to study animal models rendered completely deficient in its activity. Unfortunately, no compound had been found that could make animals completely deficient in a single deiodinase. However, cloning of cDNAs for the three deiodinases provided a novel and more specific way to achieve this goal.

Cloning the Deiodinases

In 1991, Berry *et al.* (51) used expression cloning in the Xenopus oocyte to isolate a cDNA for D1 from a rat liver cDNA library. The kinetic properties of the protein expressed in transient assay systems, tissue distribution of its messenger RNA, and changes in its level with thyroid status confirmed its identity. They also found that the mRNA for D1 contains in its coding region a UGA codon that codes for selenocysteine, a rare amino acid essential for full activity of the enzyme; enzyme activity was markedly reduced when the selenocysteine is replaced by cysteine, which has sulfur instead of selenium (SEC) in its structure (52). Reduced D1 activity and abnormal TH metabolism in humans exhibiting a mutation in the SEC insertion sequence-binding protein 2 have been reported (53).

D3 was the next deiodinase to be cloned. The group of Dr. Donald D. Brown (Carnegie Institution for Science) isolated a cDNA from a *Xenopus laevis* tail tissue cDNA library, which exhibited some homology to the mammalian D1 cDNA. A collaboration with the laboratories of Galton and St Germain resulted in the finding that this cDNA coded for a deiodinase with properties characteristic of D3 (54). The Xenopus cDNA was used as a probe to isolate D3 cDNAs from other species.

D2 was also first cloned from amphibian tissue by Davey *et al.* (55) and then used as a probe to clone a D2 cDNA from rat tissues. D2 and D3 were also shown to have selenocysteine at their active sites.

Studies in Mice Rendered Deiodinase Deficient by Targeted Disruption of the Corresponding Gene

With the sequences of cDNAs for the deiodinases now available, Schneider *et al.* were able to create mice deficient in D2 (D2KO) (56), D1 (D1KO) (57), and both D1 and D2 (D1/D2KO) (58), and a D3KO mouse was created by Hernandez *et al* (59). Surprisingly, none of the three 5′D-deficient mouse models exhibited any appreciable gross phenotype. Growth was essentially normal, reproductive capacity was seemingly unimpaired, and the serum T3 level was normal in all three models. However, the serum T4 level was significantly elevated in D1KO and D2KO mice and by almost twofold in double D1/D2KO mice (56–58), and detailed studies in individual organs revealed many tissue-specific phenotypes, most notable in the D2-deficient mice. In these mice, the phenotype included pituitary resistance to TH (56,58), reduced tissue T3 content and altered gene expression in the brain (60), impaired hearing (61), impaired BAT thermogenesis (62), and impaired bone strength and mineralization (63).

Analysis of the D1KO phenotype revealed that serum rT3 was markedly elevated (57), an abnormality also noted in two patients who had a mutation in the *DIO*1 gene (64). It has been estimated that in humans, the T4 secreted by the thyroid generates rT3 and T3 in approximately equal amounts (65). These findings, together with the knowledge that rT3 is the preferred substrate for D1, suggest that a significant fraction of rT3 normally undergoes 5′D by D1, rather than being excreted as rT3 in the feces, a mechanism that would conserve iodine should conditions warrant it. Although the serum level of T4 was elevated, the levels of T3 and TSH were unchanged, as were several indices of peripheral thyroid status, suggesting that D1 is not essential for maintenance of a normal T3 level in this species (57). However, D1 deficiency resulted in a marked decrease in excretion of iodine derived from TH, whereas excretion of iodothyronines in feces was markedly increased (57).

The D3KO mouse exhibited a much more marked gross phenotype than those of D1 and D2KO mice, and a list of both general and tissue-specific phenotypic changes can be found in a recent review (66). They include growth retardation, impaired fertility, neonatal thyrotoxicosis, adult hypothyroidism, impaired brain development and function, and impaired hearing and vision (66,67). Unlike in the D1 and D2KO mice, D3KO mice exhibit considerable perinatal lethality. Since D3 is expressed at high levels in both the placenta and uterus, this is likely due, at least in part, to exposure of fetuses to toxic levels of TH derived from the mother (66,67).

A Major Role for TH Metabolism by D2 and D3 in the Regulation of Tissue T3 Content and Action

Information obtained from the D2 and D3-deficient mice, together with the known expression profiles of D2 and D3 activities in some tissues, has provided unequivocal evidence that both these enzymes play a major role in determining the intracellular T3 content and hence action in peripheral tissues. The coordinated interplay of these two enzyme activities appears to be critically important during development, as demonstrated by several groups, in particular those of Drs. Bianco, Forrest, and Hernandez.

Contributions in this regard are detailed and referenced in a recent review (66). In this review, the data obtained by Hernandez (59,66) on the maturing hypothalamic/pituitary/thyroid (HPT) axis are used as a representative example of this concept. In both the D2KO and D3KO mice, maturation of the HPT axis is impaired. Figure 3 shows the general profiles, relative to each other, of the circulating T4 (panel A) and T3 (panel B) levels in wild-type (WT), D2KO, and D3KO mice between birth and postpartum day 30 (P30). Panel C shows the profiles of D2 and D3 activities in the hypothalamus of WT mice during the same period. In rodents, maturation of the HPT axis, the time when it reaches its set point, occurs at approximately 2 weeks of life. It is at this age that the circulating T4 and T3 levels, which are relatively low at birth, reach their peaks. In the hypothalamus, D3 activity, which is highest shortly after birth, starts to decrease steadily during neonatal life, reaching a low plateau by P20. In contrast, hypothalamic D2 activity is low at birth, but increases steadily and its peak largely coincides with those of the serum TH levels (Fig. 3). These activity profiles indicate a scenario whereby in the first few days of life, intrahypothalamic T3 content is maintained at an appropriately low level by the presence of relatively high D3 activity and minimal D2 activity, until the T3-dependent maturation period approaches, when the level of D3 activity declines and that of D2 increases, changes that enable an increase in intrahypothalamic T3 content.

The importance of this delicate interplay of two enzymes becomes evident when either of them is absent. Thus, in the D3KO mouse, the serum T3 level is markedly elevated in the

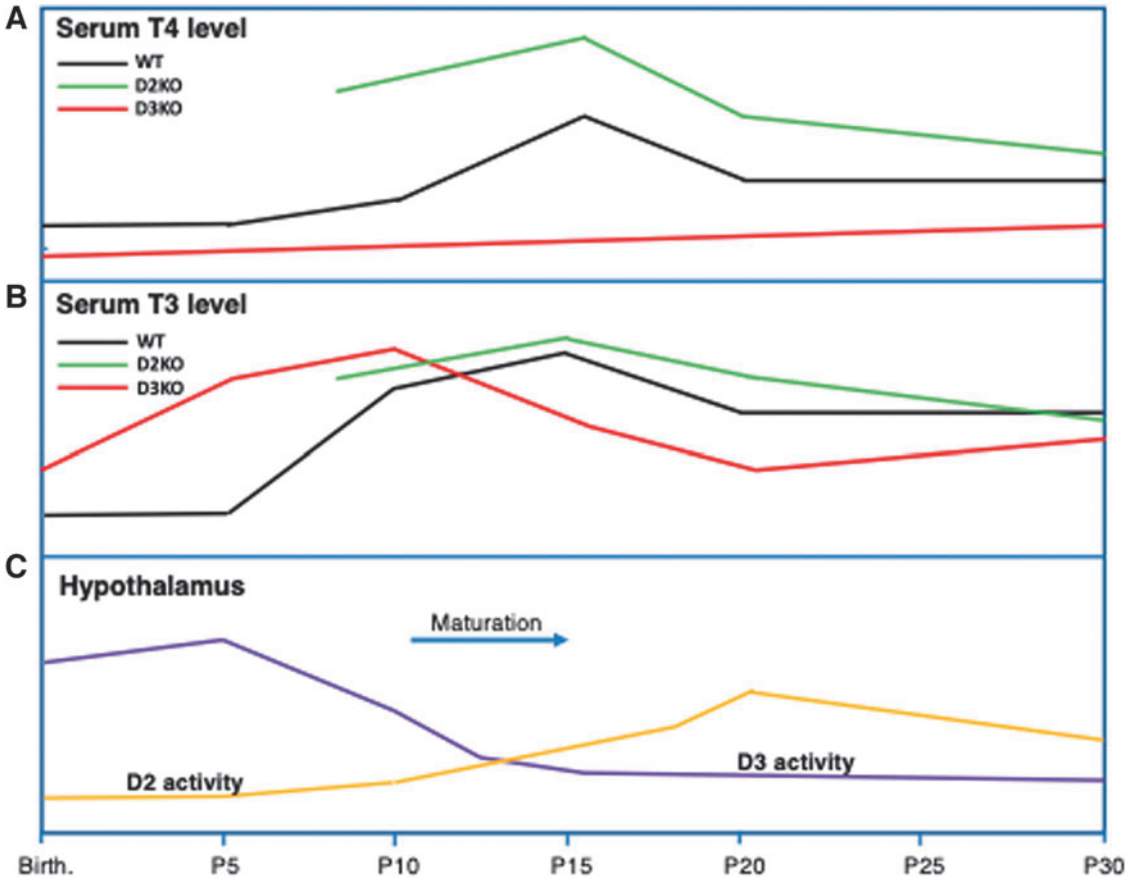

FIG. 3. Profiles of serum T4 levels **(A)** and T3 levels **(B)** in WT, D2KO, and D3KO mice between birth and P30. Profiles of D2 and D3 activities during the same period **(C)**. D2, type 2 deiodinase; D3, type 3 deiodinase; P30, postpartum day 30; WT, wild-type.

Table 1. Timeline of Advances That Have Contributed to Our Understanding of Thyroid Hormone Metabolism and Its Physiological Importance

Year	*Report*	*Reference*
1820	Iodine is shown to be important for normal function of the thyroid gland	(1)
1878	The syndrome called myxedema is noted to be associated with thyroid atrophy	(3)
1883	Thyroidectomy is shown to result in cachexia strumipriva, which resembled myxedema	(6)
1890	Symptoms of myxedema are shown to be alleviated by a graft of sheep thyroid	(8)
1896	Symptoms of myxedema ameliorated with an iodinated substance isolated from the thyroid	(10,11)
1914	An active compound, thyroxin, containing 65% iodine is purified from the thyroid	(12)
1926	The active compound is synthesized, its structure is determined, and it is renamed thyroxine	(13)
1947	[^{131}I] iodide derived by deiodination of injected [^{131}I]T4 is found in rat urine	(16)
1947	Significant radioactivity is found in the liver, bile, and GI tract after injection of [^{131}I]T4	(16)
1948	The main circulating iodinated compound is demonstrated to be T4	(14)
1952	Conjugate of T4 with GA is detected in bile	(17)
1952	The enterohepatic circulation of T4 is demonstrated	(19)
1952	T3 is identified in thyroid and plasma	(25)
1956	TH shown to undergo oxidative deamination to form acetic acid analogs *in vivo*	(22)
1960	Conjugate of T4 with sulfate is detected in plasma	(18)
1970	5′D of T4 to T3 is demonstrated in athyreotic humans	(31,32)
1972	TH nuclear receptors that have a higher affinity for T3 than T4 are discovered	(34)
1977	5D of TH is demonstrated in cultured monkey hepatocarcinoma cells	(41)
1979	First report indicating that 5′D of T4 is critical for its physiological action	(43)
1982	Evidence suggesting that there are two mechanisms for 5′D of TH is reported	(40)
1991	A cDNA for D1 is cloned, and the enzyme is shown to contain selenocysteine at its active site that is necessary for full enzyme activity	(51,52)
1994	A cDNA for D3 is cloned and the deiodinase shown to be a selenoprotein	(54)
1995	A cDNA for D2 is cloned and the deiodinase shown to be a selenoprotein	(55)
2001	A mouse completely deficient in D2 is created by targeted disruption of its gene	(56)
2002	Identification of human DIO2 SNP associated with impaired glucose metabolism	(71)
2004	3-Iodothyronamine is shown to be an endogenous active derivative of the thyroid hormone	(70)
2006	Mice completely deficient in D1 or D3 are created by targeted disruption of their genes	(57,59)
2021	Mutations in the human DIO1 gene associated with abnormal TH metabolism are reported	(66)

5′D, 5′-deiodination; 5D, 5-deiodination; D1, type 1 deiodinase; D2, type 2 deiodinase; D3, type 3 deiodinase; GA, glucuronic acid; GI, gastrointestinal; SNP, single-nucleotide polymorphism; T3, 3,5,3′-triiodothyronine; T4, thyroxine; TH, thyroid hormone.

first few days after birth and this results in elevation of brain T3 content and TH-responsive gene expression in the hypothalamus (59). The outcome of this T3 toxicity is that the set point of the HPT axis is impaired, as indicated by subsequent hypothyroidism. In the absence of D2 activity, hypothalamic T3 content is reduced and T4 content is elevated at P15, resulting in partial resistance of the HPT axis to the negative feedback effect of T4 (56,60). It is notable that a combined D3 and D2 deficiency results in partial rescue of the HPT axis impairment (68).

Current Conclusions and Unanswered Questions

To summarize the evolution of knowledge concerning TH metabolism, a timeline of the major discoveries is shown in Table 1. Perhaps the most important thing we have learned in the last 50 decades is that maintenance of a euthyroid state in humans and animals is dependent not only on regulation of the rate of secretion of TH from the thyroid gland but also on a delicate interplay among the various metabolic processes to which the hormones are subjected in peripheral tissues. These processes, together with specific TH transporters that facilitate the entry of hormones into cells (69), participate in ensuring that the appropriate amount of active hormone is maintained within a given tissue or cell. Thus, both D2 and D3 participate in regulating the intracellular content of the more active TH, T3, the former by generating it from T4 and the latter by converting both T4 and T3 to inactive metabolites.

It also appears that a major role for D1 is the 5′D of rT3, a process that serves to conserve hormonal iodine. However, there are still many questions left unanswered. We do not know the extent to which D1 is involved in converting T4 to T3 or to what extent the circulating T3 comprises T3 generated in peripheral tissues by D1 and D2 and T3 secreted by the thyroid.

Other outstanding questions include the physiological role and significance of sulfate conjugation of T4 and T3 as it affects their mode of deiodination; the role of the liver and the enterohepatic circulation in regulating circulating TH levels; the role of T4G that is stored in the liver and kidney, whether T4 has genomic actions that differ from those of T3; and to what extent other iodinated derivatives of T4 and T3, including tetrac, triac, 3,5-T2, and thyronamines (70), have physiological significance with respect to TH economy and biological effects.

Authors' Contributions

V.A.G. researched the literature and wrote the initial draft of the article. A.H. critiqued the draft and provided significant input to the last section, which comprised some of his work.

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References

1. Coindet JF 1820 Discovery of a new cure for goiter [Découverte d'un nouveau remède contre le goître]. Ann Chim Phys Paris **15:**49–59.
2. Gull WW 1874 On a cretinoid state supervening in adult life women. Trans Clin Soc Lond **7:**180–185.
3. Ord WM 1978 On myxoedema, a term proposed to be applied to an essential condition in the ''cretinoid'' affection occasionally observed in middle-aged women.'' Med Chir Trans **61:**57–78.
4. Reverdin J 1982 Accidents following total ablation goiter [Accidents consécutifs à l'ablation totale du goitre]. Rev Méd Suisse Romande **2:**539.
5. Reverdin JL, Reverdin A 1883 Note on twenty-two goiter operations [Note sur vingt-deux opérations de goitre]. Rev Med Sui Rom **3:**169–198, 233–278, 309–364.
6. Kocher T 1883 About goiter extirpation and its consequences [Ueber Kropfexstirpation und ihre Folgen]. Arch Klin Chir **29:**254–337.
7. Report of a committee of the Clinical Society of London nominated December 14th 1888, to investigate the subject of myxoedema. Trans Clin Soc Lond **21**(Supplement).
8. Bettencourt R, Serrano J-A A case of myxedema treated by the hypodermic transplant of the thyroid body of a sheep 1890 [Un cas de myxoedème traité par la greffe hypodermique du corps thyroïde d'un mouton 1890]. La Sem Méd **10:**294.
9. Murray GR 1891 Note on the treatment of myxoedema by hypodermic injections of an extract of thyroid gland of sheep. Brit Med J **1606:**796–797.
10. Baumann E 1896 About the normal occurrence of iodine in the animal body [Über das normale Vorkommen von Jod im Thierkörper]. Ztschr Phys Chem **21:**319–321.
11. Baumann E Über das Thyrojodin 1896. Munch Med Wschr **43:**309.
12. Kendall EC 1915 The isolation in crystalline form of the compound containing iodine which occurs in the thyroid gland. J Am Med Assoc **64:**2042–2043.
13. Harington CR 1926 Thyroxine: its biosynthesis and its immunochemistry. Proc R Soc Lond B **132:**223–238.
14. Taurog A, Chaikoff IL 1948 The nature of the circulating hormone. J Biol Chem **176:**639–656.
15. Laidlaw JC 1949 Nature of the circulating thyroid hormone. Nature **164:**927–928.
16. Gross J, Leblond CP 1947 Distribution of a large dose of thyroxine labeled with radioiodine in the organs of the rat. J Biol Chem **71:**309–320.
17. Taurog A, Briggs FN, Chaikoff IL 1952 I^{131}-labeled 1-thyroxine. II. Nature of the excretion product in bile. J Biol Chem **194:**655–688.
18. Michel R, de Gregorio, Lobo LC, Varrone S 1960 On the presence in rat plasma of the sulfoconjugate of thyroxin (ST4) after injection of low doses of radioactive iodine. C R Seances Soc Biol Fil **1654:**1153–1156.
19. Albert A, Keating FR 1952 The Role of the gastrointestinal tract, including the liver in the metabolism of radiothyroxine. Endocrinology **51:**427–443.
20. Galton VA 1969 The physiological role of thyroid hormone metabolism. In: James VHT (eds) Recent Advances in Endocrinology. Eighth edition. Churchill Press, England, pp 181–206.
21. Ingbar SH, Galton VA 1963 Thyroid. Ann Rev Physiol **25:** 361–380.
22. Jouan P, Michel R, Roche J, Wolf W 1956 The recovery of 3:5:3' -triiodothyroacetic acid and 3:3' -diiodothyronine from rat kidney after injection of 3:5:3' triiodothyronine. Endocrinology **59:**425–432.
23. Albright EC, Lardy HA, Larson FC, *et al.* 1957 Enzymatic conversion of thyroxine to tetraiodothyroacetic acid and of triiodothyronine to triiodothyroacetic acid. J Biol Chem **224:**387–397.
24. Galton VA, Pitt-Rivers R 1959 Thyroid hormone metabolism in the kidney. Biochem J **72:**314–318.
25. Gross J, Pitt-Rivers R 1952 Physiological activity of 3,5,3-triiodothyronine. Lancet **259:**593–594.
26. Pitt-Rivers R, Stanbury JB, Rapp B 1955 Conversion of thyroxine to 3,5,3 -triiodothyronine *in vivo*. J Clin Endocrinol Metab **15:**616–620.
27. Lassiter WE, Stanbury JB 1958 The *in vivo* conversion of thyroxine to 3,5/,3' -triiodothyronine. J Clin Endocrinol Metab **18:**903–906.
28. Albright EC, Larson FC, Tust RH 1954 *In vitro* conversion of thyroxine to triiodothyronine by kidney slices. Proc Soc Expt Biol Med **86:**37–140.
29. Albright EC, Larsen FC 1959 Metabolism of L-thyroxine by human tissue slices. J Clin Invest **38:**1899–1903.
30. Jones SL, van Middlesworth L 1960 Normal ^{131}I L-thyroxine metabolism in the presence of potassium perchlorate and interrupted by propylthiouracil. Endocrinology **67:**855–861.
31. Braverman LE, Ingbar SH, Sterling K 1970 Conversion of thyroxine to triiodothyronine in athyreotic human subjects. J Clin Invest **49:**55–864.
32. Sterling K, Brenner MA, Newman ES 1970 Conversion of thyroxine to triiodothyronine in normal human subjects. Science **169:**1099–1100.
33. Schwartz HL, Surks MI, Oppenheimer JH 1972 Quantitation of extrathyroidal conversion of l-thyroxine to 3,5,30-triiodo-L-thyronine in the rat. J Clin Invest **50:**1124–1130)
34. Oppenheimer JH, Koerner D, Schwartz HL, *et al.* 1972 Specific nuclear triiodothyronine binding sites in rat liver and kidney. J Clin Endocrinol Metab **35:**330–333.
35. Surks MI, Oppenheimer JH 1977 Concentration of L-thyroxine and L-triiodothyronine specifically bound to nuclear receptors in rat liver and kidney. J Clin Invest **60:**555–562.
36. Leonard JL, Visser TJ 1986 Biochemistry of deiodination. In: Hennemann G (ed) Thyroid Hormone Metabolism. Marcel Dekker, New York, pp 189–229.
37. Bianco AC, Salvatore D, Gereben B, *et al.* 2002 Biochemistry, cellular and molecular biology, and physiological roles of the Iodothyronine selenodeiodinases. Endo Rev **23:**38–89.
38. St Germain DL, Galton VA, Hernandez A 2009 Minireview: defining the roles of the Iodothyronine deiodinases: Current concepts and challenges. Endocrinology **150:**1097–1107.
39. Visser TJ, Van Der Does-Tobe I, Docter R, Hennemann G 1976 Subcellular localization of a rat liver enzyme converting thyroxine into triiodothyronine and possible involvement of essential thiol groups. Biochem J **157:**479–482.

40. Visser TJ, Leonard JL, Kaplan MM, *et al.* 1982 Kinetic evidence suggesting two mechanisms for iodothyronine 5'-deiodination in rat cerebral cortex. Proc Natl Acad Sci **79:** 5080–5084.
41. Wu S-Y, Green WL, Huang W-S, *et al.* 2005 Alternate pathways of thyroid hormone metabolism. Thyroid **15:**943–958.
42. Sorimachi K, Robbins J 1977 Metabolism of thyroid hormones by cultured monkey hepatocarcinoma cells: nonphenolic ring deiodination and sulfation. J Biol Chem **252:** 4458–4463.
43. Larsen PR, Dick TE, Markovitz BP, *et al.* 1979 Inhibition of intrapituitary thyroxine to 3.5.3'-triiodothyronine conversion prevents the acute suppression of thyrotropin release by thyroxine in hypothyroid rats. J Clin Invest **64:** 117–128.
44. Crantz FR, Larsen PR 1980 Rapid thyroxine to 3,5,3'-triiodothyronine conversion and nuclear 3,5,3'-triiodothyronine binding in rat cerebral cortex and cerebellum. J Clin Invest **65:**935–938.
45. Vigouroux E, Clos J, Legrand J 1979 Uptake and metablism of exogenous and endogenous thyroxine in the brain of young rats. Horm Metab Res **11:**228–232.
46. Obregon MJ, Roelfsema F, Morreale de Escobar G, *et al.* 1979 Exchange of triiodothyronine derived from thyroxine with circulating triiodothyronine as studied in the rat. Clin Endocrinol **10:**305–315.
47. Dratman MB, Crutchfield FL 1978 Synaptosomal (^{125}I)triiodothyronine after intravenous (^{125}I)thyroxine. Am J Physiol **235:**E638–E647.
48. Kaplan MM, Yaskoski KA 1981 Maturational Patterns of lodothyronine Phenolic and tyrosyl ring deiodinase activities in rat cerebrum, cerebellum, and hypothalamus. J Clin Invest **67:**1208–1214.
49. Silva JE, Larsen PR 1985 Potential of brown adipose tissue type II thyroxine 5'-deiodinase as a local and systemic source of triiodothyronine in rats. J Clin Invest **76:**2296–2305.
50. Bianco AC, Silva JE 1987 Intracellular conversion of thyroxine to triiodothyronine is required for optimal thermogenic function of brown adipose tissue. J Clin Invest **79:** 295–300.
51. Berry MJ, Banu L, Larsen PR 1991 Type I iodothyronine deiodinase is a selenocysteine-containing enzyme. Nature **349:**438–440.
52. Berry MJ, Kieffer JD, Harney JW, *et al.* 1991 Selenocysteine confers the biochemical properties characteristic of the type I iodothyronine deiodinase. J Biol Chem **266:** 14155–14158.
53. Dumitrescu AM, Liao XH, Abdullah MS, *et al.* 2005 Mutations in SECISBP2 result in abnormal thyroid hormone metabolism. Nat Genet **37:**1247–1252.
54. St Germain DL, Schwartzman R, Croteau W, *et al.* 1994 A thyroid hormone regulated gene in *Xenopus laevis* encodes a type III iodothyronine 5-deiodinase. Proc Natl Acad Sci **91:**7767–7771.
55. Davey JC, Becker KB, Schneider M J, *et al.* 1995 Cloning of a cDNA for the type II iodothyronine deiodinase. J Biol Chem **270:**26786–26789.
56. Schneider MJ, Fiering SN, Pallud SE, *et al.* 2001 Targeted disruption of the type 2 selenodeiodinase gene (DIO2) results in a phenotype of pituitary resistance to T4. Mol Endocrinol **15:**2137–2148.
57. Schneider MJ, Fiering SN, Thai B, *et al.* 2006 Targeted disruption of the type1 selenodeiodinase gene (Dio1) results in marked changes in thyroid hormone economy in mice. Endocrinology **147:**580–589.
58. Galton VA, Schneider MJ, Clark AS, *et al.* 2009 Life without thyroxine to 3,5,3'-triiodothyronine conversion: studies in mice devoid of the 5'-deiodinases. Endocrinology **150:**2957–2963.
59. Hernandez A, Martinez ME, Fiering S, *et al.* 2006 Type 3 deiodinase is critical for the maturation and function of the thyroid axis. J Clin Invest **116:**476–484.
60. Galton VA, Wood WT, St Germain EA, *et al.* 2007 Thyroid hormone homeostasis and action in the type 2 deiodinase-deficient rodent brain during development. Endocrinology **148:**3080–3088.
61. Ng L, Goodyear RJ, Woods CA, *et al.* 2004 Hearing loss and retarded cochlear development in mice lacking type 2 iodothyronine deiodinase. Proc Soc Natl Acad Sci **101:** 3474–3479.
62. de Jesus LA, Carvalho SD, Ribeiro MO, *et al.* 2001 The type 2 iodothyronine deiodinase is essential for adaptive thermogenesis in brown adipose tissue. J Clin Invest **108:** 1379–1385.
63. Bassett JH, Boyde A, Howell PG, *et al.* 2010 Optimal bone strength and mineralization requires the type 2 iodothyronine deiodinase in osteoblasts. Proc Natl Acad Sci **107:** 7604–7609.
64. Franca MM, German A, Fernandez GW, *et al.* 2021 Human type1 iodothyronine deiodinase (*DIO1*) mutations cause abnormal thyroid hormone metabolism. Thyroid **31:**202–207.
65. Engler D, Burger AG 1984 The deiodination of the iodothyronines and of their derivatives in man. Endocrine Rev **5:**151–184.
66. Hernandez A, Martinez ME, Ng L, *et al.* 2021 Thyroid hormone deiodinases: dynamic switches in developmental transitions. Endocrinology **108:**1–15.
67. Ng L, Hernandez A, He W 2009 A protective role for type 3 deiodinase, a thyroid hormone-inactivating enzyme, in cochlear development and auditory function. Endocrinology **150:**1952–1960.
68. Galton VA, de Waard E, Parlow AF, *et al.* 2014 Life without the iodothyronine deiodinases. Endocrinology **155:** 4081–4087.
69. Friesema EC, Jansen J, Milici C, *et al.* 2007 Thyroid hormone transporters. Vitam Horm **70:**137–167.
70. Scanlan TS, Suchland KL, Hart ME, *et al.* 2004 3-Iodothyronamine is an endogenous and rapid-acting derivative of thyroid hormone. Nat Med **10:**638–642.
71. Mentuccia D, Proietti-Pannunzi L, Tanner K, *et al.* 2002 Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance. Evidence of interaction with the Trp64Arg variant of the β-3-adrenergic receptor. Diabetes **51:**880–883.

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The Prevention of Iodine Deficiency: A History

Elizabeth N. Pearce[1] and Michael B. Zimmermann[2]

Iodine is an essential component of the hormones produced by the thyroid gland and is, therefore, essential for mammalian life. A landmark trial in the early 20th century definitively demonstrated that iodine supplementation could prevent what was then known as ''endemic goiter.'' Subsequent studies over the next decades demonstrated that iodine deficiency causes a spectrum of disease, including not just goiter, but also cretinism, intellectual impairment, and adverse obstetric outcomes. Salt iodization, first used in Switzerland and the United States in the1920s, has become the mainstay of iodine deficiency prevention efforts. The dramatic reduction in the global prevalence of iodine deficiency disorders (IDD) over the past 30 years represents an outstanding and under-recognized public health achievement. This narrative review provides an overview of critical scientific discoveries and advances in public health nutrition related to the prevention of IDD in the United States and worldwide. This review was written to commemorate the centennial of the founding of the American Thyroid Association.

Keywords: cretinism, endemic goiter, iodine deficiency, salt iodization

Introduction

Iodine is an essential component of thyroid hormones and is essential for mammalian life. This narrative review provides an overview of critical scientific discoveries and advances in public health nutrition related to the prevention of iodine deficiency disorders (IDD) in the United States and worldwide, with a focus on the past century (Fig. 1). This review was written to commemorate the centennial of the founding of the American Thyroid Association (ATA).

Discoveries in the 19th Century

In 1811, Courtois accidentally discovered iodine while producing saltpeter for gunpowder for Napoleon's army. He added sulfuric acid to burnt seaweed ash and produced a dark violet vapor that crystallized on cold surfaces. Gay-Lussac subsequently identified it as a new element and named it ''iodine,'' from the Greek for violet.[1] Coindet, a Geneva physician, learned of iodine's discovery and in 1813 suggested that the historical treatment of goiter with seaweed or sponges was effective because of their iodine content. He began giving oral iodine tincture (at a starting dose of 16.5 mg/day, which is enormously supraphysiological) to patients and observed shrinking of goiters with treatment.[2]

He also provoked some cases of iodine-induced thyrotoxicosis, which led to a backlash and persistent concerns that iodine treatment was inherently unsafe.[3] Boussingault, a French chemist working in South America, was the first to propose the use of iodine-containing salt to prevent goiter.[4] Chatin measured iodine in food and drinking water samples from across western Europe and concluded, in 1851, that inadequate drinking water iodine levels were the main cause of endemic goiter.[5] In response, French authorities began distributing iodine tablets and salt together with other prophylactic measures in three regions with high goiter rates. A survey of 5000 children from the region of Haute-Savoie found that this iodine treatment led to the shrinking or disappearance of 80% of goiters.[1]

By the late 1800s, scientists realized that cretinism only occurred in areas of endemic goiter but were perplexed by the fact that many patients with cretinism had an atrophic or absent thyroid gland, the opposite of goiter. In 1874, Gull was the first to describe thyroid atrophy in a myxedematous

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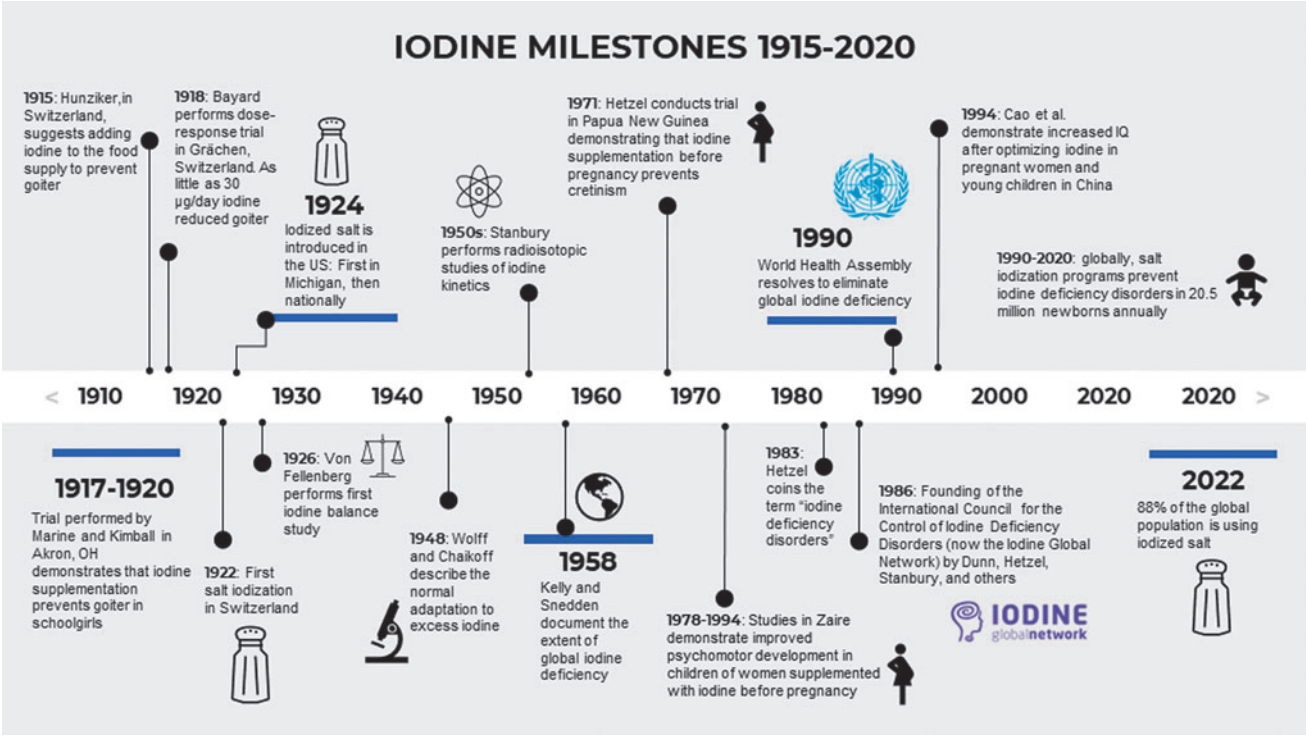

FIG. 1. A timeline of important milestones in understanding and combating the effects of iodine deficiency, 1915–2022.

patient.[6] Then, in 1878, the term ''myxedema'' was coined by Ord.[7] Myxedema resembled cretinism but was seen only in adults, usually women. It was characterized by slowness in thought and movement, facial swelling, and ''spade-like hands with skin resembling dry leather.'' Building on the work of Gull and Ord, in an 1882 address and subsequent 1883 article coauthored by his cousin, the Swiss surgeon Reverdin described the deleterious phenotype after total thyroidectomy as ''myxoedème opératoire.''[8,9]

In 1883, Swiss surgeon (and later Nobel laureate) Kocher described the results of 200 thyroidectomies (including 19 of the same cases reported by the Reverdins), terming post-thyroidectomy changes ''cachexia strumipriva,'' and noting the development of a phenotype similar to cretinism in a patient whose thyroid had been resected at age 11.[10] Semon, a British laryngologist, stated in 1883 that ''there appeared to be three conditions closely allied to each other, and having in common either absence or probably complete degeneration of the thyroid body: namely, cretinism, myxoedema, and the state after total removal of the thyroid body.''[11] In response, the Clinical Society of London established a committee that concluded, in 1888, that cretinism and myxedema were due to the ''the annihilation of the function of the thyroid body.''[12]

In the 1890s, Portuguese and British physicians began successfully treating myxedema with animal thyroid extracts.[13–15] In 1896, the link between goiter, myxedema, and iodine was established when Baumann and Roos in Germany analyzed animal thyroid glands and isolated a residual insoluble fraction that was ~10% iodine.[16] They described this compound, which was effective in treating both goiter and myxedema, as ''thyroiodine,'' and correctly postulated that the iodine had activity only when incorporated into an organic molecule.[17]

The History of Iodine Deficiency in the United States

A century ago, when the American Society for Goiter (later the ATA) was founded, endemic goiter was a substantial and highly visible public health problem in the United States. Examination of World War I military draftees demonstrated high goiter rates, particularly in the upper Northwest and Great Lakes regions, where up to 31% of candidates were disqualified from military service because their necks were too large to fit into a uniform.[18,19] Surveys carried out by the U.S. Public Health Service in the 1920s revealed goiter rates up to 70–100% in schoolchildren from parts of Minnesota, Michigan, and Wisconsin.[18] Starting in the 1920s, McClendon, a physiologist at the University of Minnesota, mapped iodine present in foods and drinking water in different regions across the United States, demonstrating that iodine intakes correlated inversely with goiter prevalence.[20]

Marine and Kimball

Marine developed an interest in goiter while training as a pathologist in Cleveland, Ohio, at the U.S. ''goiter belt'' epicenter. Starting in 1907 he did a series of experiments in dogs, pigs, cattle, and fish demonstrating that thyroidal iodine content varied inversely with the amount of hyperplasia.[21] Between 1917 and 1920, together with medical student Kimball, Marine conducted a landmark clinical trial in adolescent girls from Akron, Ohio (Fig. 2).[22] A total of 2190 girls who were treated with 200–400 mg sodium iodide taken in drinking water for 10 days twice annually were compared with 2305 girls who declined study participation. Goiter developed or worsened in only 0.2% of treated girls compared with 14% of controls. In a 1924 Harvey lecture at the New York Academy of Medicine, Marine recommended iodized salt as the best way to prevent both endemic goiter and cretinism at the population level.[23]

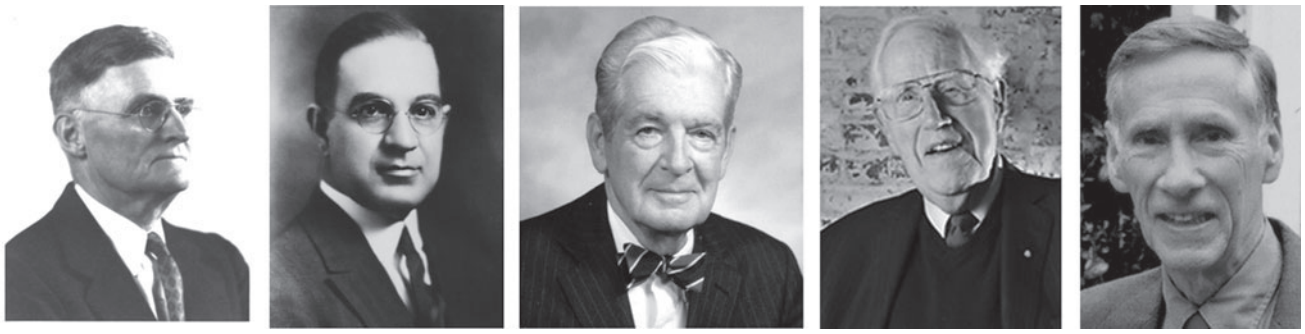

FIG. 2. Several key figures in global efforts to eliminate iodine deficiency disorders. From left to right: David Marine, Oliver Perry Kimball, John Stanbury, Sir Basil Hetzel, and John Dunn. (Photos of David Marine and Oliver P. Kimball courtesy of the National Library of Medicine; photo of Sir Basil Hetzel courtesy of the Iodine Global Network).

Salt Iodization in the United States: History and Effects

Marine noted that in the decade after his Akron trial, ''many kinds of iodine medication, including candies, tablets and chewing-gum, ran wild.''[21] However, ultimately it was iodized salt rather than iodized chewing gum that became the preferred intervention, largely due to the efforts of Cowie, a pediatrician and the chair of the Michigan State Medical Society. Salt is an excellent vehicle for iodine fortification because it is consumed by all population groups, most individuals ingest stable amounts of salt from day to day, and the salt iodization process is inexpensive and relatively simple to implement and monitor.[24]

With guidance from Marine and Kimball, Cowie spearheaded a remarkable effort to combat iodine deficiency goiter by convening stakeholders including physicians, salt producers, and grocers.[25] In 1923, Hale, a chemist at the Dow Chemical Company in Michigan, developed the methodology for mass iodized salt production, after which, in 1924, table salt iodized at 100 mg/kg was introduced in Michigan on a voluntary basis. Cowie's group developed statewide educational campaigns to create a market for the new iodized salt. These efforts initially met with vehement opposition.

Kimball later recalled that the U.S. Food and Drug Administration ''initially insisted that iodized salt cartons be labeled with a skull and crossbones because iodine is a poison'' and that ''the experiment was at first resisted by the goiter surgeons.''[26] However, in late 1924, the Morton Salt Company became the first U.S. producer to distribute iodized salt nationally. For the next two decades the Endemic Goiter Committee of the American Public Health Association (which included Marine and McClendon among its members) worked on public health education campaigns supporting salt iodization.[22] In 1948, the committee tried, without success, to pass U.S. legislation mandating iodized salt use. Although this effort failed in the United States it led to mandatory salt iodization in Canada.

An early effect of salt iodization was a transient rise in toxic nodular goiter incidence (similar to the thyrotoxicosis induced by Coindet's iodine treatments a century earlier). In 1926, Hartsock, a thyroid surgeon at the Cleveland Clinic and a leading opponent of salt iodization, reported cases of hyperthyroidism in men, which appeared to have been triggered by initiation of iodized salt use.[27] In 1934, McClure reported that although there had been a transient rise in goiter surgeries performed in Michigan hospitals for three years after salt iodization, there was a subsequent rapid 60% decline in goiter surgeries.[28]

It has been recently estimated that from 1925 to 1942 there may have been as many as 10,000 excess deaths in the United States due to iodine-induced hyperthyroidism.[29] However, salt iodization also clearly conferred major benefits. By 1951, when Brush and Altland performed a survey of Michigan schoolchildren, the prevalence of goiter had declined from 38.6% in 1924 to only 1.4%.[30] Using military data collected during World Wars I and II to compare the cognitive ability of cohorts born before and after salt iodization, it has been estimated that for the quarter of the U.S. population most deficient in iodine this intervention raised IQ by $\sim$15 points.[29]

The History of Salt Iodization in Switzerland

As early studies led to salt iodization in the United States, parallel efforts were taking place in Switzerland. The iodized salt program in Switzerland was established in 1922. Before its introduction, mountainous areas of Switzerland were affected by severe endemic goiter and cretinism. In 1800, a census of the Canton Valais ordered by Napoleon reported 4000 individuals with cretinism among 70,000 inhabitants.[1] In 1915, Hunziker, in Zurich, stated that goiter was an adaptation to low dietary iodine intake and suggested that small quantities of iodine added to the food supply would prevent the condition.[31] In 1918, a dose–response trial of iodine to treat goiter was done by Bayard in Grächen, a small village at the base of the famous Matterhorn mountain. For six months, Bayard gave iodized salt fortified at three different concentrations to families in the village.

He reported that as little as 30 μg/day of iodine reduced goiter and that diffuse goiters in children were more responsive than the nodular forms in adults.[32] von Fellenberg subsequently performed an iodine balance study that suggested that as little as 17 μg/day resulted in positive iodine balance.[33] Based on the data from Marine and Kimball, in 1919, Klinger recommended providing iodized salt for the general population and iodine supplements in Swiss schools.[1] The Swiss Goiter Committee was formed in 1922 in an advisory capacity to the Federal Office of Health. Iodized salt was introduced in the Appenzell region in 1922, thanks to the efforts of the surgeon Eggenberger, who successfully urged the local authorities to allow the sale of iodized salt. Within a few years after the introduction of iodized salt, newborn

goiter and most childhood goiter had disappeared, and no new infants had been born with cretinism.[34]

Scientific Developments from the 1940s Onward

In 1948, Wolff and Chaikoff, working at the University of California, Berkeley, first documented the homeostatic mechanism whereby a large dose of iodine transiently inhibits iodine organification in the thyroid gland.[35] Failure of the acute Wolff–Chaikoff effect may lead to iodine-induced hyperthyroidism (the Jod-Basedow phenomenon), the cause of the transient increase in rates of hyperthyroidism seen in the United States and elsewhere shortly after the initiation of salt iodization. More recent data from Denmark demonstrated that although there was a marked increase in the incidence of thyrotoxicosis after salt iodization, rates decreased to baseline by 7–8 years and after 16 years had decreased to 33% below baseline.[36]

Starting in the 1950s, Stanbury (the 1969 ATA president for whom the ATA's Stanbury Thyroid Physiology Medal is named) was an important contributor to the science of iodine metabolism as well as to iodine deficiency prevention efforts (Fig. 2). He worked first at Massachusetts General Hospital and later at the Massachusetts Institute of Technology. His interest in radioisotopic studies of iodine kinetics led to him to investigate patients with inherited forms of thyroid dysfunction, including familial iodotyrosine deiodinase deficiency (the first thyroid disorder linked to a specific enzyme defect).[37]

In 1952, he traveled to western Argentina to carry out the first collaborative international study of endemic goiter.[38] While working in Argentina, Stanbury used radioisotopic studies to demonstrate that there is increased thyroidal iodine avidity in iodine-deficient individuals and to confirm that the Jod-Basedow phenomenon in patients with long-standing iodine deficiency was caused by excess iodine.[39] His further studies in severely iodine-deficient populations in South America ultimately demonstrated that iodine deficiency impaired neurological development.

In 1952, the World Health Organization's Goiter Study Group (with Kimball as a member) developed a roadmap for global programs for the investigation and prevention of endemic goiter.[40] Subsequent to this effort, in 1958 Kelly and Snellen reviewed the world literature (>900 articles) and concluded that the world prevalence of endemic goiter at the time was close to 200 million (Fig. 2).[18]

In the late 1960s, Hetzel, an Australian physician, performed a landmark trial in an area of severe iodine deficiency in Papua New Guinea.[41] In this study, alternate families received saline or iodized oil injection. Iodine supplementation of pregnant women resulted in a 73% reduction in the prevalence of endemic cretinism at 4 years of age. In studies by Belgian investigators performed in Zaire in the 1970s,[42–44] pregnant women were randomized to receive iodized oil injection or a vitamin injection, and psychomotor development scores were measured in their children at about 6 years of age. There were significantly higher scores in the iodine group and far fewer children with low psychomotor scores.

In a study of iodized oil in area of severe iodine deficiency in China, participants were children from birth to 3 years and pregnant women.[45] Untreated children 1–3 years of age, who were studied when first seen, served as controls. The developmental quotient at 2 years of age was significantly higher in the offspring of the treated mothers and in the treated children, compared with the untreated children.[43] Several controlled studies of iodized oil given to pregnant women in the Andes led by Pretell and Stanbury showed similar benefits on the reduction of cretinism and goiter, and improved cognitive development in the offspring.[46–48]

In 1983, Hetzel coined the term ''iodine deficiency disorders.''[49] This terminology emphasized that iodine deficiency causes a spectrum of disorders, including decreased IQ with its profound effects on social and economic development, rather than just endemic goiter or cretinism (Fig. 2). Dunn (the recipient of the ATA's 1968 Van Meter prize and 1997 Paul Starr Award) traveled with his mentor, Stanbury, to South America in the 1960s and became interested in iodine deficiency. He went on to develop methods to assess population iodine status and to control the iodine content of salt, and he became active in the development of international collaborative efforts to eliminate iodine deficiency.[50]

The International Council for the Control of Iodine Deficiency Disorders (ICCIDD; now the Iodine Global Network),[51] an expert consultative group, held its inaugural meeting in 1986 in Katmandu, Nepal. Stanbury was the initial chair, Hetzel the executive director, and Dunn the Secretary (and later executive director). With funding from entities including the United Nations Children's Fund (UNICEF), the World Health Association (WHO), and U.S. Agency for International Development, ICCIDD provided technical assistance to national salt iodization programs. Largely because of advocacy from the ICCIDD and its partners, in 1990 the World Health Assembly established the elimination of IDD as a major public health goal for all countries.

Global Progress Toward Elimination of Iodine Deficiency

For the past three decades many iodized salt programs have been introduced to improve population iodine intakes. In 2022, 126 countries had legislation mandating salt iodization and another 21 had legislation allowing voluntary salt iodization.[52] UNICEF estimates that 88% of the global population currently uses iodized salt.[53] Iodine nutrition in populations is typically assessed by measuring urinary iodine concentrations (UIC) in representative cross-sectional national surveys. UIC is a good biomarker for iodine exposure because it reflects recent iodine intake from all sources (including foods such as seafood, dairy, and eggs as well as iodized salt). For the past 15 years, UIC surveys have been carried out in 152 out of 194 countries.

The number of countries with adequate iodine intake has increased from 67 in 2003 to 117 in 2022 (Fig. 3), reflecting the effectiveness of salt iodization. In contrast, excess iodine intakes have occurred in some previously iodine-deficient areas. Thirteen countries currently have excessive iodine intakes (median UIC >300 μg/L). Because a rapid increase in iodine intake may precipitate transient increase in hyperthyroidism (as seen in the United States in the 1920s), it is critical that iodized salt programs be regularly monitored to prevent both iodine deficiency and excess.[54] A modeling study recently estimated the impact of iodized salt programs: the global prevalence of clinical IDD (as assessed by the total goiter rate) fell from 13.1% to 3.2%, while IDD has been prevented in 20.5 million newborns annually.[55] The resulting

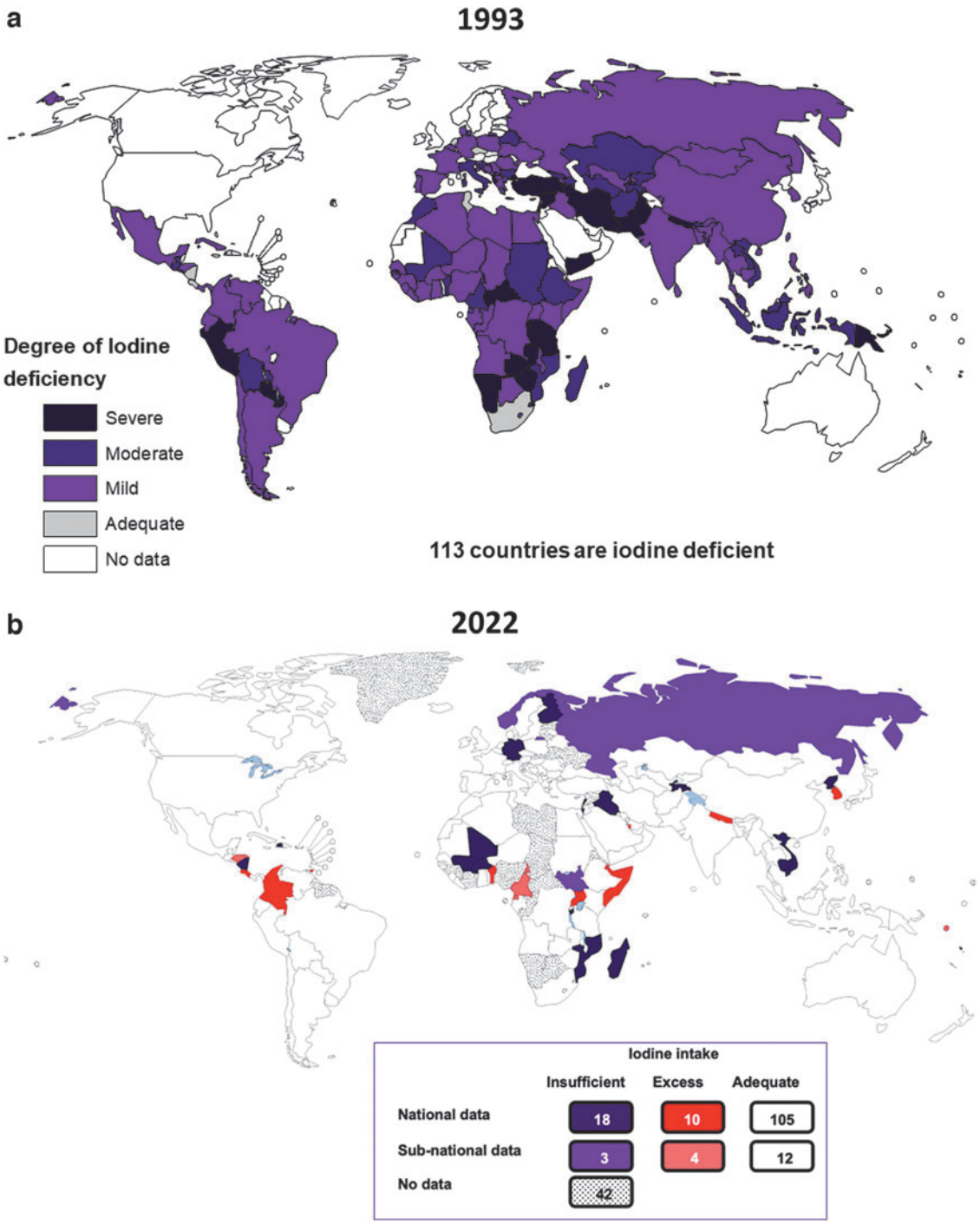

FIG. 3. Global iodine status in 1993 (**a**) and in 2022 (**b**). Data from the World Health Organization and Iodine Global Network. The 1993 data are derived from studies assessing goiter rates by palpation. The 2022 data are derived from iodine intake in the general population assessed by median urinary iodine concentration in school-age children studies conducted in 2005–2021.

improvement in cognitive development has likely provided a $33 billion global economic benefit.

Conclusions

Studies for the past century have demonstrated that iodine deficiency causes a spectrum of disease, including goiter, cretinism, intellectual impairment, and adverse obstetric outcomes. Salt iodization, first used in Switzerland and the United States in the 1920s, has become the mainstay of iodine deficiency prevention efforts. The dramatic global reduction in IDD over the past century represents an under-recognized public health achievement.

However, work remains to be done. In some regions there has been backsliding—once iodine sufficiency has been achieved resources are diverted to other public health priorities and gains are not sustained. Since the introduction of universal salt iodization in most countries, severe iodine

deficiency has been largely eradicated worldwide, but mild to moderate iodine deficiency remains prevalent, especially in pregnant women, who are at highest risk of adverse outcomes from IDD. Future studies are needed to understand how to sustain progress and to optimize iodine nutrition for the most vulnerable population groups.

Authors' Contributions

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References

1. Merke F. History and Iconography of Endemic Goitre and Cretinism [Discovery of a new cure for goiter]. Hans Huber: Berne; 1984.
2. Coindet JF. Découverte d'un nouveau remède contre le goître. Ann Chim Phys 1820;15(Ser. 2):49–59.
3. Zimmermann MB. Research on iodine deficiency and goiter in the 19th and early 20th centuries. J Nutr 2008; 138(11):2060–2063; doi: 10.1093/jn/138.11.2060
4. Boussingault JB. Recherches sur la cause qui produit le goître dans les Cordilieres de la Nouvelle-Grenade [Investigations into the cause of goiter in the Cordilleras of New Granada]. Ann Chim Phys 1833;48:41–69.
5. Chatin A. Freshwater iodine research; the presence of this body in plants and terrestrial animals [in French]. C R Acad Sci Paris 1851;31:280–283.
6. Gull WW. On a cretinoid state supervening in adult life in women. Trans Clin Soc London 1874;7:180–185.
7. Ord W. Clinical lecture on myxoedema. Br Med J 1878; 1(906):671–672.
8. Reverdin JL. Accidents following total goiter removal [in French]. Rev Méd Suisse Romande 1882;2:539–540.
9. Reverdin JL, Reverdin A. Note on twenty-two goiter operations [in French]. Rev Méd Suisse Romande 1883;3:309–364.
10. Kocher T. On goiter extirpation and its consequences [in German]. Archiv für Klinische Chirurgie 1883;29:254–337.
11. Semon F. In discussion of ''A typical case of myxoedema'' by FD Drewitt in the Proceedings of the Clinical Society of London. BMJ 1883(ii):1072–1074.
12. Ord WM. Report of a committee of the Clinical Society of London nominated December 14, 1883, to investigate the subject of myxoedema. Trans Clin Soc London 1888; 21(Suppl):1–215.
13. Bettencourt A-M, Serrano J-A. A case of myxedema treated by the hypodermic graft of the thyroid of a sheep [in French]. Sem Medicale 1890;10:294.
14. Murray GR. Note on the treatment of myxoedema by hypodermic injections of an extract of the thyroid gland of a sheep. Br Med J 1891;2(1606):796–797; doi: 10.1136/bmj.2.1606.796
15. Beadles CF. The treatment of myxoedema and cretinism, being a review of the treatment of these diseases with the thyroid gland, with a table of 100 published cases. J Ment Sci 1893;39:343–355.
16. Baumann F. On the normal occurrence of iodine in the animal body. Z Phys Chem 1896;21:319–330.
17. Roos E. Ueber die Wirkung des Thyrojodins On the action of thyroiodine [in German]. Z Phys Chem 1896;22:18–61.
18. Kelly FC, Snedden WW. Prevalence and geographical distribution of endemic goitre. Bull World Health Organ 1958;18(1–2):5–173.
19. Levin S. Discussion of goiters in 533 registrants. Mich State Med Soc 1919;18:98–104.
20. McClendon JF. The distribution of iodine with special reference to goiter. Physiol Rev 1927;7(2):189–258.
21. Marine D. Endemic goiter: A problem in preventive medicine. Ann Intern Med 1954;41(5):875–886; doi: 10.7326/0003-4819-41-5-875
22. Kimball OP, Marine D. The prevention of simple goiter in man. Second paper. 1918. Nutrition 1992;8(3):200–204.
23. Marine D. Etiology and Prevention of Simple Goiter. In: Harvey Lectures. Series 19. Lippincott: Philadelphia, PA; 1924; pp. 96–122.
24. World Health Organization. Guideline: Fortification of food-grade salt with iodine for the prevention and control of iodine deficiency disorders. World Health Organization: Geneva, Switzerland; 2014. Available from: http://apps.who.int/iris/bitstream/handle/10665/136908/9789241507929_eng.pdf?sequence=1 [Last accessed: September 3, 2022].
25. Markel H. ''When it rains it pours'': Endemic goiter, iodized salt, and David Murray Cowie, MD. Am J Public Health 1987;77(2):219–229; doi: 10.2105/ajph.77.2.219
26. Kimball OP. History of the prevention of endemic goitre. Bull World Health Organ 1953;9(2):241–248.
27. Hartsock CL. Iodized salt in the prevention of goiter: Is it safe for general use? JAMA 1926;18(18):1334–1338.
28. McClure RD. Thyroid surgery as affected by the generalized use of iodized salt in an endemic goitre region-preventive surgery. Ann Surg 1934;100(5):924–932; doi: 10.1097/00000658-193411000-00004
29. Feyrer J, Politi D, Weil DN. The cognitive effects of micronutrient deficiency: Evidence from salt iodization in the United States. J Eur Econ Assoc 2017;15(2):355–387; doi: 10.1093/jeea/jvw002
30. Brush BE, Altland JK. Goiter prevention with iodized salt: Results of a thirty-year study. J Clin Endocrinol Metab 1952;12(10):1380–1388; doi: 10.1210/jcem-12-10-1380
31. Hunziker H. Der Kropf: The goiter: an adaptation to dietary iodine deficiency [in German]. A. Franke Verlag: Bern, Switzerland; 1915.
32. Bayard O. Über das Kropfproblem [About the goiter problem]. Schweiz Med Wochenschr 1923;53:73–77.
33. von Fellenberg T. The occurrence, the cycle and the metabolism of iodine [in German]. Ergebn d Physiol 1926;25:310–314.
34. Eggenberger H. The prevention of goiter and goiter recurrence [in German]. Schweiz Med Wochenschr 1923;53: 245–249.
35. Wolff J, Chaikoff IL. The inhibitory action of excessive iodide upon the synthesis of diiodotyrosine and of thyroxine in the thyroid gland of the normal rat. Endocrinology 1948;43(3):174–179; doi: 10.1210/endo-43-3-174
36. Petersen M, Knudsen N, Carlé A, et al. Thyrotoxicosis after iodine fortification. A 21-year Danish population-based

study. Clin Endocrinol (Oxf) 2018;89(3):360–366; doi: 10.1111/cen.13751
37. Stanbury JB, Hedge AN. A study of a family of goitrous cretins. J Clin Endocrinol Metab 1950;10(11):1471–1484; doi: 10.1210/jcem-10-11-1471
38. Stanbury JB, Brownsell GL, Riggs DS, et al. Endemic Goiter. Harvard University Press: Cambridge, MA, USA; 1954.
39. Stanbury JB. Preliminary studies of iodine metabolism in patients from an area of endemic goitre. Bull World Health Organ 1953;9(2):183–196.
40. World Health Organization. Study-group on endemic goitre; final report. Bull World Health Organ 1953;9(2):293–301.
41. Pharoah PO, Buttfield IH, Hetzel BS. Neurological damage to the fetus resulting from severe iodine deficiency during pregnancy. Lancet 1971;1(7694):308–310; doi: 10.1016/s0140-6736(71)91040-3
42. Thilly CH, Delange F, Lagasse R, et al. Fetal hypothyroidism and maternal thyroid status in severe endemic goiter. J Clin Endocrinol Metab 1978;47(2):354–360; doi: 10.1210/jcem-47-2-354
43. Thilly CH, Lagasse R, Roger G, et al. Impaired fetal and postnatal development and high perinatal death rate in a severe iodine deficient area. Thyroid Research VIII. Proc 8th International Thyroid Congress, Australian Academy of Research: Sydney, Australia; 1980; pp. 20–23.
44. Thilly CH, Swennen B, Moreno-Reyes R, et al. Maternal, Fetal and Juvenile Hypothyroidism, Birthweight and Infant Mortality in the Etiopathogenesis of the IDD Spectrum in Zaire and Malawi. In: The Damaged Brain of Iodine Deficiency. (Stanbury JB. ed.) Cognizant Communication: New York, NY, USA; 1994; pp. 241–250.
45. Cao XY, Jiang XM, Dou ZH, et al. Timing of vulnerability of the brain to iodine deficiency in endemic cretinism. N Engl J Med 1994;331(26):1739–1744; doi: 10.1056/NEJM199412293312603
46. Pretell EA, Palacios P, Tello L, et al. Iodine Deficiency and the Maternal/Fetal Relationship. In: Endemic Goiter and Cretinism. Continuing Threats to World Health (Dunn JG, Medeiros-Neto GA. eds.) Pan American Health Organization Science Publication 292. WHO: Washington, DC, USA; 1974; pp. 143–155.
47. Fierro-Benitez R, Cazar R, Stanbury JB, et al. Effects on school children of prophylaxis of mothers with iodized oil in an area of iodine deficiency. J Endocrinol Invest 1988; 11(5):327–335; doi: 10.1007/BF03349050
48. Pretell EA, Caceres A. Impairment of Mental Development by Iodine Deficiency and Its Correction. A Retrospective View of Studies in Peru. In: The Damaged Brain of Iodine Deficiency. (Stanbury JB. ed.) Cognizant Communication: New York, NY, USA; 1994; pp. 187–191.
49. Hetzel BS. Iodine deficiency disorders (IDD) and their eradication. Lancet 1983;2(8359):1126–1129; doi: 10.1016/s0140-6736(83)90636-0
50. Thomas LM. John Thornton Dunn (1932–2004). In: Dictionary of Virginia Biography. Library of Virginia (1998–), Richmond, Virginia; 2016. Available from: http://www.lva.virginia.gov/public/dvb/bio.asp?b=Dunn_John_Thornton [Last accessed: July 17, 2022].
51. Iodine Global Network. Available from: https://www.ign.org/ [Last accessed: August 7, 2022].
52. Global fortification data exchange (GFDx), 2021. Available from: http://www.fortificationdata.org [Last accessed: July 29, 2022].
53. UNICEF. United Nations Children's Fund, Division of data, analysis, planning and monitoring. UNICEF Global Databases on Iodized salt: New York, USA; 2019. Available from: https://data.unicef.org/wp-content/uploads/2017/12/Global-Databases-on-Iodine.xlsx11 [Last accessed: July 29, 2022].
54. Zimmermann MB, Boelaert K. Iodine deficiency and thyroid disorders. Lancet Diabetes Endocrinol 2015;3(4):286–295; doi: 10.1016/S2213-8587(14)70225-6
55. Gorstein JL, Bagriansky J, Pearce EN, et al. Estimating the health and economic benefits of universal salt iodization programs to correct iodine deficiency disorders. Thyroid 2020;30(12):1802–1809; doi: 10.1089/thy.2019.0719

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Discoveries in Thyroid Autoimmunity in the Past Century

Sandra M. McLachlan and Basil Rapoport

This review on the 100th anniversary of the American Thyroid Association summarizes the remarkable progress attained during the past century regarding the pathogenesis and treatment of thyroid autoimmune diseases. Indeed, the general concept of autoimmune diseases in humans was established 70 years ago by thyroid investigators. Graves' disease is a paradigm for the rare occurrence of how autoimmunity can cause disease by stimulating rather than destroying an organ system. Therapeutic advances in the mid 20th century involving administration of thyroid hormones, thionamide drugs, and radioiodine have been hugely beneficial for human health. However, these approaches can only treat, but not cure, thyroid autoimmunity. Investigation of these diseases is facilitated by the identification of a limited number of specific autoantigens, whose molecular cloning has provided much information on their structure. This knowledge has led to highly sensitive and specific diagnostic tests, provided insight into novel aspects regarding the pathogenesis of thyroid autoimmunity, and has opened avenues for the development of new therapeutic agents. Immunotherapy for a *cure* as opposed to therapy of Graves' disease and Hashimoto's thyroiditis remains the holy grail for the 21st century.

Keywords: autoantibodies, Graves' disease, Hashimoto's thyroiditis, T cell and autoantibody epitopes, thyroglobulin, thyroid peroxidase

Introduction

In this year, 2023, the 100th anniversary of the American Thyroid Association, we review the remarkable progress attained during this period regarding the pathogenesis and treatment of thyroid autoimmune diseases. Advances in our knowledge have been made equally by members of our society and colleagues in many countries, often involving collaborations. Limited in length and number of citations, and covering 100 years, this review cannot be comprehensive. Our goal is to distill the essence of major, clearly established conceptual advances. Consequently, we cite reviews that readers may wish to explore, understanding that the authors of these reviews are, in many instances, not those to be credited for the discoveries (for which reason we state ''reviewed in''). Discoveries during the past century are not listed chronologically in the text but are listed in this order in Table 1.

Discovery of the Concept of Autoimmunity

Until well into the 20th century, the notion that the body was capable of attacking itself was considered extremely unlikely, hence the term ''horror autotoxicus'' coined by Paul Ehrlich in Germany. This concept, undermined by phenomena such as erythrocyte cold agglutinins, was finally put to rest by unequivocal evidence for thyroid autoimmunity reported in 1956. In this seminal year, sera from Hashimoto patients were shown to bind to thyroglobulin (Tg), a self, or auto, antigen.[1] Moreover, rabbits immunized with Tg developed antibodies to Tg, as expected, but also thyroid lymphocytic infiltrates as observed in patients with Hashimoto's thyroiditis.[2] Also in 1956, sera from Graves' patients injected into guinea pigs *in vivo* stimulated the release of radioactivity from thyroids prelabeled with radioiodine.

The prolonged release of radioactivity relative to thyrotropin (TSH) led to naming of the unidentified stimulator factor as ''Long Acting Thyroid Stimulator,'' or LATS.[3] Shortly thereafter, in 1959, antibodies in some Hashimoto patients were reported to recognize a thyroid antigen different from Tg, hence the term ''Thyroid Microsomal Antigen.''[4] In 1964, LATS was identified to be an autoantibody (IgG), a thyroid stimulating antibody (TSAb) (reviewed in Rapoport et al[5]), clinching the concept that Graves' disease, like Hashimoto's, was an autoimmune disease. The observation that transplacental transfer of TSAb and TSH receptor (TSHR) blocking autoantibodies (TBAb) could cause

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Table 1. Time Line of Selected Major Discoveries in Thyroid Autoimmunity

1927, 1953	Synthetic thyroxine and triiodothyronine and treatment of autoimmune-related hypothyroidism	[50,51]
1941	Radioiodine ablation for Graves' hyperthyroidism	Reviewed in Hertz[54]
1942	Thionamide drugs to treat Graves' hyperthyroidism	Reviewed in Burch and Cooper[52]
1956	Tg, an autoantigen in Hashimoto's disease	[1]
1956	Thyroiditis and anti-Tg in rabbits induced by immunization with Tg	[2]
1956	Long-acting thyroid stimulator	[3]
1959	Thyroid cytotoxic antibody in patient's sera	[4]
1960	Genetic predisposition to developing thyroid autoantibodies and thyroid autoimmune disease: HLA molecules (major histocompatibility complex class I, class II), cytotoxic T lymphocyte antigen 4, CD40, PTPN22, and CD25 (FoxP3); Tg variants, TSHR SNPs	Reviewed in Lee et al[29]
1964	Transplacental transfer of TSAb causing neonatal hyperthyroidism	[6]
1980	Transplacental transfer of TBAb causing neonatal hypothyroidism	[7]
1979–1988	Lymphocytes in patients' thyroid glands, but not lymph nodes or peripheral blood, spontaneously produce thyroid autoantibodies	Reviewed in Rees Smith et al[13]
1982	TSHR intramolecular cleavage into A and B subunits	[43]
1983	Aberrant human leucocyte antigen-DR isotype expression on thyroid cells in thyroid autoimmunity	[19]
1985	Cloning of human Tg	[38]
1986	TSHR synergism with the insulin-like growth factor-1 receptor	[71]
1987	Cloning of human TPO, identity with the thyroid microsomal antigen	[14]; reviewed in McLachlan and Rapoport[15]
1989–1990	Cloning of the TSHR	Reviewed in Rapoport et al[5]
1988–1991	Cloning T cells specific for TPO and Tg from diseased thyroids	[26–29]
1993	TSHR expression in orbital fibroblasts	Reviewed in Bahn[63]
1993–2000	Cloning repertoires of human monoclonal TPO and Tg autoantibody genes and their expression to define their epitopes	Reviewed in Rapoport and McLachlan[12]
1995–1999	NOD.H2h4 mice spontaneously develop thyroiditis and Tg antibodies, enhanced by iodide	Reviewed in Rapoport and McLachlan[12]
1996	First Graves' disease mouse model; mice injected with eukaryotic cells co-expressing the human TSHR and HLA	[55]
1998, 2002	Graves' disease mouse models generated by immunization with cDNA for the TSHR or its A subunit	Reviewed in Rapoport and McLachlan[12]
2003	Human monoclonal TSAb isolated from a Graves' patient	[11]
2004	Transgenic mouse expressing TPO-specific T cell receptor gene develops spontaneous Hashimoto's thyroiditis	[24]
2007	Crystal structure of portion of TSHR in complex with monoclonal TSAb defines epitope of latter	[41]
2011	Crystal structure of portion of TSHR in complex with monoclonal TBAb defines epitope of latter	[42]
2011, 2114	Intrathymic low-level TSHR expression is associated with particular TSHR SNPs and susceptibility to Graves' disease; dysregulation of thymic TSHR expression triggers Graves' disease	[30,31]
2015	Mouse model that spontaneously develops TSAb; NOD.H2h4 with the human TSHR A subunit targeted to the thyroid	[60]
2021	Thyroid autoimmunity enhanced by immune checkpoint inhibitors	Reviewed in Wright et al[35]
2022	Treatment for GO using monoclonal blocking TSHR antibody K1-70	[73]

GO, Graves' ophthalmopathy; HLA, human leucocyte antigen; SNP, single nucleotide polymorphism; TBAb, TSH receptor blocking autoantibodies; Tg, thyroglobulin; TPO, thyroid peroxidase; TSAb, thyroid stimulating antibody; TSH, thyrotropin; TSHR, TSH receptor.

neonatal hyperthyroidism[6] and hypothyroidism,[7] respectively, was an important milestone in confirming the humoral basis for Graves' disease.

Clinical Assays for Thyroid Autoantibodies

TSHR autoantibodies

Studying the action of TSH, in 1966, Pastan et al. were the first to suggest that polypeptide hormones exerted their effect by binding to a cell surface receptor.[8] Subsequently, in 1973, radiolabeled TSH binding to thyroid plasma membranes confirmed the existence of a TSHR.[9] Given the similar actions of TSH and LATS, it was intuitive that LATS, now called TSAb, also interacted with the TSHR, as subsequently demonstrated by Graves' IgG competing for radiolabeled TSH binding to this receptor.[10]

Replacement of *in vivo* animal bioassays for TSAb by detection of TSHR activation in cultured cells, initially thyrocytes then recombinant TSHR in non-thyroidal cells, as well as competition assays for TSAb binding to diverse TSHR preparations, represented major advances in the diagnosis and management of Graves' disease (reviewed in Rapoport et al[5]). Subsequently, over many years, improvements and refinements in these assays have increased their

specificity, sensitivity, and practicality facilitating automation. The most important advance followed the isolation by Sanders et al. of a human TSHR monoclonal TSAb[11] that permitted replacement of TSH with a more stable nonradioactive ligand in a binding assay.

The nomenclature of TSHR antibodies varies depending on the type of assay employed, and commercial competition has attempted to claim superiority for one over another (reviewed in Rapoport and McLachlan[12]). However, all assays that are equally sensitive and specific for Graves' hyperthyroidism are measuring TSAb (the patient is the bioassay) and, for simplicity, we will use this term. Bioassay of TSHR antibodies that block TSH action (reviewed in Rees Smith et al[13]) can be of value in hypothyroid patients. Whether ''neutral'' autoantibodies that neither activate nor block the TSHR play a role in human thyroid autoimmune disease remains an open question (reviewed in Rapoport and McLachlan[12]).

Thyroid peroxidase autoantibodies

The demonstration in 1985 that autoantibodies to the thyroid microsomal antigen interacted with purified human thyroid peroxidase (TPO) provided strong evidence that this unidentified antigen was TPO.[14] The molecular cloning of human TPO in 1987, facilitated by a report in 1986 of the nucleotide sequence of a large portion of porcine TPO, confirmed this identity (reviewed in McLachlan and Rapoport[15]). Because TPO is far less abundant than Tg and more difficult to purify from thyroid tissue, the subsequent generation of recombinant human TPO greatly facilitated the development of sensitive and specific assays for TPO autoantibodies (TPO-Ab) that are now in routine clinical use. An important issue is that human TPO-Ab are highly specific for human antigen, unlike the TSHR autoantibodies that interact well with porcine TSHR purified from readily available porcine thyroids.

Even before the availability of TPO-Ab assays, data obtained for antimicrosomal autoantibodies revealed the clinical importance of these autoantibodies in predicting the progression of Hashimoto's thyroiditis to subclinical, then clinical, hypothyroidism.[16] This conceptually vital finding provided the basis for innumerable subsequent clinical studies evaluating the relationship between TPO-Ab and autoimmune thyroiditis.

Humoral and Cell-Mediated Immunity in Autoimmune Thyroid Diseases

The availability of thyroid autoantigens, in particular recombinant human TSHR and TPO, in the 1990s led to a great burst of information regarding the interaction of these autoantigens with autoantibodies and T cells, leading to further discoveries regarding the pathogenesis and potential immunotherapy of autoimmune thyroid diseases, as discussed below. It is seldom appreciated that these thyroid autoantigens provide a great investigative advantage relative to the majority of other autoimmune diseases, which lack a single or limited number of unequivocal, specific autoantigenic targets for the immune system.

There have a number of fundamentally important immunological discoveries regarding the pathogenesis of thyroid autoimmunity including Hashimoto's thyroiditis in the past 25–30 years, which are as follows:

1. B cells (plasma cells) infiltrating Hashimoto and Graves' thyroids, but not in thyroid-draining lymph nodes or peripheral blood, spontaneously secrete microsomal (TPO) autoantibodies, Tg autoantibodies, and TSHR autoantibodies (reviewed in Rees Smith et al[13]). This insight led to the realization that cDNA libraries from Graves' thyroids would contain cDNA for thyroid-specific autoantibodies. Consequently, heavy (H) and light (L) chain genes for anti-TPO and anti-Tg autoantibodies of IgG class were isolated from H and L chain combinatorial cDNA libraries constructed from Graves' thyroids and their expression as recombinant Fab (reviewed in McLachlan and Rapoport[17]). Data obtained with these TPO and Tg autoantibody Fab (reviewed in Rapoport and McLachlan[12]) revealed the following:
   a. A limited number of H and L chain genes coded for the great majority of anti-TPO-Ab genes.
   b. The repertoires of TPO-Ab focused on two overlapping epitopes on a restricted region of the TPO molecule, that is, an immunodominant region, consistent with the previous observation of epitopic restriction with hybridoma-derived human TPO-Ab.[18] Similar observations for recognition of an immunodominant region were made for Tg autoantibodies.
   c. Recombinant TPO-Ab Fab competition for TPO-Ab in patients' sera permitted ''fingerprinting'' of TPO epitopes in an individual patient. Tg epitopes have been fingerprinted in the same way.
   d. TPO-Ab epitopic fingerprints in an individual were stable over many years despite variation of the titer and there was evidence for a familial pattern of epitopic inheritance.
2. Expression of major histocompatibility complex (MHC) class II molecules on the thyrocyte surface, most likely induced by cytokines particularly interferon-gamma. This groundbreaking discovery in the thyroid field by Bottazzo et al[19] had broad application to other autoimmune diseases by indicating that an autoimmune target tissue cell can function as a (nonprofessional) antigen-presenting cell, processing and presenting its own antigens to T cells.
3. B cells as antigen-presenting cells. For many years, with the exception of a few organ-specific autoimmune diseases caused directly by autoantibodies (e.g., Graves' disease, myasthenia gravis, and pemphigus vulgaris), in the great majority of autoimmune diseases, T cells were the dominant players, whereas B cells were the ''poor cousin,'' present but of no significance. The early observation that B cells, not only macrophages and dendritic cells, could function as ''professional'' antigen-presenting cells had little impact but is now receiving much attention in autoimmunity in general. The importance of this finding is that, unlike macrophages and dendritic cells that function as low-affinity ''vacuum cleaners'' sweeping up all antigens in the vicinity, B cells bearing IgG molecules on their surface function as high-affinity receptors for specific proteins. Following internalization of the complex, the bound antibody can influence which antigenic peptides (possibly cryptic) are

released by proteolysis and subsequently presented to T cells.[20,21] This phenomenon was first reported to be important in thyroid autoimmunity (reviewed in Quaratino et al[22]). Indeed, such antibody-mediated bias in T cell epitope presentation is a likely explanation for the finding mentioned above of TPO and Tg autoantibody recognition of immunodominant epitopic regions (reviewed in Rapoport and McLachlan[12]).

4. Although autoantibodies to both Tg and TPO are present in human disease, the latter predominate in clinical autoimmune thyroid diseases.[23] Whether these autoantibodies contribute to thyrocyte damage, are markers of thyroiditis, or simply occur in some normal individuals has been debated for many years. Early evidence for thyroid autoantibody cytotoxic effects, either complement mediated or as ''pathfinders'' for cytotoxic T cells, is now eclipsed by unequivocal data indicating that T cells in the absence of autoantibodies can be cytotoxic to thyrocytes. In a classic report in 2004, Quaratino et al. generated transgenic mice in which mouse T cells expressed a human T cell receptor cloned from intrathyroidal T cells from a patient with autoimmune thyroid disease with specificity for a cryptic TPO peptide epitope.[24] These mice spontaneously developed thyroiditis with all the clinical features of human Hashimoto's thyroiditis.
5. The cloning of antigen-specific T cells and their T cell receptors from thyroid-infiltrating T cells, with identification of their peptide epitope recognition (reviewed in Feldmann et al[25]). Much information has been obtained in humans and mice regarding Tg, TPO, and TSHR T cell epitopes and their presentation by MHC molecules (reviewed in Refs.[26–29]).
6. Central tolerance: Expression of low intrathymic levels of the TSHR, associated with particular TSHR single nucleotide polymorphisms, is associated with reduced central tolerance to the TSHR and susceptibility to developing Graves' disease.[30,31]
7. Peripheral tolerance: The appreciation that self antigens may escape induction of central tolerance in the thymus led to the concept of ''suppressor T cells'' maintaining peripheral tolerance, thereby preventing autoimmunity, as suggested with respect to the thyroid by Robert Volpe more than 30 years ago.[32] Suppressor T cells, initially met with skepticism, have now returned under a different name, ''regulatory T cells,'' or Treg.

There is now an expanding literature on Treg in thyroid autoimmunity, but primarily involving data on cells obtained from peripheral blood without evidence of thyroid antigen specificity. Clearly, a global abnormality in peripheral Treg could not be responsible for thyroid autoimmunity alone. Nevertheless, T cell reconstitution that follows therapeutic T cell depletion for a variety of conditions, or that occurs with HIV therapy, can lead to an autoimmune diathesis, very commonly Graves' disease, that may relate to a Treg imbalance (reviewed in McLachlan and Rapoport[33]). Furthermore, Treg depletion in an induced mouse model of Graves' disease suggested an important role for these cells in the progression of Graves' disease to autoimmune thyroiditis.[34]

8. Immune checkpoint inhibitors: Important insight into immune mechanisms contributing to thyroid autoimmunity has been derived from the recent use of drugs that remove immunological constraints for T cell-mediated immunotherapy of neoplasia in humans. The targets of these drugs include cytotoxic T lymphocyte antigen 4 (CTLA4), programmed cell death protein 1 (PD-1), and its ligand PD-L1 (reviewed in Wright et al[35]). Remarkably, in 1995, genetic screening of Graves' patients first reported the CTLA4 gene to be associated with this disease.[36]

Molecular Cloning of the Three Major Thyroid Autoantigens

These technical breakthroughs, although not in themselves representing conceptual advances, were critical tools for subsequent studies relating to thyroid autoimmunity (reviewed in Ludgate and Vassart).[37]

Thyroglobulin

The cloning of the cDNA for Tg in 1985 was the first molecular characterization of a thyroid autoantigen.[38] However, its great abundance and ready purification from thyroid glands, as well as its extremely large size, has limited the subsequent benefits of this molecular information in the study of thyroid autoimmunity, aside from the ability to design short synthetic peptides based on its primary amino acid sequence for investigation of T cell specificity (reviewed in Refs.[26,39]).

Thyroid peroxidase

As described above, the availability of recombinant human TPO following the molecular cloning of its cDNA in 1987 led to highly sensitive and specific clinical assays for TPO-Ab, as well as the molecular cloning and characterization of essentially the entire repertoire of H and L chains used in these autoantibodies, to our knowledge the first organ-specific autoimmune disease for which this has been accomplished.

TSH receptor

In 1989, the molecular cloning of the closely related luteinizing hormone receptor facilitated the design of nucleotide probes to screen thyroid cDNA libraries to isolate the cDNA for the dog[40] and the human TSHR (reviewed in Rapoport et al[5]). Molecular information on the TSHR, besides permitting improved clinical assays for TSHR autoantibodies (see section Clinical assays for thyroid autoantibodies, TSHR autoantibodies), has led to a number of conceptual advances regarding the pathogenesis of Graves' disease and studies exploring approaches to therapy of this disease. Crystallization of a portion (260 amino acids) of the 764 amino acid holoreceptor in complex with a human TSAb[41] and a human TBAb,[42] thereby defining their binding sites, was an advance of major importance.

Pathogenesis of Graves' Disease

Studies over four decades have provided conceptually fascinating clues regarding the pathogenesis of Graves' disease, the latter being unique among the organ-specific autoimmune diseases in that autoantibodies can activate its cognate receptor and cause glandular hyperfunction. Thus, although not explaining the loss of immune tolerance to the

TSHR, the structure of the TSHR itself contributes to the development of TSAb leading to hyperthyroidism.

The story began in 1985 with the observation by Rees Smith and colleagues that the TSHR expressed on the cell surface undergoes intramolecular cleavage into two subunits that remain linked by disulfide bonds: an extracellular A subunit and a largely intracellular B subunit.[43] In this process, a polypeptide segment of ~50 amino acids is lost, termed a ''C peptide'' because of similarity with the cleavage of proinsulin to insulin.[44] Linkage between the A and B subunits can be broken by ''maltreating'' membranes or cells by freeze-thawing[45] or hypotonic shock.[46]

However, under physiological conditions, A subunit shedding was deduced because TSAb (unlike TBAb) bound to the isolated TSHR extracellular region (ectodomain), whereas binding to the holoreceptor on the cell surface was sterically hindered.[47] Proof that the shed A subunit was the effective antigen inducing or amplifying TSHR generation in Graves' disease was provided by the observation that TSAb could only be induced in mice immunized with an adenovirus vector encoding cDNA for the isolated A subunit, unlike a holo-TSHR modified to be unable to cleave into subunits.[48] Indeed, this A subunit component of the TSHR is now in use by groups throughout the world in induced mouse models of Graves' disease (discussed further below).

These findings introduced two novel concepts. First, because of its unique structure, the TSHR is the culprit as well as the victim in the pathogenesis of Graves' disease. Indeed, unlike the TSHR, the closely related gonadotropin receptors do not cleave into subunits permitting ectodomain shedding, and there is no ''Graves'' disease of the gonads'. Second, the foregoing data provide insight into the mechanism by which TSAb activate the TSHR, thereby leading to hyperthyroidism. Thus, only the TSH holoreceptor, not the free A-subunit, can induce a signal. By overcoming partial steric hindrance to its binding, TSAb-mediated torsion in the ectodomain will lead to a shift in the seven membrane-spanning alpha-helices causing signal transduction. This conclusion is supported by data reported for the follicle stimulating hormone receptor.[49]

Clearly, additional factors are involved in the loss of immune tolerance to the TSHR. That Graves' disease commonly occurs in members of the same family (reviewed in Lee et al[29]) has led to very many studies that have identified genes that contribute to the development of Graves' disease and Hashimoto's thyroiditis, including those for the TSHR and Tg (reviewed in Lee et al[29]). Like many other autoimmune diseases, no single gene has been identified to be responsible for Graves' or Hashimoto's diseases. However, a number of immune modulatory genes appear to contribute to the development of disease, in addition to human leucocyte antigen molecules (MHC class I and class II), CTLA4, CD40, PTPN22, and CD25 (FoxP3) (reviewed in Lee et al[29]).

Therapy of Hashimoto's and Graves' Diseases

The past century has witnessed dramatic advances in the therapy of these diseases.

1. Synthetic thyroid hormones. Although used for conditions unassociated with thyroid autoimmunity, the availability of synthetic thyroxine[50] and triiodothyronine[51] has been among the most important advances in the past century for treatment of hypothyroidism in Hashimoto's thyroiditis and following thyroid ablative therapy in Graves' disease.
2. Thionamide antithyroid drugs for Graves' hyperthyroidism. The first clinical use of these drugs by Astwood in 1942 was a critical milestone in the therapy of this condition (reviewed in Burch and Cooper[52]). There is evidence that these drugs have an immunoregulatory effect in decreasing autoantibody levels.[53]
3. Radioiodine ablative therapy in Graves' disease, first used by Hertz in 1941, was another critical development in the history of thyroid autoimmunity (reviewed in Hertz[54]).
4. Immunotherapy. It must be emphasized that thionamide drugs and radioiodine can treat, but not cure, Graves' hyperthyroidism. The latter almost invariably leads to the onset of a secondary disease, hypothyroidism. A major goal for the next century will be the *cure* of Graves' disease, an approach that will require some form of immunotherapy to re-institute immune tolerance to the TSHR. Preliminary work to this end is ongoing in many laboratories and, unavoidably, requires an animal model of Graves' disease. Unfortunately, Graves' disease appear to be a uniquely human disease, and for many decades, attempts to induce this disease in sundry animal species by immunization with a variety of thyroid tissue preparations were fruitless.

In 1996, a milestone was the first success in this endeavor when Shimojo et al. reported the induction of Graves'-like disease in mice by immunization with fibroblasts transfected to co-express the TSHR and a class II MHC molecule.[55] However, a serious limitation of this model was its application only to mice strains with the same MHC molecule. This handicap was overcome by the first application by Costagliola et al. of genetic immunization of outbred mice with the cDNA for the human TSHR.[56] Although not successfully applied in other laboratories, this approach opened the door to more reproducible results using a variety of vectors, immunization techniques and, in particular (see section Pathogenesis of Graves, disease) to immunization with the cDNA for the A subunit rather than the TSH holoreceptor (reviewed in Rapoport and McLachlan[57]). In addition to the induction of hyperthyroidism, orbital changes (primarily histological) similar to those observed in human disease have been reported,[58] a potentially useful model in future studies.

In recent years, using the foregoing mouse model, there have been numerous attempts at therapy to prevent or reverse induced Graves' disease by injecting recombinant TSHR protein or TSHR-derived synthetic peptides. Although pretreatment with TSHR protein antigen can ameliorate the induction of TSAb and hyperthyroidism, injecting the antigen *after* disease induction is ineffective, ruling out the likelihood of success in treating human disease (reviewed in Rapoport and McLachlan[12]). Nevertheless, clinical trials are underway in humans using synthetic TSHR peptides with a presently uncertain outcome.[59]

In 2015, the first mouse model that *spontaneously* develops TSAb was reported, a more satisfactory animal in which to test immune therapeutical approaches to *cure* human Graves' disease.[60] This model was generated by crossing a transgenic

mouse with the *human* TSHR A subunit targeted to the thyroid with NOD.H2h4 mice that spontaneously develop Tg and TPO-Ab, but not TSAb. In this transgenic/NOD.H2h4 cross, TSAb do develop from loss of tolerance to the *human* TSHR but do not cross react with the *mouse* TSHR. That the mice remain euthyroid is an advantage for immune therapeutic investigation because thyroid hormones directly influence the immune system and variation in their levels would have a confounding effect in assessing experimental results. Moreover, the goal of immunotherapy in humans is to attenuate or eliminate TSAb generation, the direct cause of the disease.

Extrathyroidal Manifestations of Graves' Disease

There have been numerous conceptual and therapeutic advances regarding these distressing conditions. The advent of computerized tomography (CT) scanning in the 1970s supported earlier anatomical studies noting prominent orbital extraocular muscle thickening[61] on which basis these muscles were considered to be the target of the immune system. Later, CT and magnetic resonance imaging studies focused on increased volume in orbital adipose tissue and, subsequently, adipocytes became cells of greater interest in the pathogenesis of Graves' ophthalmopathy (GO). Studies in recent years have revealed orbital fibroblasts to be the most important cells in this process. In a voluminous literature, the concept emerged that orbital fibroblasts represented a distinct subgroup, including pre-adipocytes, and that cytokines secreted by thyroid-specific T cells were the proximal cause of the pathological features of GO, including hyaluronan generation and pre-adipocyte maturation (reviewed in Taylor et al[62]).

1. The revolutionary discovery in 1993 of TSHR expression on orbital fibroblasts, adipocytes, and marrow-derived fibrocytes, enhanced by sundry cytokines, introduced TSAb as an important pathogenetic factor in GO (reviewed in Bahn[63]). Evidence has since accumulated that TSAb, independent of T cells (other than receiving ''help'' for antibody production), are themselves sufficient for the characteristic pathological changes in the orbit. For example:
   a. GO and Graves' dermopathy are associated with particularly high TSAb levels.[64,65]
   b. Radioiodine therapy for Graves' hyperthyroidism increases TSAb levels and exacerbates GO.[66] In contrast, total thyroidectomy reduces TSAb levels[67] and ameliorates GO, with further benefit by thyroid remnant ablation by radioiodine (reviewed in Menconi et al[68]). Data for this conclusion are limited because of the infrequently used radical nature of this form of therapy.
   c. A TSAb monoclonal antibody increases hyaluronan production by cultured orbital fibroblasts, the former being a major component contributing to orbital edema.[69]
2. Therapy of GO. Numerous therapeutic agents have been used for this purpose in recent years, the most prominent being rituximab (anti CD20 on B cells) and teprotumumab (anti insulin-like growth factor [IGF] receptor) (reviewed in Bartalena et al[70]). The efficacy of the latter agent was anticipated because of the discovery in 1986 of synergy between IGF-1 and TSAb in activating the TSHR,[71] a phenomenon reproduced in terms of stimulation of hyaluronan production by cultured human orbital fibroblasts (reviewed in Neumann et al[72]). However, the use of a human monoclonal TSHR blocking antibody, presently in clinical trials,[73] is likely to be more specific and with fewer side effects than teprotumumab.
3. Local mechanical factors in the pathogenesis of GO and dermopathy. A paradox is that TSHR protein is expressed in human fibroblasts in many regions of the body in addition to those in the orbit and in areas of Graves' dermopathy.[74,75] Much evidence exists to provide an explanation for this paradox (reviewed in Rapoport and McLachlan[12]).

Conclusions

The past century has seen dramatic advances in knowledge regarding the pathogenesis and therapy of autoimmune thyroid diseases. Indeed, these diseases introduced the concept of autoimmunity in general. Graves' disease is a paradigm for the rare occurrence of how autoimmunity can cause disease by stimulating rather than destroying an organ system. Therapeutic advances in the mid 20th century involving administration of thyroid hormones, thionamide drugs, and radioiodine had a huge beneficial effect on human health.

More recent knowledge on autoimmune thyroid disease pathogenesis has led to new therapeutic agents being tested. However, the greater expense and risk of side effects of these approaches may lessen their competitiveness with earlier forms of therapy, with the possible exception of monoclonal antibody treatment for GO. Immunotherapy for a *cure*, a major goal for the 21st century, is unlikely to occur by targeting the immune system with agents that are not thyroid specific. Fortunately, investigation of thyroid autoimmune disease is facilitated by clearly defined antigenic targets.

Note added in proof: Since submission of this manuscript, three important studies have reported the cryo-electron microscopy structure of the TSH holoreceptor in complex with thyrotropin and TSAb,[76,77] as well as with a TBAb.[78]

Authors' Contributions

S.M.M. and B.R. are equally responsible for conceptualizing and writing the article.

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References

1. Campbell PN, Doniach D, Hudson RV, et al. Autoantibodies in Hashimoto's disease (lymphadenoid goitre). Lancet 1956;286(6947):820–821; doi: 10.1016/s0140-6736(56)92249-8
2. Rose NR, Witebsky E. Studies on organ specificity. V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. J Immun 1956; 76(6):417–427. PMID: 13332243.
3. Adams DD, Purves HD. Abnormal responses in the assay of thyrotropins. Proc Univ Otago Sch Med 1956;34:11–12. CRID: 1571135650165792384.

4. Belyavin G, Trotter WR. Investigations of thyroid antigens reacting with Hashimoto sera. Evidence for an antigen other than thyroglobulin. Lancet 1959;273(7074):648–652; doi: 10.1016/s0140-6736(59)92401-8
5. Rapoport B, Chazenbalk GD, Jaume JC, et al. The thyrotropin (TSH) receptor: Interaction with TSH and autoantibodies. Endocr Rev 1998;19(6):673–716; doi: 10.1210/edrv.19.6.0352
6. McKenzie JM. Neonatal Graves' disease. J Clin Endocrinol Metab 1964;24(7):660–668; doi: 10.1210/jcem-24-7-660
7. Matsuura N, Yamada Y, Nohara Y, et al. Familial neonatal transient hypothyroidism due to maternal TSH-binding inhibitor immunoglobulins. N Engl J Med 1980;303(13):738–741; doi: 10.1056/NEJM198009253031306
8. Pastan I, Roth J, Macchia V. Binding of hormone to tissue: The first step in polypeptide hormone action. Proc Natl Acad Sci U S A 1966;56(6):1802–1809; doi: 10.1073/pnas.56.6.1802
9. Amir SM, Carraway Jr TF, Kohn LD. The binding of thyrotropin to isolated bovine thyroid plasma membranes. J Biol Chem 1973;248(11):4092–4100. PMID: 4122527.
10. Rees Smith B, Hall R. Thyroid-stimulating immunoglobulins in Graves' disease. Lancet 1974;304(7878):427–431; doi: 10.1016/s0140-6736(74)91815-7
11. Sanders J, Evans M, Premawardhana LD, et al. Human monoclonal thyroid stimulating autoantibody. Lancet 2003; 362(9378):126–128; doi: 10.1016/s0140-6736(03)13866-4
12. Rapoport B, McLachlan SM. Reflections on thyroid autoimmunity: A personal overview from the past into the future. Horm Metab Res 2018;50(12):840–852; doi: 10.1055/a-0725-9297
13. Rees Smith B, McLachlan SM, Furmaniak J. Autoantibodies to the thyrotropin receptor. Endocr Rev 1988;9(1):106–121; doi: 10.1210/edrv-9-1-106
14. Czarnocka B, Ruf J, Ferrand M, et al. Purification of the human thyroid peroxidase and identification as the microsomal antigen in thyroid diseases. FEBS Lett 1985;190(1): 147–152; doi: 10.1016/0014-5793(85)80446-4
15. McLachlan SM, Rapoport B. The molecular biology of thyroid peroxidase: Cloning, expression and role as autoantigen in autoimmune thyroid disease. Endocr Rev 1992; 13(2):192–206; doi: 10.1210/edrv-13-2-192
16. Vanderpump MPJ, Tunbridge WMG, French JM, et al. The incidence of thyroid disorders in the community: A twenty-year follow-up of the Whickham survey. Clin Endocrinol 1995;43(1):55–68; doi: 10.1111/j.1365-2265.1995.tb01894.x
17. McLachlan SM, Rapoport B. Thyroid peroxidase as an autoantigen. Thyroid 2007;17(10):939–948; doi: 10.1089/thy.2007.0169
18. Czarnocka B, Pastuszko D, Carayon P, et al. Majority of thyroid peroxidase in patients with autoimmune thyroid disease are directed to a single TPO domain. Autoimmunity 1996;23(3):145–154; doi: 10.3109/08916939608995338
19. Bottazzo GF, Pujol-Borrell R, Hanafusa T, et al. Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. Lancet 1983;2(8359): 1115–1119; doi: 10.1016/s0140-6736(83)90629-3
20. Simitsek PD, Campbell DG, Lanzavecchia A, et al. Modulation of antigen processing by bound antibodies can boost or suppress class II major histocompatibility complex presentation of different T cell determinants. J Exp Med 1995; 181(6):1957–1963; doi: 10.1084/jem.181.6.195
21. Lanzavecchia A. How can cryptic epitopes trigger autoimmunity? J Exp Med 1995;181(6):1945–1948; doi:10.1084/jem.181.6.1945
22. Quaratino S, Osman M, Ruf J, et al. Human autoantibodies modulate the T cell epitope repertoire but fail to unmask a pathogenic cryptic epitope. J Immunol 2005;174(1):557–563; doi: 10.4049/jimmunol.174.1.557
23. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 2002;87(2):489–499; doi: 10.1210/jcem.87.2.8182
24. Quaratino S, Badami E, Pang YY, et al. Degenerate self-reactive human T-cell receptor causes spontaneous autoimmune disease in mice. Nat Med 2004;10(9):920–926; doi: 10.1038/nm1092
25. Feldmann M, Dayan C, Rapoport B, et al. T cell activation and antigen presentation in human thyroid autoimmunity. J Autoimmun 1992;5 (Supplement A):115–121; doi: 10.1016/0896-8411(92)90026-m
26. Kong YC, Morris GP, Brown NK, et al. Autoimmune thyroiditis: A model uniquely suited to probe regulatory T cell function. J Autoimmun 2009;33(3-4):239–246; doi: 10.1016/j.jaut.2009.09.004
27. Braley-Mullen H, Yu S. NOD.H-2h4 mice: An important and underutilized animal model of autoimmune thyroiditis and Sjogren's syndrome. Adv Immunol 2015;126:1–43; doi: 10.1016/bs.ai.2014.11.001
28. Fisfalen ME, Palmer EM, van Seventer GA, et al. Thyrotropin-receptor and thyroid peroxidase-specific T cell clones and their cytokine profile in autoimmune thyroid disease. J Clin Endocrinol Metab 1997;82(11):3655–3663; doi: 10.1210/jcem.82.11.4336
29. Lee HJ, Li CW, Hammerstad SS, et al. Immunogenetics of autoimmune thyroid diseases: A comprehensive review. J Autoimmun 2015;64(Nov):82–90; doi: 10.1016/j.jaut.2015.07.009
30. Colobran R, Armengol MP, Faner R, et al. Association of an SNP with intrathymic transcription of TSHR and Graves' disease: A role for defective thymic tolerance. Hum Mol Genet 2011;20(17):3415–3423; doi: 10.1093/hmg/ddr247
31. Stefan M, Wei C, Lombardi A, et al. Genetic-epigenetic dysregulation of thymic TSH receptor gene expression triggers thyroid autoimmunity. Proc Natl Acad Sci U S A 2014; 111(34):12562–12567; doi: 10.1073/pnas.1408821111
32. Volpe R. Immunoregulation in autoimmune thyroid disease. N Engl J Med 1987;316(1):44–45; doi: 10.1056/NEJM198701013160109
33. McLachlan SM, Rapoport B. Breaking tolerance to thyroid antigens: Changing concepts in thyroid autoimmunity. Endocr Rev 2014;35(1):59–105; doi: 10.1210/er.2013-1055
34. McLachlan SM, Nagayama Y, Pichurin PN, et al. The link between Graves' disease and Hashimoto's thyroiditis: A role for regulatory T cells. Endocrinology 2007;148(12):5724–5733; doi: 10.1210/en.2007-1024
35. Wright JJ, Powers AC, Johnson DB. Endocrine toxicities of immune checkpoint inhibitors. Nat Rev Endocrinol 2021; 17(7):389–399; doi: 10.1038/s41574-021-00484-3
36. Yanagawa T, Hidaka Y, Guimaraes V, et al. CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. J Clin Endocrinol Metab 1995;80(1): 41–45; doi: 10.1210/jcem.80.1.7829637
37. Ludgate M, Vassart G. The molecular genetics of three thyroid autoantigens: Thyroglobulin, thyroid peroxidase and the thyrotropin receptor. Autoimmunity 1990;7(2-3):201–211; doi: 10.3109/08916939008993392

38. Mercken L, Simons M, Swillens S, et al. Primary structure of bovine thyroglobulin deduced from the sequence of its 8,431-base complementary DNA. Nature 1985;316(6029): 647–651; doi: 10.1038/316647a0
39. Jacobson EM, Yang H, Menconi F, et al. Employing a recombinant HLA-DR3 expression system to dissect major histocompatibility complex II-thyroglobulin peptide dynamism: A genetic, biochemical, and reverse immunological perspective. J Biol Chem 2009;284(49):34231–34243; doi: 10.1074/jbc.M109.041574
40. Parmentier M, Libert F, Maenhaut C, et al. Molecular cloning of the thyrotropin receptor. Science 1989;246(4937):1620–1622; doi: 10.1126/science.2556796
41. Sanders J, Chirgadze DY, Sanders P, et al. Crystal structure of the TSH receptor in complex with a thyroid-stimulating autoantibody. Thyroid 2007;17(5):395–410; doi: 10.1089/thy.2007.0034
42. Sanders P, Young S, Sanders J, et al. Crystal structure of the TSH receptor (TSHR) bound to a blocking-type TSHR autoantibody. J Mol Endocrinol 2011;46(2):81–99; doi: 10.1530/JME-10-0127
43. Buckland PR, Rickards CR, Howells RD, et al. Photoaffinity labelling of the thyrotropin receptor. FEBS Lett 1982;145(2):245–249; doi: 10.1016/0014-5793(82)80176-2
44. Chazenbalk GD, Tanaka K, Nagayama Y, et al. Evidence that the thyrotropin receptor ectodomain contains not one, but two, cleavage sites. Endocrinology 1997;138(7):2893–2899; doi: 10.1210/endo.138.7.5259
45. Davies Jones E, Rees Smith B. A water-soluble fragment of the thyroid-stimulating hormone receptor which binds both thyroid-stimulating hormone and thyroid-stimulating hormone receptor antibodies. J Endocrinol 1984;100(1):113–118; doi: 10.1677/joe.0.1000113
46. Misrahi M, Milgrom E. Cleavage and shedding of the TSH receptor. Eur J Endocrinol 1997;137(6):599–602; doi: 10.1530/eje.0.1370599
47. Chazenbalk GD, Pichurin P, Chen CR, et al. Thyroid-stimulating autoantibodies in Graves disease preferentially recognize the free A subunit, not the thyrotropin holoreceptor. J Clin Invest 2002;110(2):209–217; doi: 10.1530/eje.0.1370599
48. Chen C-R, Pichurin P, Nagayama Y, et al. The thyrotropin receptor autoantigen in Graves' disease is the culprit as well as the victim. J Clin Invest 2003;111(12):1897–1904; doi: 10.1172/JCI17069
49. Jiang X, Fischer D, Chen X, et al. Evidence for follicle-stimulating hormone receptor as a functional trimer. J Biol Chem 2014;289(20):14273–14282; doi: 10.1074/jbc.M114.549592
50. Harington CR, Barger G. Chemistry of thyroxine: Constitution and synthesis of thyroxine. Biochem J 1927;21(1): 169–183; doi: 10.1042/bj0210169
51. Gross J, Pitt-Rivers R. 3:5:3' -triiodothyronine. 1. Isolation from thyroid gland and synthesis. Biochem J 1953;53(4): 645–650; doi: 10.1042/bj0530645
52. Burch HB, Cooper DS. Anniversary Review: Antithyroid drug therapy: 70 years later. Eur J Endocrinol 2018;179(5): R261–R274; doi: 10.1530/EJE-18-0678
53. McGregor AM, Petersen MM, McLachlan SM, et al. Carbimazole and the autoimmune response in Graves' disease. N Engl J Med 1980;303(7):302–307; doi: 10.1056/NEJM198008073030603
54. Hertz B. A tribute to Dr. Saul Hertz: The discovery of the medical uses of radioiodine. World J Nucl Med 2019;18(1): 8–12; doi: 10.4103/wjnm.WJNM_107_18
55. Shimojo N, Kohno Y, Yamaguchi K-I, et al. Induction of Graves-like disease in mice by immunization with fibroblasts transfected with the thyrotropin repector and a class II molecule. Proc Natl Acad Sci U S A 1996;93(20):11074–11079; doi: 10.1073/pnas.93.20.11074
56. Costagliola S, Many MC, Denef JF, et al. Genetic immunization of outbred mice with thyrotropin receptor cDNA provides a model of Graves' disease. J Clin Invest 2000; 105(6):803–811; doi: 10.1172/JCI7665
57. Rapoport B, McLachlan SM. TSH receptor cleavage into subunits and shedding of the A-subunit; a molecular and clinical perspective. Endocr Rev 2016;37(2):114–134; doi: 10.1210/er.2015-1098
58. Moshkelgosha S, So PW, Deasy N, et al. Cutting edge: Retrobulbar inflammation, adipogenesis, and acute orbital congestion in a preclinical female mouse model of Graves' orbitopathy induced by thyrotropin receptor plasmid-in vivo electroporation. Endocrinology 2013; 154(9):3008–3015; doi: 10.1210/en.2013-1576
59. Lane LC, Cheetham TD, Perros P, et al. New therapeutic horizons for Graves' hyperthyroidism. Endocr Rev 2020; 41(6):873–884; doi: 10.1210/endrev/bnaa022
60. Rapoport B, Aliesky HA, Banuelos B, et al. A unique mouse strain that develops spontaneous, iodine-accelerated, pathogenic antibodies to the human thyrotrophin receptor. J Immunol 2015;194(9):4154–4161; doi: 10.4049/jimmunol.1500126
61. Enzmann DR, Donaldson SS, Kriss JP. Appearance of Graves' Disease on orbital computed tomography. J Comput Assist Tomogr 1979;3(6):815–819. PMID: 583152.
62. Taylor PN, Zhang L, Lee RWJ, et al. New insights into the pathogenesis and nonsurgical management of Graves orbitopathy. Nat Rev Endocrinol 2020;16(2):104–116; doi: 10.1038/s41574-019-0305-4
63. Bahn RS. Current Insights into the Pathogenesis of Graves' ophthalmopathy. Horm Metab Res 2015;47(10):773–778; doi: 10.1055/s-0035-1555762
64. Gerding MN, van der Meer JW, Broenink M, et al. Association of thyrotrophin receptor antibodies with the clinical features of Graves' ophthalmopathy. Clin Endocrinol (Oxf) 2000;52(3):267–271; doi: 10.1046/j.1365-2265.2000.00959.x
65. Lan C, Hu L, Liao C, et al. Thyroid-stimulating hormone receptor autoimmunity and local factors in multiple risk factors are mainly involved in the occurrence of pretibial myxedema. J Clin Med Res 2020;12(11):711–723; doi: 10.14740/jocmr4352
66. Traisk F, Tallstedt L, Abraham-Nordling M, et al. Thyroid-associated ophthalmopathy after treatment for Graves' hyperthyroidism with antithyroid drugs or iodine-131. J Clin Endocrinol Metab 2009;94(10):3700–3707; doi: 10.1210/jc.2009-0747
67. Kim J, Choi MS, Park J, et al. Changes in thyrotropin receptor antibody levels following total thyroidectomy or radioiodine therapy in patients with refractory Graves' disease. Thyroid 2021;31(8):1264–1271; doi: 10.1089/thy.2020.0756
68. Menconi F, Marino M, Pinchera A, et al. Effects of total thyroid ablation versus near-total thyroidectomy alone on mild to moderate Graves' orbitopathy treated with intravenous glucocorticoids. J Clin Endocrinol Metab 2007; 92(5):1653–1658; doi: 10.1210/jc.2006-1800
69. Krieger CC, Place RF, Bevilacqua C, et al. TSH/IGF-1 receptor cross talk in Graves' ophthalmopathy pathogenesis. J Clin Endocrinol Metab 2016;101(6):2340–2347; doi: 10.1210/jc.2016-1315

70. Bartalena L, Kahaly GJ, Baldeschi L, et al. The 2021 European Group on Graves' orbitopathy (EUGOGO) clinical practice guidelines for the medical management of Graves' orbitopathy. Eur J Endocrinol 2021;185(4):G43-G67; doi: 10.1530/EJE-21-0479
71. Tramontano D, Cushing GW, Moses AC, et al. Insulin-like growth factor-I stimulates the growth of rat thyroid cells in culture and synergizes the stimulation of DNA synthesis induced by TSH and Graves'-IgG. Endocrinology 1986; 119(2):940–942; doi: 10.1210/endo-119-2-940
72. Neumann S, Krieger CC, Gershengorn MC. Targeting TSH and IGF-1 receptors to treat thyroid eye disease. Eur Thyroid J 2020;9(Suppl 1):59–65; doi: 10.1159/000511538
73. Furmaniak J, Sanders J, Sanders P, et al. TSH receptor specific monoclonal autoantibody K1-70(TM) targeting of the TSH receptor in subjects with Graves' disease and Graves' orbitopathy—Results from a phase I clinical trial. Clin Endocrinol (Oxf) 2022;96(6):878–887; doi: 10.1111/cen.14681
74. Rapoport B, Alsabeh R, Aftergood D, et al. Elephantiasic pretibial myxedema: Insight into and a hypothesis regarding the pathogenesis of the extrathyroidal manifestations of Graves' disease. Thyroid 2000;10(8):685–692; doi: 10.1089/10507250050137761
75. Cianfarani F, Baldini E, Cavalli A, et al. TSH receptor and thyroid-specific gene expression in human skin. J Invest Dermatol 2010;130(1):93–101; doi: 10.1038/jid.2009.180
76. Faust B, Billesbolle CB, Suomivuori CM, et al. Autoantibody mimicry of hormone action at the thyrotropin receptor. Nature 2022;609(7928):846–853; doi: 10.1038/s41586-022-05159-1
77. Duan J, Xu P, Luan X, et al. Hormone- and antibody-mediated activation of the thyrotropin receptor. Nature 2022; 609(7928):854–859; doi: 10.1038/s41586-022-05173-3
78. Nunez Miguel R, Sanders P, Allen L, et al. Structure of full length TSH receptor in complex with a blocking autoantibody K1-70. J Mol Endocrinol 2022; [Epub ahead of print]; doi: 10.1530/JME-22-0120

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Laboratory Thyroid Tests: A Historical Perspective

Carole Ann Spencer

Background: This review presents a timeline showing how technical advances made over the last seven decades have impacted the development of laboratory thyroid tests.
Summary: Thyroid tests have evolved from time-consuming manual procedures using isotopically labeled iodine as signals (^{131}I and later ^{125}I) performed in nuclear medicine laboratories, to automated nonisotopic tests performed on multianalyte instruments in routine clinical chemistry laboratories. The development of isotopic radioimmunoassay techniques around 1960, followed by the advent of monoclonal antibody technology in the mid-1970s, led to the development of a nonisotopic immunometric assay methodology that forms the backbone of present-day thyroid testing. This review discusses the development of methods for measuring total thyroxine and triiodothyronine, direct and indirect free thyroid hormone measurements and estimates (free thyroxine and free triiodothyronine), thyrotropin (TSH), thyroid autoantibodies (thyroperoxidase, thyroglobulin [Tg] and TSH receptor autoantibodies), and Tg protein. Despite progressive improvements made in sensitivity and specificity, current thyroid tests remain limited by between-method differences in the numeric values they report, as well as nonspecific interferences with test reagents and interferences from analyte autoantibodies.
Conclusions: Thyroid disease affects ~10% of the U.S. population and is mostly managed on an outpatient basis, generating 60% of endocrine laboratory tests. In future, it is hoped that interferences will be eliminated, and the standardization/harmonization of tests will facilitate the establishment of universal test reference ranges.

Keywords: antibodies and thyroglobulin, thyroid hormones, thyrotropin

Introduction

Figure 1 shows the milestones marking the history of laboratory thyroid testing over the last 70 years, from the early manual isotopic procedures to current automated tests made on high-throughput nonisotopic analyzers. The current high volume of thyroid testing reflects the high prevalence (>10%) of thyroid disorders in the U.S. population.[1] Over 75% of tests ordered are for thyrotropin (TSH), either with (~25%) or without (~50%) a free thyroxine (fT4) test, resulting in an annual cost approximating $1.6 billion dollars.[2,3]

Thyroid Hormone Measurements

Figure 2 shows the complexity of thyroid hormone metabolism, whereby the thyroid gland is the source of all circulating T4 but only ~20% of triiodothyronine (T3). T3 has the highest avidity for thyroid nuclear receptors—10-fold higher than T4, the T3 metabolite Triac[4] also has some metabolic activity,[5] whereas reverse T3 (rT3) is inactive.[6] T4 is the preferred measurement used to assess thyroid status because of its thyroid tissue-specific origin and because the peripheral tissue deiodinase enzymes that convert T4 to T3 are tightly regulated to maintain an optimal circulating T3 in the face of T4 fluctuations.[7,8]

T3 measurement can be especially useful for investigating the etiology of hyperthyroidism—Graves' disease versus thyroiditis because the thyroidal stimulation resulting from the TSH receptor antibodies (TRAbs) that cause Graves' hyperthyroidism, stimulate a T3 dominant secretion from the thyroid, resulting in high T3/T4 ratio compared with either that seen in euthyroidism or thyroiditis.[9] Studies showing that rT3 increases with fasting[10] and nonthyroidal illness (NTI)[11] initially conveyed a false impression that rT3 measurement was an indicator of thyroid function. However, the metabolic

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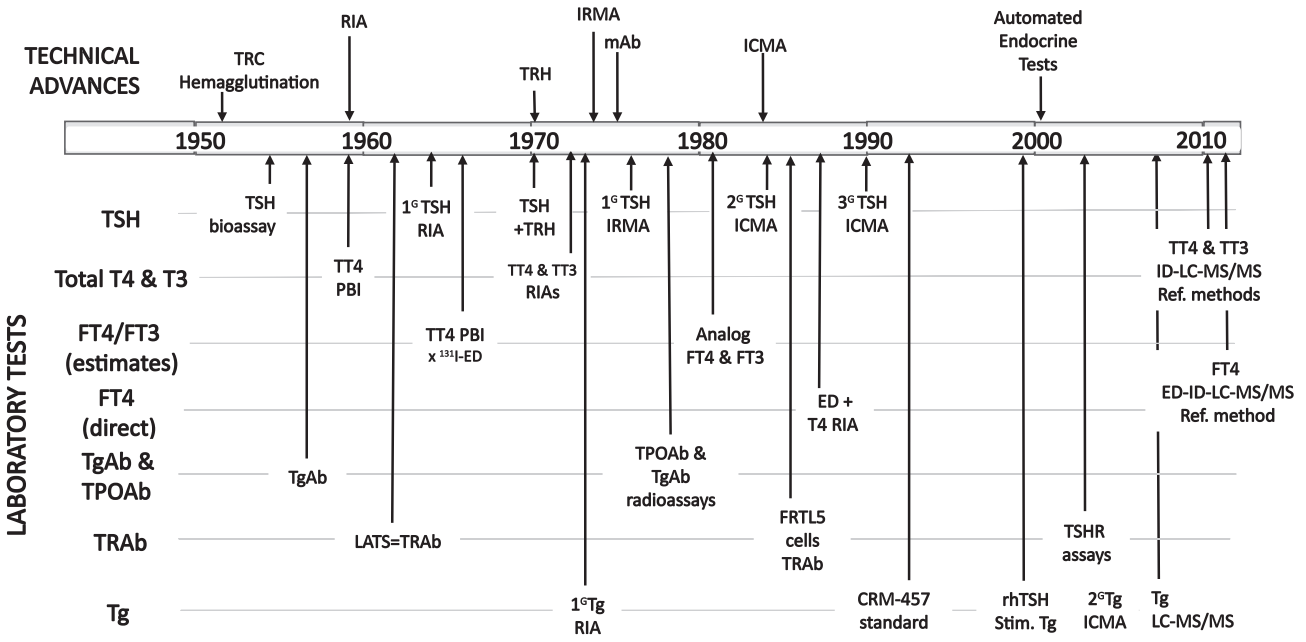

FIG. 1. Timeline for when major technical advances have influenced the development of tests for TSH, total thyroid hormones (TT4 and TT3), indirect estimates of free thyroid hormones (fT4 and fT3), direct fT4, thyroid antibodies TPOAb, TgAb and TRAb and Tg are given. fT3, free triiodothyronine; fT4, free thyroxine; Tg, thyroglobulin; TgAb, thyroglobulin autoantibody; TPOAb, thyroperoxidase autoantibody; TRAb, TSH receptor antibody; TSH, thyrotropin; TT3, total triiodothyronine; TT4, total thyroxine.

studies made in euthyroid volunteers[12] showing rT3 elevations resulted from slowed metabolic clearance and not increased production from T4, refuted the clinical utility of rT3 measurement, except in rare conditions associated with decreased sensitivity to thyroid hormones and in consumptive hypothyroidism.[13,14] Nevertheless, current misinformation on websites still encourages some to order an expensive rT3 test.[15]

Total hormone (total thyroxine and total triiodothyronine) and free hormone indices

In the 1950s, the only thyroid test was the estimation of total thyroxine (TT4) as protein-bound iodine (PBI). The PBI primarily reflected the serum T4 concentration, with some contribution from T3. Serum proteins were precipitated and ashed before the iodide content was quantified against

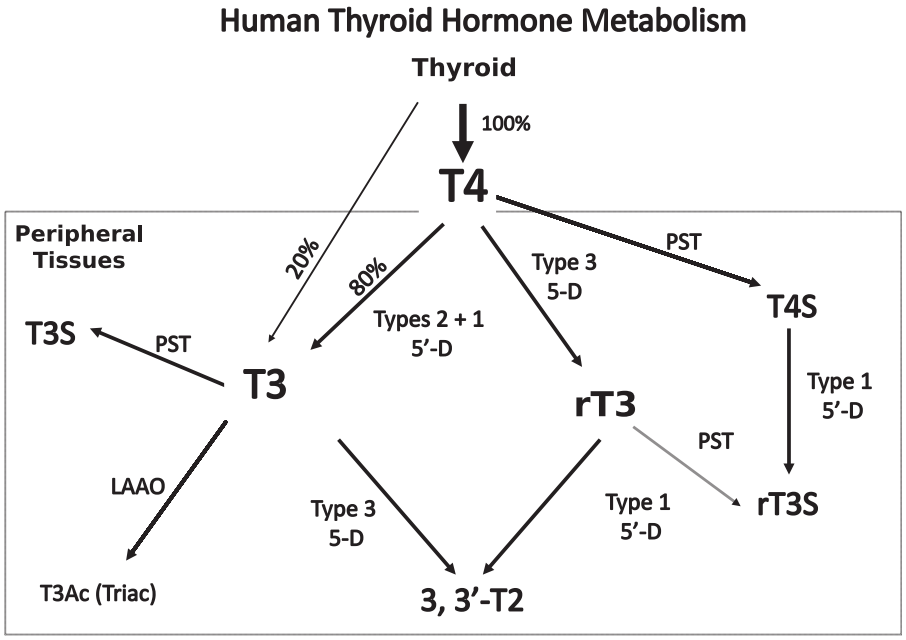

FIG. 2. The figure shows how T4 is metabolized in peripheral tissue to form the initial metabolites: T3 and rT3 as well as their sulfated derivatives. T3 is further metabolized to Triac (T3Ac) and deiodinated to form 3,3′-T2, a common metabolite of both T3 and rT3. These metabolic pathways are catalyzed by deiodinases (type 1 5′-deiodinase, type 2 5′-deiodinase and type 3 5′-deiodinase), together with PST and LAAO enzymes. LAAO, L-amino acid oxidase; PST, phosphosulfotransferases; rT3, reverse T3.

standards by colorimetry.[16] These PBI procedures typically used high temperatures and strong acids and were somewhat smelly and subject to interferences from iodine-containing therapeutic compounds. In the 1970s, laboratorians readily replaced the chemical PBI test with radioimmunoassay (RIA) methods for TT4, total triiodothyronine (TT3), and subsequently rT3,[17–19] using blockers to weaken thyroid hormone binding to plasma proteins and facilitate hormone binding to the polyclonal antibody reagents. Radioimmunological determination of T4 in dried blood samples followed and led to the first mass screening program for neonatal hypothyroidism.[20]

At present, most TT4 and TT3 measurements are made on automated platforms by nonisotopic immunoassay methods.[21,22] Although these methods correlate with the isotope-dilution liquid chromatography/tandem mass spectrometry (ID-LC-MS/MS) TT4 reference method, current TT4 immunoassays exhibit unacceptably high between-method biases and total coefficients of variation (CVs).[21,22]

It had long been recognized that thyroid hormones are mostly bound to plasma proteins, primarily thyroxine-binding globulin (TBG) with some binding to transthyretin and albumin, so that even when a patient is euthyroid, their total hormone levels could be abnormal because binding proteins were either elevated or reduced.[23] The high TBG state of pregnancy was especially problematic,[23] as were conditions associated with congenital TBG deficiency or excess, acute, and chronic NTIs, and certain drug therapies (androgens, diphenylhydantoin, heparin, and enoxaparin sodium). Congenital albumin abnormalities (dysalbuminemia or analbuminemias) were also found to interfere with both TT4, and in some cases TT3 measurements.[24,25]

The TBG dependence of TT4 led to the development of the two-test ''free hormone index'' approach, whereby the total hormone level (TT4 or TT3) was mathematically ''corrected'' for TBG abnormalities using a ''T3 uptake'' approach.[26] These T3 uptake tests indirectly measured serum TBG binding capacity as the amount of added ^{125}I-T3 tracer taken up by a thyroid hormone binding ''scavenger'' such as a resin or T3 antibody. In high TBG states the competition for tracer between TBG and the scavenger would favor binding to TBG resulting in less tracer binding the scavenger (low ^{125}I-T3 uptake). Conversely, in low TBG states less ^{125}I-T3 would bind TBG and more would bind the scavenger (high ^{125}I-T3 uptake). In 1991, the American Thyroid Association renamed these T3 uptake tests, ''Thyroid Hormone Binding Ratio'' (THBR) tests.[27]

Current THBR methods use different scavengers, formulations, calculations, and reference ranges. For practical reasons early THBR tests used ^{125}I-T3 in preference to ^{125}I-T4 because T3 has a lower affinity for TBG than T4 resulting in a higher uptake of isotope onto the scavenger,[28] necessitating a shorter isotopic count time. Current THBR tests are called ''T-uptakes'' and mainly use automated immunological formulations and nonisotopic signals to assess available TBG binding sites relative to a reference assigned a value of 1.00 or 40%.[29]

Direct fT4 measurement

The free hormone hypothesis, proposed more than 70 years ago, states that when hormones are bound to plasma proteins only the free hormone fraction (0.03% fT4 and 0.3% free triiodothyronine [fT3]) is biologically active and can enter tissues to exert hormonal action.[30,31] Unfortunately, the measurement of fT4 in the picomolar range presents a considerable technical challenge that has prompted a 50-year ongoing quest to develop rapid, direct fT4 methods that do not necessitate the physical separation of free from bound hormone using labor-intensive and expensive methods such as equilibrium dialysis,[32,33] ultrafiltration,[34] or column chromatography.[35]

In 1988, the first direct fT4 method measured T4 levels in serum dialysates using a sensitive RIA.[33] Over the last decade, equilibrium dialysis followed by ID-LC-MS/MS (ED-ID-LC-MS/MS) measurement of fT4 in the dialysate has become the fT4 reference method of choice.[36] However, direct fT4 measurement is still not broadly implemented because of high instrument costs, technical complexity, and reimbursement issues. Direct fT3 measurements are available only in research laboratories. Most fT4 and fT3 measurements performed in routine clinical chemistry laboratories use automated estimate tests.

Single test free hormone estimate tests (fT4 and fT3)

During the 1980s a range of single test fT4 and fT3 ''estimate'' methods were developed to replace the two-test index approach. The validity of these methods depends on minimizing TBG effects while maintaining equilibrium between free and bound hormones during the analysis, that is, during the analysis there should be only a minimal release of TBG-bound T4 into the free phase. These methods use two different innovative immunoassay approaches.

One-step analog tests

These competitive immunoassays use either a labeled T4 analog (e.g., a T4-albumin conjugate), or a labeled antibody to compete with free hormone in the serum for a limited number of solid-phase antibody-binding sites.[37] Although these methods were fairly successful in eliminating TBG effects, they were criticized for their sensitivity to albumin abnormalities (e.g., familial dysalbuminemic hyperthyroxinemias and analbuminemia), other abnormal thyroid hormone-binding proteins (e.g., T4 and T3 autoantibodies),[37] and interference from nonesterified fatty acids and a range of drugs that displace T4 from TBG.[24,38]

Two-step/back titration fT3 and fT4 tests

In these methods, fT4 in the serum reacts with a limited quantity of a solid-phase T4 antibody.[29,39] After washing away the unbound serum constituents, the unoccupied antibody-binding sites were quantified using labeled ligands. Because the wash step removes binding proteins, this assay design appears less susceptible to abnormal binding proteins than the analog format.[40]

The current fT4 and fT3 estimate tests are based on different constructs of these two approaches. Tests are now nonisotopic and automated for most multi-analyte instruments. However, despite >20 years of development, these tests remain sensitive to congenital albumin abnormalities,[25] iodothyronine autoantibodies,[41] heterophile antibodies (HAb),[41] and interferences with assay reagents such as biotin, as well as streptavidin and rhuthenium antibodies.[41,42] In addition, current fT4 estimate tests have significant between-method biases and negative biases relative to the ED-ID-LC-

MS/MS reference method.[43] This between-method variability precludes setting universal reference ranges for evaluating conditions such as pregnancy[44] (Fig. 3). In the future, it is hoped that manufacturers will restandardize their tests against the ED-ID-LC-MS/MS reference method.

TSH Measurements

TSH is typically the first-line test for thyroid function and is preferred to fT4.[3] The dominance of TSH testing reflects the inherent physiology of the hypothalamic–pituitary–thyroid axis, whereby small changes in fT4 are magnified 100-fold with respect to changes in TSH.[45] This log/linear TSH/fT4 relationship, that was initially established in the 1990s using the fT4 index method,[45] and later challenged by studies using fT4 analog tests,[46] has been confirmed using direct fT4 methods.[47,48] The log/linear TSH/fT4 relationship dictates that only TSH measurement will detect mild (subclinical) degrees of hypo- or hyperthyroidism.

The TSH era began in 1927 with the recognition that the pituitary secretes a thyroid stimulator.[49] Early measurements used labor-intensive bioassays that measured TSH-stimulated ^{131}I-T4 release from radiolabeled mouse or guinea pig thyroid glands.[50,51] These bioassays also identified an abnormal long-acting thyroid stimulator (LATS) in the sera of thyrotoxic patients that was distinct from TSH[52] and is now recognized as the TSH-stimulating immunoglobulin (TSI) responsible for Graves' hyperthyroidism.[53] Early immunologic studies demonstrated the species specificity of TSH antibodies and this, together with TSH radioimmunoprecipitation techniques, led to the development of the first generation of TSH RIA methods.[54,55]

However, these early RIAs were compromised by cross-reactivity with hCG resulting from TSH homology with gonadotropin hormones, necessitating the addition of a human chorionic gonadotropin (hCG) blocker,[55] and poor sensitivity ($\sim$2.0 mIU/L) that restricted their use to diagnosing primary hypothyroidism.[56] After synthetic thyrotropin-releasing hormone (TRH; thyroliberin) became available,[57] small quantities of thyroid hormone were found to inhibit TRH-stimulated TSH release[58] and the TRH-TSH response was shown to be related to the basal TSH level.[59] The dose and protocol for TRH stimulation testing became standardized and an absent TRH-stimulated TSH response became a diagnostic test for hyperthyroidism[60] during the time that assay insensitivity precluded the use of basal TSH as a diagnostic test.[60]

Subsequently, assay specificity improved and TSH assay ''quality'' became synonymous with the method's sensitivity, as evidenced by the ability to distinguish the TSH levels of hyperthyroid subjects from those of euthyroid subjects.[60] The quest to improve TSH assay sensitivity prompted test manufacturers to adopt confusing nomenclature like ''ultrasensitive''

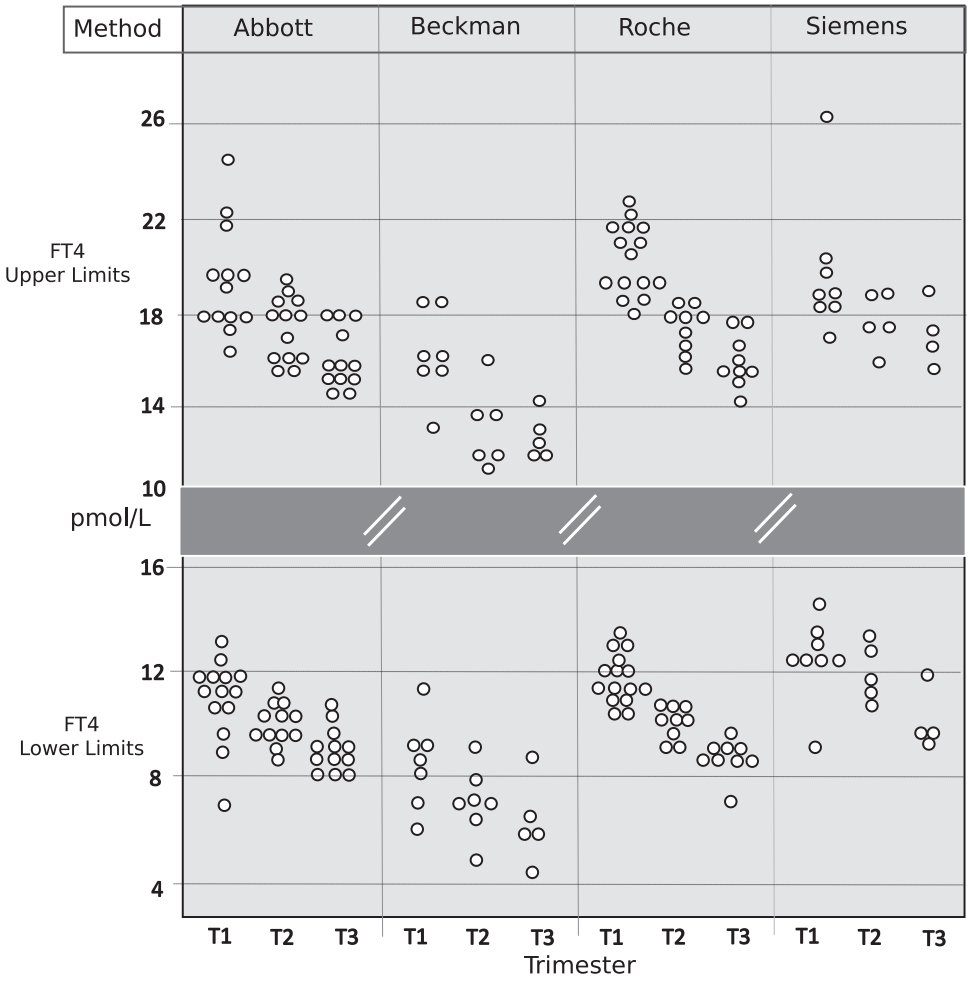

FIG. 3. Summary of 43 published studies showing the upper and lower reference limits (2.5–97.5%) for fT4 measured in each trimester of pregnancy by different methods—Abbott, Beckman, Roche, and Siemens. The data show the trend for higher fT4 in the first trimester, as expected from thyroidal human chorionic gonadotropin stimulation which is maximal in the first trimester.[152] The data is redrawn from Okosieme et al[44] with permission.

and ''supersensitive'' as marketing ploys to describe their TSH assays.[60] This led the scientific community to use a functional sensitivity (FS) parameter to reflect assay sensitivity in clinical practice. For TSH, FS was defined as the lowest analyte concentration that could be measured in human serum with a 20% between-run CV using a timespan representative of clinical practice (6–8 weeks).[61] The concept of FS was published in the 2003 National Academy of Clinical Biochemistry thyroid guidelines, together with recommended FS protocols for other thyroid tests.[61]

By the 1980s, TSH testing had been revolutionized by the advent of monoclonal antibody (mAb) production,[62] which facilitated the development of an immunometric assay (IMA) methodology.[63] IMA was inherently more sensitive than RIA, especially after replacing the isotopic ^{125}I signal with chemiluminescence[64] or fluorescence.[65] As a result, more sensitive second generation (FS = 0.1 mIU/L), third generation (FS = 0.01 mIU/L), and even a research fourth generation (FS = 0.001 mIU/L)[45,60,66] of increasingly more sensitive TSH methods were developed that could distinguish between mild (subclinical) and overt degrees of hyperthyroidism and eliminated the need for TRH testing. At present, the role for TRH testing is limited to pituitary evaluations[67] and investigations of TSH autoantibody (macro TSH) interferences.[68] However, TRH is not currently available for clinical use in the United States, although it remains available in Europe.

Currently, the third-generation TSH assays that are available on most automated instrument platforms[69] can diagnose all degrees of primary thyroid dysfunction from hyperthyroidism to hypothyroidism. The nonisotopic IMA methodology has also been adapted for blood spot testing for neonatal screening for congenital hypothyroidism[70] and point-of-care TSH testing.[71]

Despite dramatic improvements in assay sensitivity and the use of the same human pituitary extract for standardization (MRC 80/558), current TSH methods differ in their specificity for detecting different TSH glycoisoforms, not all of which are bioactive. These glycoisoforms are controlled by hypothalamic TRH, are influenced by physiological factors such as hypothyroidism, and may have altered molecular conformations that expose or mask epitopes.[72,73] As a result, different TSH numeric values are reported by different methods,[43] reflecting differences in the epitope specificities of the proprietary mAb reagents used for each IMA test. Current between-method differences preclude the establishment of universal reference ranges for different physiological states such as pregnancy (Fig. 4)[44] and aging.[1]

Furthermore, the mAb IMA reagents cannot distinguish between immunoactive and bioactive TSH, and thus cannot differentiate between primary and secondary (pituitary/hypothalamic) forms of thyroid dysfunction. It follows that TSH IMA measurement can be diagnostically misleading in

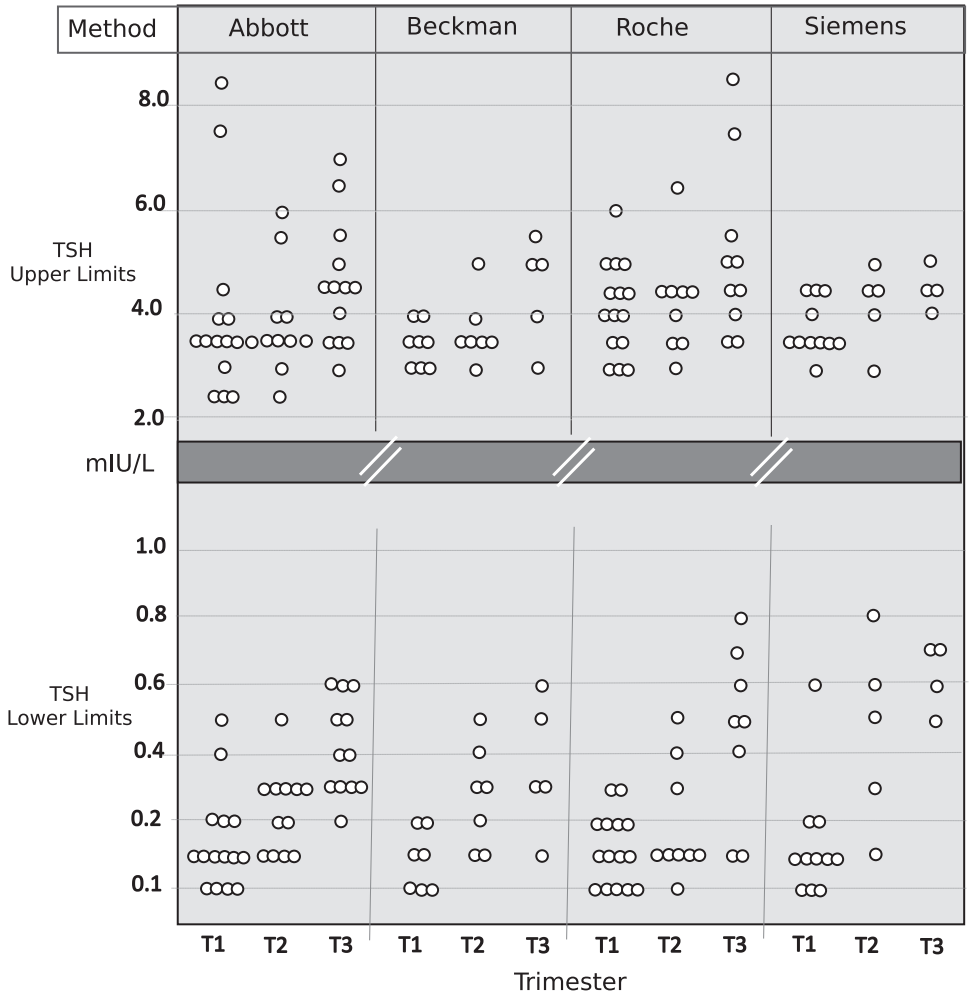

FIG. 4. Summary of 43 published studies of the upper and lower reference limits (2.5–97.5%) of TSH measured in each trimester of pregnancy by different methods—Abbott, Beckman, Roche, and Siemens. The data show the trend for lower TSH in the first trimester, as expected from thyroidal human chorionic gonadotropin stimulation which is maximal in the first trimester.[152] The data are redrawn from Okosieme et al[44] with permission.

central hypothyroidism, which is characterized by the secretion of bioinactive TSH isoforms,[74] in rare cases of TSH β subunit genetic variants[75] or pituitary tumors that may secrete TSH isoforms with enhanced bioactivity.[76] Non-TSH-related interferences are also still problematic. Although biotin interference[41] is close to being eliminated by the test manufacturers, it is more difficult to eliminate falsely high TSH levels owing to interference from HAb, such as human anti-mouse antibodies and rheumatoid factor,[77] TSH autoantibodies (macro TSH),[68] or antibodies against IMA reagents, such as rhuthenium and streptavidin.[41]

Thyroid Autoantibodies (Thyroglobulin, Antimicrosomal/Thyroperoxidase and TSH Receptor Autoantibodies)

Thyroglobulin and antimicrosomal/thyroperoxidase antibodies

By the mid 1950s the concept that an animal could not produce antibodies against its own proteins had been abandoned, after observing that the thyroglobulin autoantibodies (TgAbs) that could be elicited by injecting rabbits with an extract of one thyroid lobe caused damage in the remaining lobe resembling Hashimotos' thyroiditis.[78,79] Early methods for detecting TgAbs, and later antimicrosomal antibodies (AMA),[80] were qualitative and included precipitin reactions in gels,[78] immunofluorescence,[81] and tanned red cell (TRC) hemagglutination.[82] By the late 1970s, the qualitative TRC tests were becoming replaced by more sensitive and specific quantitative competitive binding radioassays[83] that reported high rates of positivity in patients with Graves' disease, chronic thyroiditis, and other autoimmune conditions.[84]

The thyroid peroxidase enzyme (TPO) had become recognized as the microsomal antigen, and AMA tests became TPO antibody (TPOAb) assays.[85] Subsequently, the NHANES III survey used radioassays for TPOAb and TgAb to assess the prevalence of thyroid antibodies in the U.S. population and reported that the prevalence of both antibodies approximated 10% in individuals with no apparent thyroid dysfunction.[1] Antibody prevalence was shown to increase with age, especially in women, and was influenced by ethnicity.[1,86] At present, some radioassays remain, but most TgAb and TPOAb testing is performed by methods in which the antibody in serum competes for antigens on a solid support and is detected by a nonisotopic signal.[87]

Methods are now automated on the same instrument platforms as used for the other thyroid tests and standardized against reference materials from the National Institute for Biological Standards and Control (MRC 65/093 for TgAb and MRC 19/260 for TPOAb). However, current methods vary in sensitivity, specificity, and the numeric values they report.[88] Between-method differences are less problematic for TPOAb than for TgAb, because TPOAb is typically measured once to assess the etiology of a TSH elevation[86] or as a pregnancy risk factor.[89] In contrast, both assay sensitivity and specificity are critical for detecting TgAb interference with Tg measurements[90] or for using the TgAb trend as a surrogate thyroid cancer tumor marker.[91]

TSH receptor antibodies

TRAbs were first detected by TSH bioassays as an abnormal LATS,[51,92] which were later shown to be autoantibodies targeting the TSH receptor (TSHR) that were responsible for Graves' hyperthyroidism and its extrathyroidal manifestations.[93] The first TRAb tests were competitive binding assays that measured TRAb in serum as thyroid-binding inhibiting immunoglobulins (TBII) that inhibited labeled TSH from binding to human or guinea pig thyroid membranes.[53,94,95] More recently, a TSHR-binding mAb has replaced labeled TSH, and thyroid membranes have been replaced by purified TSHR preparations[94,96] or the cloned TSHR.[97] Current TBII tests are mostly automated, nonisotopic, and standardized against the International Reference Preparation (MRC 65/122).[94,98] TRAb bioassays have evolved from labor-intensive FRTL-5 cell-based methods necessitating IgG extraction and the measurement of cAMP by RIA as the endpoint,[99] to the use of Chinese hamster ovary cells containing transfected recombinant TSHR and a cAMP-dependent luminescent signal.[100]

TBII tests do not provide information on whether the functional status of the TRAb is stimulatory or inhibitory. In contrast, bioassays have shown that not all TRAb stimulate the TSHR—some TRAbs block TSH stimulation, whereas other TRAbs bind the TSHR and are neutral with respect to TSH action.[98] Over the past four decades, these functional TRAb differences have led to a confusing lexicon of terms describing TRAb function.[98] Terms for stimulating TRAb have included TSHR-stimulating antibodies or immunoglobulins (TSAb or TSI), whereas terms for blocking TRAb include TSHR-blocking antibodies or immunoglobulins (TSB-Ab or TBI). Functional TRAb assays are useful for investigating the extrathyroidal manifestations of Hashimoto's thyroiditis and Graves' disease.[98] In pregnant patients TRAb is especially useful for distinguishing gestational transient thyrotoxicosis (no treatment required) from Graves' thyrotoxicosis (treatment required), and for assessing the risk of neonatal hypo- or hyperthyroidism.[98]

Tg Measurements

Early studies that administered ^{131}I to animals identified Tg as a thyroxine-containing iodoprotein secreted at high concentrations primarily into the thyroid lymph system and increased by TSH stimulation.[101–104] These studies established that the thyroid is a unique source of Tg and the lymphatic pathway is its major secretory route. Over the subsequent decades the thyroid-specific origin of Tg was confirmed, prompting the use of serum Tg measurement as a tumor marker for thyroid cancer. Secondary uses include the diagnosis of subacute thyroiditis and congenital thyroid conditions. Following the localization of the Tg gene to chromosome 8q24.2-q24.3 and its subsequent sequencing in 1987,[105,106] the structural complexity of the high molecular weight (19S, 660 kDa) dimeric Tg glycoprotein has been extensively investigated.[107] Recent electron cryomicroscopy studies have elucidated the complex structure of native human Tg protein resulting from complex posttranslational modifications.[108–110]

Studies have identified the hormonogenic sites[111] and the processes involved in Tg molecular maturation whereby molecular chaperones control the site-directed glycosylation, dimerization, and molecular folding critical for producing a mature Tg molecule suitable for iodination at the apical cell membrane.[107] The complexity of the Tg biosynthetic process

can lead to molecular heterogeneity and conformational abnormalities. Such abnormalities are commonly present in Tgs of neoplastic origin that are characterized by low iodine content[112,113] and abnormal glycosylation.[114–116] Such abnormalities may alter the Tg molecular conformation and mask or expose epitopes that may produce unusual interactions with immunoassay reagents and change the amount of Tg detected.[117] Currently there can be significant differences in the numeric Tg values reported for the same serum specimen using different methods, which likely reflects the heterogeneity of the Tg molecular forms in patient sera.[117–119]

Tg was first detected in blood in the 1920s using precipitation reactions with animal Tg antibodies.[120] In the 1950s, zone electrophoresis showed that Tg increased after radioiodine treatment.[121] A decade later, the first direct Tg RIA was developed, which had a sensitivity of $\sim 10\,\mu g/L$ and could only detect Tg in $\sim 60\%$ of healthy euthyroid subjects.[122] However, within a decade RIA sensitivity had improved 10-fold ($\sim 1\,\mu g/L$) and the pathophysiologic studies that followed detected Tg in most healthy euthyroid subjects[123] and found elevated Tg levels in pregnancy and cord blood[124] as well as hyperthyroidism of any cause.[123] The finding that Tg was elevated in metastatic differentiated thyroid cancer (DTC) and fell in response to successful treatment, while no elevation in Tg was seen with medullary thyroid cancer, firmly established serum Tg measurement as a DTC tumor marker.[125]

The first-generation of Tg RIA methods[126,127] had suboptimal sensitivity to detect persistent/recurrent DTC in the absence of radioiodine ablation of the surgical remnant.[128] Furthermore, sera had different numeric Tg values when measured by different methods.[129] This problem was improved by standardizing methods against the same human Tg reference material (CRM-457).[130] However, between-method differences persist, and are two- to three-fold higher than would be expected from consistently using the same method (Fig. 5),[90] likely because different methods detect different Tg molecular forms.[118] By the year 2000, the quest to improve Tg RIA sensitivity mirrored the efforts to improve TSH assay sensitivity. Recombinant human TSH (rhTSH)[131,132] was used to stimulate basal Tg 10-fold into the measurable range, much as TRH-stimulated TSH had been used to sensitize TSH detection two decades earlier.[59]

However, between 2000 and 2010 a second generation of Tg tests based on immunochemiluminometric (ICMA) methodology became available.[133] These ICMAs were inherently more sensitive (FS = 0.1 $\mu g/L$)[133] than RIA and obviated the need for expensive and inconvenient rhTSH stimulation.[134,135] At present, second-generation ICMA tests are available on virtually all the automated instrument platforms used for endocrine testing. However, because the incidence of TgAb in DTC is two-fold higher than the general population,[1,136] the major limitation of all Tg IMA methods remains interference from TgAb causing falsely low/undetectable Tg IMA in patients with structural DTC.[88,90,137,138]

By the late 1950s, TgAb were identified in sera[78] and were shown to bind ^{125}I-Tg. It followed that TgAb interference causing falsely high Tg RIA results was a well-recognized problem from the onset of RIA testing. As a result, sera containing TgAb were usually excluded from Tg studies.[122,139] In 1978, the factors determining the magnitude and direction of TgAb interference with RIA methodology were identified.[140] In response, some RIA methods were optimized to minimize interference by employing a high specific activity ^{125}I Tg tracer together with a high-affinity polyclonal antibody.[126,127,136,141] In 2008, the first LC-MS/MS Tg test was developed with the goal of overcoming TgAb interference.[142]

At present, although four LC-MS/MS Tg tests are commercially available,[138,143–145] it remains controversial whether LC-MS/MS methodology is free from TgAb interference, given that studies report paradoxically undetectable Tg LC-

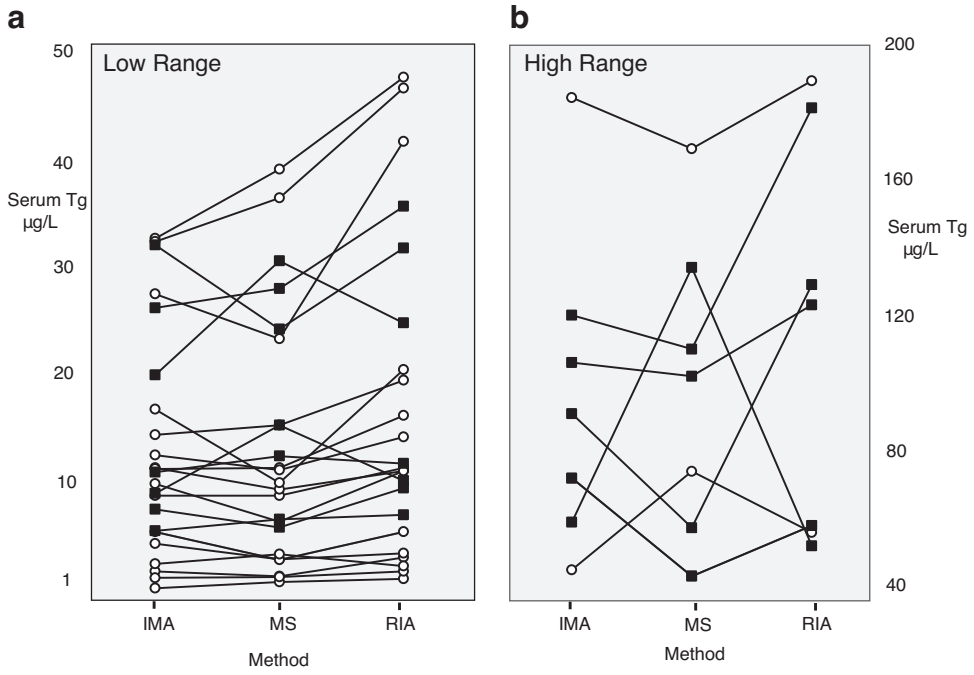

FIG. 5. Between-method variability of serum Tg measured in 34 TgAb-negative patients with metastatic differentiated thyroid cancer. Patients either had cervical lymph node metastases (open circles) or distant metastatic disease (closed squares). Patients with low range (<40 $\mu g/L$) versus high range (>40 $\mu g/L$) serum Tg are given in panels (**a**) and (**b**), respectively. The methods were IMA (Beckman), LC-MS/MS (MS) method (Mayo Medical Laboratories), and RIA (USC Endocrine Laboratory). Data are taken from Spencer et al.[90] IMA, immunometric assay; LC-MS/MS, liquid chromatography/tandem mass spectrometry; RIA, radioimmunoassay.

MS/MS tests for ~40% of TgAb-positive DTC patients with structural disease.[90,138,146] However, regardless of the Tg methodology used (RIA, IMA, or LC-MS/MS), sensitive TgAb detection remains critical for interpreting Tg measurements.[88] In fact, in recent years the TgAb trend has become recognized as a surrogate tumor marker for TgAb-positive DTC patients.[91,147] In addition to the persistent TgAb interference problem, the clinical utility of monitoring Tg and TgAb trends as DTC tumor markers remains negatively impacted by the between-method variability in current Tg[119] and TgAb[148] tests. This necessitates monitoring DTC patients using the same manufacturer's method in preferably the same laboratory.[147]

Discussion

In 1895, Magnus-Levy found that thyroid secretions stimulated the basal metabolic rate (BMR) in humans,[149,150] and that his own BMR declined with age. Such studies prompted the use of BMR the first test of thyroid function. Over the next 140 years, studies have elucidated the factors controlling the hypothalamic–pituitary–thyroid axis[151] and the etiology of the thyroid diseases that affect ~10% of the U.S. population.[1] Patients with thyroid diseases are mostly managed on an outpatient basis that generates 60% of laboratory endocrine tests.[3] Future developments will likely include the elimination of test interferences and the standardization/harmonization of tests using internationally validated reference materials. The elimination of method-related variability will facilitate the establishment of universal reference ranges.

Author's Contribution

C.A.S. is the only contributor.

Author Disclosure Statement

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References

1. Hollowell JG, Staehling NW, Hannon WH, et al. Serum thyrotropin, thyroxine, and thyroid antibodies in the United States population (1988 to 1994): NHANES III. J Clin Endocrinol Metab 2002;87(2):489–499; doi: 10.1210/jcem.87.2.8182
2. Lin DC, Straseski JA, Schmidt RL. Multi-center benchmark study reveals significant variation in thyroid testing in United States. Thyroid 2017;27(10):1232–1245; doi: 10.1089/thy.2017.0190
3. Soh SB, Aw TC. Laboratory testing in thyroid conditions—pitfalls and clinical utility. Ann Lab Med 2019; 39(1):3–14; doi: 10.3343/alm.2019.39.1.3
4. Wood WJ, Geraci T, Nilsen A, et al. Iodothyronamines are oxidatively deaminated to iodothyroacetic acids in vivo. Chembiochem 2009;10(2):361–365; doi: 10.1002/cbic.200800607
5. Zucchi R. Thyroid hormone analogues: An update. Thyroid 2020;30(8):1099–1105; doi: 10.1089/thy.2020.0071
6. Brtko J. Thyroid hormone and thyroid hormone nuclear receptors: History and present state of art. Endocr Regul 2021;55(2):103–119; doi: 10.2478/enr-2021-0012
7. Russo SC, Salas-Lucia F, Bianco AC. Deiodinases and the metabolic code for thyroid hormone action. Endocrinology 2021;162(8):bqab059; doi: 10.1210/endocr/bqab059
8. Lum SM, Nicoloff JT, Spencer CA, et al. Peripheral tissue mechanism for maintenance of serum triiodothyronine values in a thyroxine-deficient state in man. J Clin Invest 1984;73(2):570–575; doi: 10.1172/jci111245
9. Baral S, Shrestha PK, Pant V. Serum free T(3) to free T(4) ratio as a useful indicator for differentiating destruction induced thyrotoxicosis from Graves' disease. J Clin Diagn Res 2017;11(7):Oc12–Oc14; doi: 10.7860/jcdr/2017/28293.10180
10. LoPresti JS, Gray D, Nicoloff JT. Influence of fasting and refeeding on 3,3′,5′-triiodothyronine metabolism in man. J Clin Endocrinol Metab 1991;72(1):130–136; doi: 10.1210/jcem-72-1-130
11. Kaptein EM, Feinstein EI, Nicoloff JT, et al. Alterations of serum reverse triiodothyronine and thyroxine kinetics in chronic renal failure: Role of nutritional status, chronic illness, uremia, and hemodialysis. Kidney Int Suppl 1983; 16:S180–S186.
12. LoPresti JS, Eigen A, Kaptein E, et al. Alterations in 3,3′5′-triiodothyronine metabolism in response to propylthiouracil, dexamethasone, and thyroxine administration in man. J Clin Invest 1989;84(5):1650–1656; doi: 10.1172/jci114343
13. Dumitrescu AM, Refetoff S. The syndromes of reduced sensitivity to thyroid hormone. Biochim Biophys Acta 2013; 1830(7):3987–4003; doi: 10.1016/j.bbagen.2012.08.005
14. Numazawa M, Tomari K, Igaki J, et al. Advantages of monitoring rT3 and dividing LT3 dose in the treatment of consumptive hypothyroidism associated with infantile hepatic hemangioma. J Pediatr Endocrinol Metab 2022; doi: 10.1515/jpem-2022-0032
15. Schmidt RL, LoPresti JS, McDermott MT, et al. Does reverse triiodothyronine testing have clinical utility? An analysis of practice variation based on order data from a National Reference Laboratory. Thyroid 2018;28(7):842–848; doi: 10.1089/thy.2017.0645
16. Chaney A. Protein-bound iodine. Adv Clin Chem 1958; 1(2):81–109; doi: 10.1016/s0065-2423(08)60356-x
17. Chopra I. A radioimmunoassay for measurement of thyroxine in unextracted serum. J Clin Endocrinol Metab 1972;34(938–947); doi: 10.1210/jcem-34-6-938
18. Chopra IJ, Solomon DH, Beall GN. Radioimmunoassay for measurement of triiodothyronine in human serum. J Clin Invest 1971;50(10):2033–2041; doi: 10.1172/jci106696
19. Chopra IJ. A radioimmunoassay for measurement of 3,3′,5′-triiodothyronine (reverse T3). J Clin Invest 1974; 54(3):583–592; doi: 10.1172/jci107795
20. Dussault JH, Coulombe P, Laberge C, et al. Preliminary report on a mass screening program for neonatal hypothyroidism. J Pediatr 1975;86(5):670–674; doi: 10.1016/s0022-3476(75)80349-0
21. Thienpont LM, Van Uytfanghe K, Beastall G, et al. Report of the IFCC working group for standardization of thyroid function tests; part 3: Total thyroxine and total triiodothyronine. Clin Chem 2010;56(6):921–929; doi: 10.1373/clinchem.2009.140228

22. Yu S, Zhou W, Cheng X, et al. Comparison of six automated immunoassays with isotope-diluted liquid chromatography-tandem mass spectrometry for total thyroxine measurement. Ann Lab Med 2019;39(4):381–387; doi: 10.3343/alm.2019.39.4.381
23. Robbins J, Rall JE. The interaction of thyroid hormones and protein in biological fluids. Recent Prog Horm Res 1957;13:161–202; discussion 202–208.
24. Stockigt JR, Lim CF. Medications that distort in vitro tests of thyroid function, with particular reference to estimates of serum free thyroxine. Best Pract Res Clin Endocrinol Metab 2009;23(6):753–767; doi: 10.1016/j.beem.2009.06.004
25. Refetoff S, Scherberg NH, Yuan C, et al. Free thyroxine concentrations in sera of individuals with familial dysalbuminemic hyperthyroxinemia: A comparison of three methods of measurement. Thyroid 2020;30(1):37–41; doi: 10.1089/thy.2019.0058
26. Clark F, Horn DB. Assessment of thyroid function by the combined use of the serum protein-bound iodine and resin uptake of ^{131}I-triiodothyronine. J Clin Endocrinol Metab 1965;25(39–45); doi: 10.1210/jcem-25-1-39
27. Hay ID, Bayer MF, Kaplan MM, et al. American Thyroid Association assessment of current free thyroid hormone and thyrotropin measurements and guidelines for future clinical assays. The Committee on Nomenclature of the American Thyroid Association. Clin Chem 1991;37(11): 2002–2008.
28. Bikle DD. The free hormone hypothesis: When, why, and how to measure the free hormone levels to assess vitamin D, thyroid, sex hormone, and cortisol status. JBMR Plus 2021;5(1):e10418; doi: 10.1002/jbm4.10418
29. Stockigt JR. Free thyroid hormone measurement. A critical appraisal. Endocrinol Metab Clin North Am 2001; 30(2):265–289; doi: 10.1016/s0889-8529(05)70187-0
30. Recant L, Riggs DS. Thyroid function in nephrosis. J Clin Invest 1952;31(8):789–797; doi: 10.1172/jci102664
31. Ekins R. The free hormone hypothesis and measurement of free hormones. Clin Chem 1992;38(7):1289–1293.
32. Sterling K, Brenner MA. Free thyroxine in human serum: Simplified measurement with the aid of magnesium precipitation. J Clin Invest 1966;45(1):153–163; doi: 10.1172/jci105320
33. Nelson JC, Tomei RT. Direct determination of free thyroxin in undiluted serum by equilibrium dialysis/radioimmunoassay. Clin Chem 1988;34(9):1737–1744.
34. Weeke J, Boye N, Orskov H. Ultrafiltration method for direct radioimmunoassay measurement of free thyroxine and free triiodothyronine in serum. Scand J Clin Lab Invest 1986;46(4):381–389; doi: 10.3109/00365518609083686
35. Romelli PB, Pennisi F, Vancheri L. Measurement of free thyroid hormones in serum by column adsorption chromatography and radioimmunoassay. J Endocrinol Invest 1979;2(1):25–40; doi: 10.1007/bf03349272
36. Van Houcke SK, Van Uytfanghe K, Shimizu E, et al. IFCC international conventional reference procedure for the measurement of free thyroxine in serum: International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group for Standardization of Thyroid Function Tests (WG-STFT)(1). Clin Chem Lab Med 2011;49(8):1275–1281; doi: 10.1515/cclm.2011.639
37. Wellby ML, Guthrie L, Reilly CP. Evaluation of a new free-thyroxin assay. Clin Chem 1981;27(12):2022–2024.
38. Burch HB. Drug effects on the thyroid. N Engl J Med 2019;381(8):749–761; doi: 10.1056/NEJMra1901214
39. Kurtz A, Dwyer K, Ekins R. Serum free thyroxine in pregnancy. Br Med J 1979;2(6189):550–551; doi: 10.1136/bmj.2.6189.550-c
40. Thienpont LM, Van Uytfanghe K, Poppe K, et al. Determination of free thyroid hormones. Best Prac Res Clin Endocrinol Metab 2013;27(5):689–700; doi: 10.1016/j.beem.2013.05.012
41. Favresse J, Burlacu MC, Maiter D, et al. Interferences with thyroid function immunoassays: Clinical implications and detection algorithm. Endocr Rev 2018;39(5): 830–850; doi: 10.1210/er.2018-00119
42. Favresse J, Paridaens H, Pirson N, et al. Massive interference in free T4 and free T3 assays misleading clinical judgment. Clin Chem Lab Med 2017;55(4):e84–e86; doi: 10.1515/cclm-2016-0255
43. Faix JD, Miller WG. Progress in standardizing and harmonizing thyroid function tests. Am J Clin Nutr 2016;104 Suppl 3(Suppl 3):913s–917s; doi: 10.3945/ajcn.115.110379
44. Okosieme OE, Agrawal M, Usman D, et al. Method-dependent variation in TSH and FT4 reference intervals in pregnancy: A systematic review. Ann Clin Biochem 2021; 58(5):537–546; doi: 10.1177/00045632211026955
45. Spencer CA, LoPresti JS, Patel A, et al. Applications of a new chemiluminometric thyrotropin assay to subnormal measurement. J Clin Endocrinol Metab 1990;70:453–460; doi: 10.1210/jcem-70-2-453
46. Hadlow NC, Rothacker KM, Wardrop R, et al. The relationship between TSH and free T_4 in a large population is complex and nonlinear and differs by age and sex. J Clin Endocrinol Metab 2013;98(7):2936–2943; doi: 10.1210/jc.2012-4223
47. Jonklaas J, Kahric-Janicic N, Soldin OP, et al. Correlations of free thyroid hormones measured by tandem mass spectrometry and immunoassay with thyroid-stimulating hormone across 4 patient populations. Clin Chem 2009; 55(7):1380–1388; doi: 10.1373/clinchem.2008.118752
48. van Deventer HE, Mendu DR, Remaley AT, et al. Inverse log-linear relationship between thyroid-stimulating hormone and free thyroxine measured by direct analog immunoassay and tandem mass spectrometry. Clin Chem 2011;57(1):122–127; doi: 10.1373/clinchem.2010.154088
49. Uhlenhuth E, Schwartzbach S. The anterior lobe of the hypophysis as a control mechanism of the function of the thyroid gland. Br J Exp Biol 1927;5(1–5).
50. Adams DD, Purves HD. A new method of assay for thyrotrophic hormone. Endocrinology 1955;57(1):17–24; doi: 10.1210/endo-57-1-17
51. McKenzie J. The bioassay of thyrotropin in serum. Endocrinology 1958;63(3):372–382; doi: 10.1210/endo-63-3-372
52. Adams DD, Purves HD. The assessment of thyroid function by tracer tests with radioactive iodine. N Z Med J 1956;55(305):36–41.
53. Ealey PA, Marshall NJ, Ekins RP. Further studies on the response of a cytochemical bioassay to thyroid stimulators, using reference preparations of thyrotropin and long acting thyroid stimulator. J Endocrinol Invest 1984;7(1): 25–28; doi: 10.1007/bf03348371
54. Utiger RD, Odell WD, Condliffe PG. Immunologic studies of purified human and bovine thyrotropin. Endocrinology 1963;73:359–365; doi: 10.1210/endo-73-3-359
55. Odell WD, Wilber JF, Paul WE. Radioimmunoassay of thyrotropin in human serum. J Clin Endocrinol Metab 1965;25(9):1179–1188; doi: 10.1210/jcem-25-9-1179

56. Hershman J, Pittman J. Utility of the radioimmunoassay of serum thyrotrophin in man. Ann Intern Med 1971;74:481–490; doi: 10.7326/0003-4819-74-4-481
57. Hershman JM, Pittman JA Jr. Response to synthetic thyrotropin-releasing hormone in man. J Clin Endocrinol Metab 1970;31(4):457–460; doi: 10.1210/jcem-31-4-457
58. Snyder P, Utiger R. Response to thyrotropin releasing hormone (TRH) in normal man. J Clin Endocrinol Metab 1972;34:380–385.
59. Spencer C, Schwarzbein D, Guttler R, et al. TRH stimulation test responses employing 3rd and 4th generation TSH assay technology. J Clin Endocrinol Metab 1993;76: 494–499.
60. Nicoloff JT, Spencer CA. Use and misuse of the sensitive thyrotropin assays. J Clin Endocrinol Metab 1990;71:553–558; doi: 10.1210/jcem-71-3-553
61. Baloch Z, Carayon P, Conte-Devolx B, et al. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 2003;13(1):3–126; doi: 10.1089/105072503321086962
62. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975; 256(5517):495–497; doi: 10.1038/256495a0
63. Woodhead J, Addison G, Hales C. The immunoradiometric assay and related techniques. Br Med Bull 1974;30: 44–49.
64. Weeks I, Sturgess M, Siddle K, et al. A high sensitivity immunochemiluminometric assay for human thyrotrophin. Clin Endocrinol (Oxf) 1984;20(4):489–495; doi: 10.1111/j.1365-2265.1984.tb03446.x
65. Kaihola HL, Irjala K, Viikari J, et al. Determination of thyrotropin in serum by time-resolved fluoroimmunoassay evaluated. Clin Chem 1985;31(10):1706–1709.
66. Spencer CA, Schwarzbein D, Guttler RB, et al. TRH stimulation test responses employing third and fourth generation TSH assays. J Clin Endocrinol Metab 1993;76: 494–498.
67. Boronat M. Central hypothyroidism or subclinical hyperthyroidism: Can they be confused with each other? Endocrinol Diabetes Metab Case Rep 2020;2020; doi: 10.1530/edm-20-0059
68. Ohba K, Maekawa M, Iwahara K, et al. Abnormal thyroid hormone response to TRH in a case of macro-TSH and the cut-off value for screening cases of inappropriate TSH elevation. Endocr J 2020;67(2):125–130; doi: 10.1507/endocrj.EJ19-0320
69. Li J, Huang Y, Xiang G, et al. Analytical and clinical evaluation of the light-initiated chemiluminescent assays for measurement of human thyroid hormones. J Clin Lab Anal 2022;36(5):e24266; doi: 10.1002/jcla.24266
70. Caiulo S, Corbetta C, Di Frenna M, et al. Newborn screening for congenital hypothyroidism: The benefit of using differential TSH cutoffs in a 2-screen program. J Clin Endocrinol Metab 2021;106(1):e338–e349; doi: 10.1210/clinem/dgaa789
71. Di Cerbo A, Quagliano N, Napolitano A, et al. Comparison between an emerging point-of-care tool for TSH evaluation and a centralized laboratory-based method in a cohort of patients from Southern Italy. Diagnostics (Basel) 2021;11:9; doi: 10.3390/diagnostics11091590
72. Donadio S, Morelle W, Pascual A, et al. Both core and terminal glycosylation alter epitope expression in thyrotropin and introduce discordances in hormone measurements. Clin Chem Lab Med 2005;43(5):519–530; doi: 10.1515/cclm.2005.091
73. Donadio S, Pascual A, Thijssen JH, et al. Feasibility study of new calibrators for thyroid-stimulating hormone (TSH) immunoprocedures based on remodeling of recombinant TSH to mimic glycoforms circulating in patients with thyroid disorders. Clin Chem 2006;52(2):286–297; doi: 10.1373/clinchem.2005.058172
74. Beck-Beccoz P, Persani L. Variable biological activity of thyroid-stimulating hormone. Eur J Endocrinol 1994; 131(4):331–340.
75. Kaplan AI, Luxford C, Clifton-Bligh RJ. Novel TSHB variant (c.217A>C) causing severe central hypothyroidism and pituitary hyperplasia. Endocrinol Diabetes Metab Case Rep 2022;2022; doi: 10.1530/edm-22-0230
76. Persani L, Asteria C, Tonacchera M, et al. Evidence for the secretion of thyrotropin with enhanced bioactivity in syndromes of thyroid hormone resistance. J Clin Endocrinol Metab 1994;78:1035–1039.
77. Cheng X, Guo X, Chai X, et al. Heterophilic antibody interference with TSH measurement on different immunoassay platforms. Clin Chim Acta 2021;512:63–65; doi: 10.1016/j.cca.2020.11.018
78. Doniach D, Roitt IM. Auto-immunity in Hashimoto's disease and its implications. J Clin Endocrinol Metab 1957;17(11):1293–1304; doi: 10.1210/jcem-17-11-1293
79. Owen CA Jr. A review of auto-immunization in Hashimoto's disease. J Clin Endocrinol Metab 1958;18(9): 1015–1023; doi: 10.1210/jcem-18-9-1015
80. Bird T, Stephenson J. Evaluation of a tanned red cell technique for thyroid microsomal antibodies. J Clin Pathol 1973;26(8):623–627; doi: 10.1136/jcp.26.8.623
81. Nairn RC, Ghose T, Porteous IB, et al. A routine immunofluorescence method for detecting autoantibodies to thyroid colloid. J Clin Pathol 1962;15(6):594–595; doi: 10.1136/jcp.15.6.594
82. Boyden SV. The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. J Exp Med 1951;93(2):107–120; doi: 10.1084/jem.93.2.107
83. Hopwood NJ, Rabin BS, Foley TP Jr, et al. Thyroid antibodies in children and adolescents with thyroid disorders. J Pediatr 1978;93(1):57–61; doi: 10.1016/s0022-3476(78)80600-3
84. Feldt-Rasmussen U, Perrild H, Bech K, et al. Discrepancy between haemagglutination and radioimmunological techniques for measurement of serum thyroglobulin autoantibodies. Allergy 1983;38(1):49–56; doi: 10.1111/j.1398-9995.1983.tb00856.x
85. Czarnocka B, ruf J, Ferrand M, et al. Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. FEBS Lett 1985;190:147–152.
86. Spencer CA, Hollowell JG, Kazarosyan M, et al. National Health and Nutrition Examination Survey III thyroid-stimulating hormone (TSH)-thyroperoxidase antibody relationships demonstrate that TSH upper reference limits may be skewed by occult thyroid dysfunction. J Clin Endocrinol Metab 2007;92(11):4236–4240; doi: 10.1210/jc.2007-0287
87. Lupoli GA, Okosieme OE, Evans C, et al. Prognostic significance of thyroglobulin antibody epitopes in differentiated thyroid cancer. J Clin Endocrinol Metab 2015; 100(1):100–108; doi: 10.1210/jc.2014-2725

88. Spencer C, Petrovic I, Fatemi S. Current thyroglobulin autoantibody (TgAb) assays often fail to detect interfering TgAb that can result in the reporting of falsely low/undetectable serum Tg IMA values for patients with differentiated thyroid cancer. J Clin Endocrinol Metab 2011; 96(5):1283–1291; doi: 10.1210/jc.2010-2762
89. Korevaar TIM, Pop VJ, Chaker L, et al. Dose dependency and a functional cutoff for TPO-antibody positivity during pregnancy. J Clin Endocrinol Metab 2018;103(2):778–789; doi: 10.1210/jc.2017-01560
90. Spencer C, Petrovic I, Fatemi S, et al. Serum thyroglobulin (Tg) monitoring of patients with differentiated thyroid cancer using sensitive (second-generation) immunometric assays can be disrupted by false-negative and false-positive serum thyroglobulin autoantibody misclassifications. J Clin Endocrinol Metab 2014;99(12):4589–4599; doi: 10.1210/jc.2014-1203
91. Reverter JL, Rosas-Allende I, Puig-Jove C, et al. Prognostic significance of thyroglobulin antibodies in differentiated thyroid cancer. J Thyroid Res 2020;2020: 8312628; doi: 10.1155/2020/8312628
92. Adams DD, Purces HD. Abnormal responses in the assay of thyrotropin. Proc Univ Otago Med Sch 1956;34:11–15.
93. Kriss JP, Pleshakov V, Chien JR. Isolation and identification of the long-acting thyroid stimulator and its relation to hyperthyroidism and circumscribed pretibial myxedema. J Clin Endocrinol Metab 1964;24:1005–1028; doi: 10.1210/jcem-24-10-1005
94. Smith BR, Sanders J, Furmaniak J. TSH receptor antibodies. Thyroid 2007;17(10):923–938; doi: 10.1089/thy.2007.0239
95. Prentice MG, Alaghband-Zadeh J, Wise PH. Validation of cytochemical section bioassay for thyroid-stimulating immunoglobulins in treated and untreated Graves' disease. J Immunoassay 1984;5(3–4):275–290; doi: 10.1080/01971528408063012
96. Smith BR, Bolton J, Young S, et al. A new assay for thyrotropin receptor autoantibodies. Thyroid 2004;14(10): 830–835; doi: 10.1089/thy.2004.14.830
97. Parmentier M, Libert F, Maenhaut C, et al. Molecular cloning of the thyrotropin receptor. Science 1989; 246(4937):1620–1622; doi: 10.1126/science.2556796
98. Kahaly GJ, Diana T, Olivo PD. TSH receptor antibodies: Relevance & utility. Endocr Pract 2020;26(1):97–106; doi: 10.4158/ep-2019-0363
99. Tokuda Y, Kasagi K, Iida Y, et al. Inhibition of thyrotropin-stimulated iodide uptake in FRTL-5 thyroid cells by crude immunoglobulin fractions from patients with goitrous and atrophic autoimmune thyroiditis. J Clin Endocrinol Metab 1988;67(2):251–258; doi: 10.1210/jcem-67-2-251
100. Jordan NJ, Rinderle C, Ashfield J, et al. A luminescent bioassay for thyroid blocking antibodies. Clin Endocrinol (Oxf) 2001;54(3):355–364; doi: 10.1046/j.1365-2265.2001.01193.x
101. Daniel PM, Gale MM, Plaskett LG, et al. Iodoprotein in the thyroid lymph of primates. Nature 1963;198:392–393; doi: 10.1038/198392a0
102. Daniel PM, Gale MM, Pratt OE. Hormones and related substances in the lymph leaving four endocrine glands—the testis, ovary, adrenal, and thyroid. Lancet 1963; 1(7293):1232–1234; doi: 10.1016/s0140-6736(63)918634
103. Daniel PM, Plaskett LG, Pratt OE. Radioactive iodoprotein in thyroid lymph and blood. Biochem J 1966;100(3): 622–630; doi: 10.1042/bj1000622
104. Daniel PM, Plaskett LG, Pratt OE. The lymphatic and venous pathways for the outflow of thyroxine, iodoprotein and inorganic iodide from the thyroid gland. J Physiol 1967;188(1):25–44; doi: 10.1113/jphysiol.1967.sp008121
105. Baas F, Bikker H, Geurts van Kessel A, et al. The human thyroglobulin gene: A polymorphic marker localized distal to C-MYC on chromosome 8 band q24. Hum Genet 1985;69(2):138–143; doi: 10.1007/bf00293284
106. Malthiéry Y, Lissitzky S. Primary structure of human thyroglobulin deduced from the sequence of its 8448-base complementary DNA. Eur J Biochem 1987;165(3):491–498; doi: 10.1111/j.1432-1033.1987.tb11466.x
107. van de Graaf SA, Ris-Stalpers C, Pauws E, et al. Up to date with human thyroglobulin. J Endocrinol 2001;170(2): 307–321; doi: 10.1677/joe.0.1700307
108. Coscia F, Taler-Verčič A, Chang VT, et al. The structure of human thyroglobulin. Nature 2020;578(7796):627–630; doi: 10.1038/s41586-020-1995-4
109. Adaixo R, Steiner EM, Righetto RD, et al. Cryo-EM structure of native human thyroglobulin. Nat Commun 2022;13(1):61; doi: 10.1038/s41467-021-27693-8
110. Tosatto L, Coscia F. A glance at post-translational modifications in human thyroglobulin: Potential impact on function and pathogenesis. Eur Thyroid J 2022; doi: 10.1530/etj-22-0046
111. Lamas L, Anderson PC, Fox JW, et al. Consensus sequences for early iodination and hormonogenesis in human thyroglobulin. J Biol Chem 1989;264(23):13541–13545.
112. Schneider AB, Ikekubo K, Kuma K. Iodine content of serum thyroglobulin in normal individuals and patients with thyroid tumors. J Clin Endocrinol Metab 1983;57(6): 1251–1256; doi: 10.1210/jcem-57-6-1251
113. Hansson M, Grunditz T, Isaksson M, et al. Iodine content and distribution in extratumoral and tumor thyroid tissue analyzed with X-ray fluorescence and time-of-flight secondary ion mass spectrometry. Thyroid 2008;18(11): 1215–1220; doi: 10.1089/thy.2008.0020
114. Yamamoto K, Tsuji T, Tarutani O, et al. Structural changes of carbohydrate chains of human thyroglobulin accompanying malignant transformation of thyroid glands. Eur J Biochem 1984;143:133–144.
115. Kohno Y, Tarutani O, Sakata S, et al. Monoclonal antibodies to thyroglobulin elucidate differences in protein structure of thyroglobulin in healthy individuals and those with papillary adenocarcinoma. J Clin Endocrinol Metab 1985;61(2):343–350; doi: 10.1210/jcem-61-2-343
116. Shimizu K, Nakamura K, Kobatake S, et al. Discrimination of thyroglobulin from thyroid carcinoma tissue and that from benign thyroid tissues with use of competitive assay between lectin and anti-thyroglobulin antibody. Rinsho Byori 2007;55(5):428–433.
117. Heilig B, Hufner M, Dorken B, et al. Increased heterogeneity of serum thyroglobulin in thyroid cancer patients as determined by monoclonal antibodies. Klin Wochenschr 1986;64:776–780.
118. Schulz R, Bethauser H, Stempka L, et al. Evidence for immunological differences between circulating and tissue-derived thyroglobulin in men. Eur J Clin Invest 1989;19: 459–463.
119. Spencer C, LoPresti J, Fatemi S. How sensitive (second-generation) thyroglobulin measurement is changing paradigms for monitoring patients with differentiated thyroid cancer, in the absence or presence of thyroglobulin auto-

antibodies. Curr Opin Endocrinol Diabetes Obes 2014; 21(5):394–404; doi: 10.1097/med.0000000000000092
120. Hektoen L, Schulhof K. The precipitin reaction of thyroglobulin: Specificness; Presence of thyroglobulin in human thyroid veins; production by rabbit of precipitin for rabbit thyroglobulin; thyroglobulin in the foetal thyroid and in exophthalmic goiter. Proc Natl Acad Sci U S A 1925;11(8):481–484; doi: 10.1073/pnas.11.8.481
121. Robbins J. Thyroglobulin in serum after I131 therapy. I. Salting out. J Biol Chem 1954;208(1):377–386.
122. Roitt IM, Torrigiani G. Identification and estimation of undegraded thyroglobulin in human serum. Endocrinology 1967;81(3):421–429; doi: 10.1210/endo-81-3-421
123. Uller RP, Van Herle AJ. Effect of therapy on serum thyroglobulin levels in patients with Graves' disease. J Clin Endocrinol Metab 1978;46:747–755.
124. Torrigiani G, Doniach D, Roitt IM. Serum thyroglobulin levels in healthy subjects and in patients with thyroid disease. J Clin Endocrinol Metab 1969;29(3):305–314; doi: 10.1210/jcem-29-3-305
125. Van Herle AJ, Uller RP. Elevated serum thyroglobulin: A marker of metastases in differentiated thyroid carcinomas. J Clin Invest 1975;56:272–277.
126. Black EG, Hoffenberg R. Should one measure serum thyroglobulin in the presence of anti-thyroglobulin antibodies? Clin Endocrinol (Oxf) 1983;19:597–601.
127. Spencer CA, Platler BW, Nicoloff JT. The effect of 125-I thyroglobulin tracer heterogeneity on serum Tg RIA measurement. Clin Chim Acta 1985;153:105–115.
128. Haugen BR, Ladenson PW, Cooper DS, et al. A comparison of recombinant human thyrotropin and thyroid hormone withdrawal for the detection of thyroid remnant or cancer. J Clin Endocrinol Metab 1999;84:3877–3885.
129. Feldt-Rasmussen U, Schlumberger M. European interlaboratory comparison of serum thyroglobulin measurement. J Endocrinol Invest 1988;11:175–181.
130. Feldt-Rasmussen U, Profilis C, Colinet E, et al. Human thyroglobulin reference material (CRM 457) 2nd part: Physicochemical characterization and certification. Ann Biol Clin 1996;54:343–348.
131. Haugen BR, Pacini F, Reiners C, et al. A comparison of recombinant human thyrotropin and thyroid hormone withdrawal for the detection of thyroid remnant or cancer. J Clin Endocrinol Metab 1999;84(11):3877–3885; doi: 10.1210/jcem.84.11.6094
132. Mazzaferri EL, Robbins RJ, Spencer CA, et al. A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 2003;88(4): 1433–1441; doi: 10.1210/jc.2002-021702
133. Iervasi A, Iervasi G, Ferdeghini M, et al. Clinical relevance of highly sensitive Tg assay in monitoring patients treated for differentiated thyroid cancer. Clin Endocrinol (Oxf) 2007;67(3):434–441; doi: 10.1111/j.1365-2265. 2007.02907.x
134. Smallridge RC, Meek SE, Morgan MA, et al. Monitoring thyroglobulin in a sensitive immunoassay has comparable sensitivity to recombinant human tsh-stimulated thyroglobulin in follow-up of thyroid cancer patients. J Clin Endocrinol Metab 2007;92(1):82–87; doi: 10.1210/jc. 2006-0993
135. Spencer C, Fatemi S, Singer P, et al. Serum basal thyroglobulin measured by a second-generation assay correlates with the recombinant human thyrotropin-stimulated thyroglobulin response in patients treated for differentiated thyroid cancer. Thyroid 2010;20(6):587–595; doi: 10.1089/ thy.2009.0338
136. Spencer CA, Takeuchi M, Kazarosyan M, et al. Serum thyroglobulin autoantibodies: Prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 1998;83(4):1121–1127; doi: 10.1210/jcem.83.4.4683
137. Mariotti S, Barbesino G, Caturegli P, et al. Assay of thyroglobulin in serum with thyroglobulin autoantibodies: An unobtainable goal? J Clin Endocrinol Metab 1995;80: 468–472.
138. Netzel BC, Grebe SK, Carranza Leon BG, et al. Thyroglobulin (Tg) testing revisited: Tg assays, TgAb assays, and correlation of results with clinical outcomes. J Clin Endocrinol Metab 2015;100(8):E1074–E1083; doi: 10.1210/ jc.2015-1967
139. Van Herle AJ, Uller RP, Matthews NL, et al. Radioimmunoassay for measurement of thyroglobulin in human serum. J Clin Invest 1973;52(1320–1327).
140. Schneider AB, Pervos R. Radioimmunoassay of human thyroglobulin: Effect of antithyroglobulin autoantibodies. J Clin Endocrinol Metab 1978;47:126–137.
141. Spencer CA, LoPresti JS. Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. Nat Clin Pract Endocrinol Metab 2008; 4(4):223–233; doi: 10.1038/ncpendmet0757
142. Hoofnagle AN, Becker JO, Wener MH, et al. Quantification of thyroglobulin, a low-abundance serum protein, by immunoaffinity peptide enrichment and tandem mass spectrometry. Clin Chem 2008;54(11):1796–1804; doi: 10.1373/clinchem.2008.109652
143. Clarke NJ, Zhang Y, Reitz RE. A novel mass spectrometry-based assay for the accurate measurement of thyroglobulin from patient samples containing antithyroglobulin autoantibodies. J Investig Med: The Official Publication of the American Federation for Clinical Research 2012;60(8):1157–1163; doi: 10.2310/JIM.0b013e3 18276deb4
144. Kushnir MM, Rockwood AL, Roberts WL, et al. Measurement of thyroglobulin by liquid chromatography-tandem mass spectrometry in serum and plasma in the presence of antithyroglobulin autoantibodies. Clin Chem 2013;59(6):982–990; doi: 10.1373/clinchem.2012.195594
145. Shuford CM, Walters JJ, Holland PM, et al. Absolute protein quantification by mass spectrometry: Not as simple as advertised. Anal Chem 2017;89(14):7406–7415; doi: 10.1021/acs.analchem.7b00858
146. Azmat U, Porter K, Senter L, et al. Thyroglobulin liquid chromatography-tandem mass spectrometry has a low sensitivity for detecting structural disease in patients with antithyroglobulin antibodies. Thyroid 2017;27(1):74–80; doi: 10.1089/thy.2016.0210
147. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on thyroid nodules and differentiated thyroid cancer. Thyroid 2016;26(1):1–133; doi: 10.1089/thy.2015.0020
148. Spencer CA. Clinical review: Clinical utility of thyroglobulin antibody (TgAb) measurements for patients with differentiated thyroid cancers (DTC). J Clin Endocrinol

Metab 2011;96(12):3615–3627; doi: 10.1210/jc.2011-1740
149. Magnus-Levy A. Ueber den respiratorischen Gaswechsel unter dem Einfluss der Thyroidea sowie unter verschiedenen pathologischen Zuständen. Berl Klin Wochenschr 1895;32:650–652.
150. Hulbert AJ, Else PL. Basal metabolic rate: History, composition, regulation, and usefulness. Physiol Biochem Zool 2004;77(6):869–876; doi: 10.1086/422768
151. Mariotti S, Beck-Peccoz P. Physiology of the Hypothalamic-Pituitary-Thyroid Axis. In: Endotext. (Feingold KR, Anawalt B, Boyce A, et al. eds.) MDText.com, Inc., Copyright © 2000–2022, MDText.com, Inc.: South Dartmouth, MA, USA; 2000.
152. Korevaar TI, de Rijke YB, Chaker L, et al. Stimulation of thyroid function by human chorionic gonadotropin during pregnancy: A risk factor for thyroid disease and a mechanism for known risk factors. Thyroid 2017;27(3):440–450; doi: 10.1089/thy.2016.0527

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Landmark Discoveries in Maternal–Fetal Thyroid Disease Over the Past Century

Angela M. Leung

There have been significant advancements in the understanding of maternal–fetal disease over the past century. This narrative review summarizes the landmark studies that have advanced the understanding of thyroid pathophysiology and thyroid disease during preconception, pregnancy, and postpartum, written to commemorate the 100th year anniversary of the founding of the American Thyroid Association.

Keywords: gestation, iodine, lactation, preconception, pregnancy, thyroid

Introduction

The landmark studies in maternal–fetal thyroid disease reflect pivotal early experiments, noteworthy public health accomplishments, and the international collaboration of investigators studying the key questions in this field. This body of knowledge has furthered the understanding of thyroid disease management in pregnant women and their offspring. This paper is not intended to be an exhaustive review of the seminal articles in maternal–fetal thyroid disease, but highlights the stories of select interesting historical studies in the field (Fig. 1), focusing primarily on human studies published in the past several decades.

Thyroid Physiology During Pregnancy

Changes in thyroid protein binding

The vast key literature in thyroid and pregnancy over the past century illustrates the advancements that have been made to better understand the changes in thyroid physiology unique to this life stage. Although thyroxine (T4) had been isolated in 1915, early serum T4 measurements were based on circulating protein-bound iodine (PBI) levels that had emerged in the early 1900s as a diagnostic test of estimating thyroid function. Heineman et al had observed in the 1940s that serum PBI levels are increased during pregnancy,[1] heralding the observation that gestation influences thyroid status.

Shortly after the discovery of thyroid binding globulin (TBG) in 1952, Dowling et al reported that circulating TBG-bound T4 levels were higher in pregnant women than in nonpregnant women,[2] which was hypothesized to be due to an increase of at least one of the alpha globulin binding proteins induced by pregnancy. Robbins, Nelson, and others in the 1950s showed that thyroid binding increases during pregnancy.[3] The pregnancy-associated increase in TBG was later shown to be due to estrogen, which prolongs the circulating half-life of TBG.[4] Glinoer et al in the 1990s characterized the multitude of changes in thyroid economy that occur during pregnancy, including the increased T4-bound TBG levels seen in early gestation.[5]

Interpretation of serum thyroid function tests

Pregnancy is associated with unique alterations of thyroid physiology that impact the interpretation of serum thyroid function tests. As biochemical hyperthyroidism had been observed in patients with choriocarcinomas and hydatidiform moles, Hershman et al hypothesized that the normal placenta likely contained a thyroid stimulator.[6] The first clinical reports arose from studies in the 1960s showing that women with human chorionic gonadotropin (hCG)-secreting placental tumors (e.g., hydatidiform moles and choriocarcinomas) were also hyperthyroid.[7]

Later work during the 1970s to early 1990s by Braunstein and Hershman,[8] Harada et al,[9] Glinoer et al[5] using more sensitive thyrotropin (TSH) assays provided compelling evidence that even in normal pregnancy, hCG directly stimulated T4 production from the thyroid, resulting in gestational transient thyrotoxicosis in some. These early studies laid the

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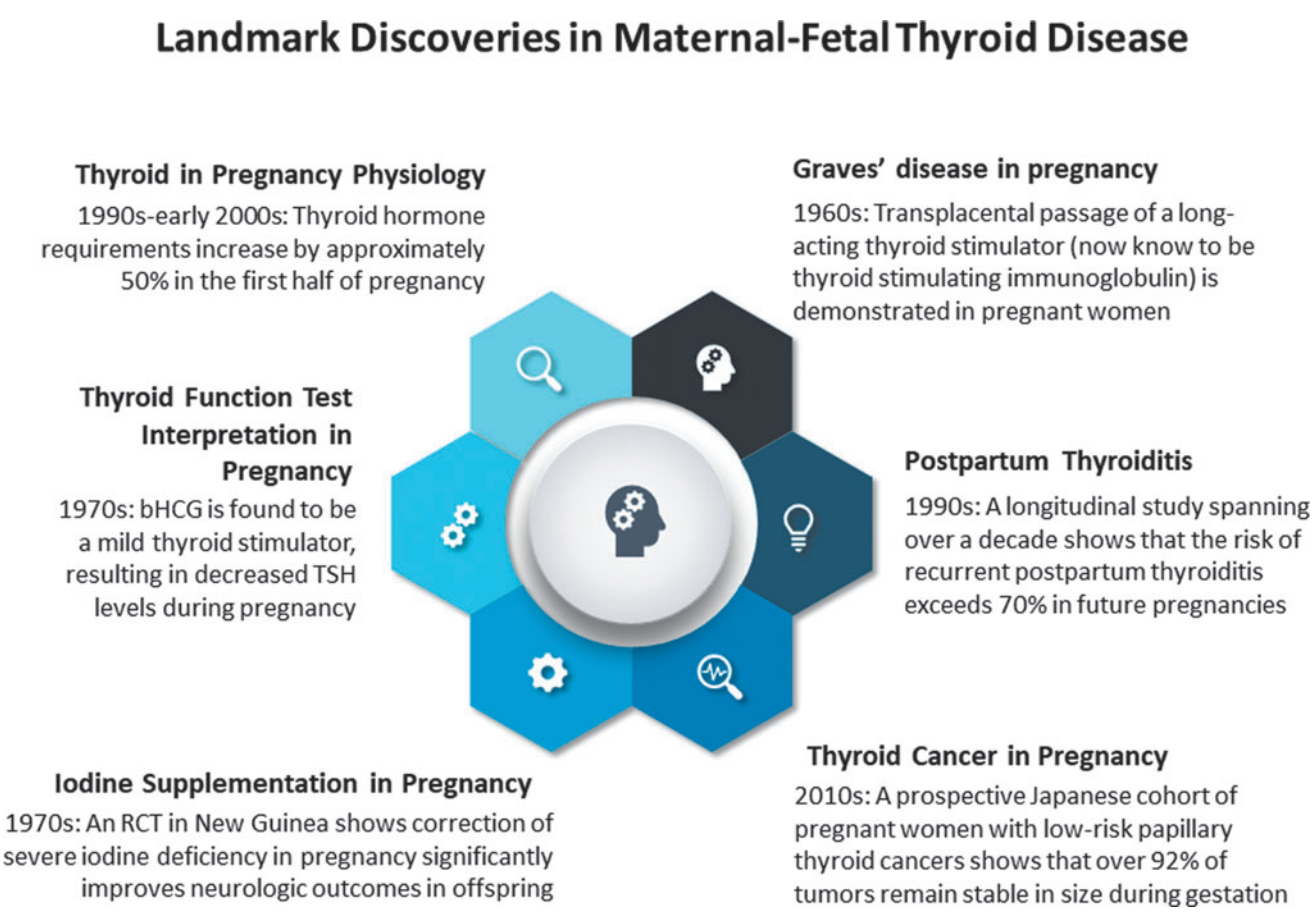

FIG. 1. Highlighted discoveries in maternal–fetal thyroid disease over the past century.

groundwork for the current American Thyroid Association (ATA) recommendations supporting a lower physiological reference limit or population-based trimester-specific reference ranges for serum TSH, primarily in the first trimester of pregnancy.[10]

Increased thyroid hormone requirements

In addition to the higher TBG-bound thyroid hormone levels induced by the rise of estrogen, other advances have further informed the need for increased thyroid hormone during pregnancy. Work by Roti et al[11] and Galton et al[12] showed that the placenta is abundant in the type III deiodinase that is responsible for converting T4 and triiodothyronine (T3) to inactive metabolites,[13] leading to faster degradation of the maternal thyroid hormone supply that is available to the fetus. Pregnancy is also associated with renal hyperfiltration and increased iodine clearance,[14] which would be exacerbated in regions of endemic iodine deficiency,[15] resulting in higher iodine needs to sustain normal thyroid hormone production for the developing fetus.

Among women with autoimmune thyroid disease, Glinoer et al showed that serum TSH levels are higher starting in the first few weeks of pregnancy and continue to rise more than in euthyroid controls throughout the entire course of gestation.[16] Seminal studies by Mandel et al in 1990[17] and Alexander et al in 2004 quantified the increased thyroid hormone requirements during pregnancy,[18] key practice-changing concepts that were championed by Larsen.[19] Thus, in order to meet the demands for a greater total body T4 pool during pregnancy, current guidelines by the ATA advise an ~30% higher dose of levothyroxine (LT4) in women treated for hypothyroidism.[10,18]

Iodine Nutrition in Preconception, Pregnancy, and Postpartum

Iodine deficiency during pregnancy

The possibility that severe iodine deficiency during pregnancy may be associated with adverse obstetric outcomes was observed in the early 1900s. Administration of iodide tincture to colts, calves, and pigs for 2 months before delivery prevented the nearly 90% postpartum mortality rate seen in animals of iodine-deficient regions.[20] In 1939, Kemp reported the anecdotal experience of several groups who had observed the benefit of prophylactically administering iodine and iron in pregnant women to decrease rates of stillbirth.[20]

Stronger scientific evidence of this association was not available until 1971, when the seminal study by Pharoah et al was published.[21] This randomized controlled trial in New Guinea, including women of childbearing age and/or pregnant women, showed a significant decrease of endemic severe iodine deficiency and associated mortality among the offspring born to families who were treated with iodized oil, compared with placebo.[21] The benefits of early iodine supplementation during pregnancy were later demonstrated by Cao et al in a pivotal cohort study performed in a severely

iodine-deficient remote province of China in 1994, in which offspring of women administered iodine supplementation before the third trimester had significantly lower risk of neurological abnormalities, compared with those born to women treated during later pregnancy.[22]

Increased iodine requirements during pregnancy and lactation

A large collective of work has shown that because of increased iodine and thyroid hormone needs for the developing fetus, as well as increased maternal renal iodine excretion,[23] dietary iodine needs are higher during pregnancy than they are for nonpregnant adults. In the 1970s, it was presumed that the iodine content in prenatal multivitamins was adequate for the increased iodide requirements during pregnancy.[24] However, data over the past decade have since shown that only ~50–60% of prenatal vitamin formulations in the United States contain any iodine, let alone optimal iodine content for gestation.[25,26]

Risks of iodine excess during pregnancy

In 1971, Senior and Chernoff described an infant with a goiter born to a mother who had received large doses of iodide throughout pregnancy.[27] Although Braverman and Ingbar had shown in the early 1960s that the normal adult thyroid in most cases is able to adjust to iodine excess,[28] the mechanism for this adaptation to the Wolff-Chaikoff effect[29] was not known until 1999, when Eng et al showed that iodine excess is associated with a temporary downregulation of the sodium iodide/symporter.[30] However, as the fetal thyroid gland is relatively immature, it cannot easily escape from the acute Wolff-Chaikoff effect,[31] thus posing the risk of fetal hypothyroidism upon an acute iodine load.

Fetal Development and Thyroid Disease

Role of maternal iodine and thyroid hormone during pregnancy

Before the recognition that adequate iodine status plays a crucial role in early growth and neurodevelopment, the diagnosis of neonatal severe iodine deficiency (termed cretinism in older texts) was challenging, with difficulties with feeding or gaining weight as the most common manifestations. Pioneering work by Stanbury et al in iodine metabolism during the 1950s[32] had established the important basis for their work during the 1950s and 1960s in understanding of the effects of endemic iodine deficiency.

A 1958 study of 49 children with cretinism reported that severe iodine deficiency was diagnosed at a mean age of 12 months,[33] with untreated children showing severe growth impairment, delayed closure of the fontanelles, periorbital edema, large protruding tongue, and cognitive impairments. The diagnosis of severe iodine deficiency during these early days was able to be made only crudely by the measurement of low serum PBI concentrations.[24]

Further studies during the 1960s were able to show that colloid formation, iodide concentration, and synthesis of thyroglobulin and T4 in the human fetal thyroid gland begin at 11 weeks gestation.[34] In addition, the fetal thyroid gland has an ~20–50 greater affinity for iodine than the maternal thyroid gland, as well as significantly increased thyroidal iodine turnover.[35] In 1969, Gitlin and Biasucci showed that fetal TSH appears as early as 14 weeks gestation,[36] but Greenberg et al shortly thereafter provided evidence that TSH is detectable and responsive to free T4 (fT4) even at 11 weeks gestation.[37]

Although it was understood that iodine, T3, and T4 all freely cross the placenta (with T3 appearing to cross more easily than T4),[38] it remained unclear even in the 1970s whether the fetus requires its own thyroid hormone supply during pregnancy or whether maternal thyroid hormone would be sufficient.[24] During the late 1980s, key animal experiments by Morreale de Escobar et al provided evidence of the maternal transfer of T4 through the placenta to the fetal rat.[39]

This was quantified by Vulsma et al, who in 1989 studied the offspring of euthyroid women with a history of underlying congenital hypothyroidism.[40] In these findings, the infants who were also affected with congenital hypothyroidism and documented to be athyreotic had ~20–50% of the normal levels of circulating thyroid hormones at birth, which also fell in accordance with the predicted serum half-life of T4, proving that transplacental crossing from the mother was their only possible source.[40]

Thyroid function newborn screening

Important studies in newborn thyroid function screening have been a significant public health accomplishment in the past century. Newborn screening programs were implemented in Switzerland in the late 1960s and rapidly became common in most developed countries during the 1970s. As it was also recognized that there is a marked increase, then fall, in neonatal serum TSH levels immediately after delivery,[41] continued refinement of strategies informed by Walfish[42] and others since has led to the adoption of regional screening algorithms.

The key report in 1979 by Fisher et al described the five oldest screening programs in North America, including a study by Dussault and colleagues in Quebec,[43] which in total had screened over one million newborns to yield an incidence rate of 1 in 3684 live births.[44] Recognition that early thyroid hormone replacement improves the cognitive and developmental impairments associated with untreated disease has virtually eliminated the adverse outcomes of congenital hypothyroidism.[45]

Hypothyroidism and Hypothyroxinemia During Pregnancy

Role of thyroid hormone in early development

The association of postnatal thyroid dysfunction and neurodevelopmental disorders has long been recognized; observations in the early 1800s noted that cretins (a pejorative, outdated historic medical term used to describe individuals with stunted growth and other physical deformities) appeared to also have substantial neurocognitive impairments.[46] Seminal studies by DeLange and colleagues were pivotal in reporting the association between maternal and fetal thyroid status, particularly in areas of endemic iodine deficiency.[47,48]

Later experiments during the 1960s showed that animals that had undergone thyroidectomy at birth have marked

morphological changes in the central nervous system,[49] as well as behavioral abnormalities; the defects appeared to be partially or wholly reversible if thyroid hormone was started shortly after birth.

Subsequent studies have focused on the influence of maternal and fetal thyroid status at earlier timepoints (i.e., prenatally) on neurodevelopment. Following experiments initially in rat, then later mouse, models, human data in the 1990s and early 2000s confirmed thyroid hormone concentrations in the fetal cortex[50] and in cord blood.[51] However, the extent to which fetal brain development is reliant on adequate thyroid hormone was still relatively uncertain even into the very late 1990s.[52]

Placental passage of thyroid hormone

For many years, the accepted thinking was that the fetal hypothalamic–pituitary thyroid development is independent of maternal thyroidal influence, even as recently as the early 1980s.[53] However, later seminal study by Obregon et al in 1984 reported that T4 and T3 were able to be measured by radioimmunoassay in rat embryos at 10–18 days gestation,[54] earlier than when the fetal rat thyroid begins to function around day 18, thus providing strong evidence that maternal thyroid hormone through placental passage is critical to fetal thyroid hormone supply. Much study has been done since to demonstrate that normal levels of thyroid hormone are essential for neuronal migration, myelination, and other structural changes of the fetal brain.[55]

Thyroid hormone replacement in maternal hypothyroidism

Several studies have investigated the associations between untreated maternal hypothyroidism and risks of adverse pregnancy complications. A 1993 study by Mestman et al showed a higher rate of gestational hypertension in hypothyroid women than in the general population.[15] Pop et al published several studies, the earliest in 1999,[56] on the association between maternal hypothyroxinemia during pregnancy and impaired infant development. In 2005, Casey et al described a 3-fold increased risk of placental abruption and nearly 2-fold risk of premature delivery in women with subclinical hypothyroidism when assessed at 20 weeks gestation.[57]

In one of the landmark studies in this field, Haddow et al in 1999 showed in a retrospective cohort series that untreated maternal hypothyroidism (ascertained at a mean gestational age of 17 ± 1 [standard deviation] weeks) was associated with decreased intelligent quotient (IQ) scores in offspring at age 7–9 years.[58] However, the role of maternal subclinical hypothyroidism (which may be associated with maternal thyroid antibody positivity) on these outcomes is less clear.

Higher TBG levels in normal pregnancy indicate a need for increased thyroid hormone production, but an increase in LT4 dose requirements in pregnant hypothyroid women was not observed in early studies. This may have been due to the generally higher LT4 doses used before the availability of a sensitive TSH assay to mask this need. In 1990, Mandel et al showed that 9 of 12 hypothyroid pregnant women required an increased dose of LT4 (by an average of 50%), compared with their prepregnancy dose, and returned to their prepregnancy requirement in the postpartum period.[17]

A subsequent study by Alexander et al showed that this increased dose requirement occurred primarily in the first half of pregnancy and plateaued at gestational week 16.[18] A more recent study by Korevaar and colleagues has demonstrated that both high and low maternal fT4 concentrations are associated with lower childhood IQ though, suggesting that overcorrection of maternal hypothyroidism may in itself also have adverse effects.[59]

Braverman et al published in 1970 their seminal article reporting that the primary source of circulating T3 in humans was the peripheral conversion of T4 to T3, leading to the recommendation of LT4 therapy for hypothyroidism,[60] including during pregnancy. It was, however, already recognized that T3 and T4 have markedly different affinities to thyroid binding proteins, suggesting that T3 may more easily cross the placenta than T4.[58]

Dumitrescu et al in the early 2000s also showed that T3 and T4 transport across the blood–brain barrier occurs through the active transporter protein, monocarboxylate transporter 8.[61] Work in the past two decades has also shown that T4, but not T3, can additionally cross into the fetal brain through organic anion transporting polypeptide 1C1 (Oatp1c1),[62] but is poorly expressed in the human fetus.[63] Morreale de Escobar et al demonstrated that maternal T3 is unable to reach the fetal brain and alleviate fetal hypothyroidism.[64] Thus, any maternal use of T3-containing regimens, including desiccated thyroid extract or synthetic T3, has the potential to reduce thyroid hormone action fetal brain, but direct data are not available.[10]

Hyperthyroidism During Pregnancy

Graves' disease during pregnancy

Although a case of neonatal hyperthyroidism born to a pregnant woman with Graves' disease had been described in 1912,[65] the role of maternal TSH receptor antibodies was not understood until several decades later. During the 1950s, Graves' disease was hypothesized to result from an undefined long-acting thyroid stimulator first described in 1956 by Adams and Purves.[66] The initial reports of transplacental passage of a maternal thyroid stimulating antibody were from the early 1960s[67] and supported an autoimmune basis of fetal Graves' disease. A retrospective cohort study of 18 women by Dirmikis and Munro in 1975 suggested that there must be a likely threshold of elevated maternal thyroid stimulating immunoglobulin (TSI) titers during pregnancy to pose a risk to the fetus.[68]

A 1983 study of 20 women showed among those who had infants with neonatal Graves' disease, all had sera containing antibodies able to induce a >500% tissue cAMP response to TSH stimulation,[69] forming the basis for recommendations advising maternal serum TSI titers be measured during midpregnancy, to assess fetal/neonatal hyperthyroidism risk.[10]

Treatment of hyperthyroidism during pregnancy

In the 1970s, it remained relatively unclear if untreated maternal thyrotoxicosis during pregnancy was associated with adverse obstetric outcomes and/or increased fetal

mortality.[24] However, a landmark article by Anselmo et al in 2004 provided evidence of a direct adverse effect of high maternal thyroid hormone levels on the fetus.[70] Offspring born to women in an Azorean family with thyroid hormone resistance (i.e., offspring were unaffected) were observed to have low birthweights and suppressed TSH levels, resulting from their exposure to high maternal thyroid hormone concentrations in utero.[70]

Treatment during the 1970s was primarily thioamides, titrated to serum PBI values. In 1986, a landmark study by Momotani et al demonstrated that targeting minimally elevated or high-normal maternal serum fT4 levels with antithyroid drugs is the most appropriate to maintain euthyroid status in the fetus.[71] Later important study by Andersen et al in the early 2010s showed the risks of congenital anomalies with antithyroid drug use and steered the field toward minimal use of these medications, along with consideration of their different side effect profiles, during gestation.[72]

In the postpartum period, there were isolated reports of cognitive impairments and goiters in children secondary to maternal propylthiouracil use during lactation. As such, mothers receiving these drugs were initially advised to not breastfeed their infants, but Low et al in 1979 showed that propylthiouracil has a breast milk–plasma ratio of only 0.1, while that for methimazole is closer to 1.0,[73] thereby providing evidence that the breast milk content of these medications is extremely low and their use during lactation is overall safe.[10]

If surgical treatment of hyperthyroidism was advised, it was understood that the thyroid surgery should be delayed until after the first trimester. It is interesting that if a total thyroidectomy was performed during pregnancy, usual postoperative thyroid hormone replacement in the 1970s was to begin three grains of DTE or its equivalent daily for the remainder of gestation,[24] advice that is no longer espoused due to the difficulties of T3 crossing the fetal blood–brain barrier.[39]

Laboratory Thyroid Antibody and Thyroid Hormone Screening in Preconception and Pregnancy

The prevalence of hypothyroidism during pregnancy ranges from 0.5% (overt hypothyroidism) to 3.47% (subclinical hypothyroidism).[10,74] Several observational studies have estimated the prevalence of serum anti-TPO or anti-Tg thyroid autoantibodies (2–17% in unselected pregnant women, depending on ethnicity).[10] The consideration of serum thyroid laboratory screening during preconception or pregnancy arose from the aforementioned study, as based on potential deleterious obstetric and neonatal effects among women with hypothyroidism or hypothyroxinemia.

Other studies have also demonstrated the obstetric risks of thyroid autoimmunity per se, even in the setting of biochemical euthyroidism. A seminal study by Stagnaro-Green et al found positive serum thyroid antibodies in 19.6% of a screened first-trimester cohort and was the first to report an association between serum anti-TPO antibody positivity in pregnant women and miscarriage,[75] while more recent pooled pregnancy data by Korevaar et al in the Consortium on Thyroid and Pregnancy show positive associations between subclinical hypothyroidism, isolated hypothyroxinemia, TPO antibody positivity, and risk of preterm birth.[76]

Some countries, including Spain, China, and Poland, employ universal thyroid screening during pregnancy, while the United States has adopted a more restricted case-finding approach aimed to detect women at highest risk.[10] Dosiou et al have proposed that universal screening for autoimmune thyroid dysfunction in first-trimester pregnancies is cost-effective, compared with no screening or even screening of only high-risk women.[77]

The potential risks versus benefits of thyroid screening before and during pregnancy require further study, particularly regarding maternal subclinical hypothyroidism if found,[78] but a small number of randomized clinical trials in the past $\sim$15 years have advanced knowledge on this topic. A 2010 Italian study by Negro et al reported no differences in adverse obstetrical or neonatal outcomes in 4562 first-trimester pregnant women, whether or not they underwent universal measurement of serum TSH, FT4, and TPO Ab levels (and treated with LT4 if a TSH >2.5 with positive TPO Ab was found, or antithyroidal medication if hyperthyroid was found).[79]

Similarly, the Controlled Antenatal Thyroid Screening study and a U.S. National Institutes of Health study showed no benefits to systematic second-trimester screening and correction of maternal subclinical hypothyroidism or hypothyroxinemia, if discovered, on child cognition at age 3–5 years.[80,81] In contrast, another study in China by Ma et al in 2016 had reported the beneficial association of thyroid function screening (and initiation of LT4 if subclinical hypothyroidism was found) and decreased miscarriage and macrosomia risks, compared with no screening.[82] Subsequent robust randomized clinical trials have not shown a benefit of LT4 use in euthyroid, thyroid antibody pregnant women to increase live birth rates.[83,84]

Postpartum Thyroid Disease

In the 1950s, Hazard described a variant of Hashimoto's thyroiditis, characterized cytologically by extensive lymphocytic infiltration without epithelial oxyphilia[85] that was seen frequently in postpartum women.[24] One of the earliest series describing postpartum thyroiditis was of six patients at Osaka University Hospital by Amino et al, who noted the spontaneous occurrence then recovery from primary hypothyroidism in the 1–3 months after delivery; all of the women had positive serum antimicrosomal antibodies.[86] Important later study by Lazarus et al in the 1990s showed that there is an approximate 70% risk of recurrent postpartum thyroiditis after an initial episode.[87]

Thyroid Nodules and Thyroid Cancer During Pregnancy

Prevalence of thyroid nodules in pregnant women

The hypothesis of a positive association between the prevalence of thyroid nodules and pregnancy was proposed by Burrow in the early 1970s.[24] From two studies in 2010 and 2014, the question of whether or not there is pregnancy-mediated hormonal stimulation to increase the risk of developing thyroid nodules remains still unclear,[88,89] but limited observational data suggest a potential association.[90] Several observational studies in the 1990s and early 2000s

reported that the prevalence of thyroid nodules during pregnancy ranges from 3% to 30%,[91] depending on iodine status and geographic region.

Impact of pregnancy in thyroid cancer risk

Similarly, whether or not pregnancy plays a role in worsening pre-existing thyroid cancer remains incompletely understood. A study by Ito et al in 2016 of the active surveillance of biopsy-proven micropapillary thyroid cancers, including in pregnant women, has allowed some reassurance; only 4 of 51 women showed growth of ≥3 mm during pregnancy, and none of the women developed nodal metastases.[92] This was in contrast to a previous analysis by Shindo et al in 2014 of a smaller cohort ($n = 9$), who showed a greater risk of tumor enlargement during pregnancy, compared with nonpregnant controls.[93]

Risks of thyroid cancer treatment in preconception, pregnancy, and postpartum

It is generally established that thyroid surgery during pregnancy should be performed during the second trimester.[10] Although the use of radioactive iodine ablation or adjuvant therapy is contraindicated during pregnancy, studies during the early 2000s have shown that radiation given many years before conception is unlikely to have any adverse consequences related to infertility, pregnancy loss, stillbirths, neonatal mortality, congenital malformations, preterm births, low birth weight, death during the first year of life, or cancers in offspring.[94,95]

Conclusion

The past century has seen significant advancements in the understanding of maternal–fetal thyroid disease. As we look toward the future, continued study will further refine how the thyroid health of mother and baby can be further optimized during this important life stage for both.

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Author's Contribution

A.M.L. contributed to conceptualization and writing.

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References

1. Heinemann M, Johnson CE, Man EB. Serum precipitable iodine concentrations during pregnancy. J Clin Invest 1948; 27(1):91–97; doi: 10.1172/JCI101928.
2. Dowling JT, Freinkel N, Ingbar SH. Thyroxine-binding by sera of pregnant women. J Clin Endocrinol Metab 1956; 16(2):280–282; doi: 10.1210/jcem-16-2-280.
3. Robbins J, Nelson JH. Thyroxine binding by serum protein in pregnancy and in the newborn. J Clin Invest 1958;37(2): 153–159; doi:10.1172/JCI103593.
4. Engbring NH, Engstrom WW. Effects of estrogen and testosterone on circulating thyroid hormone. J Clin Endocrinol Metab 1959;19(7):783–796; doi: 10.1210/jcem-19-7-783.
5. Glinoer D, de Nayer P, Bourdoux P, et al. Regulation of maternal thyroid during pregnancy. J Clin Endocrinol Metab 1990;71(2):276–287; doi: 10.1210/jcem-71-2-276.
6. Hershman JM, Higgins HP, Starnes WR. Differences between thyroid stimulator in hydatidiform mole and human chorionic thyrotropin. Metabolism 1970;19(10): 735–744; doi: 10.1016/0026-0495(70)90071-5.
7. Yoshimura M, Hershman JM. Thyrotropic action of human chorionic gonadotropin. Thyroid 1995;5(5):425; doi: 10.1089/thy.1995.5.425.
8. Braunstein GD, Hershman JM. Comparison of serum pituitary thyrotropin and chorionic gonadotropin concentrations throughout pregnancy. J Clin Endocrinol Metab 1976;42(6):1123–1126; doi: 10.1210/jcem-42-6-1123.
9. Harada A, Hershman JM, Reed AW, et al. Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. J Clin Endocrinol Metab 1979; 48(5):793–797; doi: 10.1210/jcem-48-5-793.
10. Alexander EK, Pearce EN, Brent GA, et al. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid 2017;27(3):315–389; doi: 10.1089/thy.2016.0457.
11. Roti E, Fang SL, Emerson CH, et al. Placental inner ring iodothyronine deiodination: A mechanism for decreased passage of T4 and T3 from mother to fetus. Trans Assoc Am Phys 1981;94:183–189.
12. Galton VA, Martinez E, Hernandez A, et al. Pregnant rat uterus expresses high levels of the type 3 iodothyronine deiodinase. J Clin Invest 1999;103(7):979–987; doi: 10.1172/JCI6073.
13. Salvatore D, Low SC, Berry M, et al. Type 3 lodothyronine deiodinase: Cloning, in vitro expression, and functional analysis of the placental selenoenzyme. J Clin Invest 1995; 96(5):2421–2430; doi: 10.1172/JCI118299.
14. Aboul-Khair SA, Crooks J, Turnbull AC, et al. The physiological changes in thyroid function during pregnancy. Clin Sci 1964;27:195–207.
15. Degroot LJ. Current views on formation of thyroid hormones. N Engl J Med 1965;272:355–363 CONCL; doi: 10.1056/NEJM196502182720706.
16. Glinoer D, Riahi M, Grun JP, et al. Risk of subclinical hypothyroidism in pregnant women with asymptomatic autoimmune thyroid disorders. J Clin Endocrinol Metab 1994;79(1):197–204; doi: 10.1210/jcem.79.1.8027226.
17. Mandel SJ, Larsen PR, Seely EW, et al. Increased need for thyroxine during pregnancy in women with primary hypothyroidism. N Engl J Med 1990;323(2):91–96; doi: 10.1056/NEJM199007123230204.
18. Alexander EK, Marqusee E, Lawrence J, et al. Timing and magnitude of increases in levothyroxine requirements during pregnancy in women with hypothyroidism. New Engl J Med 2004;351(3):241; doi: 10.1056/NEJMoa040079.

19. Larsen PR. Monitoring thyroxine treatment during pregnancy. Thyroid 1992;2(2):153–154; doi: 10.1089/thy.1992.2.153.
20. Kemp WN. Iodine deficiency in relation to the stillbirth problem. Can Med Assoc J 1939;41(4):356–361.
21. Pharoah PO, Buttfield IH, Hetzel BS. Neurological damage to the fetus resulting from severe iodine deficiency during pregnancy. Lancet 1971;1(7694):308; doi: 10.1016/s0140-6736(71)91040-3.
22. Cao XY, Jiang XM, Dou ZH, et al. Timing of vulnerability of the brain to iodine deficiency in endemic cretinism. N Engl J Med 1994;331(26):1739; doi: 10.1056/NEJM199412293312603.
23. Dafnis E, Sabatini S. The effect of pregnancy on renal function: Physiology and pathophysiology. Am J Med Sci 1992;303(3):184; doi: 10.1097/00000441-199203000-00011.
24. Burrow GN. The Thyroid Gland in Pregnancy. W.B. Saunders Company: Philadelphia; 1972.
25. Leung AM, Pearce EN, Braverman LE. Iodine content of prenatal multivitamins in the United States. N Engl J Med 2009;360(9):939; doi: 360/9/939 [pii] 10.1056/NEJMc0807851.
26. Lee SY, Stagnaro-Green A, MacKay D, et al. Iodine contents in prenatal vitamins in the United States. Thyroid 2017;27(8):1101–1102; doi: 10.1089/thy.2017.0097.
27. Senior B, Chernoff HL. Iodide goiter in the newborn. Pediatrics 1971;47(3):510–515.
28. Braverman LE, Ingbar SH. Changes in thyroidal function during adaptation to large doses of iodide. J Clinical Invest 1963;42(8):1216–1231; doi: 10.1172/jci104807.
29. Wolff J, Chaikoff IL. Plasma inorganic iodide as a homeostatic regulator of thyroid function. J Biol Chem 1948;174(2):555–564.
30. Eng PH, Cardona GR, Fang SL, et al. Escape from the acute Wolff-Chaikoff effect is associated with a decrease in thyroid sodium/iodide symporter messenger ribonucleic acid and protein. Endocrinology 1999;140(8):3404; doi: 10.1210/endo.140.8.6893.
31. Theodoropoulos T, Braverman LE, Vagenakis AG. Iodide-induced hypothyroidism: A potential hazard during perinatal life. Science 1979;205(4405):502–503; doi: 10.1126/science.451615.
32. Stanbury JB, Brownell GL, Riggs DS, et al. The iodine-deficient human thyroid gland; A preliminary report. J Clin Endocrinol Metab 1952;12(2):191–207; doi: 10.1210/jcem-12-2-191.
33. Lowrey GH, Aster RH, Carr EA, et al. Early diagnostic criteria of congenital hypothyroidism: A comprehensive study of forty-nine cretins. AMA J Dis Child 1958;96(2):131–143; doi: 10.1001/archpedi.1958.02060060133002.
34. Shepard TH. Onset of function in the human fetal thyroid: Biochemical and radioautographic studies from organ culture. J Clin Endocrinol Metab 1967;27(7):945–958; doi: 10.1210/jcem-27-7-945.
35. Beierwaltes WH, Crane HR, Wegst A, et al. Radioactive iodine concentration in the fetal human thyroid gland from fall-out. JAMA 1960;173:1895–1902; doi: 10.1001/jama.1960.03020350013003.
36. Gitlin D, Biasucci A. Ontogenesis of immunoreactive growth hormone, follicle-stimulating hormone, thyroid-stimulating hormine, luteinizing hormone, chorionic prolactin and chorionic gonadotropin in the human conceptus. J Clin Endocrinol Metab 1969;29(7):926–935; doi: 10.1210/jcem-29-7-926.
37. Greenberg AH, Czernichow P, Reba RC, et al. Observations on the maturation of thyroid function in early fetal life. J Clin Invest 1970;49(10):1790–1803; doi: 10.1172/JCI106397.
38. Grumbach MM, Werner SC. Transfer of thyroid hormone across the human placenta at term. J Clin Endocrinol Metab 1956;16(10):1392–1395; doi: 10.1210/jcem-16-10-1392.
39. Morreale de Escobar G, Obregon MJ, Ruiz de Oña C, et al. Transfer of thyroxine from the mother to the rat fetus near term: Effects on brain 3,5,3'-triiodothyronine deficiency. Endocrinology 1988;122(4):1521–1531; doi: 10.1210/endo-122-4-1521.
40. Vulsma T, Gons MH, de Vijlder JJ. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid agenesis. N Engl J Med 1989;321(1):13–16; doi: 10.1056/NEJM198907063210103.
41. Fisher DA, Odell WD. Acute release of thyrotropin in the newborn. J Clin Invest 1969;48(9):1670–1677; doi: 10.1172/JCI106132.
42. Walfish PG. Evaluation of three thyroid-function screening tests for detecting neonatal hypothyroidism. Lancet 1976;1(7971):1208–1210; doi: 10.1016/s0140-6736(76)92159-0.
43. Glorieux J, Dussault JH, Letarte J, et al. Preliminary results on the mental development of hypothyroid infants detected by the Quebec Screening Program. J Pediatr 1983;102(1):19–22; doi: 10.1016/s0022-3476(83)80279-0.
44. Fisher DA, Dussault JH, Foley TP, Jr, et al. Screening for congenital hypothyroidism: Results of screening one million North American infants. J Pediatr 1979;94(5):700–705; doi: 10.1016/s0022-3476(79)80133-x.
45. American Academy of Pediatrics, Rose SR, Section on Endocrinology and Committee on Genetics American Thyroid Association, et al. Update of newborn screening and therapy for congenital hypothyroidism. Pediatrics 2006;117(6):2290; doi: 117/6/2290 [pii].
46. Oppenheimer JH, Schwartz HL. Molecular basis of thyroid hormone-dependent brain development. Endocr Rev 1997;18(4):462–475; doi: 10.1210/edrv.18.4.0309.
47. Thilly CH, Delange F, Lagasse R, et al. Fetal hypothyroidism and maternal thyroid status in severe endemic goiter. J Clin Endocrinol Metab 1978;47(2):354–360; doi: 10.1210/jcem-47-2-354.
48. Delange F, Camus M, Ermans AM. Circulating thyroid hormones in endemic goiter. J Clin Endocrinol Metab 1972;34(5):891–895;doi: 10.1210/jcem-34-5-891.
49. Eayrs JT. Endocrine influence on cerebral development. Arch Biol (Liege) 1964;75:529–565.
50. Kester MH, Martinez de Mena R, Obregon MJ, et al. Iodothyronine levels in the human developing brain: Major regulatory roles of iodothyronine deiodinases in different areas. J Clin Endocrinol Metab 2004;89(7):3117–3128; doi: 10.1210/jc.2003-031832.
51. Thorpe-Beeston JG, Nicolaides KH, Felton CV, et al. Maturation of the secretion of thyroid hormone and thyroid-stimulating hormone in the fetus. N Engl J Med 1991;324(8):532–536; doi: 10.1056/NEJM199102213240805.
52. Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Role of thyroid hormone during early brain development. Eur J Endocrinol 2004;151 Suppl 3:U25–U37; doi: 10.1530/eje.0.151u025.
53. Fisher DA, Klein AH. Thyroid development and disorders of thyroid function in the newborn. N Engl J Med 1981;304(12):702–712; doi: 10.1056/NEJM198103193041205.

54. Obregon MJ, Mallol J, Pastor R, et al. L-thyroxine and 3,5,3'-triiodo-L-thyronine in rat embryos before onset of fetal thyroid function. Endocrinology 1984;114(1):305–307; doi: 10.1210/endo-114-1-305.
55. Bernal J, Guadaño-Ferraz A, Morte B. Thyroid hormone transporters-functions and clinical implications. Nat Rev Endocrinol 2015;11(9):506; doi: 10.1038/nrendo.2015.113.
56. Pop VJ, Kuijpens JL, van Baar AL, et al. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clin Endocrinol 1999;50(2):149; doi: 10.1046/j.1365-2265.1999.00639.x.
57. Casey BM, Dashe JS, Wells CE, et al. Subclinical hypothyroidism and pregnancy outcomes. Obstet Gynecol 2005;105(2):239; doi: 105/2/239 [pii] 10.1097/01.AOG.0000152345.99421.22.
58. Haddow JE, Palomaki GE, Allan WC, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 1999;341(8):549; doi: 10.1056/NEJM199908193410801.
59. Korevaar TI, Muetzel R, Medici M, et al. Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: A population-based prospective cohort study. Lancet Diabetes Endocrinol 2016;4(1):35–43; doi: 10.1016/S2213-8587(15)00327-7.
60. Braverman LE, Ingbar SH, Sterling K. Conversion of thyroxine (T4) to triiodothyronine (T3) in athyreotic human subjects. J Clin Invest 1970;49(5):855–864; doi: 10.1172/JCI106304.
61. Dumitrescu AM, Liao XH, Weiss RE, et al. Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (mct) 8-deficient mice. Endocrinology 2006;147(9):4036–4043; doi: 10.1210/en.2006-0390.
62. Mayerl S, Müller J, Bauer R, et al. Transporters MCT8 and OATP1C1 maintain murine brain thyroid hormone homeostasis. J Clin Invest 2014;124(5):1987–1999; doi: 10.1172/JCI70324.
63. Roberts LM, Woodford K, Zhou M, et al. Expression of the thyroid hormone transporters monocarboxylate transporter-8 (SLC16A2) and organic ion transporter-14 (SLCO1C1) at the blood-brain barrier. Endocrinology 2008;149(12):6251–6261; doi: 10.1210/en.2008-0378.
64. Morreale de Escobar G, Obregon MJ, Ruiz de Ona C, et al. Comparison of maternal to fetal transfer of 3,5,3'-triiodothyronine versus thyroxine in rats, as assessed from 3,5,3'-triiodothyronine levels in fetal tissues. Acta Endocrinol (Copenh) 1989;120(1):20–30; doi: 10.1530/acta.0.1200020.
65. White C. A foetus with congenital hereditary Graves's disease. Proc R Soc Med 1912;5(Obstet Gynaecol Sect):247–252.
66. Adams DD, Purves HD. Abnormal responses in the assay of thyrotropins. Proc Univ Otago Med School 1956;34:11–12.
67. McKenzie JM. Neonatal Graves' disease. J Clin Endocrinol Metab 1964;24:660–668; doi: 10.1210/jcem-24-7-660.
68. Dirmikis SM, Munro DS. Placental transmission of thyroid-stimulating immunoglobulins. Br Med J 1975;2(5972):665–666; doi: 10.1136/bmj.2.5972.665.
69. Zakarija M, McKenzie JM. Pregnancy-associated changes in the thyroid-stimulating antibody of Graves' disease and the relationship to neonatal hyperthyroidism. J Clin Endocrinol Metab 1983;57(5):1036–1040; doi: 10.1210/jcem-57-5-1036.
70. Anselmo J, Cao D, Karrison T, et al. Fetal loss associated with excess thyroid hormone exposure. JAMA 2004;292(6):691–695; doi: 10.1001/jama.292.6.691.
71. Momotani N, Noh J, Oyanagi H, et al. Antithyroid drug therapy for Graves' disease during pregnancy. Optimal regimen for fetal thyroid status. N Engl J Med 1986;315(1):24–28; doi: 10.1056/NEJM198607033150104.
72. Andersen SL, Olsen J, Wu CS, et al. Birth defects after early pregnancy use of antithyroid drugs: A Danish nationwide study. J Clin Endocrinol Metab 2013;98(11):4373–4381; doi: 10.1210/jc.2013-2831.
73. Low LC, Lang J, Alexander WD. Excretion of carbimazole and propylthiouracil in breast milk. Lancet 1979;2(8150):1011; doi: 10.1016/s0140-6736(79)92575-3.
74. Dong AC, Stagnaro-Green A. Differences in diagnostic criteria mask the true prevalence of thyroid disease in pregnancy: A systematic review and meta-analysis. Thyroid 2019;29(2):278–289; doi: 10.1089/thy.2018.0475.
75. Stagnaro-Green A, Roman SH, Cobin RH, et al. Detection of at-risk pregnancy by means of highly sensitive assays for thyroid autoantibodies. JAMA 1990;264(11):1422–1425.
76. Consortium on T, Pregnancy-Study Group on Preterm B, Korevaar TIM, et al. Association of thyroid function test abnormalities and thyroid autoimmunity with preterm birth: A systematic review and meta-analysis. JAMA 2019;322(7):632–641; doi: 10.1001/jama.2019.10931.
77. Dosiou C, Barnes J, Schwartz A, et al. Cost-effectiveness of universal and risk-based screening for autoimmune thyroid disease in pregnant women. J Clin Endocrinol Metab 2012;97(5):1536-46; doi: 10.1210/jc.2011-2884.
78. Maraka S, Ospina NM, O'Keeffe DT, et al. Subclinical hypothyroidism in pregnancy: A systematic review and meta-analysis. Thyroid 2016;26(4):580–590; doi: 10.1089/thy.2015.0418.
79. Negro R, Schwartz A, Gismondi R, et al. Universal screening versus case finding for detection and treatment of thyroid hormonal dysfunction during pregnancy. J Clin Endocrinol Metab 2010;95(4):1699; doi: 10.1210/jc.2009-2009.
80. Lazarus JH, Bestwick JP, Channon S, et al. Antenatal thyroid screening and childhood cognitive function. N Engl J Med 2012;366(6):493–501; doi: 10.1056/NEJMoa1106104.
81. Casey BM, Thom EA, Peaceman AM, et al. Treatment of subclinical hypothyroidism or hypothyroxinemia in pregnancy. N Engl J Med 2017;376(9):815–825; doi: 10.1056/NEJMoa1606205.
82. Ma L, Qi H, Chai X, et al. The effects of screening and intervention of subclinical hypothyroidism on pregnancy outcomes: A prospective multicenter single-blind, randomized, controlled study of thyroid function screening test during pregnancy. J Matern Fetal Neonatal Med 2016;29(9):1391–1394; doi: 10.3109/14767058.2015.1049150.
83. Dhillon-Smith RK, Middleton LJ, Sunner KK, et al. Levothyroxine in women with thyroid peroxidase antibodies before conception. N Engl J Med 2019;380(14):1316–1325; doi: 10.1056/NEJMoa1812537.
84. van Dijk MM, Vissenberg R, Fliers E, et al. Levothyroxine in euthyroid thyroid peroxidase antibody positive women with recurrent pregnancy loss (T4LIFE trial): A multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Diabetes Endocrinol 2022;10(5):322–329; doi: 10.1016/S2213-8587(22)00045-6.

85. Hazard JB. Thyroiditis: A review. Am J Clin Pathol 1955; 25(3):289–298; contd; doi: 10.1093/ajcp/25.3.289.
86. Amino N, Miyai K, Onishi T, et al. Transient hypothyroidism after delivery in autoimmune thyroiditis. J Clin Endocrinol Metab 1976;42(2):296–301; doi: 10.1210/jcem-42-2-296.
87. Lazarus JH, Ammari F, Oretti R, et al. Clinical aspects of recurrent postpartum thyroiditis. Br J Gen Pract 1997; 47(418):305–308.
88. Messuti I, Corvisieri S, Bardesono F, et al. Impact of pregnancy on prognosis of differentiated thyroid cancer: Clinical and molecular features. Eur J Endocrinol 2014; 170(5):659–666; doi: 10.1530/EJE-13-0903.
89. Vannucchi G, Perrino M, Rossi S, et al. Clinical and molecular features of differentiated thyroid cancer diagnosed during pregnancy. Eur J Endocrinol 2010;162(1): 145–151; doi: 10.1530/EJE-09-0761.
90. Kung AW, Chau MT, Lao TT, et al. The effect of pregnancy on thyroid nodule formation. J Clin Endocrinol Metab 2002;87(3):1010–1014; doi: 10.1210/jcem.87.3.8285.
91. Papaleontiou M, Haymart MR. Thyroid nodules and cancer during pregnancy, post-partum and preconception planning: Addressing the uncertainties and challenges. Best Pract Res Clin Endocrinol Metab 2020;34(4):101363; doi: 10.1016/j.beem.2019.101363.
92. Ito Y, Miyauchi A, Kudo T, et al. Effects of pregnancy on papillary microcarcinomas of the thyroid re-evaluated in the entire patient series at Kuma Hospital. Thyroid 2016; 26(1):156–160; doi: 10.1089/thy.2015.0393.
93. Shindo H, Amino N, Ito Y, et al. Papillary thyroid microcarcinoma might progress during pregnancy. Thyroid 2014; 24(5):840–844; doi: 10.1089/thy.2013.0527.
94. Sawka AM, Thabane L, Parlea L, et al. Second primary malignancy risk after radioactive iodine treatment for thyroid cancer: A systematic review and meta-analysis. Thyroid 2009;19(5):451–457; doi: 10.1089/thy.2008.0392.
95. Garsi JP, Schlumberger M, Rubino C, et al. Therapeutic administration of 131I for differentiated thyroid cancer: Radiation dose to ovaries and outcome of pregnancies. J Nucl Med 2008;49(5):845–852; doi: 10.2967/jnumed.107.046599.

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Radioactive Iodine: A Living History

Gilbert H. Daniels and Douglas S. Ross

Background: Before the development of antithyroid drugs in the 1940s, treatment of Graves' hyperthyroidism was primarily surgical. Surgical mortality was quite variable, but a significant minority of patients died during or after surgery.
Summary: In 1936, Karl Compton, President of the Massachusetts Institute of Technology, in a lecture attended by Massachusetts General Hospital physicians, suggested that artificially radioactive isotopes might be useful for studying metabolism. By 1942, Hertz and Roberts reported on the successful use of radioactive iodine (RAI) to treat Graves' hyperthyroidism. RAI uptake was subsequently demonstrated in well-differentiated thyroid cancer metastases. In 1948, Seidlin demonstrated stimulation of uptake in thyroid cancer metastases by thyrotropin (TSH). By 1990, 69% of endocrinologists in North America recommended RAI for Graves' hyperthyroidism. Currently RAI is less frequently used for Graves' hyperthyroidism, related to concerns about exacerbation of thyroid eye disease, about radiation exposure, and about potential adverse consequences of permanent hypothyroidism. Similarly, RAI was administered to the majority of patients with thyroid cancer for decades, but its use is now more selective.
Conclusions: RAI is a remarkable example of interinstitutional cooperation between physicians and scientists to transition from bench to bedside in only three years. It is the model for a theranostic approach to disease (the simultaneous use of a radioactive drug for diagnosis and therapy). The future of RAI is less certain; inhibition of TSH receptor stimulating antibodies in Graves' disease and more precise targeting of genes that drive thyroid oncogenesis may diminish the use of RAI. Alternatively, redifferentiation techniques may improve the efficacy of RAI in RAI-refractory thyroid cancer.

Keywords: hyperthyroidism, radioactive iodine, thyroid cancer

The Prelude

In the early 1930s, the therapeutic options for Graves' hyperthyroidism (''exophthalmic goiter'') were quite limited. The testing and development of antithyroid drugs by Astwood were a decade away (thiourea and thiouracil in 1943, propylthiouracil in 1945, and methimazole in 1949) (1). Artificially produced designer radiolabeled drugs for medical purposes could not even be imagined. Surgery was the mainstay of therapy.

Other therapies for hyperthyroidism were of uncertain efficacy. The Massachusetts General Hospital (MGH) Thyroid Clinic began studying external beam radiation in 1917, reporting cures in up to one-third of patients and improvement in another third (1924). However, the practice was largely abandoned by 1930. Hyperthyroid patients received 80–120 r (cGy) weekly for 8–10 weeks or 300 r (cGy) every other week for 8–12 cycles, either as a stand-alone therapy or (after an additional 3-week wait) as a prelude to surgery. Multiple courses of therapy might be administered. Debilitated patients were sometimes treated with radium to little effect (2,3). In contrast, modern external beam radiation for head and neck cancer delivers 5000–6000 r (cGy), causing hypothyroidism in most patients.

Pharmacological (6 mg) doses of iodine produced variable but rapid (often within hours) improvement in symptoms and basal metabolic rate (BMR), with a peak effect at 10 days. Inhibition of thyroid hormone release is the likely

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mechanism. Higher doses of iodine did not have added benefit. BMR was the definitive laboratory test for hyperthyroidism. Unfortunately, Dr. James Howard Means noted that continuation of iodine might result in ''intensification of the thyrotoxic picture'' (4).

It was also well known that iodine was contraindicated for toxic nodular goiter. Preoperative iodine therapy became standard around 1922 (see last paragraph of ''The Prelude''), which coincided with abandoning of iodine as stand-alone Graves' therapy. Currently, iodine therapy for Graves' hyperthyroidism is being revisited in Japan and other countries (5).

Subtotal thyroidectomy (defined as removal of four-fifth of the thyroid) was the definitive therapy for Graves' disease and was clearly effective, but patient mortality, initially due to uncontrollable hemorrhage and subsequently to thyroid storm and infections, was highly variable due to varying surgical skill.

Highly skilled surgeons such as the Australian surgeon Dunhill, the legendary Johns Hopkins' surgeon Halsted, and the Mayo Clinic surgeon Charles Mayo reported mortality of 2% to 6% in the early 1900s compared with a 33% mortality among those less skilled (6,7). More than 50% of the procedures were ''staged,'' with initial removal of one lobe and subsequent removal of the other. Prolonged bed rest, isolation in a dark room, circulation of ice water externally around the heart, injecting boiling water into the thyroid, and administration of thyroidectin were unsuccessful attempts to improve surgical outcome. Thyroidectin, an extract of milk or blood from thyroidectomized animals, was given orally in a futile attempt to mirror the successful use of thyroid extract in hypothyroidism.

In 1922, the addition of preoperative iodine (Lugol's solution 10 drops 2 or 3 times daily) by Plummer at the Mayo Clinic proved to be a turning point for Graves' surgery (7,8). Mortality at the Mayo Clinic decreased from 4% to 1–2% and the percentage of staged operations decreased from 50% to 2%. Pharmacological doses of iodine decreased thyroidal blood flow and inhibited thyroid hormone release. Plummer incorrectly hypothesized that iodinating putatively toxic poorly iodinated thyroxine would be beneficial. Despite preoperative use of iodine, in the 1930s, surgical mortality was still 8–10% at some institutions (9), and at 1 hospital, it varied from 2% to 7% depending upon the surgeon (2).

Discovery of Radioactive Iodine

In this context, on November 12, 1936, as part of a weekly lecture series at Harvard Medical School, Karl Compton, President of Massachusetts Institute of Technology (MIT), gave a lecture to students and faculty members entitled ''What physics can do for Biology and Medicine.'' Robley Evans, an MIT physicist, suggested discussing ''artificially radioactive isotopes'' and their potential for studying metabolism (10).

Remarkably, this was only two years after the description of the first artificially produced radioactivity by the married team of Frederic Joliot and Irene Curie (daughter of Marie Curie), publication of the first description of a cyclotron by Ernest Lawrence, and production of several short half-life ($t_{½}$) radioisotopes by Enrico Fermi who used a radon–beryllium neutron source to bombard all known available elements. All four scientists subsequently were awarded the Nobel Prize.

Much of the following information is gleaned from Sawin and Becker (9,11). Four members of the MGH Thyroid Unit attended the lecture: James Howard Means (founder of the thyroid unit and chief of medicine at the MGH), Saul Hertz (head of the thyroid clinic), Earle Chapman, and Jacob Lerman (a thyroid unit clinicians in private practice). At the end of the lecture, Dr. Hertz asked whether iodine could be made radioactive. Compton was uncertain. In December 1936 he wrote to Hertz apologizing for his tardy response and confirmed that in 1934 Enrico Fermi had produced ^{128}I, an isotope with a $t_{½}$ of 25 minutes and a beta ray spectrum. By May 1937, Hertz announced research plans to study thyroid metabolism in rabbits and ultimately to treat Graves' disease.

Subsequent therapeutic plans included treating thyroid cancer. Thus began a remarkable collaboration between MGH physicians and MIT physicists to study radioactive iodine (RAI). Evans produced ^{128}I by irradiating ethyl iodide with neutrons. Separating radioactive from nonradioactive ethyl iodide proved much easier than anticipated, as the energy of the ^{128}I ruptured the ethyl iodide bond, releasing water-soluble iodide that was easily separated from the polar water-insoluble ethyl iodide.

Hertz and Evans demonstrated uptake of iodine in rabbit thyroids (using Geiger–Mueller counter) that was increased after injection of pituitary thyrotropin (TSH) extracts, in hyperplastic thyroids, and in early pregnancy. Their results were reported in 1938 only 1.5 years after the Compton lecture (12). They emphasized the importance of using as little carrier (''cold'') iodine as possible to maximize iodine uptake.

Physiological studies with RAI were performed in humans by Hamilton at Berkeley and by Leblond in Paris. Thyroid uptake of RAI was limited because Hamilton used a large amount (14 mg) of ''cold'' iodine. During a chance encounter in 1938, Hamilton asked the future Nobel laureate physicist/chemist Glenn Seaborg whether he could produce longer lived iodine isotopes. Seaborg asked how long a half-life he would like, and Hamilton answered ''one week.'' Within 8 days Livingston and Seaborg produced 3 (fortuitously) longer lived isotopes: ^{130}I ($t_{½}$ 12 hours), ^{131}I ($t_{½}$ 8 days), and ^{126}I ($t_{½}$ 13 days). Seaborg subsequently wrote: ''And for me, it has a very personal meaning. Doctors used it to save my own mothers' life when she contracted hyperthyroidism, a disease similar to the one that killed her sister in the 1930s'' (13). As a footnote to history, the University of Michigan had previously produced ^{131}I but misidentified it as ^{131}Te.

Initial MGH/MIT clinical studies were performed with ^{130}I posted from Berkeley to the MGH by first class mail (Fig. 1). The Boston team soon realized that a cyclotron was necessary for more sophisticated studies with radioisotopes. In 1938, Compton and Roberts traveled to New York City to request funds from the New York-based John and Mary Markle Foundation. The same day they received a check for $30,000 that was sufficient to build a cyclotron. The MIT cyclotron was completed in 1940, the first cyclotron built exclusively for biological and medical use.

Initially Hertz administered ^{130}I to study Graves' patients before surgery. However, in January 1941, the first hyperthyroid patient was treated with a ''radioactive cocktail'' (i.e., RAI treatment) in a glass of water with MIT-manufactured ^{130}I (contaminated with a bit of ^{131}I). Although Hertz and colleagues assumed that the rapid discharge of

FIG. 1. Dr. Saul Hertz using a multicounter that he designed to analyze the distribution of radioactive iodine in a volunteer. With permission from the Dr. Saul Hertz Archives.

energy from ^{130}I was an advantage, ultimately the longer $t_{½}$ of ^{131}I made it the therapy of choice. At the insistence of Dr. Means, pharmacological doses of ''cold'' iodine (super saturated potassium iodide [SSKI] 6 drops twice daily) were administered 1–3 days to several weeks after RAI to ''protect the patients against the mischief from thyrotoxicosis (when a) treatment of unknown efficacy was being tried out'' (14).

The BMR returned to normal after several months. Twenty-two of the first 29 RAI-treated hyperthyroid patients had a ''successful'' outcome, defined as normalization of BMR several months after stopping ''cold'' iodine. The importance of ''cold'' iodine in the successful outcome was uncertain. The practice of administering ''cold'' iodine (SSKI twice daily beginning one week after RAI) continued at the MGH for many decades and appears to shorten the time to euthyroidism compared with RAI alone (15).

Hertz and Hamilton (who also successfully treated a series of hyperthyroid patients) presented abstracts at the American Society of Clinical Investigation meeting in 1942. The American Thyroid Association (ATA) (then the American Goiter Association) annual meetings had been cancelled for the duration of World War II. Although Dr. Hertz was particularly enthusiastic about RAI therapy for hyperthyroidism, Dr. Hamilton was more circumspect about the long-term effects of this ''potentially lethal agent.'' Preliminary results were published in 1942 (16).

When Hertz volunteered for naval duty in 1943, he asked Chapman to continue his clinical studies. Between May 1943 and March 1945, Chapman treated an additional 22 patients with RAI (primarily ^{130}I with around 10% ^{131}I). Using a higher dose of RAI (up to 79 mCi), without ''cold'' iodine, Chapman noted more radiation thyroiditis, radiation sickness, and early hypothyroidism.

Although Means asked Hertz to publish his study in 1943, Hertz declined believing that longer follow-up was necessary. In 1946, the studies of Hertz and Chapman were published separately in the same issue of the *Journal of the American Medical Association* (*JAMA*) (17,18). Fishbein, the *JAMA* editor, was suspicious of duplicate publication but was reassured by Means that these were separate studies; an editorial by Means was requested and was published anonymously (19). Coming full circle, a commentary by Dr. Compton was also included in that issue (20).

Amid accusations of misunderstanding, misappropriation, and academic fraud, the relationships between Hertz, Chapman, and Means were permanently fractured (for details see reference 9). Consequently, in 1946, Hertz moved to Boston's Beth Israel Hospital where RAI was being used to treat angina (21).

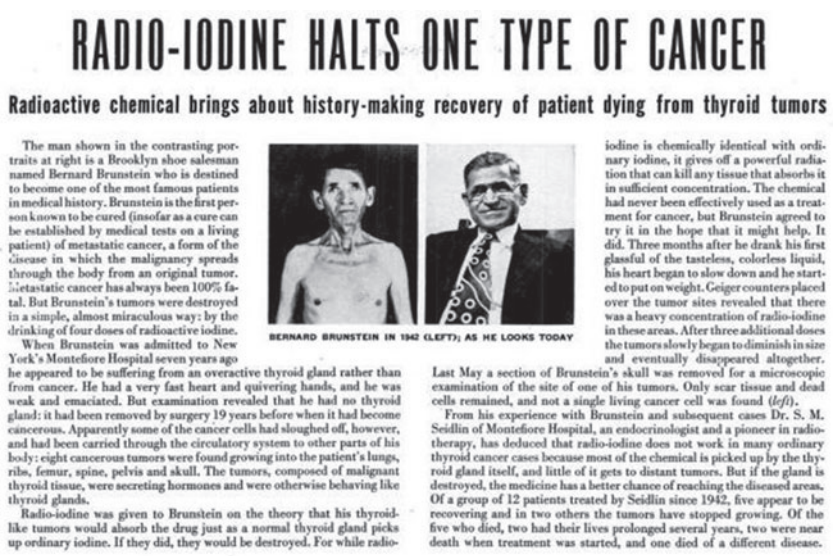
It may surprise the reader to learn that 2 of the first 29 patients were 9-year-old girls. In an era that long antedated the Geneva Conventions and institutional review boards, the guiding principles for clinical research were ''the personal ethical standard of responsible physicians'' (10). The experience of AD (one of these girls) is detailed by Daniels (22). She remembers her mother being told that the treatment might cause cancer, a concern that is currently resurfacing (see RAI for Hyperthyroidism). Seventeen years later (1958), AD had a thyroidectomy for a nodular goiter with recurrent hyperthyroidism; in 1996 she had surgery for a parathyroid adenoma causing hyperparathyroidism. Hyperparathyroidism is now a recognized long-term consequence of RAI therapy for Graves' hyperthyroidism (23). AD lived well into her 80s.

In 1942, RAI uptake was detected in a metastatic focus of well-differentiation thyroid cancer but not in coexisting poorly differentiated metastases (24). One of the coauthors of that report was Virginia Kneeland Frantz, the first woman president of the ATA (1961). A famous but unusual case of thyroid cancer was recounted in *Life Magazine* (1949) (Fig. 2) after its scientific publication (25). Post thyroidectomy, the patient had metastatic thyroid carcinoma with intractable life-threatening hyperthyroidism as well as debilitating bone pain. All the known metastatic areas took up RAI (using a Geiger counter) and several additional areas were discovered after RAI administration.

After radioiodine administration, the patient's pain resolved, he gained weight, his BMR became normal, and some tumor shrinkage and resolution of radioiodine uptake by the tumor were achieved. In 1948, Seidlin demonstrated TSH induced stimulation of RAI uptake in metastases, a seminal observation (26).

In 1946 after World War II, ^{131}I became generally available when the U.S. Atomic Energy Commission began to supply pure fission product ^{131}I. RAI was approved by the Food and Drug Administration in 1972. There are currently 20 known radioactive isotopes of iodine. It is fortuitous that 3 of these isotopes are almost perfect for their purposes: ^{131}I for therapy ($t_{½}$ 8 days), ^{123}I ($t_{½}$ 13.2 hours) for scanning, and ^{125}I ($t_{½}$ 60 days) for sealed source brachytherapy, *in vitro* iodination and protein, and immunoglobulin iodination for imaging.

^{131}I is primarily a beta emitter with a short path length that prevents damage to surrounding tissue; its gamma emissions (10%) permit post-therapy imagining. ^{123}I is an almost pure high-energy gamma emitter, whereas ^{125}I has low-energy photon emissions. Although the Berkeley group planned on testing the longer lived ^{126}I, those studies never materialized.



RADIO-IODINE HALTS ONE TYPE OF CANCER

Radioactive chemical brings about history-making recovery of patient dying from thyroid tumors

The man shown in the contrasting portraits at right is a Brooklyn shoe salesman named Bernard Brunstein who is destined to become one of the most famous patients in medical history. Brunstein is the first person known to be cured (insofar as a cure can be established by medical tests on a living patient) of metastatic cancer, a form of the disease in which the malignancy spreads through the body from an original tumor. Metastatic cancer has always been 100% fatal. But Brunstein's tumors were destroyed in a simple, almost miraculous way: by the drinking of four doses of radioactive iodine.

When Brunstein was admitted to New York's Montefiore Hospital seven years ago he appeared to be suffering from an overactive thyroid gland rather than from cancer. He had a very fast heart and quivering hands, and he was weak and emaciated. But examination revealed that he had no thyroid gland: it had been removed by surgery 19 years before when it had become cancerous. Apparently some of the cancer cells had sloughed off, however, and had been carried through the circulatory system to other parts of his body: eight cancerous tumors were found growing into the patient's lungs, ribs, femur, spine, pelvis and skull. The tumors, composed of malignant thyroid tissue, were secreting hormones and were otherwise behaving like thyroid glands.

Radio-iodine was given to Brunstein on the theory that his thyroid-like tumors would absorb the drug just as a normal thyroid gland picks up ordinary iodine. If they did, they would be destroyed. For while radio-iodine is chemically identical with ordinary iodine, it gives off a powerful radiation that can kill any tissue that absorbs it in sufficient concentration. The chemical had never been effectively used as a treatment for cancer, but Brunstein agreed to try it in the hope that it might help. It did. Three months after he drank his first glassful of the tasteless, colorless liquid, his heart began to slow down and he started to put on weight. Geiger counters placed over the tumor sites revealed that there was a heavy concentration of radio-iodine in these areas. After three additional doses the tumors slowly began to diminish in size and eventually disappeared altogether.

BERNARD BRUNSTEIN IN 1942 (LEFT); AS HE LOOKS TODAY

Last May a section of Brunstein's skull was removed for a microscopic examination of the site of one of his tumors. Only scar tissue and dead cells remained, and not a single living cancer cell was found (*left*).

From his experience with Brunstein and subsequent cases Dr. S. M. Seidlin of Montefiore Hospital, an endocrinologist and a pioneer in radiotherapy, has deduced that radio-iodine does not work in many ordinary thyroid cancer cases because most of the chemical is picked up by the thyroid gland itself, and little of it gets to distant tumors. But if the gland is destroyed, the medicine has a better chance of reaching the diseased areas. Of a group of 12 patients treated by Seidlin since 1942, five appear to be recovering and in two others the tumors have stopped growing. Of the five who died, two had their lives prolonged several years, two were near death when treatment was started, and one died of a different disease.

FIG. 2. Radioiodine halts progression of thyroid cancer.[72]

Thyroid Imaging with RAI

In the early 20th century, there was no sensitive method of detecting radioactivity. The Geiger–Mueller tube (counter) was introduced in 1928 but was still quite insensitive. In 1949 Cassen and colleagues designed the first detector (scintillation counter) to record the ''scintillations'' that occur when gamma radiation is absorbed by certain crystals (27). Automated scintillation counters were primarily used in the 1950s to image the thyroid and to quantitatively calculate the thyroid RAI uptake to plan hyperthyroidism therapy. A thyroid image could take up to 90 minutes. Subsequent modifications included a variable light source proportional to scintillations developed by Kuhl, and focused collimators developed by Newell and colleagues.

Rectilinear scanners for imaging ^{131}I, such as those developed by Picker International Corporation, were considered revolutionary but their use peaked in 1973. The rectilinear scanner was replaced by the Anger camera in the late 1970s. Currently gamma cameras are used for planar thyroid images (^{123}I), while single-photon emission computed tomography is used for three-dimensional imaging for thyroid cancer (^{131}I or ^{123}I).

RAI for Hyperthyroidism

The efficacy of RAI for hyperthyroidism is no longer in doubt. The question of safety remains an important one.

Early clinical experience with RAI confirmed that high doses were associated with late-onset hypothyroidism, whereas lower doses failed to control the hyperthyroidism in many patients (28). The hypothyroidism incidence increased progressively over time even when initial rates were relatively low. Attempts to improve the outcome included ''compensated low-dose RAI therapy'' (29), dose calculations based on the thyroid gland size and RAI uptake (30), and fixed dose RAI (31). Ultimately, however, the goal of therapy for Graves' hyperthyroidism was near complete ablation of the thyroid gland, with permanent hypothyroidism as the desired final effect, as noted by the current ATA guidelines (32).

This may be particularly important for children, in whom neoplastic change may occur in nonablated remnant thyroid tissue (33,34). In addition, while increased mortality (primarily cardiovascular) was initially reported in patients who received radioiodine for hyperthyroidism, subsequent analysis found that excess mortality was found only during the period of ongoing treatment of hyperthyroidism with thionamides, and among patients who did not become hypothyroid (35).

Patient AD already mentioned developed recurrent hyperthyroidism with a large benign nodular thyroid. Currently, hypothyroidism is considered an effect rather than a ''side effect'' of RAI therapy. Additional RAI safety concerns include worsening Graves' ophthalmopathy (36) and late-onset primary hyperparathyroidism (23).

Despite initial concerns about malignancies post-RAI, the data of Ron *et al.* from the Cooperative Thyrotoxicosis Therapy Follow-up Study (CTTFUS) reassuringly found no link between RAI and cancer mortality (37). Ron did note an increase in thyroid cancer mortality after RAI treatment for toxic nodular goiter. However, the cancer controversy was reignited by a series of publications by Kitahara and colleagues (38,39) also utilizing the CTTFUS database.

They found a dose–response relationship between RAI dose and solid cancer mortality, although the solid cancer standard mortality ratios were the same among patients treated with radioiodine, surgery, or antithyroid drugs (39). Although not all investigators confirm these data (40), we support Kim's conclusion that Kitihara's studies ''constitute a mandate for further research!'' (41).

Declining Use of RAI for Hyperthyroidism

Whereas 69% of endocrinologists in North American recommended RAI for Graves' hyperthyroidism in 1990 (42), by 2011 the number was 60% (43) with a further decline to 33% by 2020 (44). At the MGH, hyperthyroidism treatment with RAI declined by 85% between 2006 and 2019. There are several potential reasons why patients are reluctant to choose RAI. One major concern is the risk of new or worsening Graves' orbitopathy (36). The post-RAI rise in thyrotropin receptor antibodies (TRAb) is the likely culprit; in contrast TRAb tend to decline after surgery or anti-thyroid drugs (ATD) (45). Evidence to support a protective role of glucocorticoids post-RAI has not stemmed this tide (46).

The decline in RAI use largely occurred before the Kitahara publications (38,39), but our patients increasingly report a nonspecific fear of radiation. Concern among our patients regarding the adverse consequences of permanent hypothyroidism may be an additional factor; our patients frequently cite internet articles that highlight patient dissatisfaction with treatment of hypothyroidism (47).

On the positive side, more patients are pleased with the option of long-term ATD therapy (48) or the opportunity for a remission, while avoiding hypothyroidism (49). Fortunately, studies show that severe ATD toxicity (agranulocytosis and liver failure) occurs almost exclusively in the first few months of therapy, thus mitigating some fears about long-term ATD use. Additional studies confirm the long-term (>10 years) safety of ATD (50).

RAI for Toxic Adenoma and Toxic Multinodular Goiter

Unlike Graves' hyperthyroidism, the goal of radioiodine in toxic adenoma is to ablate the autonomous tissues and preserve normal thyroid function (51). Given the low RAI uptake in nonautonomous areas due to suppression of TSH, one would anticipate a low risk of hypothyroidism after RAI for toxic adenomas and toxic nodular goiters, unless pretreatment with ATD normalized the serum TSH. Thus rates of hypothyroidism after RAI depend upon absorbed dose, goiter size, and prior antithyroid drug administration; the rate of hypothyroidism in one long-term study was 8%, 28%, 46%, and 60% after 1, 5, 10, and 20 years, respectively (52).

In addition, Graves' disease develops in up to 4% of RAI-treated patients with toxic nodular goiter (53) presumably due to an immune response to released thyroid antigens. After RAI administration, toxic nodules develop suspicious ultrasound features, including calcifications with increasing (more suspicious) European-Thyroid Imaging, Reporting, and Data System scores reported by one year post-RAI (54).

RAI and Thyroid Cancer

One cannot deny the spectacular success of RAI in treating some patients with thyroid cancer, particularly those with low-volume distant metastatic disease. Despite certainty by those arguing for and against RAI in certain situations, it remains uncertain as to who should receive RAI and will likely benefit from it, and what dose is appropriate. A randomized controlled trial of RAI for important cancer outcomes (structural or radiographic evidence of recurrence, or increased thyroglobulin) has finally been published (55) but not for the lack of effort in the past. In the 1970s, Dr. Leslie DeGroot applied to the National Institute of Health to fund randomized-controlled trials of RAI for thyroid cancer. Despite a high score for these proposals, funding was denied.

It may be instructive to review some of the history of the RAI thyroid cancer controversy. Between 1960 and 1975, fewer than 10% of papillary thyroid cancer (PTC) patients at the Mayo Clinic (PTC) received RAI, based on their experience with low-risk patients who had a 99% disease-specific survival without RAI (56). Mazzaferri's *et al.*'s 1977 and 1981 landmark studies (57,58) reported decreased recurrences and mortality after RAI for PTC >1.5 cm. Subsequently almost 70% of the Mayo Clinic PTC patients received RAI, a practice not necessarily supported by their data.

A striking example of the divergent opinions is the commentaries of two North American nuclear medicine ''giants'' from Ann Arbor based on the same database: Beierwalters ''…there is no question today that we should ablate normal thyroid tissue as part of the treatment of well-differentiated thyroid carcinoma'' (59) and Sisson ''…the aggregate of evidence does not convincingly demonstrate that ablation of small remnants—and especially those remote from the primary tumor—lowers the rate of recurrent cancer'' (60).

By the 1980s, most patients with well-differentiated thyroid cancer (WDTC) larger than 1.5 cm received postoperative RAI in the United States and Europe. However, since 1999 this practice has declined in the United States. For example, data from California report that RAI for localized disease declined from 54.6% (1999) to 29.6% by 2015 (61), whereas at the Mayo Clinic it declined from 62% in 1990 to 25% in 2020 (62).

The decline in RAI for thyroid cancer was likely fueled by several concerns: the very low risk of death in low-risk patients, the uncertain benefit of RAI in preventing recurrences (63), and the concern about second primary malignancies related to high-dose RAI (64). In addition, acute RAI side effects (nausea and vomiting [rare] and painful salivary glands) and chronic side effects (permanent dry mouth, nasolacrimal gland dysfunction, recurrent sialadenitis, and delayed reproduction) need consideration. These issues led the ATA to argue against RAI for low-risk thyroid cancer (65), whereas European nuclear medicine specialists did not support this approach (66), refusing to endorse the ATA guidelines.

A randomized phase 3 trial assessed evidence for recurrence after adjunctive radioiodine remnant ablation for low-risk patients compared with no radioiodine; after 3 years no radioiodine was noninferior to radioiodine (55). Nonetheless, despite increasing evidence for little efficacy in low-risk patients, the controversy continues. In the absence of randomized controlled trials, the data support the use of radioiodine for intermediate and high-risk patients (63). The recent Martinique Conferences are an attempt to bridge the divide (67).

What is the Legacy of RAI?

The early history of RAI is a model for remarkably efficient translational research, from bench (1938) to bedside (1941) in less than three years. It is also a model for interinstitutional research cooperation. RAI remains an important therapeutic option for hyperthyroidism including postsurgical recurrent hyperthyroidism. RAI is effective in shrinking obstructing goiters when surgery is to be avoided (68).

The term theranostics (use of the same or similar radioactive compound for diagnosis and therapy (69) is new, but RAI is the paradigm for a theranostic approach to disease. Desire to image the thyroid was the major impetus for the specialty of nuclear medicine, although other radionuclide imaging preceded RAI. Along with estrogen for prostate cancer (1941), RAI was one of the first targeted therapies for cancer.

What Might the (Distant) Future Bring for RAI?

Ideal therapy for Graves' disease would specifically (and safely) inhibit the production of TRAb, thus protecting the

''innocent bystander'' (thyroid) and effectively treating the hyperthyroidism, potentially making RAI obsolete for that purpose.

Ongoing and future studies will determine who will truly benefit from RAI treatment for thyroid cancer. In addition, future advances will determine whether RAI use will increase as we explore ways to ''redifferentiate'' WDTC cancers (70) that frequently have lost their ability to concentrate iodine, or whether radioiodine use will decrease as advanced targeted therapies become more effective and less toxic.

Coda

In October 2021 (80 years after the initial RAI treatment), largely due to the efforts of his daughter, Barbara Hertz (71), the American Chemical Society honored Dr. Hertz and the medical use of RAI with a National Historical Chemical landmark designation at the MGH. Based on the legacy of RAI, it is ironic that when Hertz submitted his study for the American Goiter Association Van Meter Award (1946), he received only honorable mention.

Authors' Contributions

Both authors researched, wrote portions of this article, and reviewed the entire article.

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References

1. Burch HB, Cooper DS 2018 Anniversary review antithyroid drug therapy: 70 years later. Eur J Endocrinol **17:**261–273.
2. Thompson WO 1940 Toxic goiter: the present status of treatment. Canad Med Assoc J **42:**224–227.
3. Redisch W, Perloff WH 1940 The medical treatment of hyperthyroidism. Endocrinology **26:**221–228.
4. Means JH 1948 The Thyroid and Its Diseases. Second edition. J.B. Lippincott Co., Philadelphia, PA, pp. 341–363.
5. Okamura K, Sato K, Fujikawa M, *et al.* 2014 Remission after potassium iodide therapy in patients with Graves' hyperthyroidism exhibiting thionamide-associated side-effects. J Clin Endocrinol Metab **99:**3995–4002.
6. Giddings AEB 1998 The history of thyroidectomy. J R Soc Med **91(Suppl. 33):**3–6.
7. Becker WF 1977 Presidential address: pioneers in thyroid surgery. Ann Surg **185:**493–504.
8. Mayo CH, Plummer HS 1924 The results of iodin administration in exophthalmic goiter. Trans Am Surg Assoc **42:** 541–553.
9. Sawin CT, Becker DV 1997 Radioiodine and the treatment of hyperthyroidism: the early history. Thyroid **7:**163–176.
10. Evans RD 1975 Early history (1936–1946) of nuclear medicine in thyroid studies at Massachusetts General Hospital. Med Phys **2:**105–109.
11. Becker DV, Sawin CT 1996 Radioiodine and thyroid disease: the beginning. Semin Nucl Med **26:**155–164.
12. Hertz S, Roberts AM, Evans RD 1938 Radioactive iodine as an indicator in thyroid physiology. Proc Soc Exp Biol Med **38:**510–513.
13. Seaborg GT, Seaborg E 2001 Adventures in the Atomic Age from Watts to Washington. Farrar, Straus and Giroux: New York, NY, p. 38.
14. Means JH 1955 Historical background of the use of radioactive iodine in medicine. New Engl J Med **22:**936–940.
15. Ross DS, Daniels GH, DeStefano P, *et al.* 1983 Use of adjunctive potassium iodide after radioactive iodine (131 I) treatment of Graves' hyperthyroidism. J Clin Endocrinol Metab **57:**250–253.
16. Hertz S, Roberts A 1942 Application of radioactive iodine in therapy of Graves' disease. J Clin Invest **21:**624.
17. Hertz S, Roberts A 1946 Radioactive iodine in the study of thyroid physiology VII. The uses of radioactive iodine therapy in hyperthyroidism. JAMA **131:**81–86.
18. Chapman EM, Evans RD 1946 The treatment of hyperthyroidism with radioactive iodine. JAMA **131:**86.
19. Editorial The use of radioactive iodine in thyrotoxicosis 1946 JAMA **131:**140–141.
20. Compton KT 1946 Some contributions from the sister sciences. JAMA **131:**77–80.
21. Blumgart HL, Freedberg AS, Kurland GS 1950 Hypothyroidism produced by radioactive iodine (I131) in the treatment of euthyroid patients with angina pectoris and congestive heart failure; early results in various types of cardiovascular diseases and associated pathologic states. Circulation **1:**1105–1141.
22. Daniels GH 2013 Radioactive Iodine: a slice of history. Thyroid **23:**253–258.
23. Law RH, Quan D, Stefan AJ, *et al.* 2021 Hyperparathyroidism subsequent to radioactive iodine therapy for Graves' disease. Head Neck **43:**2994–3000.
24. Keston AS, Ball RP, Frantz VK, *et al.* 1942 Storage of radioactive iodine in a metastasis from thyroid carcinoma. Science **95:**362–363.
25. Seidlin SM, Marinelli LD, Oshry E 1946 Radioactive iodine therapy; effect on functioning metastases. JAMA **132:**838–847.
26. Seidlin SM, Oshry E, Yalow AA 1948 Spontaneous and experimentally induced uptake of radioactive iodine in metastases from thyroid carcinoma: a preliminary report. J Clin Endocrinol **8:**423–432.
27. Blahd WH 1996 Ben Cassen and the development of the rectilinear scanner. Semin Nucl Med **26:**165–170.
28. Alexander EK, Larsen PR 2002 High dose of (131)I therapy for the treatment of hyperthyroidism caused by Graves' disease. J Clin Endocrinol Metab **87:**1073–1077.
29. Sridama V, McCormick M, Kaplan E, *et al.* 1984. Long-term follow-up study of compensated low-dose 131I therapy for Graves' disease. N Engl J Med **311:**426–432.
30. Smith RN, Wilson GM 1967 Clinical trial of different doses of 131-I in treatment of thyrotoxicosis. Br Med J **1:**129–132.
31. Peters H, Fischer C, Bogner U, *et al.* 1995 Radioiodine therapy of Graves' hyperthyroidism: standard vs. calculated 131 iodine activity. Results from a prospective, randomized, multi-centre study. Eur J Clin Invest **25:**186–193.
32. Ross D, Burch HB, Cooper DS, *et al.* 2016 American Thyroid Association Guidelines for Diagnosis and Management of Hyperthyroidism and Other Causes of Thyrotoxicosis. Thyroid **26:**1343–1421.

33. Dobyns BM, Sheline G, Workman JB, *et al.* 1974 Malignant and benign neoplasms of the thyroid in patients treated for hyperthyroidism: a report of the Cooperative Thyrotoxicosis Therapy Follow-up Study. J Clin Endocrinol Metab **38:**976–998.
34. Rivkees SA, Dinauer C 2007 An optimal treatment for pediatric Graves' disease is radioiodine. J Clin Endocrinol Metab **92:**797–800.
35. Boelaert K, Maisonneuve P, Torlinska B, *et al.* 2013 Comparison of mortality in hyperthyroidism during periods of treatment with thionamides and after radioiodine. J Clin Endocrinol Metab **98:**1869–1882.
36. Tallstedt L, Lundell G, Torring O, *et al.* 1992 Occurrence of ophthalmopathy after treatment for Graves' hyperthyroidism. The Thyroid Study Group. N Engl J Med **326:** 1733–1738.
37. Ron E, Doody MM, Becker DV, *et al.* 1998 Cancer mortality following treatment for adult hyperthyroidism. Cooperative Thyrotoxicosis Therapy Follow-up Study. JAMA **280:**347–355.
38. Kitahara CM, Berrington de Gonzalez A, Bouville A, *et al.* 2019 Association of radioactive iodine treatment with cancer mortality in patients with hyperthyroidism. JAMA Intern Med **179:**1034–1042.
39. Kitahara CM, Preston DL, Sosa JA, *et al.* 2020 Association of radioactive iodine, antithyroid drugs and surgical treatments with solid cancer mortality in patients with hyperthyroidism. JAMA Netw Open **3:**e209660.
40. Gronich N, Lavi I, Rennert G, *et al.* 2020 Cancer risk after radioactive iodine treatment for hyperthyroidism: a cohort study. Thyroid **30:**243–250.
41. Kim BW 2021 Does radioactive iodine therapy for hyperthyroidism cause cancer? J Clin Endocrinol Metab **107:** e448–e457.
42. Solomon B, Glinoer D, Lagasse R, *et al.* 1990 Current trends in the management of Graves' disease. J Clin Endocrinol Metab **70:**1518–1524.
43. Burch HB, Burman KD, Cooper DS 2012 A 2011 survey of clinical practice patterns in the management of Graves' disease. J Clin Endocrinol Metab **97:**4549–4558.
44. Brito JP, Payne S, Singh Ospina N, *et al.* 2020 Patterns of use, efficacy, and safety of treatment options for patients with Graves' disease: a nationwide population-based study. Thyroid **30:**357–364.
45. Laurberg P, Wallin G, Tallstedt L, *et al.* 2008 TSH-receptor autoimmunity in Graves' disease after therapy with anti-thyroid drugs, surgery, or radioiodine: a 5-year prospective randomized study. Eur J Endocrinol **158:** 69–75.
46. Bartalena L, Marcocci C, Bogazzi F, *et al.* 1998 Relation between therapy for hyperthyroidism and the course of Graves' ophthalmopathy. N Engl J Med **338:** 73–78.
47. Peterson SJ, Cappola AR, Castro MR, *et al.* 2018 An online survey of hypothyroid patients demonstrates prominent dissatisfaction. Thyroid **28:**707–721.
48. El Kawkgi OM, Ross DS, Stan MN 2021 Comparison of long-term antithyroid drugs versus radioactive iodine or surgery for Graves' disease: a review of the literature. Clin Endocrinol (Oxf) **95:**3–12.
49. van Kinschit CMJ, Soekhavi VR, de Bekker-Grob EW, *et al.* 2021 Preferences of patients and clinicians for treatment of Graves' disease: a discrete choice experiment. Eur J Endocrin **184:**803–812.
50. Azizi F 2020 Long-term treatment of hyperthyroidism with antithyroid drugs: 35 years of personal clinical experience. Thyroid **30:**1451–1457.
51. Bonnema SJ, Hegedus L 2012 Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev **33:**920–980.
52. Ceccarelli C, Bencivelli W, Vitti P, *et al.* 2005 Outcome of radioiodine-131 therapy in hyperfunctioning thyroid nodules; a 20 years retrospective study. Clin Endocrinol (Oxf) **62:**331–335.
53. Meller J, Siefker U, Hamann A, *et al.* 2006 Incidence of radioiodine induced Graves' disease in patients with multinodular toxic goiter. Exp Clin Endocrinol Diabetes **114:** 235–239.
54. Schenke SA, Weustemann J, Zimny M, *et al.* 2020 Ultrasound assessment of autonomous thyroid nodules before and after radioiodine therapy using Thyroid Imaging, Reporting and Data System (TIRADS). Diagnostics (Basel) **10:**1038.
55. Leboulleux S, Bournaud C, Chougnet CN, *et al.* 2022 Thyroidectomy without radioiodine in patients with low-risk thyroid cancer. N Engl J Med **386:**923–932.
56. Hay I 1990 Papillary thyroid carcinoma. Endocrinol Metab Clin N Am **19:**545–576.
57. Mazzaferri EL, Young Rl, Oertel JE, *et al.* 1977 Papillary thyroid carcinoma: the impact of therapy in 576 patients. Medicine **56:**171–196.
58. Mazzaferri EL, Young RL 1981 Papillary thyroid carcinoma: a 10-year follow-up report of the impact of therapy in 576 patients. Am J Med **70:**511–518.
59. Beierwaltes WH, Rabbani R, Dmuchowski C, *et al.* 1984 An analysis of ''ablation of thyroid remnants'' with I-131 in 511 patients from 1947–1984: experience at University of Michigan. J Nucl Med **25:**1287–1293.
60. Sisson JC. 1983 J Applying the radioactive eraser: I-131 to ablate normal thyroid tissue in patients from whom thyroid cancer has been resected. J Nucl Med **24:**743–745.
61. Park KW, Wu JX, Du L, *et al.* 2015 Decreasing use of radioactive iodine for low-risk thyroid cancer in California, 1999 to 2015. J Clin Endocrinol Metab **103:**1095–1101.
62. Hay ID, Kaggal S, Iniguez-Ariza NM, *et al.* 2021 Inability of radioiodine remnant ablation to improve postoperative outcome in adult patients with low-risk papillary thyroid carcinoma. Mayo Clin Proc **96:**1727–1745.
63. Sawka AM, Thephamongkhol K, Brouwers M, *et al.* 2004 Clinical review 170: a systematic review and meta-analysis of the effectiveness of radioactive iodine remnant ablation for well-differentiated thyroid cancer. J Clin Endocrinol Metab **89:**3668–3676.
64. Sawka AM, Thabane L, Parlea L, *et al.* 2009 Second primary malignancy risk after radioactive iodine treatment for thyroid cancer: a systematic review and meta-analysis. Thyroid **19:**451–457.
65. Haugen BR, Alexander EK, Bible KC, *et al.* 2016 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid **26:**1–133.
66. Luster M, Aktolun C, Amendoeira I, *et al.* 2019 European perspective on 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: proceedings of an interactive international symposium. Thyroid **29:**7–26.

67. Tuttle RM, Ahuja S, Avram AM, *et al.* 2019 Controversies, consensus, and collaboration in the use of 131I therapy in differentiated thyroid cancer: a joint statement from the American Thyroid Association, the European Association of Nuclear Medicine, the Society of Nuclear Medicine and Molecular Imaging, and the European Thyroid Association. Thyroid **29:**461–470.
68. Bonnema SJ, Bertelsen H, Mortensen J, *et al.* 1999 The feasibility of high dose iodine 131 treatment as an alternative to surgery in patients with a very large goiter: effect on thyroid function and size and pulmonary function. J Clin Endocrinol Metab **84:**3636–3641.
69. Ehrhardt JD Jr, Güleç S 2020 A review of the history of radioactive iodine theranostics: The origin of nuclear ontology. Mol Imaging Radionucl Ther **29:**88–97.
70. Lamartina L, Anizan N, Dupuy C, *et al.* 2021 Redifferentiation-facilitated radioiodine therapy in thyroid cancer. Endocr Relat Cancer **28:**T179–T191.
71. Hertz B 2019 A tribute to Dr. Daul Hertz: the discovery of the medical uses of radioiodine. World J Nucl Med **18:** 8–12.
72. No Authors Listed 1949 Radio-iodine halts one type of cancer: Radioactive chemical brings about history-making recovery of patient dying from thyroid tumors. Life Magazine **27:**54–56.

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Discoveries Around the Hypothalamic–Pituitary–Thyroid Axis

Jerome M. Hershman[1,*] and Paolo Beck-Peccoz[2]

Many members of the American Thyroid Association played prominent roles in discovering the various aspects of the hypothalamic–pituitary–thyroid axis. This axis is fundamental for maintaining the normal serum levels of circulating thyroid hormones (THs) and thus the euthyroid state. The pituitary glycoprotein hormone, thyrotropin (TSH), controls the activity of the thyroid gland. Thyrotropin-releasing hormone and the negative feedback mechanism of circulating TH regulate the synthesis and the secretion of TSH. The dynamic interplay of these two dominant mechanisms has essential effects on TSH release. Therefore, the finding of abnormal serum levels of TSH often indicates the presence of a disorder of thyroid gland function. A summary of key historical discoveries in the understanding of the hypothalamic–pituitary axis is presented.

Keywords: HPT axis, negative feedback mechanism, disorder of thyroid gland function, TSH, TRH, Thyroid hormones

Introduction

The normal secretion of thyroid hormones (THs) is essential for the regulation of their physiological effects on brain development and function, growth, basal metabolic rate, thermogenesis, energy expenditure, muscular contraction, heart rate stimulation and the expression, and activities of numerous proteins involved in lipid and carbohydrate metabolism. The hypothalamic–pituitary–thyroid (HPT) axis synchronizes the secretion of normal levels of TH (thyroxine [T4] and triiodothyronine [T3]) and there is a negative feedback mechanism between circulating TH, pituitary thyrotropin (TSH), and hypothalamic thyrotropin-releasing hormone (TRH) (Fig. 1).

The association between the pituitary gland and endocrine disease was clearly recognized in humans by Harvey Cushing who, performing complete hypophysectomy, reported a characteristic series of symptoms including bradycardia, ataxia, hypothermia, loss of consciousness, hypogonadism, and reduced growth rate. Studies in animal models showed that lesions of the pituitary and/or the hypothalamus resulted in hypothyroidism.[1] In contrast, injection of pituitary and/or hypothalamic extracts resulted in increased circulating TH.[2] In humans, gene mutations or polymorphisms, as well as pituitary or hypothalamic lesions of one or more components of the HPT axis, may cause thyroid disorders.

The concept of the HPT axis was established in the middle of the 20th century.[3–6] The discovery of the various components of the HPT axis took place in an inverted order: first TH T4,[7,8] second the TSH,[9,10] and lastly, TRH.[11,12]

In all these steps, the thyroidologist members of the American Thyroid Association (ATA) played a crucial role in the discovery of the various hormones, their chemistry, their action, and the mechanisms involved in the negative feedback regulation.

Thyrotropin-Releasing Hormone

In 1948, Geoffrey W. Harris conducted a series of experiments and concluded: ''the hypothalamus served as the key link between the endocrine and nervous systems in reacting to the surrounding environment.''[1] In 1951, Monte Greer examined the inhibition of thyroxine synthesis and TSH overproduction in response to the antithyroid drug thiouracil and clearly showed that a hypothalamic lesion prevented these responses.[3] In 1955, Saffran et al suggested that an unknown substance from the hypothalamus was able to regulate the secretion of the pituitary hormones and they coined the term ''releasing factors.''[13]

TRH is synthetized and secreted from neuron belonging to the hypothalamic paraventricular nucleus (PVN), which

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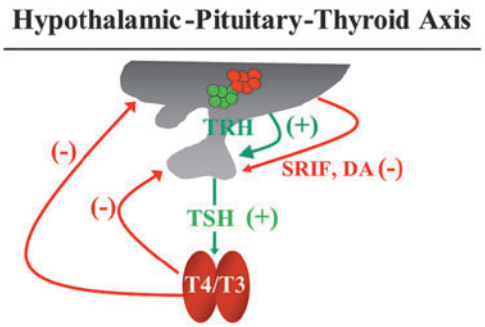

FIG. 1. The HPT axis. TRH stimulates (+) pituitary thyrotropes to secrete TSH, which, in turn, stimulate the thyroid gland to produce THs (T4 and T3). THs control the secretion of TRH and TSH through a negative feedback mechanism (–). TSH secretion from the pituitary is inhibited (–) by the hypothalamic hormone somatostatin (SRIF) and dopamine (DA). HPT, hypothalamic–pituitary–thyroid; T4, thyroxine; T3, triiodothyronine; TH, thyroid hormone; TRH, thyrotropin-releasing hormone; TSH, thyrotropin.

projects axons to the median eminence (ME). In the ME, TRH is processed from nerve terminals located close to the portal vessels and to the tanycytes.[14]

The chemical structure of TRH was discovered in 1970 in the laboratories of two Nobel Prize winners, Andrew V. Schally and Roger Guillemin (Fig. 2). TRH is a tripeptide [L-glutaminyl-L-histidyl-L-prolinamide (Gln-His-Pro (NH_2)] derived from a precursor (pre-proTRH) of 242 amino acids. The precursor includes six copies of TRH and is synthetized in the medial portion of the PVN.[11,12,15] Several ultrastructural studies showed that transformation of pre-proTRH into mature TRH is performed directly in the neuronal body by the prohormone convertases 1 and 2.[16]

A TRH-degrading ectoenzyme ([TRH-DE] pyroglutamyl peptidase II) present in the membrane of the tanycytes can inactivate TRH before it enters the portal vessels.[17] Therefore, TRH-DE modulates the amount of TRH that enters in

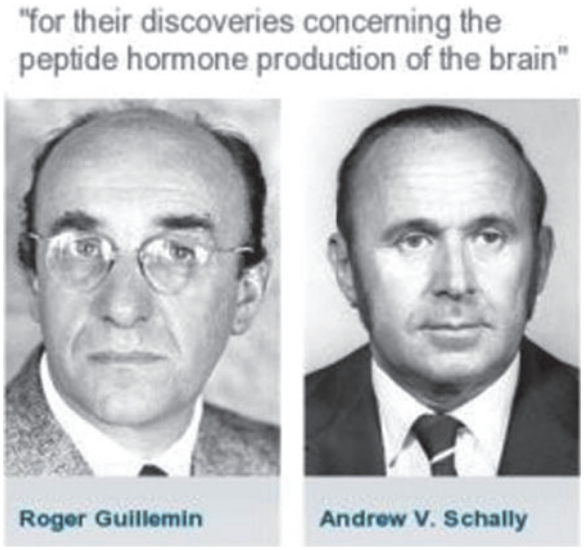

FIG. 2. Nobel Prize winners Roger Guillemin and Andrew V. Schally who discovered the chemical structure of TRH in 1970.

the pituitary gland. A fast degradation of circulating TRH, which has a half-life of about five minutes, is catalyzed by various enzymes. These include pyroglutamyl-aminopeptidase 1 and 2, thyroliberinase, and proline endopeptidase, which are partially controlled by TH, and have a direct action on the expression of the TRH gene.[18] John P. Wilber and Bruce D. Weintraub discovered that the gene encoding TRH was located on chromosome 3.[19]

The control of TRH secretion is mainly due to the feedback mechanism of circulating levels of T4 and T3[20] and the hypothalamic peptide somatostatin, which inhibits its TRH secretion.[21] TRH stimulates both *in vivo* and *in vitro* the secretion of TSH and controls the correct glycosylation of TSH, which is fundamental for the normal biological activity of TSH.[22] Moreover, TRH is effective in stimulating the secretion of prolactin from pituitary lactotrope cells.

The mentioned actions are due to the binding of TRH to its type 1 receptor (TRH-R1) that belong to the family of G-protein coupled receptors (GPCR), which activate the pathway of Ca^{2+}-dependent protein kinases.[23,24] Biallelic inactivating mutations in the 5′-part of the TRH receptor gene are responsible for congenital central hypothyroidism.[25]

Finally, the clinical use of synthetic TRH represented a novel and useful test for the investigation of thyroid diseases. Most of the first data were published by American thyroidologists,[26,27] who demonstrated that the TRH-stimulation test is of special clinical value in discriminating between primary and central hypothyroidism and in assessing the functional pituitary TSH reserve in the presence of pituitary and hypothalamic lesions. Furthermore, the findings in patients with hyperthyroidism suggested a possible clinical utility of the TRH test in the differentiation of compensated and decompensated autonomous adenomas, particularly for borderline cases.

Furthermore, in patients scintigraphically suspected to have autonomous thyroid nodules, a normal response to the TRH test helped to exclude this diagnosis and avoid further diagnostic procedures, such as the T3 suppression test. Nonetheless, the TRH test is currently obsolete due to the ultrasensitive immunometric measurement of TSH and it is only used in the research setting for the phenotyping of patients with unexplained alterations in the HPT axis.

Thyrotropin

In 1916, two American scientists, Smith and Allen, identified a thyrotropic substance in the anterior pituitary of tadpoles.[9,10,28] A few years later, Leo Loeb and Max Aron independently showed that injection of a pituitary extract induced thyroid hyperplasia in guinea pigs.[29] Thereafter, successful research was conducted to separate and isolate the various hormones of the anterior pituitary. The thyrotropic substance was purified and characterized by Anderson and Collip in 1935.[30]

In 1973, TSH was found to be a glycoprotein and was assigned a molecular weight between 26 and 30 kDa by Pierce et al.[31] A few years later, Pierce and Cornell showed that TSH is a heterodimeric glycoprotein hormone constituted by two subunits, α and β.[32] The α-subunit is common to the other glycoprotein hormones from the pituitary (luteinizing hormone [LH] and follicle stimulating hormone

[FSH]) and from the placenta (chorionic gonadotropin), while the β-subunit confers functional and immunological specificity to the TSH molecule.

Recombinant DNA technology was then used to understand the tissue-specific developmentally regulated expression of the four different glycoprotein hormones: CG, LH, FSH, and TSH. The isolation of a full-length cDNA encoding the common α-subunit demonstrates that there is a single human gene for this protein, expressed in the pituitary and placenta, for the production of the various glycoprotein hormones. From this, it is concluded that the control for the expression of glycoprotein hormones probably resides in the β-subunit genes.

In the following years, many laboratories in the United States were able to clone the cDNA encoding TSH subunits of various species and to characterize their structure and expression.[33–35] Moreover, it was demonstrated that the hypothalamic hormones, dopamine and TRH, can act directly on pituitary cells to modulate the transcription rate of the TSH subunit genes[36] and that the carbohydrate moiety of TSH is essential for full bioactivity of the hormone.[37] Finally, biallelic mutations of the TSH β-subunit gene can lead to central hypothyroidism characterized by a hyperplastic pituitary gland, high serum glycoprotein hormone α-subunit, and variable circulating TSH levels.[38]

TSH secretion is pulsatile[39] and shows a circadian rhythm both in rat and in humans.[40] Interestingly, Ridgway and collaborators demonstrated that TSH pulsatility is significantly synchronized with prolactin (PRL) pulsatility[41] (Fig. 3). Hershman and colleagues were able to show that, during the afternoon, TSH concentrations are at the lowest levels and reach a zenith between 10 PM and 2 AM. They also documented that circadian variations are under a dopaminergic control.[42] Moreover, the same authors showed a sleep-related inhibition of TSH release. In fact, after the onset of sleep, TSH levels decrease, which is prevented by keeping the subject awake.[43,44] Finally, circannual variation in TSH secretion has been recently documented.[45]

TRH stimulates the secretion of TSH, while somatostatin and dopamine have inhibitory effects on TSH secretion.[21,42] TSH response to TRH is partially inhibited by corticosteroids, whereas estrogens strengthen the response of TSH to TRH.[46,47] Nevertheless, the most important mechanism for the regulation of TSH secretion is the negative feedback by TH, in particular T3, mediated by the β 2 isoform of TH receptors.[48] TH needs a series of transporters to enter in and to exit from the various cells of human body. Among them, two are the most important transporters: the membrane monocarboxylate transporters and organic anionic transport proteins.[49]

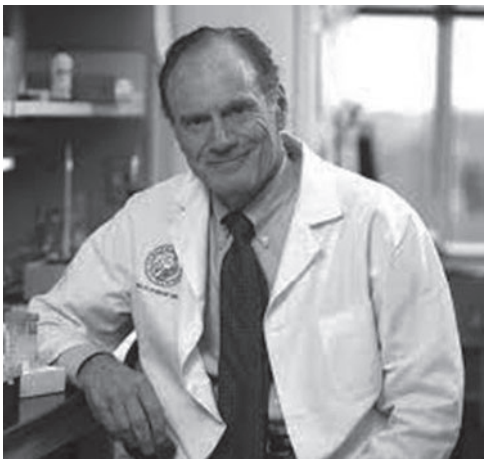

FIG. 3. E. Chester Ridgway (1942–2014) who left us too soon.

TH tissue concentrations are regulated by the activity of 2 deiodinases (Dio2 and 3) in a tissue-specific manner.[50] In the hypothalamus, pituitary, and target tissues such as skeletal muscle, white and brown adipose tissue, Dio2 is responsible for T4 conversion to T3, while Dio3 converts T4 to reverse T3, and T3 to 3,5-diiodothyronine. In the thyrotropes and in the paraventricular hypothalamic nuclei, Silva and Larsen showed that T3 generated from T4 inhibits the transcription of the TSH β-subunit and the TRH gene.[51]

The physiological TSH actions occur by binding to its specific cognate GPCR, the TSH receptor, localized on the basal membrane of thyroid cells. This binding results in the activation of cyclic adenosine monophosphate (cAMP)-dependent mechanisms throughout coupling to the α-subunit of the stimulatory G protein, which triggers the synthesis and secretion of TH. Nonetheless, other intracellular effectors, such as calcium and diacylglycerol, may promote some of the biological effects of TSH.

Loss-of-function mutations in the gene encoding the TSH receptor produce the so-called resistance to TSH action, which is characterized by elevated levels of biologically active serum TSH, absence of goiter, and mild-to-severe hypothyroidism.[52] In contrast, monoallelic gain-of-function somatic mutations of the TSH receptor are a major cause of toxic thyroid adenomas, whereas patients with sporadic or familial nonautoimmune hyperthyroidism have *de novo* or inherited monoallelic germline mutations of the TSH receptor.[53]

It is noteworthy that TSH stimulates every step involved in TH synthesis. In fact, TSH controls the synthesis of the Na^+/I symporter (NIS), which is fundamental for the iodide uptake. The stimulation of colloid endocytosis and thyroglobulin proteolysis occurs even earlier than the induction of NIS synthesis.[54] Moreover, TSH stimulates H_2O_2 production, iodide incorporation into tyrosine residues of thyroglobulin, and its exocytosis in the follicular lumen. Finally, chronic stimulation of the thyroid by TSH increases the transcriptional and translational activities of thyroid cells inducing hyperplasia and goiter.

To attain normal bioactivity, TSH needs proper glycosylation, a process that requires the interaction of TRH with its receptor on thyrotropes.[38] In patients with central hypothyroidism, normal or even slightly elevated serum levels of TSH may be observed, despite below normal serum levels of free T4. Studies performed in Weintraub's laboratory showed that partially purified TSH from patients with central hypothyroidism has reduced biological activity. Moreover, chronic administration of oral TRH to these patients normalized the glycosylation process, enhancing both its TSH receptor binding affinity and its capacity to activate adenyl cyclase.[55]

In contrast, enhanced TSH bioactivity is invariably found in sera from patients with TH resistance. Moreover, variations of TSH bioactivity (mostly related to different TSH

glycosylation) have been observed in normal subjects during the nocturnal TSH surge, in normal fetuses during the last trimester of pregnancy, in primary hypothyroidism, in patients with TSH-secreting pituitary adenomas, and in nonthyroidal illnesses.[56]

Studies on TSH molecular structure and biological activity have led to the synthesis of small molecules with either antagonist or superagonist activity on the TSH receptor.[57] As reported from Gershengorn's laboratory, a nonpeptidic antagonist, therefore, devoid of intrinsic immunogenicity, would be particularly useful in the treatment of different forms of hyperthyroidism, such as Graves' disease, Graves' orbitopathy, TSH-secreting pituitary adenomas, and activating mutations of the TSH receptor. As far as the application of a super agonist is concerned, it may be useful in patients with well-differentiated thyroid cancer who may undergo radioiodine ablation of residual cancer or thyroid remnant, thus preventing prolonged periods of symptomatic hypothyroidism due to TH withdrawal.

Nonetheless, it is worth noting that today in these patients, TSH stimulation is elicited by the administration of recombinant human TSH, a current standard of care that was initially investigated more than 30 years ago.

Wondisford et al showed the significant role of the pituitary-specific transcription factor Pit-1 in the regulation of human TSH β-subunit expression.[58] Indeed, expression of an inactive mutant of Pit-1 in a clonal rat pituitary-cell line (GH3) decreases TRH stimulation of TSH, whereas transfection of Pit-1 in cell lines lacking this factor restores cAMP induction of the TSH β gene. Taken together these results strongly support a significant role of Pit-1 in the regulation of TSH gene expression.

In fact, mutations of PIT-1 in humans cause clinical syndromes of combined pituitary hormone deficiency, where TSH, GH, and PRL secretions are compromised due to the loss of the pituitary cells that produce these hormones. The significant role of Pit-1 in the control of TSH synthesis and secretion has been demonstrated by the ''anti-Pit-1 antibody syndrome,'' in which circulating Pit-1 antibodies are associated with combined deficiencies of GH, prolactin, and TSH.[59]

Finally, the use of ultrasensitive immunometric assays for TSH measurement makes this key diagnostic test for thyroid dysfunction more precise. Nonetheless, even in the presence of a normal function of the HPT axis, artifacts in TSH measurement methods may be present and should be considered to prevent an incorrect diagnosis. Artificially elevated levels of TSH may result from the presence of endogenous anti-mouse γ globulin antibodies, as well as presence of large molecular-sized TSH that is mostly a complex of TSH and IgG (''macro TSH'').

Moreover, many hormone immunoassays use the biotin streptavidin interaction to immobilize immune complexes. The intake of high dose biotin can interfere with immunoassays using the biotin streptavidin interaction, thus generating falsely low or falsely high measurements of hormones, depending on the type of immunoassay used.

The presence of assay interference should be investigated especially in patients with normal levels of circulating free THs, lack of signs and symptoms of hypothyroidism, absence of antithyroid autoantibodies, and/or a normal thyroid ultrasound examination.

The HPT Axis in Pathological Conditions

Serum levels, pulse amplitude, and nocturnal peak of TSH are blunted in many nonthyroidal conditions, including starvation, moderate and severe illnesses, neuropsychiatric disorders, effects of drugs, and many others.

In fasted obese men, Hershman and coworkers showed that serum TSH, T4, and T3 are lower than in normal subjects and a blunted response of TSH to TRH is present.[60] These findings suggest that the thyrotropes remain responsive during short-term fasting and that the decrease in TSH is due to decreased hypothalamic TRH synthesis and release.[61] It is interesting to note that serum leptin concentrations are low in fasted persons. Since leptin has a direct action to modulate HPT axis by regulating TRH gene expression, the fall in circulating leptin levels probably resets the set point for TH negative feedback on TRH biosynthesis, thereby allowing adaptation to starvation.[62]

The variations in circulating serum TSH that are present during fasting are much more critical during severe illness. Serum TSH is usually reduced, as are serum THs, particularly circulating T3, although in rare cases elevated serum TSH levels may be found.[63] Decrease of nocturnal TSH secretion and pulse amplitude, as well as impaired TSH response to TRH, may explain these results. Indeed, recent studies showed low levels of TRH mRNA in the PVN of patients who died of nonthyroidal disease.[64]

Moreover, changes in neuroendocrine pathways have been suggested, but the activation of proinflammatory cytokines may have a critical role in the suppression of TSH secretion in nonthyroidal illness of acutely ill patients. Cytokines, such as interleukin (IL)-1β, tumor necrosis factor-α, and IL-6, are elevated in serum of patients with nonthyroidal illnesses, and they may exert a marked inhibitory activity on TRH–TSH synthesis and secretion. However, the patients do not have clinical signs and symptoms of hypothyroidism. Interestingly, during recovery from illness, an increase in serum TSH may be observed, even if circulating THs are still reduced.

Abnormal sialylation of TSH molecules may reduce the hormone bioactivity, thus explaining this apparent thyroid cell resistance to TSH. Lastly, the HPT axis almost always returns to normal after complete recovery, indicating the transient nature of these changes.

Similar alterations of the HPT axis found in nonthyroidal illness syndrome can be present in some neuropsychiatric disorders. In patients with anorexia nervosa or depressive illness, both serum TSH and its response to TRH may be blunted. The etiology of these changes is not known, though it has been speculated that they are a consequence of abnormal TRH secretion. Moreover, the same situation may be found in patients with chronic alcoholism and in those with opioid addiction.

In conclusion, many members of the ATA played important roles in discovering the various aspects of the HPT axis. These discoveries have formed the foundation of our understanding of the HPT axis as well as the pathogenesis of thyroid diseases. Furthermore, these discoveries have informed current strategies for diagnosis and treatment of thyroid diseases and future research directions.

Authors' Contributions

J.M.H. and P.B.-P. conceived, wrote, and edited this article.

Author Disclosure Statement

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References

1. Harris GW. Neural control of the pituitary gland. Physiol Rev 1948;28:139–179.
2. Ortiga-Carvalho TM, Chiamolera MI, Pazos-Moura CC, et al. Hypothalamus-pituitary-thyroid axis. Compr Physiol 2016;6:1387–1428.
3. Greer MA. Evidence of hypothalamic control of the pituitary release of thyrotropin. Proc Soc Exp Biol Med 1951; 77:603–608.
4. D'Angelo SA. Role of the hypothalamus in pituitary-thyroid interplay. J Endocrinol 1958;17:286–299.
5. Florsheim WH. Influence of hypothalamus on pituitary-thyroid axis in the rat. Proc Soc Exp Biol Med 1959;100: 73–75.
6. Reichlin S. Neuroendocrinology. N Engl J Med 1963;269: 1296–1303.
7. Kendall EC. The isolation in crystalline form of the compound containing iodin, which occurs in the thyroid its chemical nature and physiologic activity. JAMA 1915;250: 2045–2046.
8. Harington CR, Barger G. Chemistry of thyroxine: Constitution and synthesis of thyroxine. Biochem J 1927;21: 169–183.
9. Smith PE. Experimental ablation of the hypophysis in the frog embryo. Science 1916;44:280–282.
10. Allen BM. The results of extirpation of the anterior lobe of the hypophysis and of the thyroid in Rana pipiens larvae. Science 1916;44:755–758.
11. Schally AV, Müller EE, Arimura A, et al. Releasing factors in human hypothalamic and neurohypophysial extracts. J Clin Endocrinol Metab 1967;27:755–762.
12. Folkers K, Chang JK, Currie BL, et al. Synthesis and relationship of L-glutaminyl-L-histidyl-L-prolinamide to the thyrotropin releasing hormone. Biochem Biophys Res Commun 1970;839:110–113.
13. Saffran M, Schally AV, Benfey BG. Stimulation of the release of corticotropin from the adenohypophysis by a neurohypophysial factor. Endocrinology 1955;57:439–444.
14. Toni R, Jackson IM, Lechan RM. Neuropeptide-Y-immunoreactive innervation of thyrotropin-releasing hormone-synthesizing neurons in the rat hypothalamic paraventricular nucleus. Endocrinology 1990;126:2444–2453.
15. Burgus R, Dunn TF, Desiderio D, et al. Characterization of ovine hypothalamic hypophysiotropic TSH-releasing factor. Nature 1970;226:321–325.
16. Sanchez VC, Goldstein J, Stuart RC, et al. Regulation of hypothalamic prohormone convertases 1 and 2 and effects on processing of prothyrotropin-releasing hormone. J Clin Invest 2004;114:357–369.
17. Rodríguez-Rodríguez A, Lazcano I, Sánchez-Jaramillo E, et al. Tanycytes and the control of thyrotropin-releasing hormone flux into portal capillaries. Front Endocrinol (Lausanne) 2019;10:401.
18. Sánchez E, Vargas MA, Singru PS, et al. Tanycyte pyroglutamyl peptidase II contributes to regulation of the hypothalamic-pituitary-thyroid axis through glial-axonal associations in the median eminence. Endocrinology 2009; 150:2283–2291.
19. Yamada M, Wondisford FE, Radovick S, et al. Assignment of human preprothyrotropin-releasing hormone (TRH) gene to chromosome 3. Somat Cell Mol Genet 1991;17:97–100.
20. Bruhn TO, Rondeel JM, Jackson IM. Thyrotropin-releasing hormone gene expression in the anterior pituitary. IV. Evidence for paracrine and autocrine regulation. Endocrinology 1998;139:3416–3422.
21. Arimura A, Schally AV. Increase in basal and thyrotropin-releasing hormone (TRH)-stimulated secretion of thyrotropin (TSH) by passive immunization with antiserum to somatostatin in rats. Endocrinology 1976;98:1069–1072.
22. Carlson HE, Hershman JM. The hypothalamic-pituitary-thyroid axis. Med Clin North Am 1975;59:1045–1053.
23. Engel S, Gershengorn MC. Thyrotropin-releasing hormone and its receptors—A hypothesis for binding and receptor activation. Pharmacol Ther 2007;113:410–419.
24. Straub RE, Frech GC, Joho RH, et al. Expression cloning of a cDNA encoding the mouse pituitary thyrotropin-releasing hormone receptor. Proc Natl Acad Sci U S A 1990;87: 9514–9518.
25. Bonomi M, Busnelli M, Beck-Peccoz P, et al. A family with complete resistance to thyrotropin-releasing hormone. N Engl J Med 2009;360:731–734.
26. Haigler ED Jr, Pittman JA, Jr, J. Hershman JM, et al. Direct evaluation of pituitary thyrotropin reserve utilizing synthetic thyrotropin-releasing hormone. J Clin Endocrinol Metab 1971;33:573–581.
27. Foley TP Jr, Owings J, Hayford JT, et al. Serum thyrotropin responses to synthetic thyrotropin-releasing hormone in normal children and hypopituitary patients. A new test to distinguish primary releasing hormone deficiency from primary pituitary hormone deficiency. J Clin Invest 1972; 51:431–437.
28. Magner J. Historical note: Many steps led to the 'discovery' of thyroid-stimulating hormone. Eur Thyroid J 2014;3:95–100.
29. Sawin CT. Leo Loeb, Max Aron, and Thyrotropin. In: A Biographical History of Endocrinology. (Loriaux LD. eds.) John Wiley & Sons; 2016; pp. 207–211.
30. Anderson EM, Collip JB. Studies on the physiology of the thyreotropic hormone of the anterior pituitary. J Physiol 1934;82:11–25.
31. Pierce JG, Carsten ME, Wynston LK. Purification and chemistry of the thyroid-stimulating hormone. Ann N Y Acad Sci 1960;86:612–624.
32. Cornell JS, Pierce JG. The subunits of human pituitary thyroid-stimulating hormone. Isolation, properties, and composition. J Biol Chem 1973;248:4327–4333.
33. Gurr JA, Catterall JF, Kourides IA. Cloning of cDNA encoding the pre-beta subunit of mouse thyrotropin. Proc Natl Acad Sci USA 1983;80:2122–2126.
34. Burnside J, Buckland PR, Chin WW. Isolation and characterization of the gene encoding the alpha-subunit of the rat pituitary glycoprotein hormones. Gene 1988;70: 67–74.
35. Wondisford FE, Radovick S, Moates JM, et al. Isolation and characterization of the human thyrotropin beta-subunit gene. Differences in gene structure and promoter function from murine species. J Biol Chem 1988;263:12538–12542.
36. Shupnik MA, Ridgway EC, Chin WW. Molecular biology of thyrotropin. Endocr Rev 1989;10:459–475.

37. Amir SM, Kubota K, Tramontano D, et al. The carbohydrate moiety of bovine thyrotropin is essential for full bioactivity but not for receptor recognition. Endocrinology 1987;120:345–352.
38. Bonomi M, Proverbio MC, Weber G, et al. Hyperplastic pituitary gland, high serum glycoprotein hormone alpha-subunit, and variable circulating thyrotropin (TSH) levels as hallmark of central hypothyroidism due to mutations of the TSH beta gene. J Clin Endocrinol Metab 2001;86: 1600–1604.
39. Greenspan SL, Klibanski A, Ridgway EC. Pulsatile secretion of thyrotropin in man. J Clin Endocrinol Metab 1986; 63:661–668.
40. Fukuda H, Greer MA, Roberts L, et al. Nyctohemeral and sex-related variations in plasma thyrotropin, thyroxine and triiodothyronine. Endocrinology 1975;97:1424–1431.
41. Samuels MH, Veldhuis J, Ridgway EC. Copulsatile release of thyrotropin and prolactin in normal and hypothyroid subjects. Thyroid 1995;5:369–372.
42. Sowers JR, Catania RA, Hershman JM. Evidence for dopaminergic control of circadian variations in thyrotropin secretion. J Clin Endocrinol Metab 1982;54:673–675.
43. Parker DC, Pekary AE, Hershman JM. Effect of normal and reversed sleep-wake cycles upon nyctohemeral rhythmicity of plasma thyrotropin: Evidence suggestive of an inhibitory influence in sleep. J Clin Endocrinol Metab 1976;43:318–329.
44. Parker DC, Rossman LG, Pekary AE, et al. Effect of 64-hour sleep deprivation on the circadian wave form of thyrotropin (TSH): Further evidence of sleep-related inhibition of TSH release. J Clin Endocrinol Metab 1987;64: 157–161.
45. Yoshihara A, Noh JY, Watanabe N, et al. Seasonal changes in serum thyrotropin concentrations observed from big data obtained during six consecutive years from 2010 to 2015 at a single Hospital in Japan. Thyroid 2018;28:429–436.
46. Boler J, Enzmann F, Folkers K, et al. The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline amide. Biochem Biophys Res Commun 1969;37:705–710.
47. Fröhlich E, Wahl R. The forgotten effects of thyrotropin-releasing hormone: Metabolic functions and medical applications. Front Neuroendocrinol 2019;52:29–43.
48. Sawin CT, Hershman JM, Chopra IJ. The comparative effect of T4 and T3 on the TSH response to TRH in young adult men. J Clin Endocrinol Metab 1977;44:273–278.
49. Groeneweg S, van Geest FS, Peeters RP, et al. Thyroid Hormone Transporters. Endocr Rev 2020;41:146–201.
50. Russo SC, Salas-Lucia F, Bianco AC. Deiodinases and the metabolic code for thyroid hormone action. Endocrinology 2021;162:1–13.
51. Silva JE, Larsen PR. Pituitary nuclear 3,5,3'-triiodothyronine and thyrotropin secretion: An explanation for the effect of thyroxine. Science 1977;198:617–620.
52. Beck-Peccoz P, Persani L, Calebiro D, et al. Syndromes of hormone resistance in the hypothalamic-pituitary-thyroid axis. Best Pract Res Clin Endocrinol Metab 2006;20:529–546.
53. Kopp P. The TSH receptor and its role in thyroid disease. Cell Mol Life Sci 2001;58:1301–1322.
54. Dunn JT, Dunn AD. Update on intrathyroidal iodine metabolism. Thyroid 2001;11:407–414.
55. Beck-Peccoz P, Amr S, Menezes-Ferreira MM, et al. Decreased receptor binding of biologically inactive thyrotropin in central hypothyroidism. Effect of treatment with thyrotropin-releasing hormone. N Engl J Med 1985;312: 1085–1090.
56. Beck-Peccoz P, Persani L. Variable biological activity of thyroid-stimulating hormone. Eur J Endocrinol 1994;131: 331–340.
57. Gershengorn MC, Neumann S. Update in TSH receptor agonists and antagonists. J Clin Endocrinol Metab 2012;97: 4287–4292.
58. Steinfelder HJ, Hauser P, Nakayama Y, et al. Thyrotropin-releasing hormone regulation of human TSHB expression: Role of a pituitary-specific transcription factor (Pit-1/GHF-1) and potential interaction with a thyroid hormone-inhibitory element Proc Natl Acad Sci USA 1991;88:3130–3134.
59. Yamamoto M, Iguchi G, Takeno R, et al. Adult combined GH, prolactin, and TSH deficiency associated with circulating PIT-1 antibody in humans. J Clin Invest 2011;121: 113–119.
60. Carlson HE, Drenick EJ, Chopra IJ, et al. Alterations in basal and TRH-stimulated serum levels of thyrotropin, prolactin, and thyroid hormones in starved obese men. J Clin Endocrinol Metab 1977;45:707–713.
61. Blake NG, Eckland DJ, Foster OJ, et al. Inhibition of hypothalamic thyrotropin-releasing hormone messenger ribonucleic acid during food deprivation. Endocrinology 1991;129:2714–2718.
62. Legradi G, Emerson CH, Ahima RS, et al. Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. Endocrinology 1997;138:2569–2576.
63. Brent GA, Hershman JM, Braunstein GD. Patients with severe nonthyroidal illness and serum thyrotropin concentrations in the hypothyroid range. Am J Med 1986;81:463–466.
64. Fliers E, Guldenaar SE, Wiersinga WM, et al. Decreased hypothalamic thyrotropin-releasing hormone gene expression in patients with nonthyroidal illness. J Clin Endocrinol Metab 1997;82:4032–4036.

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History of Thyroid Ultrasound

Robert A. Levine

From low-resolution images in the 1960s to current high-resolution technology, ultrasound has proven to be the initial imaging modality of choice for thyroid application. Point-of-care ultrasound has brought the technology to the thyroid specialist. Combined with physical examination, it provides real-time information regarding goiter, thyroid nodules, and thyroid cancer. Ultrasound-guided fine-needle aspiration biopsy has become the accepted norm, with biopsies rarely performed using palpation alone. Advantages of ultrasound-guided biopsy include precise placement of the needle within the nodule, selective sampling of areas with suspicious features, and accurate direction of the biopsy needle to actively growing viable cells in the periphery of the nodule. Education of endocrinologists in thyroid ultrasound began in the late 1990s and by 2016 more than 6000 clinicians had completed an ultrasound course. Concurrent with this rapid expansion of use of thyroid ultrasound was a rise in the diagnosis of small papillary carcinomas, which might have otherwise remained indolent and undetected. The 2009 American Thyroid Association Guidelines for the Management of Thyroid Nodules and Thyroid Cancer recommended biopsy for all solid hypoechoic nodules measuring larger than 1 cm. Attempting to decrease the frequency of biopsies of low-risk nodules, subsequent guidelines have focused on identifying and selectively biopsying those thyroid nodules at higher risk of clinically significant carcinoma based on ultrasound appearance. A major role for thyroid ultrasound has been in both preoperative staging and mapping to help determine the extent of surgery, as well as postoperative monitoring for locoregional soft tissue or lymph node metastases. With the recognition that the increase in papillary carcinoma was predominantly a result of early diagnosis of small often indolent cancers, active surveillance has become a promising management strategy for papillary thyroid microcarcinomas. Thyroid ultrasound is essential to active surveillance of thyroid cancer. Easy access to high-quality ultrasound studies is a requirement for a successful active surveillance program. Thyroid ultrasound has been used to facilitate interventional procedures, including treatment of thyroid nodules, treatment of recurrent thyroid cancer, and therapy of papillary thyroid microcarcinoma.

Keywords: thyroid ultrasound, history, thyroid nodules, thyroid cancer, thyroid guidelines

Introduction

The thyroid is well suited to ultrasound study because of its superficial location, vascularity, size, and echotexture.[1] Ultrasound of the thyroid was not available before the 1960s. Now it is the modality of choice for most thyroid imaging, and ''point-of-care'' ultrasound has made it accessible to the thyroid specialist. It has a proven value in evaluation of goiter and thyroiditis, thyroid nodules, population evaluation for iodine deficiency, and as an adjunct to thyroid biopsy. Comprehensive cervical ultrasound is essential in pre- and postoperative thyroid cancer care. Ultrasound skills are a prerequisite for interventional procedures, including alcohol and thermal ablation. Consistent high-quality thyroid ultrasound is indispensable in the active surveillance of thyroid cancer.

An article published in 2004 reviewed the development of ultrasound technology.[1] Since then, there have been minor refinements in technology, but significant changes in utilization and application of thyroid ultrasound. This article will discuss the rapid growth of thyroid ultrasound for the past two decades, its role in the overdiagnosis of thyroid cancer, and resulting efforts to use thyroid ultrasound in a more appropriate and rational manner. It will focus on the central role of ultrasound in the management of thyroid cancer.

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Early Use of Ultrasound

Medical ultrasound was developed based on technology used in sonar navigation, using a ''pulse-echo'' technique. Early in the 1950s, the first medical applications of imaging by pulse-echo reflection were performed. Later in that decade John Julian Wild reported the observation that gastric malignant tumors were more echogenic than normal gastric tissue.[1] Early in the 1960s the first studies utilizing two-dimensional B-mode ultrasound scanning in gynecology were published.

Ultrasound for Thyroid Imaging

The earliest use of ultrasound for thyroid imaging began in the late 1960s. In 1967, Fujimoto et al reported data on 184 patients studied with low-resolution B-Mode ultrasound and reported that abnormal thyroid tissue showed increased echogenicity. Areas of absent echoes suggested cystic composition and a pattern of strong internal echoes within a thyroid nodule was felt to correspond to carcinoma.[2]

In 1971, Manfred Blum published a series of A-mode ultrasound images, demonstrating the ability of ultrasound to distinguish solid from cystic nodules and accurately measure their dimensions.[3]

Development of grayscale display provided greatly improved images, and in 1974 Crocker et al published a report on the grayscale imaging of thyroid cancer. Despite very low-resolution images they described ''low amplitude sparse and disordered echos'' characteristic of thyroid malignancy.[4] Subsequent postacquisition processing of the image, including edge enhancement and noise reduction, greatly improved the quality of the displayed image. Compound spatial imaging became available early in the 21st century. This technology combines multiple images obtained from different angles and reconstructs them into a single image.

The result is much less noise, sharper definition, and a more realistic appearing ultrasound displayed (Fig. 1). Additional advances in technology, including higher frequency transducers and more rapid frame rates have further improved image quality. While the development of matrix array transducers has both improved image quality and enabled three-dimensional (3D) image acquisition, 3D imaging has not demonstrated clinical advantage and has not entered common use. Similarly, elastography and contrast-enhanced ultrasound remain promising techniques, but have not yet been proven to be practical in clinical use.

Ultrasound Guidance for Fine-Needle Aspiration of Thyroid Nodules

In 1977, Walfish et al recommended combining ultrasound guidance with fine-needle aspiration biopsy.[5] In the majority of patients with a prior ''nondiagnostic'' biopsy, an adequate sample could be obtained when ultrasound-guided biopsy was performed.[6] Advantages of ultrasound-guided biopsy include precise placement of the needle within the nodule and accurate direction of the biopsy needle to actively growing viable cells in the periphery of the nodule. Ultrasound guidance enables targeting of the solid components of complex nodules, allows selective sampling of areas with suspicious features, and helps avoid introducing the needle through large blood vessels. Ultrasound-guided fine-needle aspiration biopsy has become the standard, with biopsies rarely performed using palpation alone.

Point-of-Care Thyroid Ultrasound Performed by Endocrinologists

Despite growth of point-of-care ultrasound in obstetrics and gynecology, before the turn of the century thyroid ultrasound imaging remained in the domain of radiology. Radiology reports were often sparse, rarely providing three dimensional measurements, and often lacking details regarding the characteristics of nodules, or the presence or absence of lymphadenopathy.

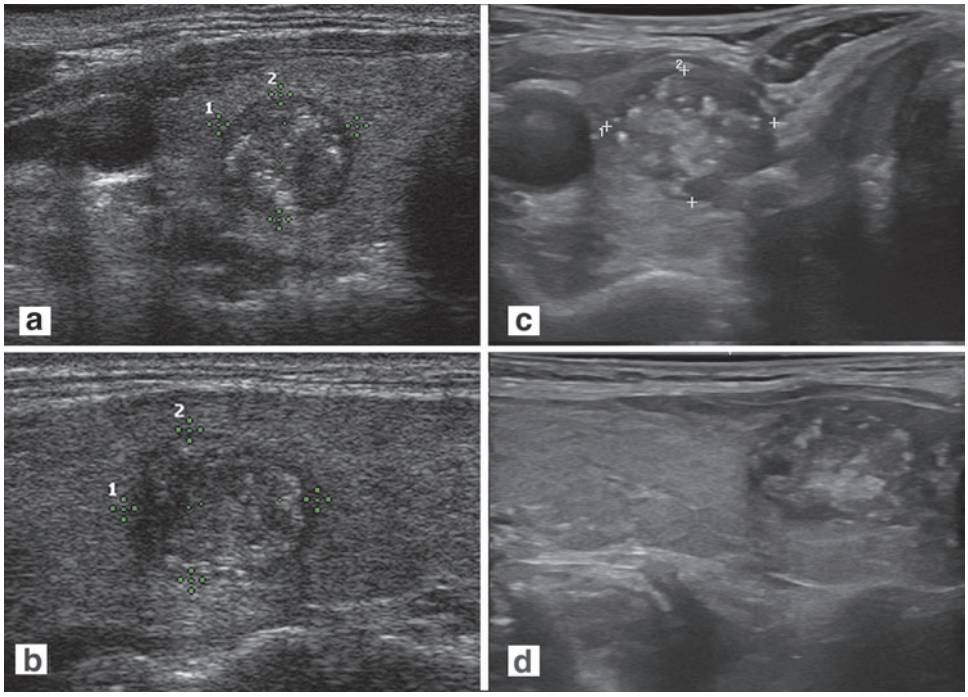

FIG. 1. Compound spatial imaging. (**a**, **b**) Ultrasound images of a papillary carcinoma taken in 2003 on a high-resolution ultrasound machine before the utilization of spatial compound imaging. (**c**, **d**) Images from a different patient with papillary carcinoma taken in 2021 using a machine with compound imaging. The newer images have less speckle and provide a more realistic and detailed image.

In 2000, Susan Mandel and her colleagues reported that thyroid ultrasound altered the clinical management in 63% of patients referred to the thyroid nodule clinic at the Brigham and Women's Hospital.[7]

In 2004, Baskin wrote an editorial titled ''Thyroid Ultrasound—just do it.''[8] Citing the above report,[7] the editorial reviewed indications for ultrasound imaging and discussed the value of combining ultrasound with fine-needle aspiration biopsy. Encouraging point-of-care thyroid ultrasound, he stated that ''clinicians must become proficient in the use of ultrasound and ultrasound-guided FNA as extensions of the physical examination. We cannot afford to deprive our patients of the many benefits of these procedures.''

Education and Expansion of Use

Point-of-care thyroid ultrasound performed by endocrinologists grew rapidly in the late 20th century. Education in thyroid ultrasound began at several major medical centers and the American Association of Clinical Endocrinologists (AACE) offered the first educational course specific to thyroid ultrasound in 1998. Under the direction of Baskin and Daniel Duick, 53 endocrinologists were taught to perform diagnostic ultrasound and ultrasound-guided fine-needle aspiration biopsy. Three hundred endocrinologists were trained for the next two years.[9] With the support of its CEO, Donald C. Jones, AACE took the lead in thyroid ultrasound education, offering multiple two-day courses every year, as well as short courses linked to the annual meeting. The Endocrine Society and American Thyroid Association also began holding short courses at their annual meetings.

Endocrine University was established in 2002 by AACE. A four-day course, including two days of didactic and ''hands on'' instruction in thyroid ultrasound and biopsy was provided to senior endocrine fellows. Held at the Mayo Clinic in Rochester, Minnesota, under the direction of Gharib,[10] more than 3000 endocrine fellows have attended Endocrine University and received training in thyroid ultrasound. By 2016, more than 6000 participants had completed an AACE ultrasound course.[9]

Cautionary Tales—Overdiagnosis of Thyroid Cancer

In 2008, Cronan opined in Radiology, ''Thyroid Nodules: Is it time to turn off the ultrasound machines?''[11] addressing the controversy of evaluating nonpalpable nodules with ultrasound. With technology now permitting detection of 2–3 mm nodules, the editorial discussed the impact of identifying small nodules. Acknowledging that 67% of the population evaluated with ultrasound have a small incidental nodule, he indicated that this would represent a potential reservoir of 150 million Americans with thyroid nodules. He noted that there had been a 2.4-fold increase in the reported incidence of thyroid nodules for the prior three decades, and a 3-fold increase in thyroid aspiration biopsies between 1995 and 2005.

Despite most guidelines at that time recommending biopsy only if nodules exceeded 1 cm, he stated that he was routinely asked to perform biopsy on 5–10 mm nodules. He projected that if all thyroid nodules in the American population were evaluated, and all cancers detected were treated, the surgical cost alone ''would conservatively reach 30 billion dollars.''(sic) It became clear that rather than turning off ultrasound machines, guidelines were needed regarding the evaluation of sub-centimeter nodules and diagnosis of subclinical thyroid cancer.

The overdiagnosis of thyroid cancer was addressed in landmark articles by Davies and Welsh in 2006 and 2014. In 2006, they reported on the increasing incidence of thyroid cancer in the United States, noting that there had been a doubling of thyroid cancer diagnoses in the prior decade, while the mortality rate for thyroid cancer had not significantly changed. Eighty-seven percent of the increase was due to tumors 2 cm or smaller, many nonpalpable and found either by ultrasound screening or as an incidental lesion on CT or MRI.[12]

In a subsequent 2014 report they again analyzed the results of Surveillance, Epidemiology, and End Results (SEER) data, indicating that there had been close to a threefold increase in incidence of thyroid cancer between 1975 and 2009. Despite the increase in cancer diagnosis the mortality rate had remained stable and extremely low (Fig. 2).[13] Figure 2, graciously provided by Davies, extends the data, and appears to show stabilization of the incidence of thyroid cancer diagnoses for the past decade since their prior report.

In 2014, Ahn et al reported on an ''epidemic'' of thyroid cancer in South Korea. They indicated that following institution of fee-for-service thyroid cancer screening with ultrasound, the number of patients undergoing surgery for thyroid cancer in South Korea rose from $\sim$1000 in 2001 to over 11,000 in 2012.[14] In March 2014, a physician coalition for prevention of overdiagnosis of thyroid cancer called for a cessation of ultrasound screening for thyroid cancer, with a 35% drop in thyroid cancer surgery in only 1 year.[15]

Analyzing data from the SEER database, Haymart et al reported that increased use of thyroid ultrasound was associated with an increase in thyroid cancer diagnosis, including localized papillary thyroid cancer with size $\leq$1 cm. They estimated that between 2002 and 2013 at least 6594 patients over the age of 64 years in the United States were diagnosed with thyroid cancer due to increased use of thyroid ultrasound.[16]

Improved Guidance for Use of Thyroid Ultrasound

With the recognition that thyroid cancer incidence was rising faster than any other cancer type in the United States, and epidemiological evidence indicating that the majority of cases were small indolent subclinical papillary carcinomas unlikely to result in morbidity or mortality, efforts were made to mitigate the problem of overdiagnosis.[17] Guidelines from the United States Preventative Services Task Force recommended against screening for thyroid cancer by ultrasound or palpation.[18] Nonetheless, it was apparent that more specific ultrasound guidelines were needed to address the escalating diagnosis and surgery of small thyroid cancers.

The 2009 American Thyroid Association Guidelines for the management of Thyroid Nodules and Thyroid Cancer recommended that biopsy be performed on all nodules over 5 mm in patients with a history suggesting a high risk for thyroid cancer. Biopsy was otherwise recommended for *all* solid hypoechoic nodules measuring larger than 1 cm.[19] AACE, in collaboration with Associazione Medici Endocrinologi, and European Thyroid Association published guidelines in 2010 recommending biopsy of nodules of *any* size

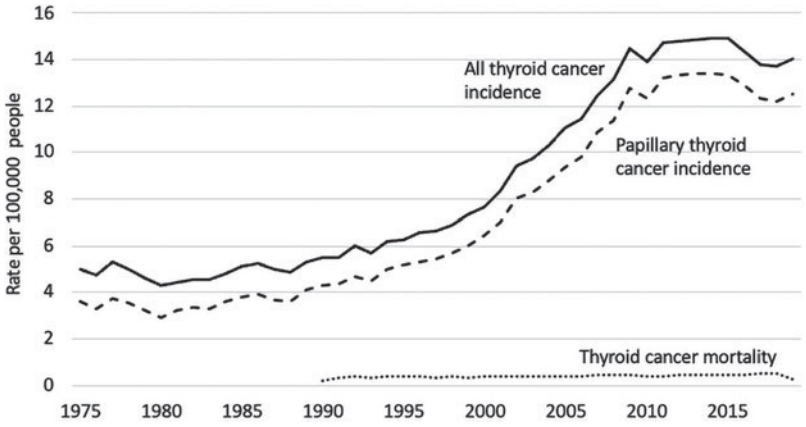

FIG. 2. Thyroid Cancer Incidence and Mortality in the United States, 1975–2019. This illustrates a rapid rise in diagnosis of thyroid cancer between 1995 and 2009, while the mortality remained low and essentially unchanged. Most of the increase in carcinoma diagnosed was due to small papillary carcinomas. There appears to be stabilization of the increase for the past decade, following improved guidelines for biopsy of thyroid nodules. Data are from The SEER Program (www.seer.cancer.gov) SEER*Stat Database: Incidence and Mortality SEER Research Data, 8 Registries, November 2021 Submission, Underlying mortality data provided by NCHS (www.cdc.gov/nchs). Released April 2022, based on the November 2021 submission. Provided by Louise Davies, MD, MS. Department of Veterans Affairs, White River Junction, Vermont. NCHS, National Center for Health Statistics; SEER, Surveillance, Epidemiology, and End Results.

with marked hypoechogenicity, irregular margins, anteroposterior dimension greater than transverse dimension, microcalcifications, or ''chaotic arrangement of intramodular vascular images.''[20] In 2011, the Korean Society for Thyroid Radiology recommended biopsy of *all* nodules exhibiting any feature suspicious for malignancy, acknowledging that nodules smaller than 5 mm are difficult to biopsy.[21]

It was apparent that the approach to thyroid nodules and cancer should be aimed at detecting clinically significant lesions rather than identifying all incidental and indolent lesions. Between 2015 and 2017 several sets of guidelines were developed in an attempt to identify those thyroid nodules at higher risk of clinically significant carcinoma based on ultrasound appearance. These guidelines took two distinct approaches: pattern recognition and quantitative scoring systems based on structural criteria.

The nodule section of the 2015 American Thyroid Association Guidelines for the Management of Thyroid Nodules and Thyroid Cancer, led by Haugen and Mandel, proposed a novel pattern recognition approach, dividing nodule patterns into five categories: high, intermediate, low, very low suspicion, and benign, with size criteria for biopsy in each category. Biopsy of nodules <1 cm was not recommended, even with suspicious criteria or high-risk history.[22]

In 2009, Horvath et al proposed a Thyroid Imaging Reporting and Data System (TI-RADS), based on the concepts of the breast imaging system of the American College of Radiology.[23] There have been at least seven TI-RADS proposed: Chilian TI-RADS, Kwak TI-RADS, Korean TI-RADS, European-TI-RADS, American College of Radiology (ACR)-TI-RADS, and Chinese TI-RADS. As with the 2015 ATA Guidelines described earlier, each of these systems utilizes features of nodules such as composition, shape, echogenicity, vascularity, margins, and inclusions/calcifications to predict the probability of malignancy, and provide guidance regarding the need for biopsy.[24]

In 2017, the American College of Radiology modified prior systems and proposed the ACR TI-RADS. Assigning points for five criteria of composition, echogenicity, shape, margins, and presence of echogenic foci, nodules were categorized as benign, not suspicious, mildly suspicious, moderately suspicious, or highly suspicious, with criteria for the size of the nodule prompting biopsy. Similarly, biopsy was recommended of high suspicion nodules only if the size exceeded 1 cm, with recommendation that sub-centimeter nodules be followed with sequential ultrasound examinations.[25]

The Korean TI-RADS used a similar algorithm beginning with the composition of the nodule (solid, partially cystic, purely cystic, or spongiform) with the presence or absence of any suspicious ultrasonographic features, including microcalcifications, nonparallel orientation, speculated, or microlobulated margins. Nodules were divided into high, intermediate, or low suspicion, and benign categories. Again, each category was then assigned a size cutoff for biopsy, with recommendation to biopsy high suspicion nodules only if larger than 1 cm, but ''selective biopsy'' of smaller nodules, including those with suspicious cervical lymph nodes or suspicion of extrathyroidal extension.[26] In 2021, the Korean Society of Thyroid Radiology guidelines were revised, with changes in the nodule size thresholds for biopsy, and addition of more precise guidance regarding diagnosis of suspicious lymph nodes and extrathyroidal extension.[27]

Studies to validate and compare each of the leading guidelines demonstrate good performance of each in reducing biopsies of benign nodules, and either performing biopsy or active surveillance of malignant nodules.[24,28]

Several organizations have promoted guidelines to standardize the performance of ultrasound examination and the reporting of thyroid, parathyroid, and comprehensive cervical ultrasound examinations. The American Institute of Ultrasound Medicine has periodically published such guidelines. Of note, in the 2013 revision a recommendation first appeared that all cervical ultrasound examinations should include a brief investigation of the lateral neck for the presence or absence of suspicious or abnormal appearing lymph nodes.[29]

With the recognition that high-quality ultrasound imaging is necessary for long-term management of thyroid nodules and thyroid cancer, the Thyroid Head and Neck Cancer Foundation (THANC) developed a consensus statement on ultrasound evaluation of thyroid nodules and lymph nodes, aiming to standardize thyroid and cervical ultrasound reports with regard to content, terminology, and organization.[30] Recommendations were made regarding the performance and recording of thyroid ultrasound examinations, including characterization of the thyroid, clinically significant nodules, and cervical lymph nodes.

Central Role of Ultrasound in Active Surveillance of Thyroid Cancer

With the recognition that the increase in papillary carcinoma was predominantly a result of early diagnosis of small often indolent cancers, active surveillance became a promising management strategy for papillary thyroid microcarcinomas.[31] In active surveillance, delaying surgery in favor of observation with serial ultrasound requires precise ultrasound measurements of nodules, as well as determination of the absence of involved cervical lymph nodes. Initial trials of active surveillance from Japan indicated that nonsurgical management of papillary thyroid microcarcinoma was safe and cost-effective when compared with immediate surgery with postsurgical surveillance. Reports from Ito et al in 2003 were promising[31] and supported by subsequent long-term studies.[32,33] Active surveillance of select sub-centimeter cancers was incorporated into the 2015 ATA guidelines.[22]

A successful active surveillance program requires availability of high-quality ultrasound studies. Studies are typically repeated initially at six-month intervals, and precise measurements are needed along with skilled evaluation of the cervical lymph node basins. Appropriate tumor characteristics and location, and the absence of suspicious cervical lymph nodes are essential criteria for active surveillance (Fig. 3). As stated by Ito, ''The quality and consistency of ultrasound for longitudinal evaluation of the thyroid and cervical node basins is a significant factor in active surveillance and poses a challenge for the implementation of this type of program outside of high-volume thyroid centers.''[31]

The Thyroid Cancer Care Collaborative offered guidance in selection of appropriate candidates, monitoring, and record keeping for patients entering active surveillance. Providing an imaging and cytology module, they helped form a framework for active surveillance.[34] Subsequently, Tuttle et al reported on 291 patients enrolled in an active surveillance program at Memorial Sloan Kettering Cancer Center.[35] Classifying patients as ''ideal, appropriate, or inappropriate'' for active surveillance[36] (Fig. 3), they increased the maximum acceptable tumor dimension to 1.5 cm. They stressed the importance of availability of ultrasound expertise as a prerequisite for such a program. In a subsequent article reviewing active surveillance, the importance of patient selection and of a multidisciplinary team was emphasized.[37]

Recently, an investigation of clinician acceptance of active surveillance found that while most endocrinologists support its use, it is vastly underutilized, with concerns regarding lack of sufficient long-term evidence and medicolegal concerns remaining significant barriers.[38]

Thyroid Ultrasound in Pre- and Postoperative Surveillance of Thyroid Cancer

An essential role for thyroid ultrasound has been in both preoperative staging and mapping to determine the extent of surgery, and postoperative monitoring for locoregional soft tissue or lymph node metastases.

In 2003, investigators at the MD Anderson Cancer Center reported performance of a preoperative comprehensive cervical ultrasound examination in patients undergoing surgery

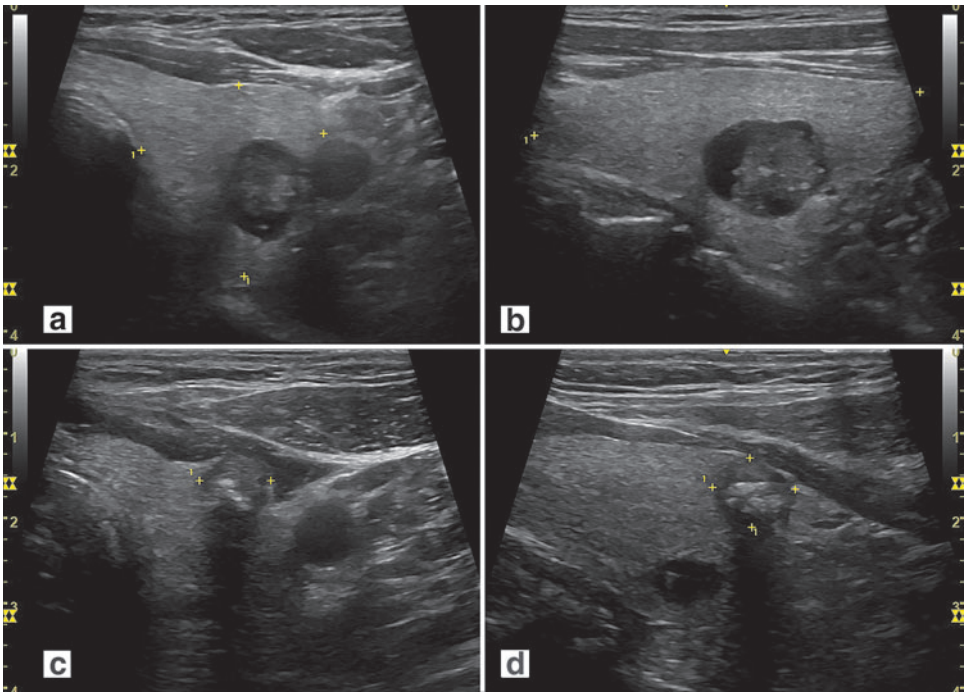

FIG. 3. Ideal and inappropriate candidates for active surveillance. The distinction between ideal, appropriate, and inappropriate candidates for active surveillance includes ultrasound characteristics, as well as patient characteristics and characteristics of the medical team. (**a**, **b**) A tumor with ideal ultrasonographic characteristics for active surveillance. It shows a solitary thyroid nodule with no evidence of extranodular extension. The tumor is surrounded by >2 mm of normal thyroid parenchyma, and there is no evidence of extrathyroidal extension. (**c, d**) A tumor felt to be inappropriate for active surveillance. The location is close to the thyroid capsule, with possible extrathyroidal extension into the overlying strap muscles.[33]

for thyroid cancer, in whom clinical evaluation suggested that the neck was free of metastatic disease. Abnormal lymph nodes or soft tissue metastases were detected by ultrasound in 39% of patients, altering the operative procedure in these patients, greatly improving the probability of complete resection at surgery.[39] The following year a retrospective study performed by the same group reported that in patients undergoing reoperation for persistent or recurrent thyroid cancer, reoperation was judged to have been possibly preventable in 39% in whom incomplete initial surgery was performed without an adequate preoperative ultrasound study.[40]

Similarly, in 2006 clinicians at the Mayo Clinic reported that preoperative ultrasound detected nonpalpable lymph node metastasis in 32.9% of 702 patients undergoing ultrasound papillary thyroid cancer surgery.[41]

The 2009 and 2015 ATA guidelines recommend a comprehensive cervical ultrasound examination be performed in all patients undergoing surgery for suspicion of thyroid cancer.[19,22] Such studies should include details of the tumor, including location within the gland and evidence of the presence or absence of extracapsular or extrathyroidal extension. A comprehensive cervical lymph node survey should be performed in all thyroid cancer cases. The 2015 guidelines additionally recommended cross-sectional imaging studies (CT and MRI) for patients with clinical suspicion for advanced disease, including invasive primary tumor, or clinically apparent lymph node involvement.[22] A preoperative map should be prepared, illustrating size and location of the thyroid lesion(s) and abnormal or suspicious lymph nodes (Fig. 4).

The 2015 ATA guidelines recommended that after surgery for well-differentiated thyroid cancer, cervical ultrasound to evaluate the thyroid bed and central and lateral cervical node compartment should be performed at 6–12 months, and then periodically, depending on the patient's risk for recurrent disease and thyroglobulin status, and indicated that suspicious lymph nodes greater than 8–10 mm in the smallest diameter should undergo biopsy only ''if a positive result would change management.'' Per these guidelines, ''Non-suspicious and small nodes (less than 8–10 mm in the smallest diameter) can be monitored with neck ultrasound.''[22]

With the demonstrated value of ultrasound in detection of cervical lymph node metastases, the management of recurrent/persistent nodal disease was addressed in 2015 by the American Thyroid Association surgical affairs committee writing task force.[42] Similar recommendations were presented in a Head and Neck Society consensus statement in

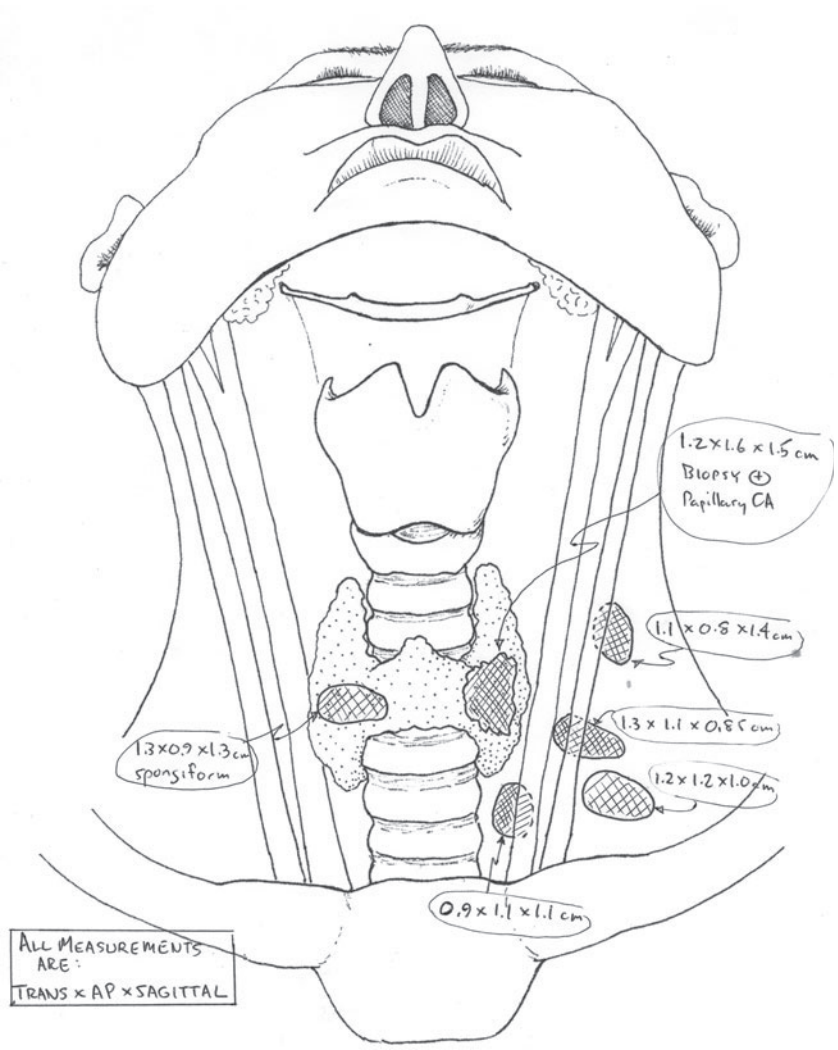

FIG. 4. This map has been prepared using ultrasound and CT images obtained in the preoperative evaluation. It demonstrates the primary thyroid lesion, as well as involved cervical lymph nodes. Preparation of such a map allows detailed preoperative surgical planning.

2016.[43] Both of these articles clearly indicated that ultrasound is the initial imaging study of choice for detection of structural recurrence, discussed active surveillance of select nodal disease, and suggested that a multidisciplinary approach is essential to management decisions. As with active surveillance of thyroid nodules and thyroid microcarcinoma, high-quality ultrasound performed by experienced operators is essential to active surveillance of recurrent disease.

Interventional Uses of Thyroid Ultrasound

Thyroid ultrasound has been used to facilitate interventional procedures, including treatment of thyroid nodules and recurrent thyroid cancer, and therapy of papillary thyroid microcarcinoma.

The earliest ultrasound-assisted interventional procedure was alcohol ablation of hyperfunctioning thyroid nodules. However, it became clear that the predominant role for alcohol ablation was in large cystic nodules. Subsequently, thermal ablation, including laser and radio frequency ablation was introduced. Papini et al described long-term efficacy of ultrasound-guided laser ablation for benign thyroid nodules in 2014.[44] The European Thyroid Association published guidelines for thermal ablation of benign thyroid nodules in 2020.[45]

The guidelines recommended restricting procedures to benign lesions with either symptomatic or cosmetic concerns. The Asian Conference on Tumor Ablation Task Force published similar guidelines in 2021.[46] Both guidelines emphasized the importance of ultrasound in patient selection, recommended that nodules with high-risk ultrasonographic features not be considered for procedure, and recommended one or two benign biopsies be obtained before intervention. Both guidelines also stated that experience with microwave ablation and high-intensity focused ultrasound was insufficient to recommend their current use. The European guidelines commented on nearly similar clinical efficacy and safety of laser and radiofrequency ultrasound-guided procedures.[45]

With the safety and efficacy of thermal ablation for benign thyroid nodules demonstrated, studies have been performed evaluating nonsurgical treatment of papillary thyroid microcarcinoma. Reports have shown long-term efficacy of ultrasound-guided laser ablation for papillary thyroid microcarcinoma[47] and comparable four-year clinical outcome between radio frequency ablation and thyroid lobectomy for low-risk papillary thyroid microcarcinoma.[48]

Recently, a consensus statement was issued by the American Head and Neck Society Endocrine Surgery Section with the Asia Pacific Society of Thyroid Surgery, Associazione Medici Endocrinologi, British Association of Endocrine and Thyroid Surgeons, European Thyroid Association, Italian Society of Endocrine Surgery Units, Korean Society of Thyroid Radiology, Latin American Thyroid Society, and Thyroid Nodules Therapies Association. It discussed the indications, techniques, complications, and expectation of successful treatment, and recommended follow-up for percutaneous ablation techniques. It provided a manual for best practice application of ablation techniques. It emphasized that advanced training in ultrasound and an ''…established skill set in the performance of ultrasound and ultrasound guided procedures is a prerequisite for the safe application of ultrasound guided ablation technologies.''[49]

Certification and Endocrine Certification in Neck Ultrasound

In 2006, several commercial insurance plans indicated that they would not reimburse providers for the performance of thyroid ultrasound unless they were certified either by American Institute of Ultrasound in Medicine (AIUM) or the ACR. A subsequent joint venture between AACE and AIUM resulted in development of the Endocrine Certification in Neck Ultrasound (ECNU) program in 2007. Certification was provided through AACE, with agreement by AIUM. Certification required completion of a written examination, documentation of appropriate case volume, and a case-based validation of competency with submission of relevant cases to be reviewed and critiqued by ECNU-certified physicians.

The ECNU handbook included guidelines for reporting thyroid ultrasound results utilizing standards from ACR and AIUM. These included indications for the procedure, descriptions of the overall thyroid, and nodules, all measurements reported in three dimensions, the presence or absence of involved suspicious cervical lymph nodes, and recommendation of action based on the study.

Physicians were certified under the ECNU program for the next 13 years. However, in December 2019, AACE restructured their thyroid education program and announced that they would no longer offer certification or recertification to individual physicians.[50] All physicians previously certified received permanent certification with the prior 10-year recertification requirement rescinded. Currently, practices, departments, or institutions can be can be recognized by either AIUM or the ACR if clinician-performed ultrasounds fall under the auspices of the radiology department.

The issues of continuing education in thyroid ultrasound, certification, and validation of competency remain significant challenges for the future of clinician-performed point-of-care thyroid ultrasound.

Conclusion

From its first availability in the 1960s with low-resolution images, to current high-resolution imaging, ultrasound has a crucial role in the evaluation and treatment of thyroid disorders. It is invaluable to endocrinologists as an extension of the physical examination, providing real-time information regarding goiter and thyroid nodules. It is a proven adjunct to fine-needle aspiration biopsy. Comprehensive central and lateral cervical ultrasound evaluation is essential before thyroid cancer surgery, and in the subsequent follow-up of thyroid cancer patients.

Active surveillance of papillary microcarcinoma or evaluation for thyroid cancer requires precise measurements of lesions and skill at cervical lymph node evaluation. Thyroid ultrasound is instrumental to interventional studies, including chemical and thermal ablation of benign and malignant thyroid tumors as well as recurrent thyroid cancer. While overuse of thyroid ultrasound may have played a role in the overdiagnosis of small indolent clinically insignificant thyroid cancers, recent data suggest that improved guidelines may have resulted in a stabilization of the prior rise in incidence (Fig. 2).

Moving into the future, radiomics (quantification and extraction of minable data from images) and artificial intelligence/machine learning will clearly play a large role

in minimizing user dependence as a limiting feature of ultrasound imaging. As we transition from an old paradigm of seeking and destroying all thyroid cancer, to a new paradigm of finding and selectively treating significant thyroid cancer, ultrasound will continue to play a critical role.

Dedication

Dedicated to the memory of Daniel Duick, MD, FACP, MACE, ECNU (1941-2022). A great clinician, teacher, and mentor.

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References

1. Levine RA. Something old and something new: A brief history of thyroid ultrasound technology. Endocr Pract 2004;10(3):227–233; doi: 10.1177/0096144204265201
2. Fujimoto F, Oka A, Omoto R, et al. Ultrasound scanning of the thyroid gland as a new diagnostic approach. Ultrasonics 1967;5(3):177–180; doi: 10.1016/s0041-624x(67)80065-9
3. Blum M, Weiss B, Hernberg J. Evaluation of thyroid nodules by A-mode echography. Radiology 1971;101(3): 651–656; doi: 10.1148/101.3.651
4. Crocker EF, McLaughlin AF, Kossoff G, et al. The gray scale echographic appearance of thyroid malignancy. J Clin Ultrasound 1974;2(4):305–306; doi: 10.1148/101.3.651
5. Walfish PG, Hazani E, Strawbridge HT, et al. Combined ultrasound and needle aspiration cytology in the assessment and management of hypo functioning thyroid nodule. Ann Intern Med 1977;87(3):270–274; doi: 10.7326/0003-4819-97-3-270_1
6. Gharib H. Fine-needle aspiration biopsy of thyroid nodules: Advantages, limitations, and effect. Mayo Clin Proc 1977; 69(1):44–49; doi: 10.1016/s0025-6196(12)61611-5
7. Marqusee E, Benson CB, Frates MC, et al. Usefulness of ultrasonography in the management of nodular thyroid disease. Ann Intern Med 2000;133(9):696–700; doi: 10.7326/0003-4819-133-9-200011070-00011
8. Baskin HJ. Thyroid ultrasound—Just do it. Thyroid 2004; 14(2):91–92; doi: 10.1089/105072504322880319
9. Levine RA, Sistrunk JW. History of thyroid ultrasound. In: Thyroid and Parathyroid Ultrasound and Ultrasound-Guided FNA, 4th ed. Springer; New York, NY; 2018; pp. 1–14; doi: 10.1007/978-3-319-67238-0_1
10. Gharib H, Hodgson SF. Endocrine University: Evolution of a novel educational program. Endocr Pract 2006;12(2): 223–226; doi: 10.4158/ep.12.2.223
11. Cronan JJ. Thyroid nodules: Is it time to turn off the US machines? Radiology 2008;247(3):602–604; doi: 10.1148/radiol.2473072233
12. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973–2002. JAMA 2006; 295(18):2164–2167; doi: 10.1001/jama.295.18.2164
13. Davies L, Welch HG. Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surg 2014; 140(4):317–322; doi: 10.1001/jamaoto.2014.1
14. Ahn HS, Kim HJ, Welch HG. Korea's Thyroid-Cancer ''Epidemic''—Screening and overdiagnosis. N Engl J Med 2014;371(19):1765–1767; doi: 10.1056/nejmp1409841
15. Ahn SA, Welch HG. South Korea's Thyroid-Cancer ''Epidemic''—Turning the tide. N Engl J Med 2015; 373(24):2389–2390; doi: 10.1056/nejmc1507622
16. Haymart MR, Banerjee M, Reyes-Gastelum D, et al. Thyroid ultrasound and the increase in diagnosis of low-risk thyroid cancer. J Clin Endocrinol Metab 2019;104(3):785–792; doi: 10.1210/jc.2018-01933
17. Roman BR, Morris LG, Davies L. The Thyroid Cancer Epidemic, 2017 Perspective. Curr Opin Endocrinol Diabetes Obes 2017;24(5):332–336; doi: 10.1097/med.0000000000000359
18. Davies L, Morris LGT. The USPSTF Recommendation of Thyroid Cancer Screening: Don't ''Check your Neck.'' JAMA Otolaryngol Head Neck Surg 2017;143(8):755–756; doi: 10.1001/jamaoto.2017.0502
19. Cooper DS, Doherty GM, Haugen BR, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009;19(11):1167–1214; doi: 10.1089/thy.2009.0110
20. Gharib H, Papini E, Paschke R, et al. AACE/AME/ETA Task Force on Thyroid Nodules. American Association of Clinical Endocrinologists, Associazione Medici Endocrinologi, and European Thyroid Association Medical Guidelines of Clinical Practice for the Diagnosis and Management of Thyroid Nodules. Endocr Pract 2010;16(3): 1–43; doi: 10.1007/bf03346587
21. Moon WJ, Baek JH, Jung SL, et al. Korean Society of Thyroid Radiology. Ultrasonography and the ultrasound-based management of thyroid nodules: Consensus statement and recommendations. Korean J Radiol 2011;12(1): 1–14; doi: 10.3348/kjr.2011.12.1.1
22. Haugen BR, Alexander EK, Bible KC, et al. American Thyroid Association Management Guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26(1):1–133; doi: 10.3410/f.725851631.793516477
23. Horvath E, Majlis, Rossi R, et al. An ultrasonogram reporting system for thyroid nodules stratifying cancer risk for clinical management. J Clin Endocrinol Metab 2009; 94(5):1748–1751; doi: 10.1210/jc.2008-1724
24. Russ G, Trimboli, Buffet C. The new era of TIRADSs to stratify the risk of malignancy of thyroid nodules: Strength, weaknesses and pitfalls. Cancers (Basel) 2021;13(17): 4316–4337; doi: 10.3390/cancers13174316
25. Tessler FN, Middleton WD, Grant EG, et al. ACR Thyroid Imaging, Reporting and Data System (TI-RADS): White Paper of the ACR TI-RADS Committee. J Am Coll Radiol 2017;14(5):587–595; doi: 10.1016/j.jacr2017.12.035
26. Shin JH, Baek JH, Chung J, et al. Ultrasonography diagnosis and imaging-based management of thyroid nodules: Revised Korean Society of Thyroid Radiology consensus statement and recommendations. Korean J Radiol 2016; 17(3):370–395; doi: 10.3348/kjr.2016.17.3.370
27. Ha EJ, Chung SR, Na DG, et al. 2021 Korean thyroid imaging reporting and data system and imaging-based management of thyroid nodules: Korean Society of Thyroid Radiology Consensus Statement and Recommendations.

Korean J Radiol 2021;22(12): 2094–2123; doi: 10.53347/rid-68338
28. Middleton WD, Teefey SA, Reading CC, et al. Comparison of Performance Characteristics of American College of Radiology TI-RADS, Korean Society of Thyroid Radiology TIRADS, and American Thyroid Association Guidelines. AJR Am J Roentgenol 2018;210(5):1148–1154; doi: 10.2214/ajr.18.18822
29. AIUM practice parameter for the performance of a thyroid and parathyroid ultrasound examination. J Ultrasound Med 2016;35(9):1–11; doi: 10.7863/jum.2016.35.9.4
30. Su HK, Dos Reis LL, Lupo MA, et al. Striving toward standardization of reporting of ultrasound features of thyroid nodules and lymph nodes: A multidisciplinary consensus statement. Thyroid 2014;24(9):1341–1349; doi: 10.1089/thy.2014.0110
31. Ito Y, Uruno T, Nakano K, et al. An observation trial without surgical treatment in patients with papillary microcarcinoma of the thyroid. Thyroid 2003;13(4):381–387; doi: 10.1089/105072503321669875
32. Ito Y, Miyauchi A, Kobayashi K, et al. Prognosis and growth activity depend on patient age in clinical and subclinical papillary thyroid carcinoma. Endocr J 2014;61(3):205–213; doi: 10.1507/endocrj.ej13-0403
33. Davies L, Chang CH, Sirovich B, et al. Thyroid cancer active surveillance program retention and adherence in Japan. JAMA Otolaryngol Head Neck Surg 2021;147(1):77–84; doi: 10.1001/jamaoto2020.4200
34. Haser GC, Tuttle RM, Su HK, et al. Active surveillance for papillary thyroid microcarcinoma: New challenges and opportunities for the health care system. Endocr Pract 2016;22(5):602–611; doi: 10.1089/thy.2016.0109
35. Tuttle RM, Fagin JA, Minkowitz G, et al. Natural history and tumor volume kinetics of papillary thyroid cancers during active surveillance. JAMA Otolaryngol Head Neck Surg 2017;143(10):1015–1020; doi: 10.1001/jamaoto.2017.1442
36. Brito JP, Ito Y, Miyauchi A, et al. A clinical framework to facilitate risk stratification when considering an active surveillance alternative to immediate biopsy and surgery in papillary microcarcinoma. Thyroid 2016;26(1):144–149; doi: 10.1089/thy.2015.0178
37. Lohia S, Hanson M, Tuttle RM, et al. Active surveillance for patients with very low-risk thyroid cancer. Laryngoscope Investig Otolaryngol 2020;5(1):175–182; doi: 10.1002/lio2.356
38. Roman BR, Brito JP, Saucke MC, et al. National survey of endocrinologists and surgeons regarding active surveillance for low-risk papillary thyroid cancer. Endocr Pract 2021;27(1):1–7; doi: 10.1016/j-eprac.2020.11.003
39. Kouvaraki MA, Shapiro SE, Fornage BD, et al. Role of preoperative ultrasonography in the surgical management of patients with thyroid cancer. Surgery 2003;134(6):946–954; doi: 10.1016/s0039-6060(03)00424-0
40. Kouvaraki MA, Lee JE, Shapiro SE, et al. Preventable reoperations for persistent and recurrent papillary thyroid carcinoma. Surgery 2004;136(6):1183–1191; doi: 10.1016/j.surg.2004.06.045
41. Stulak JM, Grant CS, Farley DR, et al. Value of preoperative ultrasonography in the surgical management of initial and reoperative papillary thyroid cancer. Arch Surg 2006;141(5):489–494; doi: 10.1001/archsurg.141.5.489
42. Turfano RP, Clayman G, Heller KS, et al. Management of recurrent/persistent nodal disease in patients with differentiated thyroid cancer: A critical review of the risks and benefits of surgical intervention versus active surveillance. Thyroid 2015;25(1):15–27; doi: 10.1089/thy.214.0098
43. Scharph J, Tuttle RM, Wong R, et al. Comprehensive management of recurrent thyroid cancer: An American Head and Neck Society Consensus Statement. Head Neck 2016;38(12):1862–1869; doi: 10.1002/hed.24513
44. Papini E, Rago T, Gambelunghe G, et al. Long term efficacy of ultrasound guided laser ablation for benign solid thyroid nodules. Results of a three-year multi center prospective randomized trial. J Clin Endocrinol Metab 2014;99(10):3653–3659; doi: 10.1210/jc.2014-1826
45. Papini E, Monpeyssen H, Frasoldati A, et al. European Thyroid Association Clinical Practice Guidelines for the use of image guided ablation in benign thyroid nodules. Eur Thyroid J 2020;9(4):172–185; doi: 10.1159/000508484
46. Ha EJ, Baek JH, Che Y, et al. Radiofrequency ablation of benign thyroid nodules: Recommendations from the Asian Conference on Tumor Ablation Task Force. Ultrasonography 2021;40(1):75–82; doi: 10.14366/usg.20112
47. Kim HJ, Chung SM, Kim H, et al. Long-term efficacy of ultrasound guided laser ablation for papillary thyroid microcarcinoma: Results of a 10-year retrospective study. Thyroid 2021;31(11):1723–1729; doi: 10.1089/thy.2021.0151
48. Yan L, Zhang M, Song Q, et al. Ultrasound-guided radio frequency ablation versus thyroid lobectomy for low-risk papillary thyroid microcarcinoma: A propensity matched cohort study of 884 patients. Thyroid 2021;31(11):1662–1672; doi: 10.1089/thy.2021.0100
49. Orloff LA, Noel JE, Stack BC Jr, et al. Radiofrequency ablation and related ultrasound-guided ablation technologies for treatment of benign and malignant thyroid disease: An international multidisciplinary consensus statement of the American Head and Neck Society Endocrine Surgery Section with the Asia Pacific Society of Thyroid Surgery, Associazione Medici Endocrinologi, British Association of Endocrine and Thyroid Surgeons, European Thyroid Association, Italian Society of Endocrine Surgery Units, Korean Society of Thyroid Radiology, Latin American Thyroid Society, and Thyroid Nodules Therapies Association. Head Neck 2021;44(3):633–660; doi: 10.1002/hed.26960
50. Sistrunk JW. Thyroid ultrasound education in the United States without the Endocrine Certification in Neck Ultrasound (ECNU) Program—What's next? Clin Thyroidol 2020;32(2):51–53; doi: 10.1089/ct.2020;32.51–53

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History of Thyroid Surgery in the Last Century

Lisa A. Orloff[1] and Sareh Parangi[2]

Before the 20th century, thyroid surgery was regarded as ''horrid butchery'' such that no honest and sensible surgeon would ever engage in it. Yet, by the mid-20th century, thyroidectomy had become a respected, life-saving, safe, and increasingly practiced operation. From Kocher to Wells and onward into the 21st century, the evolution of thyroid surgery has continued, enhanced by the integration of endocrinology, genetics, immunology, physiology, technology, training, and multidisciplinary care. The ability to personalize and optimize the care of thyroid disorders has been progressively achieved through shared insights and discoveries, highlights of which are described herein.

Keywords: thyroid gland, surgery, thyroidectomy, history, thyroid carcinoma, Theodor Kocher

> The extirpation of the thyroid gland for goiter typifies perhaps better than any other operation the supreme triumph of the surgeon's art.—William Halsted (1852–1922).[1]

THE LAST ONE hundred years have encompassed a fascinating array of developments related to thyroid surgery, ranging from diagnostic and physiologic insights to therapeutic tools and technology to modified guidelines and philosophies. Through reflections on historical outcomes, progressive scientific growth, and a trend toward patient-centered care, thyroid surgery has been emblematic of the modern precision health care movement.

A discussion of the highlights of the last century would be incomplete without briefly acknowledging key advances before the 20th century that enabled safe thyroid surgery to be performed in the first place. Although space limitations preclude a more detailed review of every important advance with an impact on thyroid surgery, many of these topics are addressed in other invited articles in this American Thyroid Association (ATA) Centennial series.

Before the revolutionary developments in anesthesia, antisepsis, and hemostatic instruments in the mid-19th century, thyroid surgery was vehemently condemned due to its high mortality rate. When the brilliant Swiss surgeon–scientist, Dr. Theodor Kocher (1841–1917) (Fig. 1), performed his first thyroid surgery in 1872, the mortality rate from thyroidectomy was as high as 70%. In place of the pain, suffocation, sanguination, and sepsis associated with thyroid surgery,[2] Kocher's utter lack of fuss and confusion characteristic of so many other clinics of that time[3] established meticulous, calm, and logical stepwise care of patients.

The discovery of human parathyroid glands by Ivar Sandström (published in 1880) (Fig. 2) and recognition that their preservation prevented tetany (by Gley, 1891 and others) led to further refinements in techniques. The principles of clamp-and-tie blood vessel control, extracapsular dissection, and identification and preservation of the recurrent laryngeal nerves were espoused by Kocher and Mikulicz and further advanced by Halsted, Lahey, and others who followed.

However, Kocher's disheartened observation that patients who survived total thyroidectomy for goiter, only to be doomed to be cretins, were ''saved for a life not worth living'' led to his commitment to performing subtotal thyroidectomy or thyroid lobectomy only, while seeking to understand the function, and not just the anatomy, of the thyroid gland.[4] Through his pursuit of a deep understanding of thyroid physiology, combined with his appreciation of regional anatomy, Kocher became the first surgeon to be awarded the Nobel Prize in 1909; by this time, he had transformed thyroidectomy and reduced its mortality risk to <1%.[5,6]

A master educator, Kocher laid the foundation for the role of surgeons in advancing the art and science of thyroid surgery and thyroidology for the next century. The contemporary and ensuing generation of thyroid surgeons, including American surgeons, William Halsted (1852–1922), Harvey Cushing (1869–1939), George Crile (1864–1943), Charles Mayo (1865–1939), and Frank Lahey (1880–1953), and the Australian surgeon, Thomas Dunhill (1876–1957), worked collegially to expand and enhance the techniques, sometimes across large geographic barriers and multiple world wars.

For example, Dr. Dunhill visited some of the top thyroid surgery centers in the United States and England, where he

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FIG. 1. Emil Theodor Kocher (1841–1917) whose work revolutionized thyroid surgery and physiology and who was the first surgeon to be awarded the Nobel Prize in 1909 (image from https://en.wikipedia.org/w/index.php?title=Emil_Theodor_Kocher&oldid=1105433419).

shared results of his approach to thyrotoxic goiter, with 230 cases and only 4 deaths in patients treated with unilateral lobectomy and contralateral subtotal thyroidectomy.[7–9] Dunhill also recognized that earlier intervention rather than last-resort surgery after progressive deterioration from thyrotoxicosis was a key ingredient to minimizing complications.

The American Association for the Study of Goiter, which later became the ATA and whose original members were mostly surgeons, was founded in 1923. In his presidential address to the Association in 1929, the surgeon, Seymour D. Van Meter, stated: ''Who thinks a matter out is of no importance whatsoever. The important thing is that the problem should be solved. This was the theory upon which the originators of our Association based their hopes of progress and ultimate success in the solution of the unsolved phases of goiter.''[10]

Today's surgical societies have collaborated to ensure that this same determination and meticulous attention to detail are the mainstays of subspecialty training in thyroid surgery. The evolution of a competent thyroid surgeon now includes extensive education in the preoperative, intraoperative, and postoperative care of patients with thyroid diseases, incorporating technical and scientific adjuncts. Given the breadth of new developments in thyroid surgery, it is no surprise that fellowships and additional training have been designed in these areas by our professional societies.

The surgical management of both structural and functional disorders of the thyroid advanced in conjunction with insights in endocrinology. After observed benefits from injection of thyroid extract (1891, by George Murray), consumption of sheep thyroid (1890s), and isolation of thyroxine (T4) (1915, by Kendall), the chemical structure of thyroxine was identified in 1926 (Harington).[11] The first commercial thyroxine was produced in 1949, and triiodothyronine (T3) was isolated soon thereafter in 1952–1953. The availability of both synthetic and desiccated (animal) T4 and T3 resulted in the ability to replace the thyroid hormone and thus avoid hypothyroidism (cretinism), even following total thyroidectomy.

In the 1940s, the antithyroid action of thionamide drugs was recognized,[12] which provided both a new alternative to surgery for thyrotoxicosis and a dramatic decrease in the risk of a postoperative thyroid storm when surgery was undertaken. The additional therapeutic alternative of radioactive iodine (^{131}I) for hyperthyroidism was simultaneously identified, and early success helped spawn the development of theranostics (the combination of drugs or techniques to simultaneously or sequentially diagnose and treat medical conditions).

With thyroidectomy having become a safe, respected, and more widely practiced procedure, the following decades saw developments in preoperative tools that influenced patient selection for surgery. Imaging studies evolved. For a generation in the mid-20th century, the radionuclide ^{123}I thyroid scan provided information about thyroid function (''hot'' vs. ''cold'' nodularity), yet crude anatomic resolution.

Low-resolution ultrasound provided better anatomic detail, but fairly primitive characterization of thyroid nodules beyond size and composition. Expansion in imaging modalities to include cross-sectional scans (CT and MRI), high-resolution B-mode ultrasonography (as well as Doppler sonography, including carotid artery studies), and ultimately [^{18}F]Fluorodeoxyglucose-positron emission tomography/computed tomography scanning led to an increase in detection of thyroid nodularity.

Increased identification of even small nodules through imaging, early (and exaggerated) connotation of malignancy with cold nodules, and availability of the exogenous thyroid hormone led to a steady increase in the practice of thyroid surgery for diagnostic and therapeutic purposes. Total thyroidectomy became commonplace. Fortunately, patient selection and risk stratification were aided by the evolution of expertise in thyroid cytology obtained through fine-needle aspiration (FNA) biopsy.

The widespread adoption of thyroid FNA in the 1980s resulted in a reduction in thyroid surgery for benign disease and an increase in yield of malignancy on surgical pathology of thyroidectomy specimens.[13] The Bethesda System for Reporting Thyroid Cytology (original publication in 2009, updated in 2017, and version 3 expected in 2023) later helped to standardize the reporting of thyroid nodule biopsies[14,15] and to imply cancer risk, according to assignment of samples to one of six categories. A majority of thyroid nodules can be definitively characterized as either benign or malignant by cytology, with high sensitivity (≤3% false-negative rate). Still, 20–30% of FNAs yield indeterminate cytology[16] and until the advent of molecular testing, they would have resulted in a recommendation for diagnostic thyroid lobectomy at a minimum.

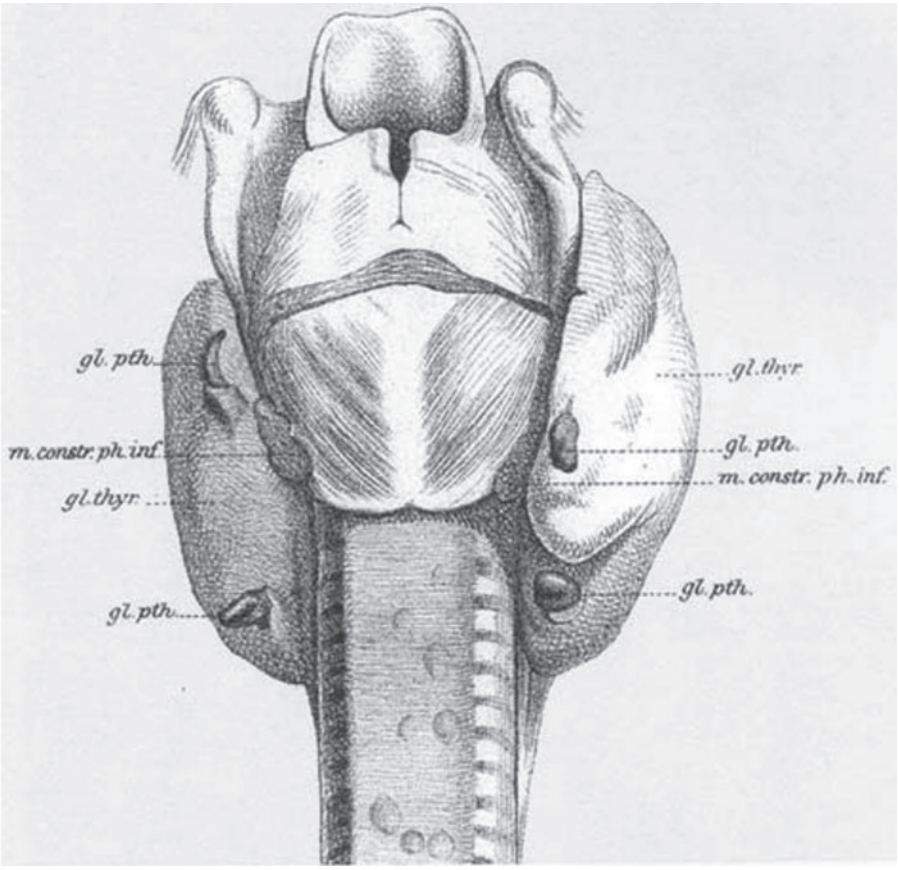

FIG. 2. The parathyroid glands, drawn by Ivar Sandström (1852–1889), a Swedish medical student at the University of Uppsala. Owing to his meticulous dissections of animal and human cadavers, he was the first to describe a gland as ''hardly as big as a hemp seed, which was enclosed in the same connective tissue as the thyroid, but could be distinguished therefrom by the light colour.'' Reprinted from Johansson, 2015, Online Open Access; doi: 10.3109/03009734.2015.1027426.

The double-edged sword of refinement in ultrasound resolution, combined with a well-intentioned, but oversimplified, belief that early cancer detection is advantageous, contributed to a steady increase in the rate of diagnosis of thyroid cancer, the vast majority of which was well-differentiated and early-stage disease.[17] Throughout the first decade of the 21st century, thyroid cancer was hailed as the fastest growing cancer diagnosis, yet mortality rates from thyroid cancer were noted to be low and essentially stable.

As a result, guidelines for the evaluation and management of thyroid nodules have been, and continue to be, progressively modified in an effort to reduce unnecessary biopsies and overdiagnosis of nonclinically significant cancers.[18–20] Ultrasound-based risk stratification systems for incidental thyroid nodules have been developed throughout the world to refine criteria for performing FNA or ultrasound surveillance.[19,21–25] These systems have also standardized the reporting of ultrasound examinations and hence several bear names incorporating the theme of TI-RADS (Thyroid Imaging Reporting and Data System).

In parallel, active surveillance (reserving intervention for evidence of disease progression or patient preference) is a modern approach taken to address small, low-risk thyroid cancers, which relies on serial ultrasonography. Initiated in Kuma, Japan, in 1993,[26] active surveillance has proven to be a safe alternative to immediate surgery in carefully selected healthy patients with papillary thyroid microcarcinoma and is progressively gaining acceptance around the globe.[22,27,28] In addition, *in situ* ablation of papillary microcarcinomas is now under investigation.[29]

Clinician-performed ultrasonography has enabled interpretation of features in the context of known patient history, symptomatology, and physical findings (Fig. 3a). Ultrasound examination of the neck beyond the thyroid itself has enhanced preoperative planning and postoperative surveillance tailored to the individual patient's condition, and ultrasound-guided FNA may be incorporated as needed.

All of these advancements have resulted in a trend toward de-escalation of treatment for low-risk, differentiated (mainly papillary) thyroid cancer; more selective use of total thyroidectomy plus postoperative ^{131}I ablation; and increasing acceptance of thyroid lobectomy with no ^{131}I ablation for early-stage (low-risk) disease, especially when clinically apparent lymph node metastases are not present.

The application of thermal and chemical ablation techniques to benign thyroid disease as well as for palliation in select cases of metastatic malignancy has grown in popularity and acceptance in the 21st century. Cystic thyroid nodules have been treated with alcohol and other sclerosing agents for decades.[30] Now, volume reduction of large, solid, and mixed benign thyroid nodules by thermal or chemical means has been embraced, pioneered in Asia and Europe and propagated around the globe (Fig. 3b–d). Radiofrequency, laser, microwave, and high-intensity focused ultrasound are all being employed. These techniques will continue to provide alternatives to traditional thyroidectomy and all of them rely on image guidance, predominantly through ultrasound.[29]

In spite of these many nonsurgical advances, the global volume of thyroid surgical procedures has continued to grow. From 1996 to 2006, the total number of thyroidectomies

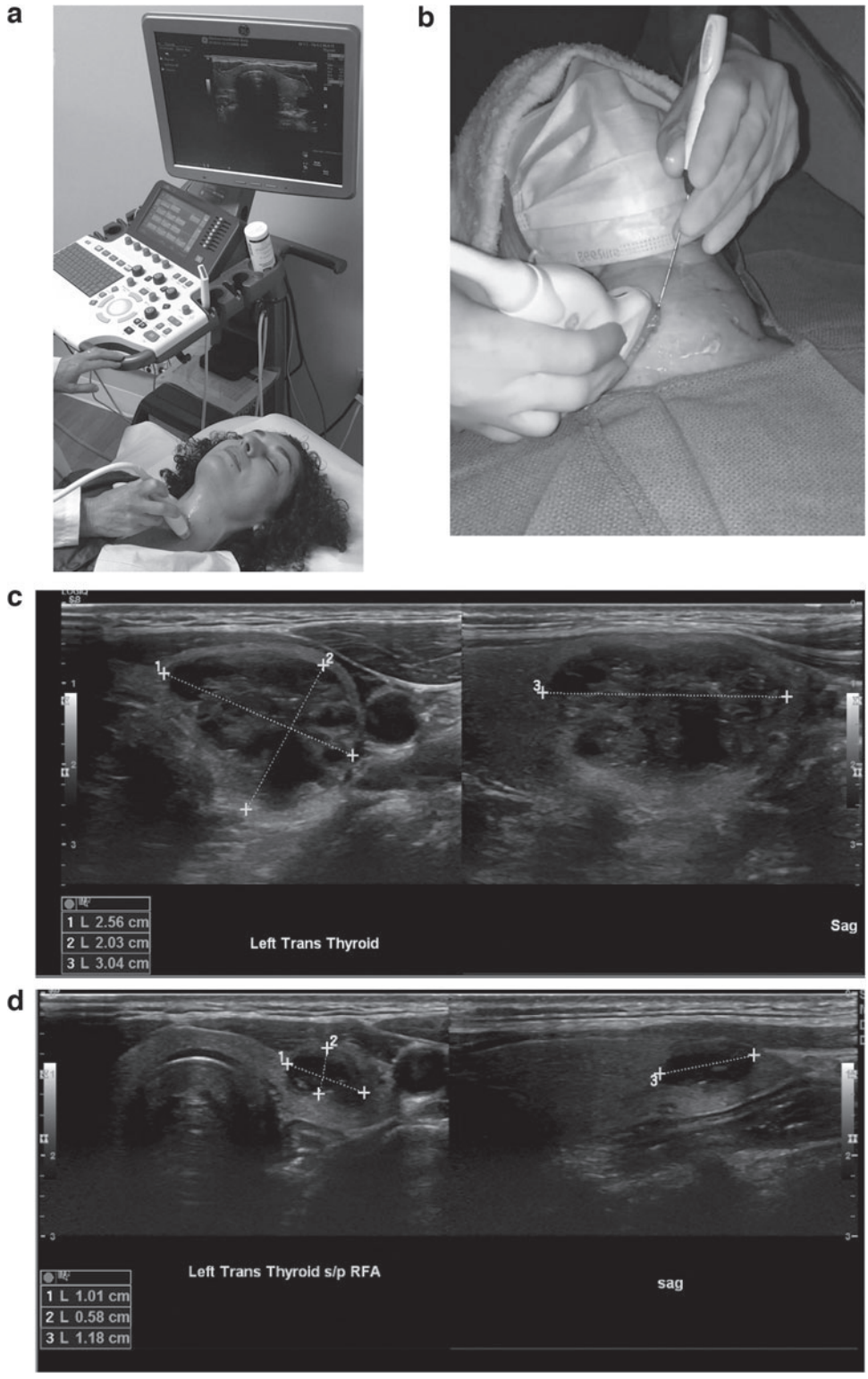

FIG. 3. **(a–d)** Surgeon-performed ultrasonography **(a)** has resulted in enhanced diagnostic imaging, surgical planning, procedural guidance such as RFA **(b)**, and nonsurgical management and volume reduction of benign thyroid nodules. **(c)** Pretreatment ultrasound of benign left thyroid nodule, transverse and sagittal views. **(d)** Six months after RFA, transverse and sagittal ultrasound views. Image 3a used with permission from the ultrasound examination model. RFA, radiofrequency ablation.

performed in the United States increased by 39%, from 66,864 to 92,931 cases per year. Outpatient procedures increased by 61%, while inpatient procedures increased by 30%.[31] By 2016, the number of annual thyroidectomies had increased to 169,000.[32]

These figures predate the COVID pandemic that began in 2019, which has further influenced a shift toward ambulatory and outpatient care. In addition to surgical safety, factors such as economics, insurance influences, patient expectations, desire for avoidance of infection, and ability for telehealth and communication have all led to shorter hospital stays and more outpatient procedures.[33] The impact of newer ablative technologies for benign thyroid nodules on surgical statistics has yet to be quantified.

Meanwhile, an array of surgical devices and approaches have been combined to make thyroid surgery safer, faster, less visible, less painful, more precise, and more individualized. Since the origins of thyroid surgery, the vulnerability of the laryngeal nerves has been recognized and regarded with respect. Nerve avoidance, superseded by identification and protection, has been a surgeon's top priority. Progress in hemostasis that prevented exsanguination also enabled identification and dissection of the laryngeal nerves, aided by lighting and magnification.[34]

Examination of the larynx, beginning with indirect mirror laryngoscopy, as first described by Manuel Garcia in 1854,[35] evolved to incorporate fiberoptics, stroboscopy, video recording, and even ultrasonography. Most literature citing rates of vocal fold paralysis after thyroidectomy, even into the current century, has been based not on laryngeal examination, but on perceptual voice assessment, which significantly underrecognizes nerve dysfunction.

Nevertheless, in the quest to prevent laryngeal nerve injury in the first place, nerve monitoring technology evolved and has grown in adoption.[36] Endotracheal tube-based surface electrodes and pulsed intermittent or continuous laryngeal nerve stimulation provide objective functional and prognostic information during thyroid surgery, which aids intraoperative decision-making and perioperative management.[37,38]

Energy-based devices for hemostasis have been developed and increasingly applied in thyroid surgery.[39–41] Current vascular sealing tools include ultrasonic, bipolar radiofrequency, and hybrid energy systems. Use of these devices in no way lessens the responsibility for knowledge of regional anatomy and avoidance of thermal injury to delicate structures, especially the laryngeal nerves, parathyroid glands, trachea, esophagus, and the skin. The cost of such devices appears to have been offset by a reduction in operative time, operative blood loss and transfusion need, and hospital length of stay. Furthermore, these devices, along with endoscopic and robotic equipment, have planted the seed that has enabled minimal access thyroid surgery to blossom.

The three broad categories of minimal access thyroid procedures include completely endoscopic procedures (either direct or remote access) with carbon dioxide insufflation; partly endoscopic gasless procedures; and nonendoscopic mini-incision procedures.[39] Remote access to the thyroid has been gained through axillary, breast, facelift, and most recently transoral vestibular approaches. The dominant motivation for such approaches is cosmetic: avoidance of a visible neck scar. However, additional potential advantages include reduced overall trauma, scarring, and postoperative pain in carefully selected candidates with appropriate thyroid and overall anatomy.

Since Kocher's time, the preservation of parathyroid glands and their blood supply has been a paramount goal in thyroid and central neck surgery. The techniques of anticipation, meticulous dissection, and distal vessel ligation beyond the point of parathyroid perfusion remain the gold standard. Parathyroid autotransplantation has been practiced since at least the 1920s.[42] Capitalizing on the biochemistry and ultrashort half-life (3–5 minutes) of the parathyroid hormone (PTH),[43] rapid intraoperative PTH testing was developed initially for parathyroidectomy surgery to treat hyperparathyroidism, but subsequently as a means of assessing normal parathyroid function during and after thyroid surgery.

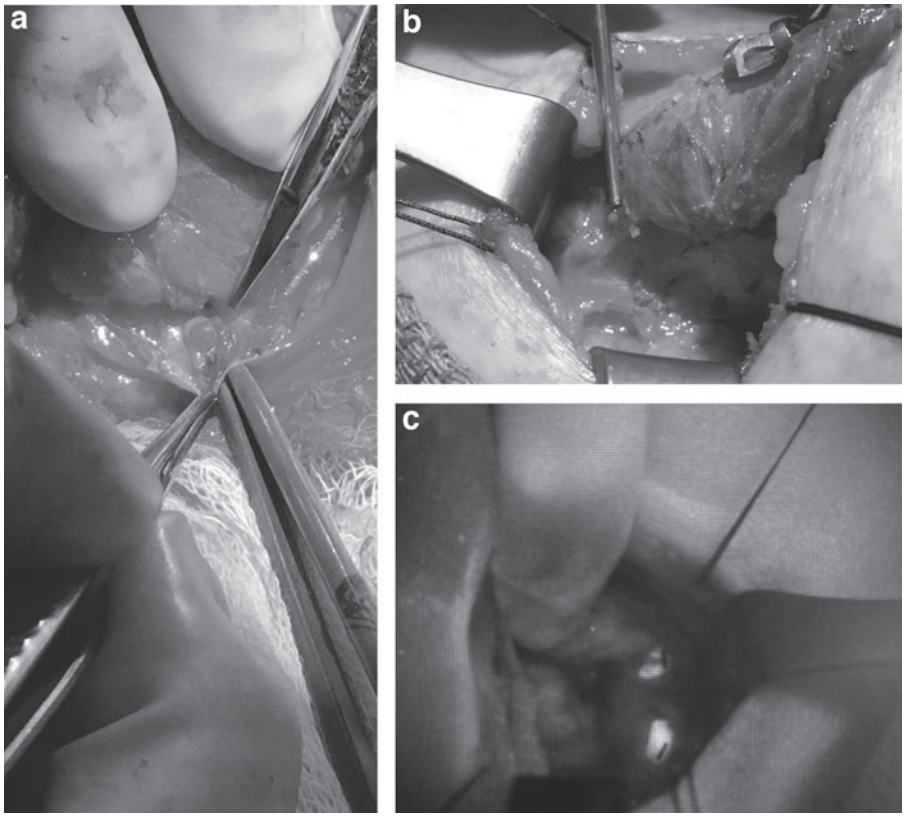

FIG. 4. **(a–c)** Parathyroid gland identification **(a)** can be enhanced by using autofluorescence-detecting devices. **(b)** Probe-based autofluorescence detection confirming left inferior parathyroid gland *in situ* before thyroid removal. **(c)** Camera-based autofluorescence detection of left superior and inferior parathyroid glands after total thyroidectomy.

In 2011,[44] the first reports of near-infrared (NIR) parathyroid autofluorescence were published. Since then, camera-based and probe-based devices have been designed, with FDA approval gained in 2018 in the United States for real-time parathyroid gland identification during surgery (Fig. 4a–c). These devices have been coupled with contrast enhancement (using intravenous indocyanine green) to detect fluorescence not only in the glands themselves but also in their perfusing blood vessels. NIR fluorescence detection has the potential to help identify and preserve parathyroid glands, reduce the operating time, and prevent hypoparathyroidism.

Use of molecular genetics to fine-tune either the need for thyroid surgery or the appropriate extent of thyroid surgery has become an important aspect of the art and science of thyroid surgery in the last 30 years. Medullary thyroid carcinoma (MTC) was the first cancer in which surgeons investigating the role of genetic changes were led to proposing that surgery based on a germline mutation be performed before surgical manifestation of disease.[45–47] Subsequent knowledge about genetic–phenotypic correlation in patients with mutations of the rearranged during transfection (RET) proto-oncogene led to published guidelines on the surgical management of patients with either sporadic or hereditary MTC.[48,49]

Prophylactic thyroidectomy met all the criteria for applying knowledge of hereditary cancer syndromes: the genetic mutation known to cause MTC with complete or near-complete penetrance became testable with high accuracy, the thyroid gland could be removed with minimal morbidity and virtually no mortality, there was a hormone to replace the function of the organ being surgically removed, and there was a reliable marker (calcitonin) to determine if the treatment was curative.

Prophylactic thyroidectomy, defined as removing the thyroid before MTC is clinically apparent, became the standard of care. As genetic–phenotypic heterogeneity has become recognized in families with MTC, the recommended age for prophylactic thyroidectomy in MTC patients has become dependent on the specific knowledge about the mutation and its natural history.[48,50,51]

The historical context of the discovery of the RET proto-oncogene and the role of surgeons in the paradigm shift toward suggesting surgery based on germline genetic testing rather than as a treatment of an existing tumor is critical to understand.[9] Multiple endocrine neoplasia type 2 and its subgroupings (MEN2a, MEN2b, and familial MTC) have MTC as a prime feature. In the 1950s, the surgeon, Dr. Samuel Wells, meticulously documented features of various MEN syndromes within multiple kindred as he worked to characterize calcitonin as a marker of MTC and enable early detection.

By using pentagastrin to stimulate calcitonin production from the hyperplastic C cells of the thyroid, he was able to diagnose MTC and treat it surgically before development of lymph node-positive disease. Wells recognized that while some patients were cured by this method, many still died of distant metastatic disease. In 1985, Takahashi, while searching for ''transforming'' genes by looking at fragments that could result in transformation of normal fibroblasts, identified a gene he named ''REarranged during Transfection'' or RET.[47]

By 1987, the RET proto-oncogene had been mapped to chromosome 10, and genetic linkage analysis had mapped the MEN2a locus to the same chromosome.[52] By 1993, Wells together with Dr. Helen Donis-Keller identified seven RET mutations by screening multiple families with all three MEN2 types. Over the next few years, additional mutations were found by various groups and these mutations were noted to activate the tyrosine kinase.

This eventually led to seminal work by Chi and Wells who proposed the first set of predictive genetic tests for MEN2 based on the identified mutations in the RET proto-oncogene.[45,53] By 1995, Wells showed that prophylactic surgery driven by knowledge of a patient's genetics was the

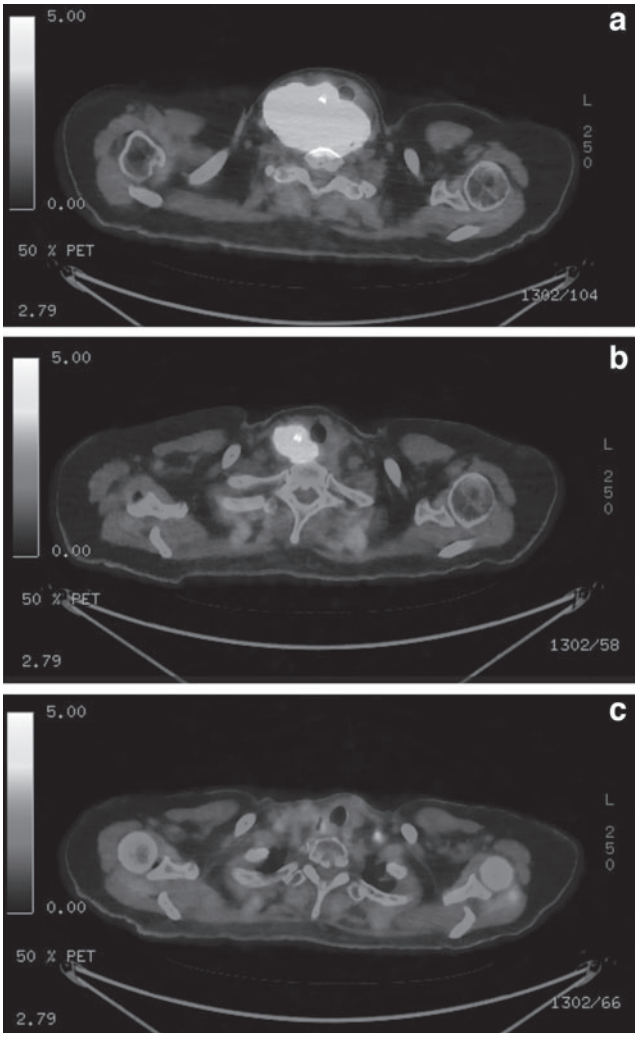

FIG. 5. **(a–c)** Anaplastic thyroid carcinoma (ATC), once nearly universally and rapidly lethal, has recently become a survivable malignancy thanks to combination therapy with neoadjuvant systemic agents, surgery, external beam radiation, and/or immunotherapy. **(a)** ATC at presentation, showing carotid artery encasement; esophageal invasion; positive test for $BRAF^{V600E}$, TERT, and TP53 mutations; PD-L1 expression; and association with pulmonary metastases. **(b)** Three months after neoadjuvant therapy with BRAF-targeting dabrafenib and trametinib. The primary tumor was then resected. **(c)** Nine months after surgery and 6 months after completion of external beam radiation therapy, with ongoing dabrafenib, trametinib, and pembrolizumab immunotherapy. The patient remained alive and well at the most recent follow-up, more than 1.5 years after surgery. PD-L1, programmed death-ligand 1.

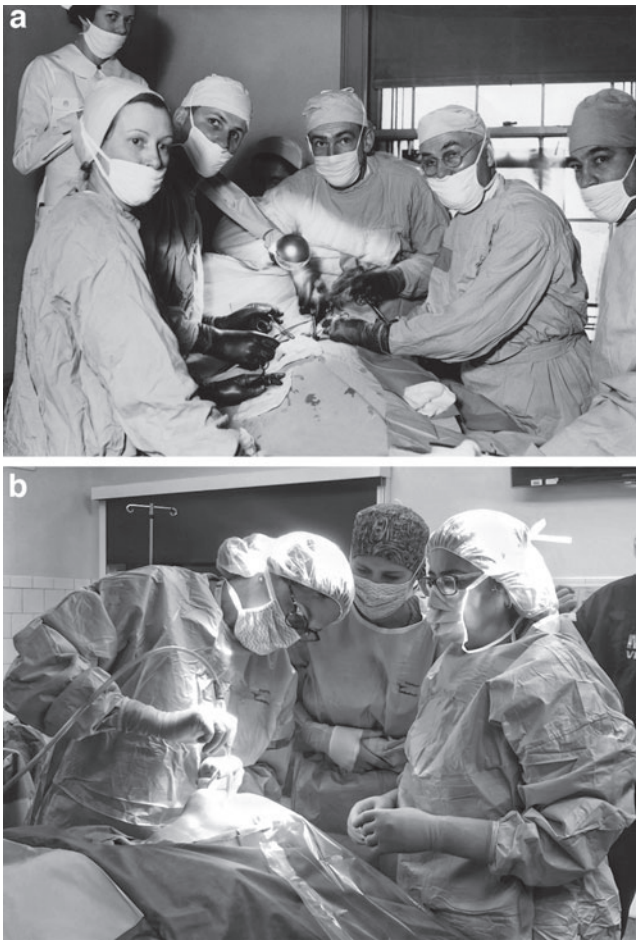

FIG. 6. **(a, b)** Evolution of thyroid surgery in the last century. **(a)** Dr. George Crile Sr. (with glasses, second from right) performs his 25,000th thyroid surgery with his son Dr. George Crile Jr. (third from left) at the Cleveland Clinic in 1936. The sphere of light held by the nurse helped illuminate the field of surgery (reprinted with permission, Cleveland Clinic Foundation ©2022; all rights reserved). **(b)** Dr. Antonia Stephen (left), Dr. Sareh Parangi (right), and Dr. Maria Troulis (middle) performing their first scarless transoral thyroid surgery to remove a thyroid lobe at Massachusetts General Hospital in 2018.

key to long-term survival and opened the door to other future prophylactic surgeries based on genotype.[54] These were the first forays into personalizing thyroid surgery based on germline genetic testing.

Today, the evaluation of thyroid nodules, especially those with indeterminate cytology, has led surgeons to further innovations in the use of nongermline mutational analysis. Before the molecular characterization of thyroid nodule FNA cytology, most patients with indeterminate nodules underwent diagnostic thyroid surgery, usually lobectomy, for what ultimately proved most often to be benign disease. Molecular testing now available to surgeons has allowed for better risk stratification and has dramatically reduced the need for diagnostic surgery.

Unfortunately, due to high costs of these genetic tests, this innovation is not currently accessible worldwide. Surgeons have come to better understand the mutational landscape of thyroid nodules and thyroid cancers. Some mutations such as RAS are known to be ''weak drivers'' since they are not uniquely found in malignant neoplasms of the thyroid, whereas certain other mutations such as RET, NTRK, ALK, BRAF, TERT, and p53 are now known as ''strong drivers'' of cancers, with none generally being expressed in normal thyroid epithelial cells.[55]

At the other end of the risk spectrum, the use of molecularly driven care has allowed surgeons to comanage patients with anaplastic thyroid carcinoma (ATC) with novel approaches. While most patients with ATC are still presenting with locally unresectable disease and distant spread at time of diagnosis, molecular profiling of suspected ATC is now recommended at diagnosis—including testing for BRAF and NTRK mutations and programmed death-ligand 1 expression by pathologic immunostaining or by molecular testing.[56]

Furthermore, the use of blood-based liquid biopsy for circulating (cell-free) tumor DNA shows great promise for even more rapid profiling of aggressive thyroid cancer variants.[57,58] Patients with ATC who have BRAF-mutated tumors may undergo neoadjuvant therapy with BRAF/MEK inhibitors and in some cases with the addition of immune checkpoint inhibitors, leading to potential dramatic tumor responses with enough shrinkage that tumors may become surgically resectable[59–62] (Fig. 5a–c).

Even nonresectable or disseminated disease may achieve a prolonged palliative response to targeted systemic therapy with or without external beam radiation compared with nontargeted therapy with cytotoxic chemotherapeutic agents. Perhaps these developments in the integration of genetics, immunology, physiology, technology, research, training, and multidisciplinary care epitomize better than any other advances in the last century the ability of risk stratification and personalization of thyroid care (Fig. 6).

Kocher's words remain as true today as they did in his time, yet with contemporary optimism, thanks to ancillary innovations in traditional thyroid surgery in isolation: ''A surgeon is a doctor who can operate and who knows when not to.''[4]

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Authors' Contributions

L.A.O. and S.P. were involved in conceptualization, original draft preparation, and writing—reviewing and editing.

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References

1. Halsted W. The Operative Story of Goiter: The author's operation. Johns Hopkins Hosp Rep 1920;19:71.

2. Zeiger MA, Shen WT, Felger EA. The Supreme Triumph of the Surgeon's Art: A Narrative History of Endocrine Surgery. University of California, Medical Humanities Consortium: San Francisco, CA; 2013.
3. Wiese ER, Gilbert JE. Theodore Kocher. Ann Med Hist 1931;3(5):521–529.
4. Sarkar S, Banerjee S, Sarkar R, et al. A review on the history of 'Thyroid Surgery'. Indian J Surg 2016;78(1):32–36; doi: 10.1007/s12262-015-1317-5
5. Chiesa F. The 100 years Anniversary of the Nobel Prize Award winner Emil Theodor Kocher, a brilliant far-sighted surgeon. Acta Otorhinolaryngol Ital 2009;29(6):289.
6. Welbourn RB. The History of Endocrine Surgery. Praeger: New York; 1990.
7. Hannan SA. The magnificent seven: A history of modern thyroid surgery. Int J Surg 2006;4(3):187–191; doi: 10.1016/j.ijsu.2006.03.002
8. Vellar ID. Thomas Peel Dunhill: Pioneer thyroid surgeon. Aust N Z J Surg 1999;69(5):375–387; doi: 10.1046/j.1440-1622.1999.01577.x
9. Pasieka JL, Lee JA, SpringerLink. Surgical Endocrinopathies: Clinical Management and the Founding Figures. Springer International Publishing (Imprint), Springer: Cham; 2015.
10. Van Meter S. Origin, aims and policy of the American Association for the Study of Goiter. Am J Surg 1929;7(1):1–3.
11. Niazi AK, Kalra S, Irfan A, et al. Thyroidology over the ages. Indian J Endocrinol Metab 2011;15(Suppl 2):S121–S126; doi: 10.4103/2230-8210.83347
12. Burch HB, Cooper DS. Anniversary review: Antithyroid drug therapy: 70 Years later. Eur J Endocrinol 2018;179(5):R261–R274; doi: 10.1530/eje-18-0678
13. Miller TR, Abele JS, Greenspan FS. Fine-needle aspiration biopsy in the management of thyroid nodules. West J Med 1981;134(3):198–205.
14. Cibas ES, Ali SZ. The 2017 Bethesda System for Reporting Thyroid Cytopathology. Thyroid 2017;27(11):1341–1346; doi: 10.1089/thy.2017.0500
15. Baloch Z, LiVolsi VA. Fifty years of thyroid pathology: Concepts and developments. Hum Pathol 2020;95:46–54; doi: 10.1016/j.humpath.2019.09.008
16. Frates MC, Benson CB, Charboneau JW, et al. Management of thyroid nodules detected at US: Society of Radiologists in Ultrasound consensus conference statement. Radiology 2005;237(3):794–800; doi: 10.1148/radiol.2373050220
17. Sanabria A, Kowalski LP, Shah JP, et al. Growing incidence of thyroid carcinoma in recent years: Factors underlying overdiagnosis. Head Neck 2018;40(4):855–866; doi: 10.1002/hed.25029
18. Cooper DS, Doherty GM, Haugen BR, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009;19(11):1167–1214; doi: 10.1089/thy.2009.0110
19. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26(1):1–133; doi: 10.1089/thy.2015.0020
20. Haddad RI, Bischoff L, Ball D, et al. Thyroid Carcinoma, Version 2.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2022;20(8):925–951; doi: 10.6004/jnccn.2022.0040
21. Tessler FN, Middleton WD, Grant EG, *et al.* ACR Thyroid Imaging, Reporting and Data System (TI-RADS): White Paper of the ACR TI-RADS Committee. J Am Coll Radiol 2017;14(5):587–595; doi: 10.1016/j.jacr.2017.01.046
22. Molinaro E, Campopiano MC, Pieruzzi L, et al. Active surveillance in papillary thyroid microcarcinomas is feasible and safe: Experience at a Single Italian Center. J Clin Endocrinol Metab 2020;105(3):e172–e180; doi: 10.1210/clinem/dgz113
23. Shin JH, Baek JH, Chung J, et al. Ultrasonography diagnosis and imaging-based management of thyroid nodules: Revised Korean Society of Thyroid Radiology Consensus Statement and Recommendations. Korean J Radiol 2016;17(3):370–395; doi: 10.3348/kjr.2016.17.3.370
24. Gharib H, Papini E, Garber JR, et al. American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi Medical Guidelines for Clinical Practice for the Diagnosis and Management of Thyroid Nodules—2016 Update. Endocr Pract 2016;22(5):622–639; doi: 10.4158/ep161208.Gl
25. Zhou J, Yin L, Wei X, et al. 2020 Chinese guidelines for ultrasound malignancy risk stratification of thyroid nodules: The C-TIRADS. Endocrine 2020;70(2):256–279; doi: 10.1007/s12020-020-02441-y
26. Ito Y, Miyauchi A, Kudo T, et al. Trends in the implementation of active surveillance for low-risk papillary thyroid microcarcinomas at Kuma Hospital: Gradual increase and heterogeneity in the acceptance of this new management option. Thyroid 2018;28(4):488–495; doi: 10.1089/thy.2017.0448
27. Tuttle RM, Fagin JA, Minkowitz G, et al. Natural history and tumor volume kinetics of papillary thyroid cancers during active surveillance. JAMA Otolaryngol Head Neck Surg 2017;143(10):1015–1020; doi: 10.1001/jamaoto.2017.1442
28. Oda H, Miyauchi A, Ito Y, et al. Incidences of unfavorable events in the management of low-risk papillary microcarcinoma of the thyroid by active surveillance versus immediate surgery. Thyroid 2016;26(1):150–155; doi: 10.1089/thy.2015.0313
29. Orloff LA, Noel JE, Stack Jr BC, et al. Radiofrequency ablation and related ultrasound-guided ablation technologies for treatment of benign and malignant thyroid disease: An international multidisciplinary consensus statement of the American Head and Neck Society Endocrine Surgery Section with the Asia Pacific Society of Thyroid Surgery, Associazione Medici Endocrinologi, British Association of Endocrine and Thyroid Surgeons, European Thyroid Association, Italian Society of Endocrine Surgery Units, Korean Society of Thyroid Radiology, Latin American Thyroid Society, and Thyroid Nodules Therapies Association. Head Neck 2022;44(3):633–660; doi: 10.1002/hed.26960
30. Hahn SY, Shin JH, Na DG, et al. Ethanol ablation of the thyroid nodules: 2018 Consensus Statement by the Korean Society of Thyroid Radiology. Korean J Radiol 2019;20(4):609–620; doi: 10.3348/kjr.2018.0696
31. Sun GH, DeMonner S, Davis MM. Epidemiological and economic trends in inpatient and outpatient thyroidectomy

in the United States, 1996–2006. Thyroid 2013;23(6):727–733; doi: 10.1089/thy.2012.0218
32. Meltzer C, Hull M, Sundang A, et al. Association between annual surgeon total thyroidectomy volume and transient and permanent complications. JAMA Otolaryngol Head Neck Surg 2019;145(9):830–837; doi: 10.1001/jamaoto.2019.1752
33. Francis DO, Randolph G, Davies L. Nationwide variation in rates of thyroidectomy among US Medicare Beneficiaries. JAMA Otolaryngol Head Neck Surg 2017;143(11):1122–1125; doi: 10.1001/jamaoto.2017.1746
34. Sapalidis K, Papanastasiou A, Fyntanidou V, et al. Comparison between magnification techniques and direct vision in thyroid surgery: A systematic review and meta-analysis. Medicina (Kaunas) 2019;55(11):725; doi: 10.3390/medicina55110725
35. Lapeña JF. Mirrors and reflections: The evolution of indirect laryngoscopy. Ann Saudi Med 2013;33(2):177–181; doi: 10.5144/0256-4947.2013.177
36. Randolph GW, Dralle H, Abdullah H, et al. Electrophysiologic recurrent laryngeal nerve monitoring during thyroid and parathyroid surgery: International standards guideline statement. Laryngoscope 2011;121 Suppl 1:S1–S16; doi: 10.1002/lary.21119
37. Schneider R, Randolph GW, Dionigi G, et al. International neural monitoring study group guideline 2018 part I: Staging bilateral thyroid surgery with monitoring loss of signal. Laryngoscope 2018;128 Suppl 3:S1–S17; doi: 10.1002/lary.27359
38. Wu CW, Dionigi G, Barczynski M, et al. International neuromonitoring study group guidelines 2018: Part II: Optimal recurrent laryngeal nerve management for invasive thyroid cancer-incorporation of surgical, laryngeal, and neural electrophysiologic data. Laryngoscope 2018;128 Suppl 3:S18–S27; doi: 10.1002/lary.27360
39. Bakkar S, Papavramidis TS, Aljarrah Q, et al. Energy-based devices in thyroid surgery—An overview. Gland Surg 2020;9(Suppl 1):S14–S17; doi: 10.21037/gs.2019.08.05
40. Garas G, Okabayashi K, Ashrafian H, et al. Which hemostatic device in thyroid surgery? A network meta-analysis of surgical technologies. Thyroid 2013;23(9):1138–1150; doi: 10.1089/thy.2012.0588
41. Dionigi G, Wu CW, Kim HY, et al. Safety of energy based devices for hemostasis in thyroid surgery. Gland Surg 2016;5(5):490–494; doi: 10.21037/gs.2016.09.01
42. Sitges-Serra A, Lorente-Poch L, Sancho J. Parathyroid autotransplantation in thyroid surgery. Langenbecks Arch Surg 2018;403(3):309–315; doi: 10.1007/s00423-018-1654-5
43. Leiker AJ, Yen TW, Eastwood DC, et al. Factors that influence parathyroid hormone half-life: Determining if new intraoperative criteria are needed. JAMA Surg 2013;148(7):602–606; doi: 10.1001/jamasurg.2013.104
44. Solórzano CC, Thomas G, Berber E, et al. Current state of intraoperative use of near infrared fluorescence for parathyroid identification and preservation. Surgery 2021;169(4):868–878; doi: 10.1016/j.surg.2020.09.014
45. Donis-Keller H, Dou S, Chi D, et al. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet 1993;2(7):851–856; doi: 10.1093/hmg/2.7.851
46. Mulligan LM, Kwok JB, Healey CS, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 1993;363(6428):458–460; doi: 10.1038/363458a0
47. Takahashi M, Ritz J, Cooper GM. Activation of a novel human transforming gene, ret, by DNA rearrangement. Cell 1985;42(2):581–588; doi: 10.1016/0092-8674(85)90115-1
48. Wells SA, Jr., Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid 2015;25(6):567–610; doi: 10.1089/thy.2014.0335
49. Tuttle RM, Ball DW, Byrd D, et al. Medullary carcinoma. J Natl Compr Cancer Netw 2010;8(5):512–530; doi: 10.6004/jnccn.2010.0040
50. Sosa JA, Tuggle CT, Wang TS, et al. Clinical and economic outcomes of thyroid and parathyroid surgery in children. J Clin Endocrinol Metab 2008;93(8):3058–3065; doi: 10.1210/jc.2008-0660
51. Elisei R, Romei C, Renzini G, et al. The timing of total thyroidectomy in RET gene mutation carriers could be personalized and safely planned on the basis of serum calcitonin: 18 Years experience at one single center. J Clin Endocrinol Metab 2012;97(2):426–435; doi: 10.1210/jc.2011-2046
52. Simpson NE, Kidd KK, Goodfellow PJ, et al. Assignment of multiple endocrine neoplasia type 2A to chromosome 10 by linkage. Nature 1987;328(6130):528–530; doi: 10.1038/328528a0
53. Chi DD, Toshima K, Donis-Keller H, et al. Predictive testing for multiple endocrine neoplasia type 2A (MEN 2A) based on the detection of mutations in the RET proto-oncogene. Surgery 1994;116(2):124–132; discussion 132–133.
54. Wells Jr SA, Chi DD, Toshima K, et al. Predictive DNA testing and prophylactic thyroidectomy in patients at risk for multiple endocrine neoplasia type 2A. Ann Surg 1994;220(3):237–247; discussion 247–250; doi: 10.1097/00000658-199409000-00002
55. Shonka Jr DC, Ho A, Chintakuntlawar AV, et al. American Head and Neck Society Endocrine Surgery Section and International Thyroid Oncology Group consensus statement on mutational testing in thyroid cancer: Defining advanced thyroid cancer and its targeted treatment. Head Neck 2022;44(6):1277–1300; doi: 10.1002/hed.27025
56. Bible KC, Kebebew E, Brierley J, et al. 2021 American Thyroid Association Guidelines for Management of patients with anaplastic thyroid cancer. Thyroid 2021;31(3):337–386; doi: 10.1089/thy.2020.0944
57. Qin Y, Wang JR, Wang Y, et al. Clinical utility of circulating cell-free DNA mutations in anaplastic thyroid carcinoma. Thyroid 2021;31(8):1235–1243; doi: 10.1089/thy.2020.0296
58. Gouda MA, Ong E, Huang HJ, et al. Ultrasensitive detection of BRAF V600E mutations in circulating tumor DNA of patients with metastatic thyroid cancer. Endocrine 2022;76(2):491–494; doi: 10.1007/s12020-022-03004-z
59. Wang JR, Zafereo ME, Dadu R, et al. Complete surgical resection following neoadjuvant dabrafenib plus trametinib in BRAF(V600E)-mutated anaplastic thyroid carcinoma. Thyroid 2019;29(8):1036–1043; doi: 10.1089/thy.2019.0133
60. Cabanillas ME, Ferrarotto R, Garden AS, et al. Neoadjuvant BRAF- and immune-directed therapy for anaplastic thyroid carcinoma. Thyroid 2018;28(7):945–951; doi: 10.1089/thy.2018.0060

61. Busaidy NL, Konda B, Wei L, et al. Dabrafenib versus dabrafenib + trametinib in BRAF-mutated radioactive iodine refractory differentiated thyroid cancer: Results of a Randomized, Phase 2, Open-Label Multicenter Trial. Thyroid 2022;32(10):1184–1192; doi: 10.1089/thy.2022.0115
62. Maniakas A, Dadu R, Busaidy NL, et al. Evaluation of overall survival in patients with anaplastic thyroid carcinoma, 2000–2019. JAMA Oncol 2020;6(9):1397–1404; doi: 10.1001/jamaoncol.2020.3362

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A Historical Reflection on Scientific Advances in Understanding Thyroid Hormone Action

Gregory A. Brent[1,2]

Background: Thyroid hormone (TH) has actions in every tissue of the body and is essential for normal development, as well as having important actions in the adult. The earliest markers of TH action that were identified and monitored clinically, even before TH could be measured in serum, included oxygen consumption, basal metabolic rate, serum cholesterol, and deep tendon reflex time. Cellular, rodent, amphibian, zebrafish, and human models have been used to study TH action.

Summary: Early studies of the mechanism of TH action focused on saturable-specific triiodothyronine (T3) nuclear binding and direct actions of T3 that altered protein expression. Additional effects of TH were recognized on mitochondria, stimulation of ion transport, especially the sodium potassium ATPase, augmentation of adrenergic signaling, role as a neurotransmitter, and direct plasma membrane effects. The cloning of the thyroid hormone receptor (THR) genes in 1986 and report of the THR crystal structure in 1995 produced rapid progress in understanding the mechanism of TH nuclear action, as well as the development of modified THR ligands. These findings revealed nuances of TH signaling, including the role of nuclear receptor coactivators and corepressors, repression of positively stimulated genes by the unliganded receptor, THR isoform-specific actions of TRα (THRA) and TRβ (THRB), and THR binding DNA as a heterodimer with retinoid-x-receptor (RXR) for genes positively regulated by TH. The identification of genetic disorders of TH transport and signaling, especially Resistance to Thyroid Hormone (RTH) and monocarboxylate transporter 8 (*Mct8*) defects, has been highly informative with respect to the mechanism of TH action.

Conclusions: The impact of THR isoform, post-translational modifications, receptor cofactors, DNA response element, and selective TH tissue uptake, on TH action, have clinical implications for diagnosing and treating thyroid disease. Additionally, these findings have led to the development of novel TH and TH analogue therapies for metabolic, neurological, and cardiovascular diseases.

Keywords: thyroid hormone receptor, thyroid hormone action, nuclear receptor coregulators, Resistance to Thyroid Hormone, thyroid hormone transport

Introduction

This invited review on the history of thyroid hormone (TH) action is one of a series of articles celebrating the American Thyroid Association centennial. Our understanding of TH action is the result of investigators working across multiple disciplines and experimental models, for at least 140 years. This review will focus on studies of the nuclear thyroid hormone receptor (THR), our understanding before the report of cloning of THR in 1986, and the impact of the cloning on our understanding of TH action and genetic disorders of TH action. Some of the clinical implications of insights into TH action will also be described. TH metabolism and local ligand availability, a key regulator of TH action, will be discussed in a separate review.

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Early Observations of TH Action

The earliest attempts to measure TH action centered on the metabolic actions, assessing the increase or decrease in basal metabolic rate as a manifestation of TH status, body temperature, body weight, body fat composition, oxygen consumption, and the actions on circulating lipid levels, especially serum total and low-density lipoprotein cholesterol.[1] The recognition that oxygen consumption in patients with thyroid disease directly increased or decreased with the level of TH was reported as early as 1895 by Magnus-Levy, 20 years before thyroxine (T4) was isolated (reviewed in Magnus-Levy[2]).

Additional actions of TH tracked clinically were the effects on the heart (chronotropic and inotropic), as well as deep tendon reflex relaxation time. Mitochondria were thought to be an essential site of TH action, based on TH stimulation of oxygen consumption in tissues.[3,4] In most tissues, except the brain, oxygen consumption increased or decreased with an increase or decrease in TH levels. Early studies and models recognized the importance of TH action in development, including the absolute requirement for TH to promote metamorphosis in frogs.[5] Interestingly, TH was recognized as essential for both of the key features of amphibian transition from aquatic to terrestrial life, tail resorption and limb generation.

The varied clinical manifestations of TH deficiency and TH excess, across almost every organ system in the body (Table 1), led many to consider that TH worked through a range of activating pathways.[6–9] Although several TH targets were identified and studied, including mitochondria, ion transporters, and membrane receptors, the bulk of the action was recognized as mediated by nuclear THRs. TH is one of only two tyrosine-derived hormones, the other being catecholamines. Catecholamines circulate without being bound to proteins and act at membrane receptors, similar to the peptide hormone family. TH, which is hydrophobic, circulates bound to protein and would ultimately be shown to act through nuclear receptors, similar to steroid hormones.[10]

Models and Mechanisms of TH Action

The study of circulating serum proteins that bind T4 and triiodothyronine (T3) was an early focus that later informed studies of the TH nuclear receptor (reviewed in Pappa et al[11]). It was found that there were individuals born with absent, or defective, TH binding proteins, thyroxine binding globulin, transthyretin (TTR), and albumin. Although a high fraction of circulating T4 and T3 is bound to proteins, the pituitary and hypothalamus regulate the ''free fraction'' of T4 and T3, such that the tissues are euthyroid despite serum total T4 and T3 concentrations outside the reference range in affected individuals. The characteristics of T3 and T4 binding revealed by TH binding protein mutations, however, were later used to guide investigation of nuclear TH binding.[11]

A major milestone in studies of TH action was the demonstration by Tata in 1963 that inhibitors of RNA production and protein synthesis, actinomycin D and puromycin, blocked the biological actions of TH on growth and basal metabolic rate in a rodent model.[12] This challenged the popular view at that time of a direct action of TH on mitochondria and oxidative phosphorylation and favored an action that required protein synthesis. A series of follow-up studies by Tata, utilizing a range of animal, cellular, and amphibian models, including radioactive labeling techniques, directly demonstrated TH-induced nuclear protein synthesis, with a time course and pattern consistent with TH nuclear action (reviewed in Tata[13]). Significant progress was made during this time by other laboratories, identifying the properties of the nuclear receptors for many steroid hormones, including estrogen, progesterone, and glucocorticoid.

These studies showed a mechanism of action with hydrophobic ligands passing into the cytoplasm, binding to receptors, the receptor–ligand complex entering the nucleus, binding specific DNA elements, and modifying gene transcription.[10] Specific nuclear T3 binding in growth hormone 1 pituitary cells was shown by Samuels in 1973.[14] A series of studies from a number of investigators in the mid-1970s, including Oppenheimer, Surks, Dillmann, Silva, Samuels, Refetoff, DeGroot, Tata, and Baxter, further characterized T3 nuclear binding in cells and tissues, linked T3 nuclear binding to gene expression of messenger RNA (mRNA) and protein, showed that T3, rather than T4, was the predominant bound ligand, and that tissues varied with respect to capacity for T3 binding, concentration highest in anterior pituitary, and then in descending order, in the liver, kidney, heart, and brain (reviewed in Sterling[3,4] and Oppenheimer[15]).

Table 1. Physiological and Biochemical Actions of Thyroid Hormone

Growth and development	*Metabolic*
Regulates the rate of postnatal growth of mammalian tissues	Regulation of basal metabolic rate in homeotherms
Development of fetal brain and bone	Control of oxidative phosphorylation and energy metabolism
Regulation of morphogenesis, developmental gene switching (e.g., in cardiac development), and apoptosis in amphibian larval metamorphosis	Regulation of cholesterol metabolism
Regulation of synthesis of mitochondrial respiratory enzymes and membranes	Regulation of lipogenesis and lipolysis
Skeletal muscle	Activation of brown adipose tissue in adaptive thermogenesis
White and brown adipose tissue	Calcium and phosphorus metabolism
	Nitrogen (urea, creatine) metabolism
	Movement of water and Na^+ ions across cell membranes

Adapted from Tata.[13]
Na^+, sodium.

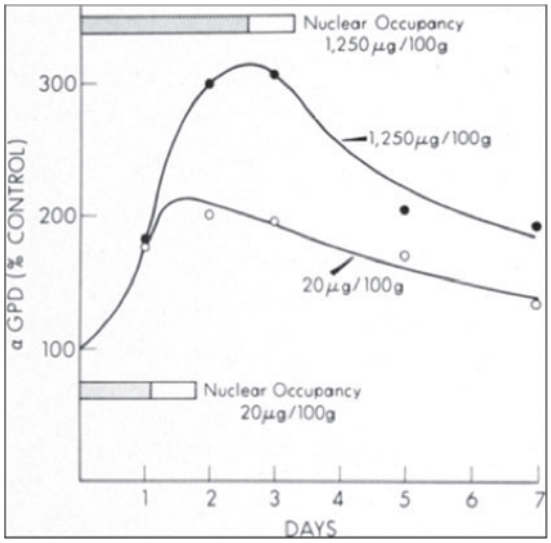

FIG. 1. Effect of high and low doses of T3 on alpha-GPD activity and on nuclear occupancy by T3 stimulation of hepatic alpha-GPD activity in euthyroid rats given a single, low-dose or high-dose intravenous injection of T3. The peak induction of alpha-GPD was closely linked to the duration of T3 nuclear occupancy, shown in the horizontal shaded bars. Nuclear occupancy by T3 was closely linked to the magnitude and duration of enzyme stimulation. From Oppenheimer.[15(p1065)] Copyright © (1975) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society. GPD, glycerophosphate dehydrogenase; T3, triiodothyronine.

Nuclear saturation by T3 was directly linked to the magnitude and duration of stimulation of a TH-responsive enzyme, α-glycerophosphate dehydrogenase, in the liver, which also correlated with oxygen consumption[15] (Fig. 1). Nuclear extracts were labeled with radioactive T3, and the T3 binding protein was isolated by gel electrophoresis, indicating a single species and a receptor size of about 60 kDa, similar to the size of other described steroid nuclear receptors.

Studies of TH action, before the cloning of the nuclear THR genes, are described in reviews by Oppenheimer in 1975[15] and Sterling in 1979[3,4] (Table 2). The predominant mechanism of action was thought to be nuclear (Fig. 2A), but TH action was also recognized in mitochondria, adrenergic signaling, neural transmitters, and membrane actions. Some investigators focused almost exclusively on TH stimulation of thermogenesis by active sodium transport through upregulation of the sodium potassium ATPase (Na^+-K^+-ATPase). TH stimulation of oxygen consumption in the skeletal muscle was significantly reduced by treatment with ouabain, which inhibits the Na^+-K^+-ATPase.[16] Even those promoting a primary action of TH on ion transport, however, recognized that TH nuclear action on protein synthesis was the initial step, and these proteins stimulated by TH secondarily influenced ion transport.

Cloning of the THR Genes

A major turning point in understanding TH action occurred in 1986, with publication of the cloning of the THR genes.[17,18] The work leading to this point represented the contribution of many laboratories and investigators using a wide range of models and tools to characterize T3 nuclear binding. Many of the key steps were the result of findings from studies of other steroid hormone signaling pathways.[10,19] Ultimately, however, TH had many unique features and actions that informed subsequent studies, especially in terms of DNA binding characteristics, nuclear receptor cofactors, and with respect to nutrient signaling and cross talk with other nuclear receptors regulating fat and carbohydrate metabolism.[1,6,7,9]

The rapid progress in the field was also shaped by clinical/translational investigation, identifying individuals with defects in the thyroid signaling pathway. This was most notable for studies of Resistance to Thyroid Hormone (RTH), but also patients with defects of TH transport.[20,21] Molecular biology tools became available to clone genes of interest by a range of techniques, and multiple members of the steroid receptor family were cloned. Two groups, the laboratories of

Table 2. Pathways of Thyroid Hormone Signaling from the 1970s

Site of TH action	*Examples of TH actions*	*Laboratories (specific references included in source articles)*
Nuclear transcription	Stimulation of growth hormone expression in pituitary cells, malic enzyme, and alpha GPD	Samuels, Oppenheimer, DeGroot and Refetoff, Baxter
Mitochondrial activation	Uncoupling oxidative phosphorylation	Tapley, Bronk, Hoch, Babior and Ingbar, Sterling
Na^+-K^+-ATPase (sodium pump)	Increased oxygen consumption in rat liver slices, inhibited by ouabain	Edelman, Ishmael-Beggi
Augmentation of adrenergic pathway	Increase in the number of membrane adrenergic receptors	Tsai, Lefkowitz, Malbon and Fain, Bilezikian and Loeb
Neurotransmitter	Play a role as a tyrosine-promoting catecholamine synthesis	Drakman
Plasma membrane effects	Transport of amino acids across membranes and incorporated into cartilage and bone	Adamson and Ingbar, Segal, Gross, Goldfine

Adapted from Sterling[3,4] and Oppenheimer.[15]
GPD, glycerophosphate dehydrogenase; Na^+-K^+-ATPase, sodium potassium ATPase; TH, thyroid hormone.

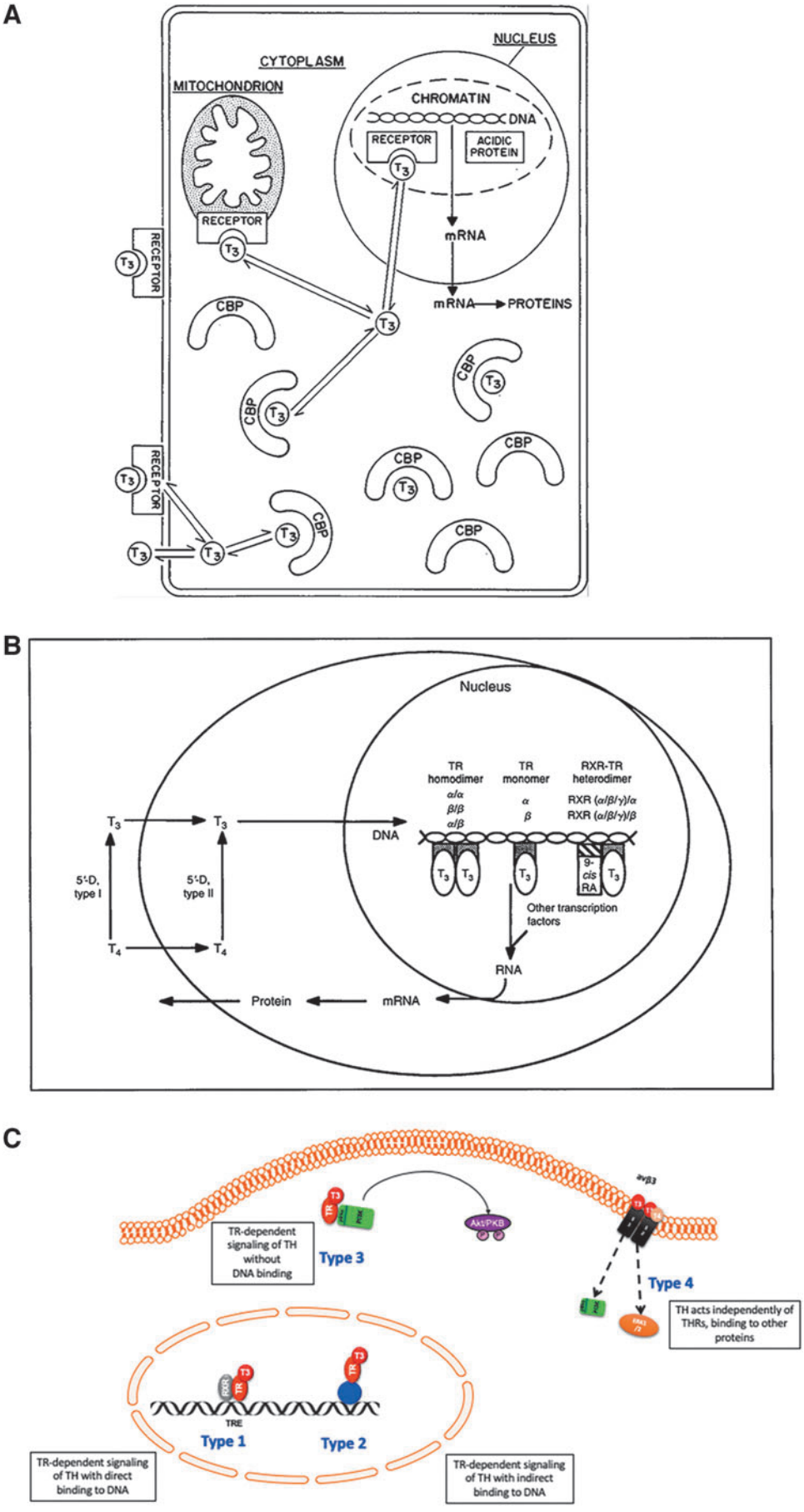

FIG. 2. Models for TH action on the target cell 1979, 1994, and 2021. **(A)** 1979—The unbound hormone (T4 or T3, the sequence of events is thought to be similar) diffuses or is transported into the cell, bound by CBP in a reversible equilibrium with unbound cytoplasmic T3. T3 acts on receptors in the mitochondria and nucleus, binding to DNA, stimulating mRNA production, which is translated to proteins. From Sterling.[3(p120)] Copyright © (1979) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society. **(B)** 1994—Nuclear action of TH (T3) bound to TRα and TRβ, bound to DNA response elements as homodimers, monomers, or heterodimers with RXR. Local activation of thyroxine T4 to T3 shown by the 5′-deiodinases types 1 and 2. From Brent.[9(p848)] Copyright © (1994) Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society. **(C)** 2021—The range of TH action, types 1, 2, 3, and 4, as described in Table 3 (figure modified [with permission] from Jonklaas et al,[61] classification of TH action as described in Flamant et al[60]). CBP, cytosol-binding protein; mRNA, messenger RNA; RXR, retinoid-x-receptor; T4, thyroxine; TH, thyroid hormone; THRs, thyroid hormone receptors; TR, TH receptors.

Evans and Vennström, working independently, published the cloning of the THR in the same issue of *Nature*.[17,18]

The THR had similarities to an erythroblastic leukemia virus, v-erb-A, and so, the initial clone was referred to as cellular erb-A (c-erb-A). The structure of the THR was similar to those of other steroid nuclear receptors already cloned and included an N-terminal domain, DNA binding domain, and a carboxy-terminal ligand binding domain. Studies showed that these domains were ''cassettes'' that could be interchanged among the nuclear receptors and maintain their specificity of the nuclear receptor of origin.[19] A progesterone receptor DNA binding domain, for example, could be substituted for the estrogen receptor DNA binding domain, and the chimeric receptor would bind estrogen but recognize the progesterone DNA response element.[22] TR was later shown to have these same interchangeable components.

The first cloned steroid hormone receptors, estrogen, progesterone, androgen, and glucocorticoid, were predominantly in the cytoplasm bound to heat shock protein, which was displaced by ligand, entered the nuclear, and bound as homodimers to palindromic DNA response elements.[19,22] In contrast, THR and other ''type 2'' steroid and steroid-like receptors reside predominantly in the nucleus bound to DNA, form heterodimers with retinoid-x-receptor (RXR) that bind to direct repeat elements, with various spacing that gives specificity, bind to corepressor complexes that repress gene expression, displaced by ligand, coactivator complexes are recruited, and gene activation occurs.[23,24]

Before full identification of the THR protein or cloning of the gene, it was possible to prepare nuclear protein extract and characterize DNA binding. Genes known to be directly stimulated by TH were limited, but it was known that growth hormone levels were very low in hypothyroid rodents. The rat growth hormone gene was shown to be stimulated by TH and glucocorticoids.[3,4] The earliest natural TH response element (TRE) was characterized using these methods and reported a sequence characterized as a ''direct repeat'' of 6 base pairs, with the sequence AGGTCA, and a 4 base pair ''gap''[25,26] (Fig. 2B). Other TH-responsive genes were subsequently shown to have some variations in half site arrangement and sequence, but the direct repeat with two half sites separated by a 4 base pair gap, AGGTCANNNNAGGTCA, was considered the consensus site.[27]

A similar DNA response element was identified that mediated the response to retinoic acid and TH.[28] This finding was an important divergence from the DNA response element that mediated binding of the other steroid receptors studied at that time, estrogen, progesterone, glucocorticoid, and mineralocorticoid. These receptors also bound to 6 base pair ''half site'' sequences, but these ''half sites'' were arranged as palindromes thought to facilitate homodimer binding, placing the common interface for covalent binding in direct proximity. A direct repeat DNA response element would bind a homodimers oriented in tandem with ''opposite'' receptor domains in close proximity, not something that had been previously reported for steroid receptors.

The resolution of this puzzle led to identification of a second class of nuclear hormone receptors, RXR, and expanded the mechanisms to modulate various TH signals. Early studies showed that the addition of nuclear extract enhanced THR binding and that these ''heterodimers'' were resistant to disruption by the addition of T3.[29] The identity of the heterodimer was later shown to be RXR, a member of the retinoid receptor family, which heterodimerized with THR and enhanced binding and transcription[30] (Fig. 2B), as well as heterodimerizing with retinoic acid receptor, vitamin D receptor, and nutrient receptors, such as peroxisome proliferator-activated receptors alpha and gamma.[31]

THR Isoforms and THR Gene Products

It was recognized, as with some other steroid and steroid-like receptors, that there were two different THR isoforms, alpha (TRα) and beta (TRβ), with different expression developmentally, and in adult tissues, TRβ predominant in the pituitary and liver, TRα in the brain and skeletal muscle, and both TRα and TRβ in the heart. There were also several splice product variants of unknown significance. Much of the original descriptions examined mRNA expression in various tissues. The limited availability of high-affinity antibodies that reliably distinguished the THR isoforms limited research in this area.

The most readily available tool was to examine mRNA expression, but there was recognition of the potential discordance between mRNA and protein. The availability of specific antibodies was especially a challenge to distinguish the various isoforms. Perhaps, the most puzzling is TRα2, a TRα isoform with a truncated ligand domain resulting in loss of T3 binding and shown functionally to antagonize T3 action.[32] Despite high levels of TRα2 mRNA expressed, especially in the brain, the expression of the TRα2 protein has been difficult to demonstrate.

A wide range of genetic rodent models with THRB and THRA gene inactivation and mutations showed consistent phenotypes that generally matched the distribution of THR isoforms.[6,7] The initial knockout mice for THRB had a phenotype similar to that seen in humans with RTHβ, goiter, elevated serum T4, and nonsuppressed thyrotropin (TSH),[33] subsequent evaluation revealed a significant hearing defect.[34] A wide range of mice with dominant negative mutations of the TR genes that reduced ligand affinity and disrupted signaling by the wild-type TR, generally had a more robust phenotype.[35]

There were also distinct phenotypes with mutations of THRA, although the metabolic phenotype varied depending on the THRA mutation that was being modeled.[1] The ultimate evidence of THR isoforms having distinct physiological function was the clinical report of individuals with mutations of the THRA gene.[36,37] Since TRα is not expressed significantly in the pituitary or hypothalamus, there was no impairment of T3 feedback, so serum TH levels remained largely normal, but there was evidence of reduced TH action with reduced growth, hypometabolism, bony defects, and constipation. Mutations of the THRA gene have been associated with skin tags and melanocytic nevi.[38]

Early characterization of the THRA gene and associated transcripts identified a related protein that did not bind T3 or was involved in TH signaling. ReverbA is transcribed from the opposite strand of the THRA gene, did not bind T3, and the function was unknown.[39] This THR-related gene was subsequently shown to be a key link in the regulation of the circadian clock, serving as a heme sensor that is a negative regulator of the circadian clock and suppresses hepatic gluconeogenic gene expression and glucose output.[40]

Basal Gene Repression by Unliganded THR

Despite some early skepticism about the validity of a direct repeat TRE in the rat growth hormone gene, others identified genes regulated by TH with a similar TRE configuration.[7] Although *in vitro* synthesized THR at high concentration could be shown to bind a direct repeat in a gel retardation assay, a major breakthrough was the recognition that the addition of nuclear extract dramatically increased binding.[29] The predominant protein in nuclear extract that was ultimately identified to bind THR was another nuclear receptor, RXR.[30] Another feature of this receptor family, first identified with THR, was that unliganded receptor bound DNA and repressed gene expression of positively regulated genes.

This was first shown with transient transfection assays and could be demonstrated as a receptor ''dose'' effect.[41] There was an underlying concern, however, that this repressive effect was an artifact of the transfection assay and that the high expression of heterologous promoters used in transient transfection could be consuming transcription factors that were artifactually lowering gene expression, a phenomenon referred to as ''squelching.''

A wide range of subsequent studies confirmed that this repression was mediated by unliganded receptor, but more importantly, an underlying mechanism was identified, which dramatically expanded the ways in which TH signaling could be modulated. Corepressor binds to unliganded DNA-bound THR and represses gene expression. Addition of ligand disrupts corepressor binding and promotes binding of coactivator to THR, stimulating gene expression. The other receptors that form heterodimers with RXR have similar properties. Ultimately, a family of corepressors, the most prominent that binds THR, nuclear receptor corepressor (NCoR), and small mediator for retinoid/thyroid hormone, were identified and shown to be very significant in TH signaling (reviewed in Astapova and Hollenberg[42]).

This was perhaps the most surprising property of TH that the clinical manifestations of hypothyroidism are not only the absence of ligand and reduced thyroid induced gene expression but also basal repression of genes based on occupancy of the response element by unliganded THR, which is bound to corepressor. The prediction from this finding is that the physiological consequences of reduced ligand in the presence of receptor would be partially restored toward normal in the absence of receptor. This was supported by a report of a mouse model with deletion of both THRA and THRB, which had a unique phenotype of retarded growth and bone maturation, reduced female fertility, and a hyperactive pituitary–thyroid axis.[43] Further evidence of the repressive role of TRα was a model with knockout of THRA, which makes up 80% of THR in the cerebellum that reversed the defects of hypothyroidism.[44]

The role of NCoR was shown by an *in vitro* model expressing a mutant NCoR that does not bind to THR, showed that TH-dependent gene expression in the liver was upregulated when the mice were euthyroid, and expressed in the normal range when hypothyroid, demonstrating that NCoR physiologically regulated THR-dependent genes.[45] The repressive action of unliganded THR on positively regulated genes also led to an important connection of THR and related factors, to the effects of THR to mediate differentiation in development and then when those differentiation actions were disrupted, to promote oncogenesis.

Human Disorders of Genetic Defects of TH Signaling and Transport

A range of genetic syndromes of reduced TH action have provided important insights into mechanisms.[20,46] The syndrome, RTHβ, with clinical manifestations of goiter, elevated serum T4 concentration, and a nonsuppressed TSH, with variable bone and metabolic manifestations was initially described by Refetoff et al in 1967,[47] has provided key information on the physiological role of THR. Once the THR genes were cloned, the THRB gene was linked to RTH.[48] The initial family reported was ultimately shown to be homozygous for deletion of the THRB gene, but the majority of reported patients are heterozygous for a ''dominant negative'' mutation, most in the ligand binding domain, which results in interaction with corepressor, which is not relieved by the usual levels of ligand.[49,50] Animal models that selectively mutate the primary THR corepressor, NCoR, were shown to partially normalize thyroid signaling in models of RTH with a dominant negative THRB mutation.[51]

A number of investigators identified membrane transporters for TH.[21] The specific transport of TH with high specificity was shown for the monocarboxylate transporter 8 (Mct8).[52] The linkage of *Mct8* gene mutation to a previously described X-linked disorder, with intellectual deficit, hypotonia, spastic paraplegia, and hypermetabolism, demonstrated that the Mct8 transporter was essential for normal T3 action on brain development in humans.[53,54] The importance of the OATP1C1 transporter was demonstrated by the report of an adolescent girl with an OATP1C1 gene mutation and evidence for dementia and neurodegeneration, with some response to treatment with the TH analog Triac, which does not require a transporter to enter neurons.[55]

Developmental Actions of TH

Sensory development, the inner ear and retina, have requirements for TH to progress normally (reviewed in Ng et al[56]). This developmental process revealed a key principle of TH action in development, apparent from the earliest amphibian studies of TH in frog metamorphosis, where premature exposure to TH induced early metamorphosis (reviewed in Tata[5]). TH availability in development is regulated by the deiodinase enzymes. Expression of the inactivating enzyme, 5-deiodinase type 3, reduces T3 levels and expression of the activating enzyme, 5′-deiodinase type 2, increases T3 levels. Premature or delayed exposure to T3 can result in developmental defects. This seems to be important for TH action in sensory development and the brain.

Although many studies of TH action have used cellular, rodent, and human models, key findings have come from amphibian models as well as more recently zebra fish (reviewed Tata[5]). *Xenopus* has THRA and THRB, but there are gene duplications of both. Early models that tracked TH action showed distinct requirements for both key features of metamorphosis, tail resorption and limb development. Zebra fish also contain THRA and THRB and have been very useful developmental models.

Ligands and Analogues

The structural characterization of the ligand binding domain crystal structure in 1995[57] led to important insights. Distinct from T3 binding to the serum protein, TTR, which did not produce any conformational change in the protein, the prediction from the crystal structure of the ligand bound TRα was that the central hydrophobic core would be present in the absence of ligand, and the addition of T3 would induce a conformational change. Key residues in the ligand binding pocket also matched mutations associated with RTH, which included mutations of residues with direct contact with ligand as well those that disrupt stabilization of the bound confirmation.

Characterization of the THR ligand binding pocket also led to the design of compounds that were more selective for activation of TRβ or TRα. Most of the initial focus was on compounds that favored TRβ binding, with the therapeutic intent of targeting the beneficial metabolic actions of TH, weight loss, and lowering of cholesterol, without the adverse actions on the bone, heart, and skeletal muscle. GC1, or sobetirome, was among the earliest with a favorable profile of metabolic actions.[58] A broad range of compounds were identified with significant success, especially cholesterol lowering in statin-refractory patients; although the effects of these compounds on the bone and cartilage in animal toxicity testing have limited widespread use, there are a range of clinical applications being investigated.[59]

Newer Findings in TH Action

The thyroid nuclear receptor is the fundamental pathway of TH action, but the impact of DNA response element sequence and location, coactivator and corepressor interactions, relative THR isoform expression, TH transport, and local ligand availability have all led to a recognition of factors that modulate TH signaling, especially at the local tissue level. An expanded description of modes of TH action[60] (Table 3; Fig. 2C) is more sophisticated mechanistically but recognizes a very similar range of actions as described in TH action studies from the 1970s (Table 2; Fig. 2A). Recognition of the wide range of TH action is especially relevant in consideration of thyroid replacement therapies.[61]

Noncanonical TH signaling pathways include TRα and TRβ interfacing in the cytoplasm with signaling pathways, such as PI3 kinase, and these actions are blocked by THR mutations in regions outside the ligand binding domain.[62] Direct tags of THR genes in rodent models showed that, in comparison to the original mRNA studies, TRα is the predominant isoform in most tissues, except the liver and pituitary, even in those tissues where THRA and THRB mRNA levels were similar.[63] Whole genome approaches have confirmed the core DNA response direct repeat consensus element sequence for the majority of TH signaling but also indicates TH signaling, which does not require direct DNA binding, especially for TH-mediated negative regulation.[64]

The dogma of a direct ligand-induced switch from corepressor to coactivator is challenged by a study in the liver, showing that there is a ligand-induced ''shift,'' a predominance of coactivator or corepressor, rather than a complete ''switch.''[64] THR resides predominantly in the nucleus, but cytoplasmic to nuclear TH transport may regulate some TH signaling.[65] THR undergoes post-translational modification by phosphorylation[66] and sumoylation,[67] which also impacts signaling and may respond to nutrient signals and signal the fed or fasted state. Nongenomic actions of TH, including direct histone modifications as well as membrane, mitochondria, and other targets, are being increasingly described and documented.[6]

Clinical Applications of TH Action, Present and Future

TH has significant actions on stem cell differentiation and has been shown to stimulate stem cells in white and brown adipose tissue, pancreatic islet cells, skeletal muscle satellite cells, hepatocytes, and intestinal epithelial cells.[68] TRα has a specific role in skeletal muscle stem cells (satellite cells) essential for skeletal muscle development and regeneration after injury.[69] TH promotes recovery after brain injury as well as use in a range of neurological diseases.[70] TH is essential for bone development, and differential expression of THR isoforms underlies the clinical manifestations of reduced and excess TH.[71]

TH action on the heart is central to the clinical manifestations of thyroid excess and deficiency and has also been a

Table 3. Updated Nomenclature for Modes of Triiodothyronine Action

T3 action type	*Examples of mechanism*
Type 1: THR-dependent signaling of TH with direct binding to DNA	• THR monomer or homodimer binding to response elements • THR binding to enhancer elements in which half-sites are organized as an everted repeat with a six-nucleotide spacer or an inverted repeat without a spacer • THR binding with other heterodimer partners such as RAR
Type 2: THR-dependent signaling of TH with indirect binding to DNA	• TRs can interact with a number of chromatin-associated proteins, which can tether THRs to specific genomic locations, even if THRs do not directly contact DNA
Type 3: THR-dependent signaling of TH without DNA binding	• Cytoplasmic THRs can interact with kinases normally found at the plasma membrane, activate them, and serve as a substrate for phosphorylation
Type 4: THR-independent TH signaling	• TH can act without binding to THR. Integrin $\alpha V\beta 3$ may serve as a membrane receptor of T4 and T3, as has been shown in the context of cancer. • TH can also influence actin *in vitro* polymerization

Adapted from Flamant et al.[60]
RAR, retinoic acid receptor; T3, triiodothyronine; T4, thyroxine; THR, thyroid hormone receptor.

site with demonstration of the full range of genomic and nongenomic actions of TH.[72] Few agents are available to treat nonalcoholic fatty liver disease, but several clinical trials of T3, T4, as well as TH analogues, such as resmetirom, specifically acting or activated in the liver, show significant promise for treatment.[73] The liver-selective actions of resmetriom, a TRβ-selective analogue, are accomplished both by cell-specific transport and by TR isoform preference.[74]

Author's Contributions

G.A.B. prepared this invited review and is solely responsible for all writing and editing and approval of the submitted version.

Author Disclosure Statement

G.A.B. confirms that there is no conflict of interest in relation to this work.

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References

1. Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev 2014;94(2):355–382; doi: 10.1152/physrev.00030.2013
2. Magnus-Levy A. Energy metabolism in health and disease. J Hist Med Allied Sci 1947;2(3):307–320; doi: 10.1093/jhmas/ii.3.307
3. Sterling K. Thyroid hormone action at the cell level (first of two parts). N Engl J Med 1979;300(3):117–123; doi: 10.1056/NEJM197901183000304
4. Sterling K. Thyroid hormone action at the cell level (second of two parts). N Engl J Med 1979;300(4):173–177; doi: 10.1056/NEJM197901253000405
5. Tata JR. Amphibian metamorphosis as a model for the developmental actions of thyroid hormone. Mol Cell Endocrinol 2006;246(1–2):10–20; doi: 10.1016/j.mce.2005.11.024
6. Cheng SY, Leonard JL, Davis PJ. Molecular aspects of thyroid hormone actions. Endocr Rev 2010;31(2):139–170; doi: 10.1210/er.2009-0007
7. Brent GA. Mechanisms of thyroid hormone action. J Clin Invest 2012;122:3035–3043.
8. Mendoza A, Hollenberg AN. New insights into thyroid hormone action. Pharmacol Ther 2017;173:135–145; doi: 10.1016/j.pharmthera.2017.02.012
9. Brent GA. The molecular basis of thyroid hormone action. N Engl J Med 1994;331(13):847–853; doi: 10.1056/NEJM199409293311306
10. O'Malley BW. Mechanisms of action of steroid hormones. N Engl J Med 1971;284(7):370–377; doi: 10.1056/NEJM197102182840710
11. Pappa T, Ferrara AM, Refetoff S. Inherited defects of thyroxine-binding proteins. Best Pract Res Clin Endocrinol Metab 2015;29(5):735–747; doi: 10.1016/j.beem.2015.09.002
12. Tata JR. Inhibition of the biological action of thyroid hormones by actinomycin D and puromycin. Nature 1963; 197:1167–1168; doi: 10.1038/1971167a0
13. Tata JR. The road to nuclear receptors of thyroid hormone. Biochim Biophys Acta 2013;1830(7):3860–366; doi: 10.1016/j.bbagen.2012.02.017
14. Samuels HH, Tsai JS. Thyroid hormone action in cell culture: Demonstration of nuclear receptors in intact cells and isolated nuclei. Proc Natl Acad Sci U S A 1973;70(12): 3488–3492; doi: 10.1073/pnas.70.12.3488
15. Oppenheimer JH. Initiation of thyroid hormone action. N Engl J Med 1975;292(20):1063–1068; doi: 10.1056/Nejm197505152922006
16. Edelman IS, Ismail-Beigi F. Thyroid thermogenesis and active sodium transport. Recent Prog Horm Res 1974;30(0):235–257.
17. Sap J, Munoz A, Damm K, et al. The c-erb-A protein is a high-affinity receptor for thyroid hormone. Nature 1986; 324(6098):635–640; doi: 10.1038/324635a0
18. Weinberger C, Thompson CC, Ong ES, et al. The c-erb-A gene encodes a thyroid hormone receptor. Nature 1986; 324(6098):641–646; doi: 10.1038/324641a0
19. Evans RM. The steroid and thyroid hormone receptor superfamily. Science 1988;240(4854):889–895; doi: 10.1126/science.3283939
20. Refetoff S, Dumitrescu AM. Syndromes of reduced sensitivity to thyroid hormone: Genetic defects in hormone receptors, cell transporters and deiodination. Best Pract Res Clin Endocrinol Metab 2007;21(2):277–305; doi: 10.1016/j.beem.2007.03.005
21. Groeneweg S, Visser WE, Visser TJ. Disorder of thyroid hormone transport into the tissues. Best Pract Res Clin Endocrinol Metab 2017;31(2):241–253; doi: 10.1016/j.beem.2017.05.001
22. Giguere V, Yang N, Segui P, et al. Identification of a new class of steroid hormone receptors. Nature 1988;331(6151): 91–94; doi: 10.1038/331091a0
23. Mangelsdorf DJ, Thummel C, Beato M, et al. The nuclear receptor superfamily: The second decade. Cell 1995;83(6): 835–839.
24. Shulman AI, Mangelsdorf DJ. Retinoid x receptor heterodimers in the metabolic syndrome. N Engl J Med 2005; 353(6):604–615; doi: 10.1056/NEJMra043590
25. Koenig RJ, Brent GA, Warne RL, et al. Thyroid hormone receptor binds to a site in the rat growth hormone promoter required for induction by thyroid hormone. Proc Natl Acad Sci U S A 1987;84(16):5670–5674.
26. Glass CK, Franco R, Weinberger C, et al. A c-erb-A binding site in rat growth hormone gene mediates transactivation by thyroid hormone. Nature 1987;329(6141): 738–741; doi: 10.1038/329738a0
27. Brent GA, Larsen PR, Harney JW, et al. Functional characterization of the rat growth hormone promoter elements required for induction by thyroid hormone with and without a co-transfected beta type thyroid hormone receptor. J Biol Chem 1989;264(1):178–182.
28. Umesono K, Giguere V, Glass CK, et al. Retinoic acid and thyroid hormone induce gene expression through a common responsive element. Nature 1988;336(6196):262–265; doi: 10.1038/336262a0
29. Yen PM, Darling DS, Carter RL, et al. Triiodothyronine (T3) decreases binding to DNA by T3-receptor homodimers but not receptor-auxiliary protein heterodimers. J Biol Chem 1992;267(6):3565–3568
30. Hallenbeck PL, Marks MS, Lippoldt RE, et al. Heterodimerization of thyroid hormone (TH) receptor with H-2RIIBP (RXR beta) enhances DNA binding and TH-dependent transcriptional activation. Proc Natl Acad Sci U S A 1992;89(12): 5572–5576; doi: 10.1073/pnas.89.12.5572
31. Marks MS, Hallenbeck PL, Nagata T, et al. H-2RIIBP (RXR beta) heterodimerization provides a mechanism for combi-

natorial diversity in the regulation of retinoic acid and thyroid hormone responsive genes. EMBO J 1992;11(4):1419–1435; doi: 10.1002/j.1460-2075.1992.tb05187.x
32. Koenig RJ, Lazar MA, Hodin RA, et al. Inhibition of thyroid hormone action by a non-hormone binding c-erbA protein generated by alternative mRNA splicing. Nature 1989;337(6208):659–661; doi: 10.1038/337659a0
33. Forrest D, Hanebuth E, Smeyne RJ, et al. Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor beta: Evidence for tissue-specific modulation of receptor function. EMBO J 1996;15(12):3006–3015.
34. Forrest D, Erway LC, Ng L, et al. Thyroid hormone receptor beta is essential for development of auditory function. Nat Genet 1996;13(3):354–357; doi: 10.1038/ng0796-354
35. Cheng SY. Isoform-dependent actions of thyroid hormone nuclear receptors: Lessons from knockin mutant mice. Steroids 2005;70(5–7):450–454; doi: 10.1016/j.steroids.2005.02.003
36. Bochukova E, Schoenmakers N, Agostini M, et al. A mutation in the thyroid hormone receptor alpha gene. N Engl J Med 2012;366(3):243–249; doi: 10.1056/NEJMoa1110296
37. van Mullem A, van Heerebeek R, Chrysis D, et al. Clinical phenotype and mutant TRalpha1. N Engl J Med 2012;366(15):1451–1453; doi: 10.1056/NEJMc1113940
38. Di Cicco E, Moran C, Visser WE, et al. Germ line mutations in the thyroid hormone receptor alpha gene predispose to cutaneous tags and melanocytic nevi. Thyroid 2021;31(7):1114–1126; doi: 10.1089/thy.2020.0391
39. Lazar MA, Hodin RA, Darling DS, et al. A novel member of the thyroid/steroid hormone receptor family is encoded by the opposite strand of the rat c-erbA alpha transcriptional unit. Mol Cell Biol 1989;9(3):1128–1136; doi: 10.1128/mcb.9.3.1128-1136.1989
40. Yin L, Wu N, Curtin JC, et al. Rev-erbalpha, a heme sensor that coordinates metabolic and circadian pathways. Science 2007;318(5857):1786–1789; doi: 10.1126/science.1150179
41. Brent GA, Dunn MK, Harney JW, et al. Thyroid hormone aporeceptor represses T3-inducible promoters and blocks activity of the retinoic acid receptor. New Biol 1989;1(3):329–336.
42. Astapova I, Hollenberg AN. The in vivo role of nuclear receptor corepressors in thyroid hormone action. Biochim Biophys Acta 2013;1830(7):3876–3881; doi: 10.1016/j.bbagen.2012.07.001
43. Gothe S, Wang Z, Ng L, et al. Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. Genes Dev 1999;13(10):1329–1341.
44. Morte B, Manzano J, Scanlan T, et al. Deletion of the thyroid hormone receptor alpha 1 prevents the structural alterations of the cerebellum induced by hypothyroidism. Proc Natl Acad Sci U S A 2002;99(6):3985–3989; doi: 10.1073/pnas.062413299
45. Astapova I, Lee LJ, Morales C, et al. The nuclear corepressor, NCoR, regulates thyroid hormone action in vivo. Proc Natl Acad Sci U S A 2008;105(49):19544–19549; doi: 10.1073/pnas.0804604105
46. Refetoff S, Bassett JH, Beck-Peccoz P, et al. Classification and proposed nomenclature for inherited defects of thyroid hormone action, cell transport, and metabolism. J Clin Endocrinol Metab 2014;99(3):768–770; doi: 10.1210/jc.2013-3393
47. Refetoff S, DeWind LT, DeGroot LJ. Familial syndrome combining deaf-mutism, stuppled epiphyses, goiter and abnormally high PBI: Possible target organ refractoriness to thyroid hormone. J Clin Endocrinol Metab 1967;27(2):279–294.
48. Usala SJ, Bale AE, Gesundheit N, et al. Tight linkage between the syndrome of generalized thyroid hormone resistance and the human c-erbA beta gene. Mol Endocrinol 1988;2(12):1217–1220; doi: 10.1210/mend-2-12-1217
49. Sakurai A, Takeda K, Ain K, et al. Generalized resistance to thyroid hormone associated with a mutation in the ligand-binding domain of the human thyroid hormone receptor beta. Proc Natl Acad Sci U S A 1989;86(22):8977–8981.
50. Usala SJ, Tennyson GE, Bale AE, et al. A base mutation of the C-erbA beta thyroid hormone receptor in a kindred with generalized thyroid hormone resistance. Molecular heterogeneity in two other kindreds. J Clin Invest 1990;85(1):93–100; doi: 10.1172/JCI114438
51. Fozzatti L, Lu C, Kim DW, et al. Resistance to thyroid hormone is modulated in vivo by the nuclear receptor corepressor (NCOR1). Proc Natl Acad Sci U S A 2011;108(42):17462–17467; doi: 10.1073/pnas.1107474108
52. Friesema EC, Ganguly S, Abdalla A, et al. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. J Biol Chem 2003;278(41):40128–40135; doi: 10.1074/jbc.M300909200
53. Friesema EC, Grueters A, Biebermann H, et al. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. Lancet 2004;364(9443):1435–1437; doi: 10.1016/S0140-6736(04)17226-7
54. Dumitrescu AM, Liao XH, Best TB, et al. A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. Am J Hum Genet 2004;74(1):168–175; doi: 10.1086/380999
55. Stromme P, Groeneweg S, Lima de Souza EC, et al. Mutated thyroid hormone transporter OATP1C1 associates with severe brain hypometabolism and juvenile neurodegeneration. Thyroid 2018;28(11):1406–1415; doi: 10.1089/thy.2018.0595
56. Ng L, Kelley MW, Forrest D. Making sense with thyroid hormone—The role of T(3) in auditory development. Nat Rev Endocrinol 2013;9(5):296–307; doi: 10.1038/nrendo.2013.58
57. Wagner RL, Apriletti JW, McGrath ME, et al. A structural role for hormone in the thyroid hormone receptor. Nature 1995;378(6558):690–697; doi: 10.1038/378690a0
58. Trost SU, Swanson E, Gloss B, et al. The thyroid hormone receptor-beta-selective agonist GC-1 differentially affects plasma lipids and cardiac activity. Endocrinology 2000;141(9):3057–3064.
59. Baxter JD, Webb P. Thyroid hormone mimetics: Potential applications in atherosclerosis, obesity and type 2 diabetes. Nat Rev Drug Discov 2009;8(4):308–320; doi: 10.1038/nrd2830
60. Flamant F, Cheng SY, Hollenberg AN, et al. Thyroid hormone signaling pathways: Time for a more precise nomenclature. Endocrinology 2017;158(7):2052–2057; doi: 10.1210/en.2017-00250
61. Jonklaas J, Bianco AC, Cappola AR, et al. Evidence-based use of levothyroxine/liothyronine combinations in treating hypothyroidism: A consensus document. Thyroid 2021;31(2):156–182; doi: 10.1089/thy.2020.0720
62. Hones GS, Geist D, Moeller LC. Noncanonical action of thyroid hormone receptors alpha and beta. Exp Clin En-

docrinol Diabetes 2020;128(6–07):383–387; doi: 10.1055/a-1088-1187
63. Minakhina S, Bansal S, Zhang A, et al. A direct comparison of thyroid hormone receptor protein levels in mice provides unexpected insights into thyroid hormone action. Thyroid 2020;30(8):1193–1204; doi: 10.1089/thy.2019.0763
64. Shabtai Y, Nagaraj NK, Batmanov K, et al. A coregulator shift, rather than the canonical switch, underlies thyroid hormone action in the liver. Genes Dev 2021;35(5–6):367–378; doi: 10.1101/gad.345686.120
65. Allison LA. Getting there: Thyroid hormone receptor intracellular trafficking. J Biol Chem 2021;296:100677; doi: 10.1016/j.jbc.2021.100677
66. Minakhina S, De Oliveira V, Kim SY, et al. Thyroid hormone receptor phosphorylation regulates acute fasting-induced suppression of the hypothalamic-pituitary-thyroid axis. Proc Natl Acad Sci U S A 2021;118(39):e2107943118; doi: 10.1073/pnas.2107943118
67. Ke S, Liu YY, Karthikraj R, et al. Thyroid hormone receptor beta sumoylation is required for thyrotropin regulation and thyroid hormone production. JCI Insight 2021;6(16):e149425; doi: 10.1172/jci.insight.149425
68. Pascual A, Aranda A. Thyroid hormone receptors, cell growth and differentiation. Biochimica et biophysica acta 2013;1830(7):3908–3916; doi: 10.1016/j.bbagen.2012.03.012
69. Milanesi A, Lee JW, Kim NH, et al. Thyroid hormone receptor alpha plays an essential role in male skeletal muscle myoblast proliferation, differentiation, and response to injury. Endocrinology 2016;157(1):4–15; doi: 10.1210/en.2015-1443
70. Liu YY, Brent GA. The role of thyroid hormone in neuronal protection. Compr Physiol 2021;11(3):2075–2095; doi: 10.1002/cphy.c200019
71. Williams GR, Bassett JHD. Thyroid diseases and bone health. J Endocrinol Invest 2018;41(1):99–109; doi: 10.1007/s40618-017-0753-4
72. Cappola AR, Desai AS, Medici M, et al. Thyroid and cardiovascular disease: Research agenda for enhancing knowledge, prevention, and treatment. Thyroid 2019;29(6):760–777; doi: 10.1089/thy.2018.0416
73. Sinha RA, Singh BK, Yen PM. Direct effects of thyroid hormones on hepatic lipid metabolism. Nat Rev Endocrinol 2018;14(5):259–269; doi: 10.1038/nrendo.2018.10
74. Hones GS, Sivakumar RG, Hoppe C, et al. Cell-specific transport and thyroid hormone receptor isoform selectivity account for hepatocyte-targeted thyromimetic action of MGL-3196. Int J Mol Sci 2022;23(22):13714; doi: 10.3390/ijms232213714

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Progress in Thyroid Cancer Genomics: A 40-Year Journey

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Background: Very little was known about the molecular pathogenesis of thyroid cancer until the late 1980s. As part of the Centennial celebration of the American Thyroid Association, we review the historical discoveries that contributed to our current understanding of the genetic underpinnings of thyroid cancer.
Summary: The pace of discovery was heavily dependent on scientific breakthroughs in nucleic acid sequencing technology, cancer biology, thyroid development, thyroid cell signaling, and growth regulation. Accordingly, we attempt to link the primary observations on thyroid cancer molecular genetics with the methodological and scientific advances that made them possible.
Conclusions: The major genetic drivers of the common forms of thyroid cancer are now quite well established and contribute to a significant extent to how we diagnose and treat the disease. However, many challenges remain. Future work will need to unravel the complexity of thyroid cancer ecosystems, which is likely to be a major determinant of their biological behavior and on how they respond to therapy.

Keywords: thyroid cancer, genetics, oncogenes, tumor suppressors, next-generation sequencing, TCGA

Introduction

The objective of this review was to chart the history of the major discoveries leading to our current understanding of the genomic evolution of thyroid cancer and to place this information in the context of the mechanistic and technological breakthroughs that made these discoveries possible. Due to the limitations of the format of the article, we are unable to credit the work of all investigators who contributed to this field, and for this, we apologize to those who have been overlooked.

Clonal Composition of Thyroid Neoplasms

Although it may seem self-evident to current readers, until the late 1980s, it was not clear whether all thyroid tumors were clonal and thus derived from a single cell transformed through a mutation that conferred it with a fitness advantage. This was certainly the case for nodules arising within multinodular goiters (MNG) that were considered at the time to be mostly hyperplastic, and therefore at lower risk for malignancy. The technology that allowed this to be clarified arose from work by Philip Fialkow in hematological malignancies. Dr. Fialkow was a geneticist who showed that leukemias were clonal in women who were heterozygous for a variant in the X chromosome-linked *G6PD* gene, which resulted in G6PD proteins with distinct electrophoretic mobility (1).

Since one of the two X chromosomes is randomly inactivated in cells through DNA methylation (2), normal tissues in women heterozygous for the G6PD variant would contain a mixture of cells randomly expressing either the paternal- or maternal-derived copy of the gene, resulting in both G6PD proteins to be present in equal proportion in tissue extracts, whereas if a tumor had arisen from a single cell, all tumor cells would produce the same G6PD protein. Vogelstein refined the power of this approach by using restriction DNA enzymes that differentially digested X chromosome alleles based on their methylation status, thus enabling distinction of polyclonal from clonal cell populations by Southern blotting of DNA (3).

With this technology, several groups showed that thyroid adenomas and carcinomas were clonal and that clonal tumors also arose with some frequency within MNG (4–6). This strategy to investigate clonality is confounded in the thyroid

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context by evidence that normal thyroid epithelium is organized into large stem-cell derived monoclonal patches, such that a variable subset of normal thyroid tissue extracts showed monoclonal patterns by X-chromosome inactivation analysis (6–8), requiring tumors from these patients to be excluded from the analysis.

Discovery of Thyroid Cancer Genes Through the NIH-3T3 Cell Focus Assay

The search for genes involved in thyroid cancer pathogenesis initially relied on a method that leveraged the ability of tumor viruses to transform mouse fibroblast cell lines *in vitro* (9). Since many human oncogenes are structurally related to these viruses, a method was devised to identify human cancer genes based on the ability of shredded human tumor DNA to transform mouse NIH-3T3 cells, through sequential rounds of DNA isolation of transformants containing human DNA sequences and re-transfection into NIH-3T3 cells (10). Transformed fibroblast cells could be identified and isolated under the microscope because they were no longer inhibited by cell contact and tended to grow on top of each other and form little nodules on the culture dish.

Using this approach, Takahashi isolated human DNA encoding the C-terminal region of REcombined during Transfection (RET), which encompasses its kinase domain, from NIH-3T3 cells transfected with human lymphoma DNA, which was fused to an unrelated human DNA fragment. Since the *RET* rearrangement was not present in normal cells or in the original lymphoma DNA, he concluded that the two genes had REcombined during Transfection (hence the term RET) (11). In 1989, two Italian groups identified *bona fide* rearrangements of *RET* in papillary thyroid cancers (PTCs) using the NIH-3T3 cell focus assay (12,13) and also identified *NTRK1* fusions as PTC oncogenes (13). The groups of Suarez and Lemoine also employed the NIH-3T3 cell focus assay to identify *RAS* mutations in thyroid cancer (14,15). Hence, three common drivers of differentiated thyroid cancers were first identified through this screening method.

The Mostly Futile Search for Copy Number Alterations in PTC

The search for tumor suppressor genes was primarily performed by screening genomic DNA for regions of loss of heterozygosity (LOH), based on the evidence that genetic lesions of both copies of these genes were required to inactivate their function, one of which often took place through copy number loss (16). By using polymorphic DNA markers, regions of recurrent copy number loss could then point out to the location of a tumor suppressor, as performed, for instance, for the identification of *RB1* in retinoblastoma (17), and *APC*, *TP53*, and *DCC* in colorectal cancers (18). This approach was not revealing in the early studies of thyroid cancer, mostly because they primarily focused on small series of PTC, a thyroid cancer type that we now know to be diploid in about 75% of cases, and associated with infrequent recurrent copy number abnormalities (19).

Search for Genetic Drivers Based on Knowledge of Thyroid Cell Physiology

A prior understanding of the mechanisms of thyrotropin (TSH)-induced cell signaling and growth rendered the key nodes in the TSH receptor (TSHR) signaling pathway as attractive candidate oncoproteins for thyroid neoplasms. Shortly after the Nobel prize winning discovery by Sutherland of cAMP as a ''second messenger'' (20), TSH and other hormones were found to signal through a cAMP-dependent pathway (21). The TSHR complementary DNA (cDNA) was cloned in 1989 and found to be a member of the G protein-coupled receptor family (22). In 1990, activating somatic mutations of *GNAS*, the gene encoding Gsα, a GTPase that signals to activate cAMP generation, were discovered in pituitary somatotroph tumors and autonomously functioning thyroid adenomas (AFTA) (23,24).

Activating mutations of *TSHR* were subsequently found to be the most common driver of AFTA (25,26). Mutations of *GNAS* and *TSHR* are rare in thyroid cancer, consistent with the evidence that AFTA have a low risk of malignant transformation (19,27). Recently, a recurrent hot spot mutation (c.1712A>G; p.Gln571Arg) in the enhancer of zeste homolog 1 (*EZH1*) gene was found in 27% of AFTA in association with *TSHR* or *GNAS* mutations (28). *EZH1* codes for a catalytic subunit of the polycomb complex, which plays important roles in chromatin remodeling. This cooperative event may be important in clonal expansion of these tumors.

Polymerase Chain Reaction-Based Screens for Candidate Genes

Rearrangements of *RET* and *RAS* point mutations were subsequently confirmed by several groups primarily using a candidate gene, polymerase chain reaction (PCR)-based technology (29,30), which, together with Sanger sequencing, greatly accelerated the pace of cancer gene discovery. In the early 1990s, *TP53* mutations as hallmarks of anaplastic thyroid cancer (ATC) were reported by several groups by using targeted PCR-based approaches that surveyed specific hot spot exons of the gene, based on data reported in other cancer types (31–33).

In some studies, the PCR products were sequenced directly, and in others, they underwent a preliminary screening strategy using RNase protection assays, which can identify base substitutions by hybridizing PCR-amplified single-strand DNA to a radiolabeled RNA probe encoding the wild-type sequence. DNA-RNA mismatches are detected by cleavage of the unhybridized single-strand RNA with RNAse 1, which allows triage of samples for Sanger DNA sequencing. These screening approaches could be scaled up to evaluate multiple samples on a single gel, since at the time, Sanger DNA sequencing gels were read manually, which was a slow cumbersome process. *TP53* mutations were the first genetic lesion present in anaplastic but not in differentiated thyroid cancers, which began to delineate events involved in tumor microevolution.

In 1993, a major breakthrough was achieved in the understanding of the genetic basis of familial and sporadic forms of medullary thyroid carcinomas (MTC). A study by Mulligan *et al.* identified germline-activating mutations of the *RET* gene, previously found to be activated by rearrangement in PTC, to be responsible for multiple endocrine neoplasia (MEN) type 2A (MEN2A), a syndrome with MTC as its main clinical manifestation (34).

Specifically, sequencing of *RET*, located within a 480-kb region on chromosome 10q11.2 to which the putative

MEN2A gene had been previously localized by genetic and physical mapping techniques, revealed missense point mutations and small in-frame insertions or deletions in the conserved cysteine residues at the boundary of the RET extracellular and transmembrane domains in the tumor and blood samples from MEN 2A families (34). Soon thereafter, activating mutations in the intracellular tyrosine kinase domain of RET, typically at M918T, were identified in patients with MEN2B and sporadic MTC (35,36). Discovery of *RET* mutations opened a new era for managing patients with familial MTC, enabling early diagnosis and guiding prophylactic thyroid surgeries for the affected family members (37).

In the year 2000, the *PAX8-PPARG* fusion was identified as a common genetic alteration in follicular thyroid carcinomas (FTC), the second most common type of thyroid cancer after PTC (38). The fusion was identified by mapping the genes located at the breakpoints of the recurrent t(2;3)(q13;p25) chromosomal rearrangement, which had been previously detected by cytogenetic analysis in several FTC. Soon thereafter, it became apparent that *PAX8-PPARG* fusions do not co-occur with *RAS* mutations, another very common driver in these tumors, with each of these drivers being responsible for the development of ~40% of FTC (39). This fusion was also found in a proportion of follicular variant PTC and follicular adenomas, providing the evidence that these tumors were biologically related to FTC (40).

2003 was a particularly significant year for thyroid cancer genetics due to the identification of *BRAF* mutations, primarily $BRAF^{V600E}$, as the most common driver event in PTC, as well as in advanced thyroid cancers (41–44). A year earlier, *BRAF* had been characterized as an oncogene in melanomas, colorectal, and ovarian cancers (45). The gene codes for a cytoplasmic serine/threonine kinase that signals along the mitogen-activated protein kinase (MAPK) pathway. The V600E mutation, initially annotated as V599E, was found to lead to the strongest phosphorylation of downstream ERK1/2 compared with the much less common mutations found in the residues of the activation segment of the protein surrounding residue 600 or in the P loop (45). In thyroid cancer, $BRAF^{V600E}$ was found in 29–69% of PTC (41–44), and it quickly became apparent that this mutation was restricted to PTC and poorly differentiated thyroid carcinoma (PDTC) and ATC arising from pre-existing papillary carcinoma (46).

From the first reports, it was evident that *BRAF* mutations did not overlap with *RAS* mutations or *RET/PTC* rearrangements in PTC, establishing that activation of the MAPK pathway by any of its effectors is sufficient for driving the development of PTC (41,44). Subsequently, genetic alterations in different nodes of the MAPK pathway were found to confer tumors with distinct PTC phenotypes. Whereas PTC carrying $BRAF^{V600E}$ or *RET/PTC* fusions typically had prominent papillary growth characteristics of classical type PTC, *RAS*-driven tumors displayed almost exclusively a follicular growth pattern with abundant colloid, characteristic of the follicular variant PTC (47). These discoveries increased the proportion of thyroid cancers with known genetic drivers and established the central role of MAPK activation in thyroid cancer initiation, thus laying the foundation for the development of genetic panels for thyroid cancer diagnosis and oncoprotein-targeted therapies that blossomed during the ensuing decades.

In 2013, two back-to-back articles in *Science* reported recurrent somatic mutations at specific nucleotides in the promoter for *TERT* in patients with melanomas (48,49). These mutations were also present in the germline in a rare familial form of melanoma (48). The *TERT* gene encodes for the telomerase reverse transcriptase, a component of the telomerase complex that prevents telomere shortening during cell division, a key process enabling cell immortalization in patients with cancer. The *TERT* promoter mutations in melanoma generate *de novo* consensus DNA binding sites for the ETS family of transcription factors, which mediate in part the transcriptional program activated by the MAPK signaling pathway, which in melanomas is driven by activating mutations of *BRAF* or *RAS*.

Since these same driver mutations are highly prevalent in thyroid cancers, several groups quickly found that *TERT* promoter mutations were also present in thyroid cancers, primarily in more advanced forms of differentiated thyroid cancer, as well as PDTC and ATC (50–53). To the best of our knowledge, this was the first highly recurrent somatic mutation affecting a non-coding sequence in cancer. Moreover, it was later shown to carry important prognostic implications in patients with differentiated thyroid cancer (54). This has been demonstrated most clearly in the context of coexisting $BRAF^{V600E}$ mutation (55), although *TERT* promoter mutations are also enriched in advanced cancers driven by oncogenic fusions and mutations of *RAS* (56).

Activation of the phosphatidylinositol 3-kinase (PI3K) pathway is also implicated in thyroid carcinogenesis, which occurs mostly via inactivating mutations or deletions of the *PTEN* tumor suppressor gene, which encodes for a dual-specificity phosphatase for phosphatidylinositol (3,4,5)-trisphosphate, as well as activating mutations and copy gains of *PIK3CA*, the catalytic subunit of phosphoinositide 3-kinase, and activating mutations of the phosphatidylinositol (3,4,5)-trisphosphate substrate *AKT1* (57–59). Germline inactivating mutations of *PTEN* predispose to development of MNG, follicular adenomas, and differentiated thyroid cancers (60,61). Somatic mutations of these three genes are enriched in advanced differentiated thyroid cancers, including radioiodine-refractory cancers (58), as well as in PDTC and ATC, where they often coexist with mutations of *BRAF* or *RAS* (56,58,62).

Gene Expression Studies in Differentiated Thyroid Cancer

In addition to the expansion of knowledge on driver alterations occurring in genomic DNA of cancer cells, the broad availability of microarray technologies in the late 1990s and early 2000s enabled exploration of global gene expression changes in the disease. High-density cDNA microarrays allowed quantification of messenger RNA (mRNA) transcripts for each of thousands of genes by hybridizing tumor cDNA with gene-specific oligonucleotide probes spotted on a solid surface. The first studies using cDNA microarrays demonstrated profound changes in gene expression profiles of thyroid cancer compared with normal thyroid tissue and follicular adenomas, with consistent patterns of mRNA expression changes among PTC or FTC analyzed in the same study (63,64). However, further reports demonstrated significant differences in gene expression

profiles between specific histological variants of PTC, such as classical papillary and follicular variants (65,66), and between PTC driven by *BRAF*^{V600E}, *RAS* mutations, and *RET/PTC* fusions (67,68).

In the early 2000s, microRNAs (miRNAs) were recognized as a distinct class of small non-coding RNA molecules affecting post-transcriptional regulation of gene expression in different human diseases including cancer (69). In 2005, He *et al.* provided the first demonstration of upregulation of specific miRNAs in PTC compared with normal thyroid tissue (70). Soon thereafter, upregulation and downregulation of specific miRNAs were found in other types of differentiated and dedifferentiated thyroid cancers (71,72). Similar to mRNA profiles, significant differences in expression of individual miRNA were observed in PTC driven by *BRAF*^{V600E}, *RET/PTC*, and *RAS* mutations (73).

Advent of Next-Generation Sequencing Technologies: The Cancer Genome Atlas Study of PTC

Sanger sequencing technology was the primary method for DNA sequencing for more than 30 years following its original discovery in 1977 (74). Its throughput was markedly improved following the introduction of capillary electrophoresis (CE)-based sequencing instruments in 1987, which became the primary tools used for the completion of the Human Genome Projects (75). However, it was not until the advent of massively parallel next-generation sequencing (NGS) technologies that the field of cancer genomics was truly revolutionized, by enabling large-scale studies of whole exome, whole genome, and RNA sequencing of tumor samples. The Cancer Genome Atlas (TCGA) program, a collaborative effort of the United States National Cancer Institute with the National Human Genome Research institute, was launched in 2006 and played a central role in shepherding landmark comprehensive genomic, epigenomic and transcriptomic analyses of the major types of human cancer using NGS.

TCGA elected to focus on in-depth studies of the most common type of cancer of each main cell lineage, which in the case of thyroid follicular cells was PTC. This large team science effort included a group of disease experts, some of whom unsuccessfully advocated for inclusion of the more aggressive types of thyroid cancer in the analysis because the major drivers of PTC were already known, which was not the case for PDTC and ATC. Ultimately, the decision to focus solely on the most common disease entity in our view proved to be far-sighted, as the homogeneity of the cohort allowed for robust integrative analyses of multidimensional molecular data, a rich trove of information that was published in 2014 and that to this day continues to be mined by the research community to fuel new discoveries (19). TCGA PTCs proved to be mostly diploid, to have a very low mutation burden, and were confirmed to be driven mostly by clonal nonoverlapping mutations of *BRAF*, *RAS*, and fusions of *RET* and *NTRK*.

However, whole exome, and in a smaller subset, whole-genome sequencing identified new thyroid cancer genes, among others *EIF1AX*, *CHEK2* and *PPM1D*, as well as a more expanded repertoire of fusion oncoproteins (19). RAS mutants and BRAF^{V600E} activate the MAPK pathway, but they do so with different intensity. Oncogenic RAS constitutively signals through RAF dimers, which are subject to negative feedback by ERK, resulting in a relative attenuation of the MAPK pathway flux. By contrast, BRAF^{V600E} signals as a monomer and is partially unresponsive to negative feedback by ERK, resulting in a higher MAPK signaling output (76) (Fig. 1). Accordingly, *BRAF*-mutant PTCs are transcriptionally distinct from their *RAS*-mutant counterparts.

The integration of the driver mutations with the transcriptomic data from RNA sequencing allowed the derivation of a transcriptomic score, termed BRAF-RAS score (BRS), which categorized individual tumors based on their gene expression alignment with either a BRAF^{V600E} or an *RAS*-mutant tumor. The BRS associates strongly with PTC pathological features, metastatic tropism, and differentiation state (Fig. 2). BRAF-like PTCs are typically classic or tall cell variants of PTC, more likely to show infiltrative border, metastasize to lymph nodes before spreading to distant sites, and be relatively refractory to radioactive iodine (RAI).

By contrast, RAS-like PTCs are follicular variants, tend to be encapsulated, can invade blood vessels and metastasize hematogenously bypassing regional lymph nodes, and are more RAI-avid. These concepts are now beginning to be incorporated into clinical practice. Other TCGA molecular platforms, such as miRNA sequencing, DNA methylation profiling, and proteomics, were also highly revealing and collectively have resulted in a deeper and more fundamental understanding of the biology of these common cancers.

Genetics of Oncocytic (Hurthle Cell) Tumors

Until recently, the molecular pathogenesis of oncocytic (Hurthle) cell tumors, composed of cells with innumerable abnormal mitochondria, remained poorly understood. Although loss-of-function mitochondrial DNA mutations in complex I genes had been commonly observed in these cells (77,78), most of these tumors lacked the nuclear DNA drivers seen in other types of thyroid cancer. In 2012, Corver *et al.* reported that most oncocytic carcinomas had a highly unusual pattern of chromosomal copy number alterations called genome near-haploidization (79). It involved either the loss of one of the two copies of most chromosomes resulting in monosomy or alternatively uniparental disomy, in which the remaining chromosome was duplicated so that the copies were both either paternal or maternally-derived. Such changes affected multiple chromosomes but spared chromosome 7, which always retained heterozygosity (79).

In 2018, two back-to-back publications provided comprehensive genomic characterization of oncocytic carcinomas using whole exome and whole transcriptome sequencing among other techniques (80,81). They showed that these tumors were characterized by (i) recurrent homoplasmic mutations of mitochondrial genes, primarily those encoding complex I enzymes of the electron transport chain; (ii) widespread chromosomal copy number alterations resulting in genome near-haploidization (which was found in most of these tumors); and (iii) nuclear DNA mutations in a small proportion of these cancers, which included *RAS* mutations, typically seen in diploid tumors, as well as *TERT* and *TP53* mutations, seen in tumors with and without chromosomal copy number alterations (80,81) (Fig. 3).

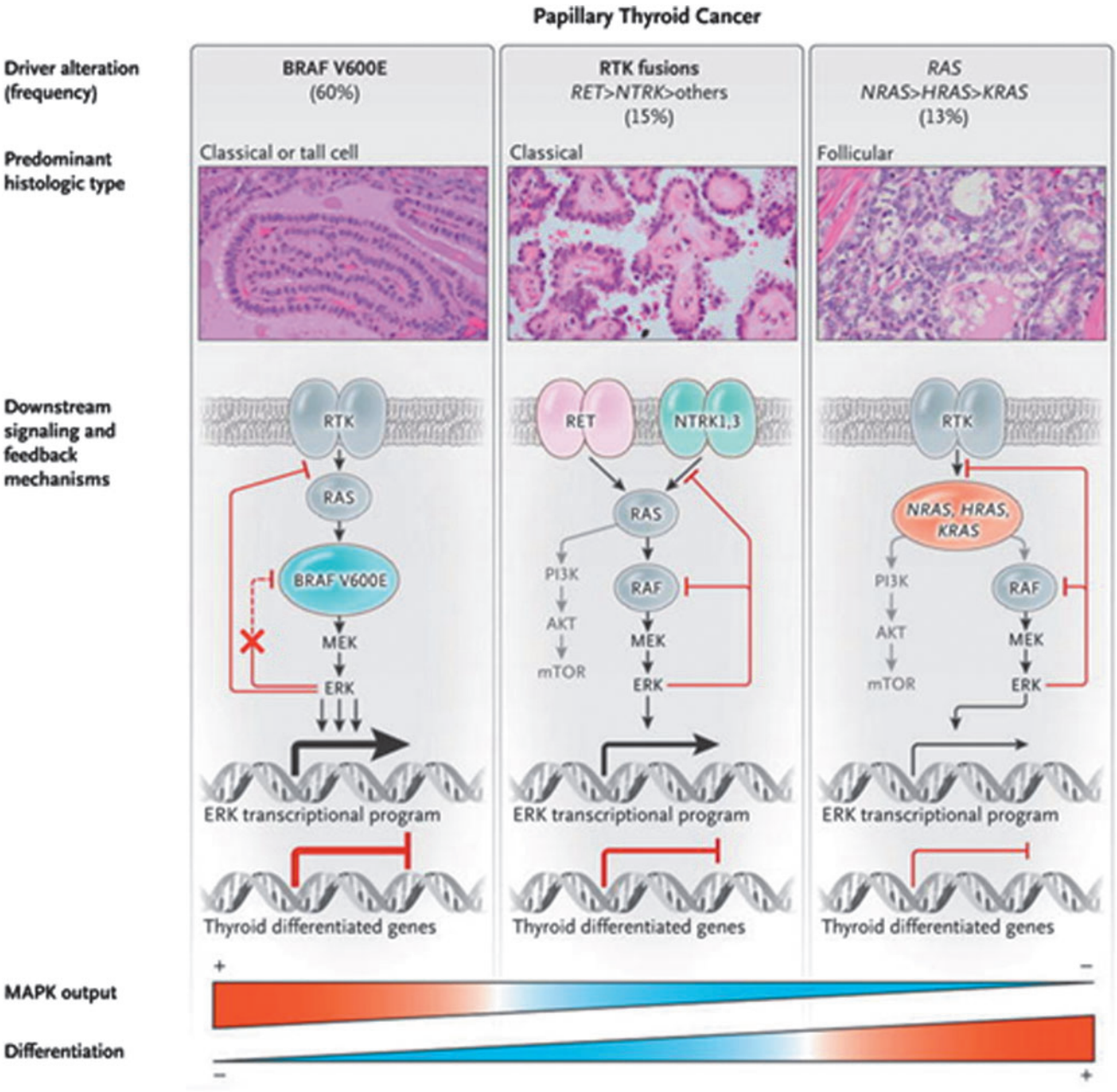

FIG. 1. Functional consequences of driver mutations in PTCs: RTK fusions, RAS mutants, and $BRAF^{V600E}$ activate the MAPK pathway, but they do so with different intensity. RTK fusions such as RET/PTC and ETV6-NTRK3 and mutant RAS proteins signal through RAF dimers, which are subject to negative feedback by ERK, resulting in a constitutively active but dampened flux through the MAPK pathway. By contrast, $BRAF^{V600E}$ signals as a monomer and is partially unresponsive to negative feedback by ERK, resulting in a higher MAPK signaling output. The expression of genes required for thyroid cell differentiation and thyroid hormone biosynthesis is inversely correlated with the transcriptional output of the MAPK pathway. MAPK, mitogen-activated protein kinase; PTC, papillary thyroid cancer; RET, REcombined during Transfection; RTK, receptor tyrosine kinase. From N Engl J Med, Fagin JA, Wells SA Jr., Biological and Clinical Perspectives on Thyroid Cancer, v375:1054–1067. Copyright © (2016) Massachusetts Medical Society. Reprinted with permission.

Genome near-haploidization was subsequently noted not to be restricted to oncocytic carcinomas, as it is present in about one third of oncocytic adenomas, which likely serve as precursor lesions for oncocytic carcinomas (82). The unique genetic mechanisms of oncocytic tumor development provided strong support for reclassification of these tumors as an independent pathologic entity and not, as previously thought, as variants of FTC and adenoma.

Cancer Exome Sequencing Panels Propel Discovery of Genetic Drivers of PDTC and ATC

PDTC and ATC are uncommon types of thyroid cancer, yet they are of paramount interest because of their association with poor clinical outcomes. So far neither TCGA nor other large cancer genome sequencing consortia have focused on advanced thyroid cancers. An early whole-genome

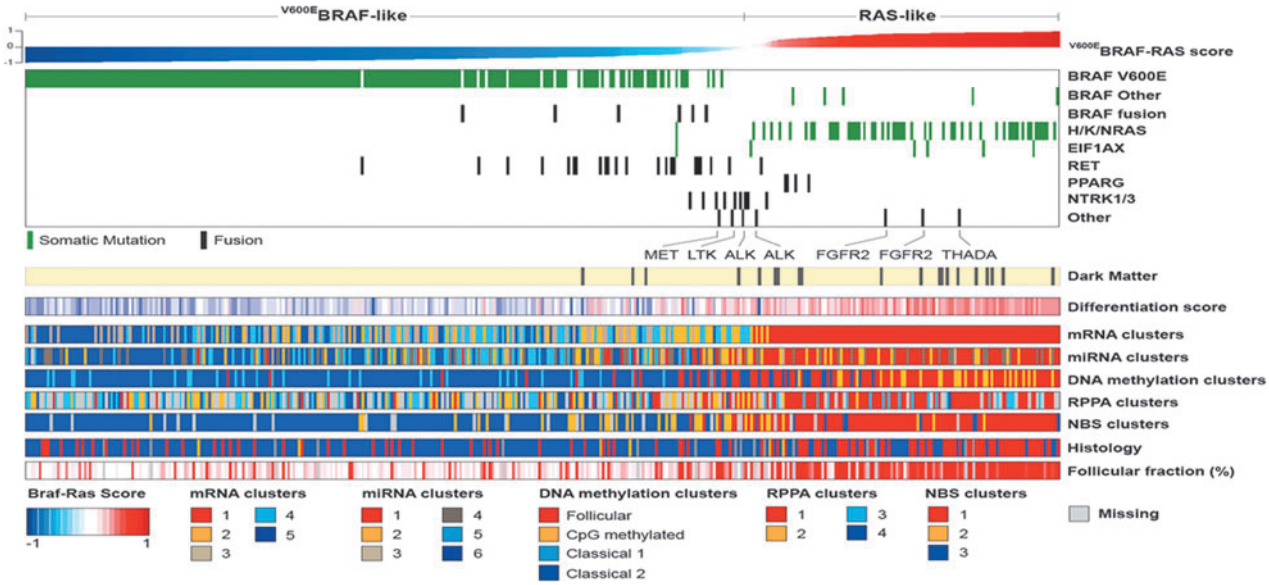

FIG. 2. BRAF-like and RAS-like papillary carcinomas: The transcriptomic, microRNA, and methylome profiles of $BRAF^{V600E}$-mutant and RAS-mutant PTC are distinct. A 71-gene signature, termed the BRS, was generated by TCGA to classify tumors as either BRAF-like or RAS-like. PTCs harboring other drivers can be categorized based on this signature: for example, RET fusions tend to be BRAF-like, whereas NTRK fusions lie somewhere in between. The BRS correlates with the histological characteristics of the tumors, their differentiation state, as well as their metastatic tropism. BRS, BRAF-RAS score; TCGA, The Cancer Genome Atlas. Reprinted with permission from Cell 2014;159:676–690 (ref. 19).

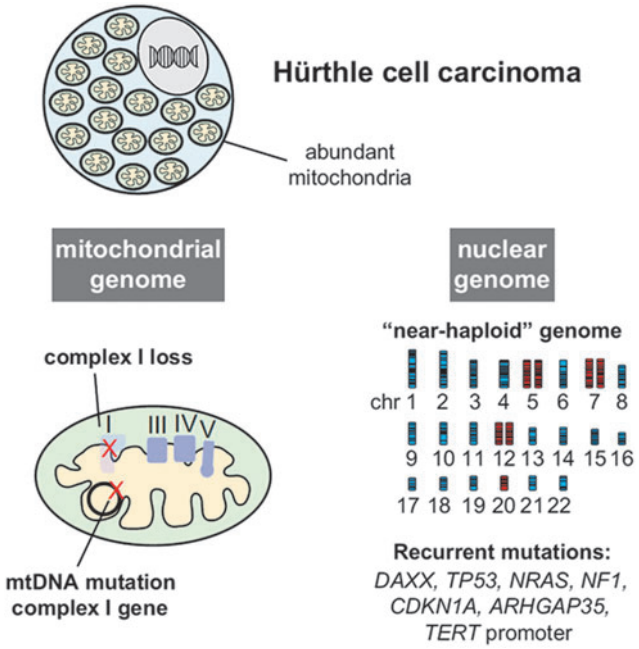

FIG. 3. Genetics of Hurthle cell carcinomas: Hurthle cell (oncocytic) tumors are driven by alterations of the nuclear and mitochondrial genomes. Mutations of mtDNA primarily involve loss-of-function complex I gene mutations. Most of these cancers also develop widespread loss of one of the two chromosome alleles leading to a near-haploid genome. Nuclear DNA mutations are present in a smaller fraction of Hurthle cell cancers, with *TP53* and *TERT* mutations found more commonly in widely invasive cancers. mtDNA, mitochondrial DNA. Reprinted with permission from Cancer Cell 2018;34:242.e5–255.e5 (ref. 81).

sequencing study of 22 ATC identified MAPK pathways drivers as common recurrent events, as well as mutations of *MTOR*, *NF1*, *NF2*, *MLH1*, *MLH3*, *MSH5*, *MSH6*, *ERBB2*, *EIF1AX*, and *USH2A* (83). The era of precision medicine in cancer ushered a need for rapid genomic analysis of patient tumor samples because many mechanism-based treatments targeted specific oncogenic drivers of the disease. Although initially these assays were needed for implementation of clinical trials of these agents, many of these drugs have since become part of the standard of care for cancers of different lineages, including thyroid cancers.

To enable these treatments, cancer centers (84,85) and commercial entities (86) developed NGS panels covering the coding regions of a large number of genes known to be mutated across cancers of different types. Coupled to single institution small series of whole exome studies (87), the cumulative data arising from cancer exome profiling of large numbers of patient tumors have provided a deeper understanding of the genetics of advanced thyroid cancers (56,88). Aside from the role of MAPK pathway drivers across the entire spectrum of the disease, PDTC and ATC have a higher frequency of *TERT* promoter and *TP53* mutations, as well as lesions of genes encoding for effectors in the PI3K/AKT/MTOR pathway and in chromatin remodeling complexes (Fig. 4). PDTC are also enriched for loss-of-function mutations of *RBM10* (89), which encodes for an RNA binding protein that regulates alternative splicing of cassette exons (90).

Whereas mutations of *EIF1AX*, a component of the translation preinitiation complex, are often seen in benign neoplasms and in about 30% of PTC as isolated events (19), in advanced thyroid cancers, they are almost invariably associated with *RAS* and *TERT* mutations and confer patients with decreased disease-specific survival (56,91). Interestingly, many of the mutations found in PDTC and ATC are present as subclonal events in PTC, supporting their role as

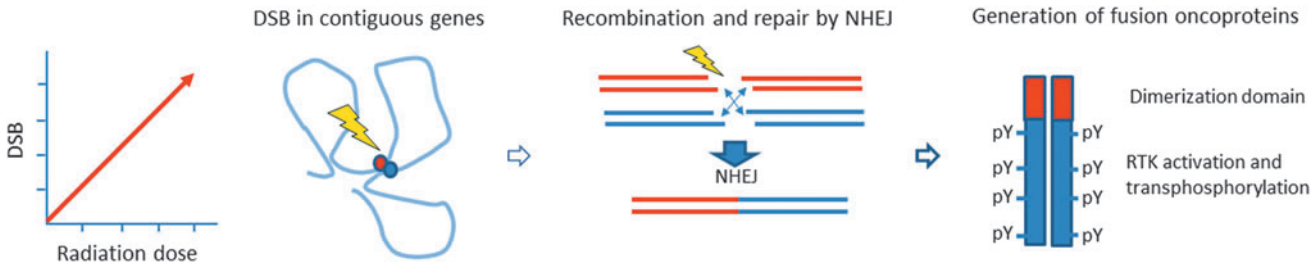

FIG. 4. Mechanisms of radiation-induced thyroid cancer: Gene fusions are genetic hallmarks of thyroid cancer associated with exposure to ionizing radiation. Radiation exposure induces a dose-dependent increase in DNA double-strand breaks, short deletions, and simple/balanced structural variants, but not in single nucleotide variants. The radiation dose-dependent generation of fusion oncogenes is favored by spatial proximity of the participating genes and carries a signature of NHEJ at the fusion points. The most prevalent fusions involve RTK genes, which are commonly activated by recombination with a gene fragment encoding a protein dimerization domain. This drives homodimerization and activation by transphosphorylation of the cytoplasmic kinase domain of the RTK. NHEJ, nonhomologous end joining.

determinants of tumor microevolution (19,56). In addition, PDTC and ATC are associated with copy number abnormalities, including among others chromosome 1q and 20q gain, and 13q loss, which associate with worse clinical outcomes (56). Loss of Chr 22q is one of few recurrent copy number abnormalities in PTC (19), and it is also highly prevalent in PDTC and ATC. PTCs with 22q LOH have a metastatic tropism to bone (92). Interestingly, three thyroid cancer tumor suppressor genes are encoded in 22q: *CHEK2* (19), *SMARCB1* (56,93), and *NF2* (94,95).

Identification of Rare Thyroid Tumor Genes

In recent years, the landscape of genetic drivers of thyroid tumors has continued to fill in. Newly identified driver alterations activating the MAPK pathway include *ROS1* fusions and *MEK1* mutations in PTC (96,97). *GLIS* fusions, typically *PAX8-GLIS3*, have been found to be a genetic hallmark of a rare type of hyalinizing trabecular tumor (HTT), a rare thyroid tumor (98). They are characterized by a trabecular growth, prominent nuclear features characteristic of PTC and pronounced hyalinization, and had been suspected to be a variant of PTC. The identification of *GLIS* fusions in virtually all HTT, but not in PTC, provided the evidence that HTT is a distinct tumor unrelated to PTC.

Mutations in *DICER1* gene coding for an endoribonuclease responsible for processing miRNAs were identified in thyroid cancers arising in children and adults, including those with sporadic tumors or tumors associated with an inherited DICER1 syndrome (99–101). DICER1 somatic missense mutations cluster in metal-ion binding residues in the DICER1 RNAse IIIb domain, whereas germline mutations are typically nonsense mutations scattered along the gene. *DICER1* mutations do not overlap with other common genetic alterations such as *BRAF* or *RAS*, supporting a distinct mechanism of transformation (100,101) Whereas the vast majority of *DICER1*-positive thyroid cancers are low-risk follicular variant PTC or FTC, rare cases of DICER1-driven PDTC occur in children and young adults, some of which develop in the setting of DICER1 syndrome (102). *DICER1* joins the list of genes, which includes *RET*, *PRKR1A*, and *PTEN*, that when mutated in the germline predispose to thyroid neoplasia and that are also mutated in sporadic tumors (103).

Molecular Basis of Radiation-Associated Thyroid Cancer

Exposure to ionizing radiation is the only well-established risk factor for sporadic thyroid cancer. This includes exposure to medical external beam radiation as well as accidental exposures to radioiodine or γ-radiation after a nuclear weapon explosion or nuclear reactor accident (104). The link between radiation exposure and thyroid cancer, mostly PTC, has been known since 1950 after its demonstration in children exposed to therapeutic external beam radiation for benign conditions of the head and neck (105). It was not until the dramatic increase in incidence of thyroid cancer in children and young adults exposed to radiation after the accident at the Chernobyl nuclear power plant in April 1986 (106) that the genetic basis of radiation-induced thyroid cancer was uncovered.

Early candidate gene studies showed that PTC in children exposed to radiation after Chernobyl had a very high prevalence of *RET* fusions, mostly *RET/PTC1* and *RET/PTC3* (107–110). Subsequent studies employing large cohorts of patients with post-Chernobyl PTC confirmed a high prevalence of *RET* and other fusions, such as *NTRK1*, in contrast to a low prevalence of *BRAF* point mutations in these cancers (111,112). The first fusion of *BRAF* in any cancer type (*AKAP9-BRAF*) was discovered in post-Chernobyl cancers, consistent with the association of these tumors with genomic rearrangements (113). Beyond post-Chernobyl cancers, *RET* fusions were also found to be prevalent in thyroid cancers from patients who received therapeutic external beam radiation (114).

RET/PTC1 and *RET/PTC3* fusions could be induced experimentally by irradiating human thyroid tissue grafted into mice and in cultured human thyroid cells (115). Following DNA damage induced by ionizing radiation, *RET* fusions may be facilitated by close spatial proximity of chromosomal regions containing fusion partners in normal human thyroid cells in interphase (116). After the introduction of NGS-based genotyping, the association between gene fusions and radiation exposure was further supported by the high prevalence in Ukrainian children born before Chernobyl, in contrast with a higher frequency of point mutations in children born after the accident living in the same areas (117), as well as the demonstration of radiation dose dependence of gene fusions in children with post-Chernobyl cancer (118).

These findings have been confirmed and expanded in the recent large-scale genomic analysis of >400 post-Chernobyl

thyroid cancers, demonstrating radiation dose-related increases in small clonal deletions and in fusion drivers that bear characteristics of nonhomologous end-joining repair of double-strand DNA breaks (119) (Fig. 5). Radiation dose dependence of these alterations was more pronounced for younger individuals at the time of exposure. The study also found that, similar to sporadic cancers, transcriptomic and epigenomic profiles were strongly associated with driver alterations, although they did not correlate with radiation dose (119). A related study of children born after the Chernobyl accident to parents exposed to radiation reported no significant increase in germline *de novo* mutations, suggesting minimal effect of the parents' radiation exposure on health of subsequent generations (120).

Genetic Predisposition to Thyroid Cancer

In addition to familial MTC in patients with MEN syndromes, several familial diseases increase the risk of nonmedullary thyroid cancer. The association between familial adenomatous polyposis/Gardner syndrome and thyroid cancer was established in the 1960s, and in 1991, this syndrome was linked to a germline mutation of the *APC* gene (121). Thyroid cancers developing in these patients have distinct microscopic features initially described as cribriform-morular variant PTC (122), recently renamed as cribriform-morular thyroid carcinoma.

Thyroid nodularity and cancer are a common manifestation of the Cowden syndrome, and several other rare syndromes collectively known as the PTEN hamartoma tumor syndrome, which is caused by germline mutations of the *PTEN* tumor suppressor gene (60,123). An increased risk of thyroid cancer has also been found in patients affected by the Carney complex caused by inactivating mutations in the *PRKAR1A* gene (124,125), and by Werner syndrome linked to the *WRN* gene (126). More recently, thyroid nodules and cancer have been found to be a manifestation of DICER1 syndrome caused by inactivating mutations in the *DICER1* gene, which is essential for maturation of miRNAs (127,128).

Translation of Genomics into Clinical Practice: Molecular Diagnostics of Thyroid Nodules

The information on genomic alterations in thyroid cancer gained over the past four decades has been translated into clinical practice for patients with thyroid nodules and cancer. Fine-needle aspiration (FNA) cytology of sonographically suspicious nodules provides a reliable diagnosis of benign or malignant nodule in ~80% of cases (129). In the remaining 20%, the cytology is indeterminate (130). In the premolecular era, most nodules with indeterminate cytology would prompt diagnostic lobectomy, which yielded cancer in only about one third of cases. The ability to detect gene mutations in thyroid FNA samples was demonstrated in the

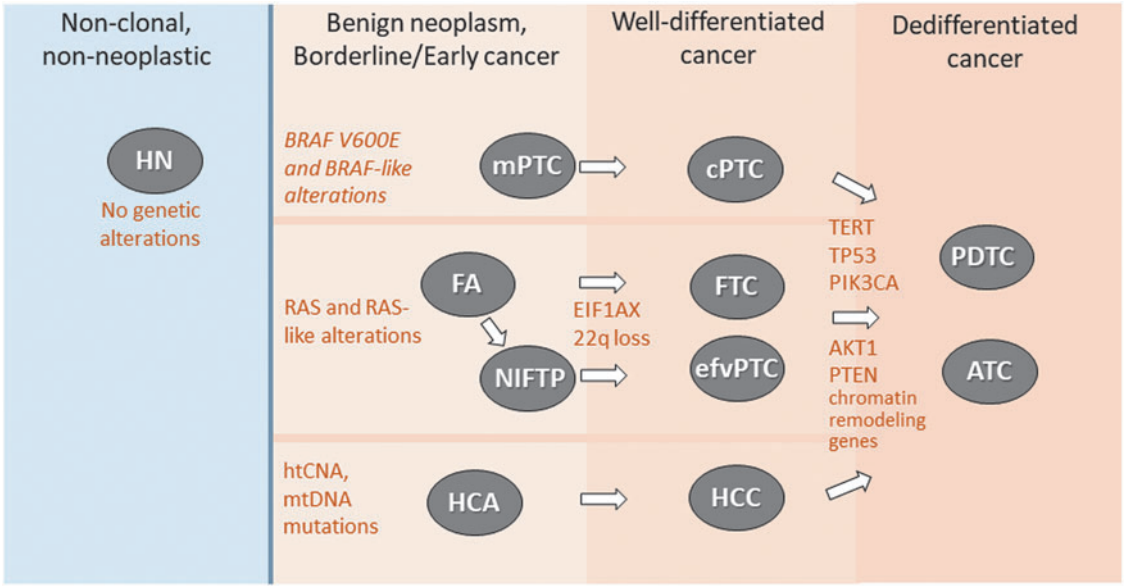

FIG. 5. Molecular classification of thyroid nodules. Most thyroid nodules are HN that carry no clonal genetic alterations. Follicular cell-derived thyroid tumors develop via three distinct molecular pathways initiated by $BRAF^{V600E}$-like alterations, RAS-like alterations, or mtDNA mutations and chromosomal copy number abnormalities leading to genome haploidiation-type copy number alterations (htCNA). RAS-like encapsulated follicular-patterned tumors, including FTC, invasive efvPTC, and htCNA/mtRNA-driven encapsulated HCC likely develop from benign/preinvasive precursors: that is, FA/NIFTP and HCA, respectively. BRAF-like cPTC lack a benign precursor and develop by growth of a micropapillary thyroid cancer (mPTC). Progression of RAS-like tumors to well-differentiated cancer frequently involves *EIF1AX* mutations and/or 22q loss, whereas conversion of cancers initiated by all three molecular pathways to PDTC and ATC involve the accumulation of additional mutations in *TERT*, *TP53*, PI3K pathway mutations, and alterations of chromatin remodeling genes. ATC, anaplastic thyroid cancer; cPTC, classic papillary thyroid carcinomas; efvPTC, encapsulated follicular variant papillary thyroid carcinoma; FA, follicular adenoma; FTC, follicular thyroid carcinoma; HCA, Hurthle cell adenoma; HCC, Hurthle cell (oncocytic) cancer; HN, hyperplastic nodules; htCNA, genome haploidiation-type copy number alterations; mPTC, micropapillary thyroid cancer; mtRNA, mitochondrial RNA; NIFTP, noninvasive follicular tumor with papillary-like nuclear features; PDTC, poorly differentiated thyroid carcinoma; PI3K, phosphatidylinositol 3-kinase.

early 1990s (131). However, it took more than a decade for the progress in technology coupled to a more comprehensive understanding of the genetic drivers in thyroid cancers to enable the development of the first-generation genetic tests for diagnosing PTC and FTC in FNA samples.

Initial experience with panels of six to seven genes (*BRAF*, *NRAS*, *HRAS*, *KRAS*, *RET/PTC1*, *RET/PTC3*, *PAX8-PPARG*) analyzed by single-gene PCR-based assays was reported in 2009 and 2010 (132,133). These relatively limited panels had a high specificity but lacked the sensitivity required to avoid diagnostic surgery. In the ensuing years, second-generation molecular diagnostic tests have been introduced into clinical practice, which include larger panels composed of different combinations of DNA, RNA, and miRNA markers (134–138). These tests, offered commercially and broadly available in the United States, had either high sensitivity or high specificity but did not achieve both.

Finally, over the last 5 years, most comprehensive DNA/RNA or RNA/NGS-based tests have been extensively validated to offer high sensitivity and fairly high specificity for detecting follicular cell-derived thyroid cancers as well as MTC and parathyroid nodules that masquerade cytologically as follicular cell-derived nodules (139,140). The introduction of molecular tests has resulted in a significant decrease in the rate of diagnostic surgeries for patients with indeterminate cytology thyroid nodules (141).

Molecular Diagnostics as a Guide for Systemic Therapy of Advanced Metastatic Thyroid Cancers

The first generation of effective systemic therapies for RAI-refractory metastatic differentiated thyroid cancer and MTC is small-molecule multikinase inhibitors such as lenvatinib, sorafenib, and pazopanib that act primarily by inhibiting VEGF receptor activation and inhibiting angiogenesis, regardless of the nature of the oncogenic driver of the disease (142,143). Similarly, the multikinase inhibitors vandetanib and cabozantinib, which do target RET kinase activity, also benefit RET-wild-type MTC patients because of their inhibition of VEGFR (144,145). Hence, when these drugs were the only available treatment option for patients, determination of tumor genotype did not meaningfully contribute to disease management.

The landscape of thyroid cancer therapies has evolved dramatically in the past 5 years, such that tumor genotype is now critical to determine the best treatment option for patients with advanced locally recurrent or metastatic disease (146). The recent approval of the highly selective RET kinase inhibitors selpercatinib and pralsetinib requires tumor genotyping, as they are indicated solely for MTC with *RET* mutations or cancers driven by *RET* fusions. The rationale for tumor genotyping is further buttressed by the fact that thyroid cancers harboring other receptor tyrosine kinase (RTK) fusions, such as of *NTRK3*, *NTRK1* or *ALK*, are candidates for treatment with selective inhibitors of these mutant oncoproteins. The first line of systemic treatment for patients with $BRAF^{V600E}$-mutant ATC is the combination of the RAF kinase inhibitor dabrafenib with the MEK inhibitor trametinib, which has also shown efficacy in the neoadjuvant setting. The recent FDA approval for combined RAF and MEK inhibition now extends to any $BRAF^{V600E}$-mutant cancers, regardless of their origin.

Summary and Future Directions

Differentiated thyroid cancers, MTC, and Hurthle cell (oncocytic) cancer (HCC) are arguably among the best characterized human cancer types with respect to the genetic drivers responsible for their development. This has already had a transformational impact on diagnostics of thyroid nodules and on treatment for the subset of these patients whose tumors recur, become refractory to radioiodine, or develop distant metastatic disease. We have also gained information on the more complex genomic and transcriptomic features of PDTC and ATC. Although the progress has been remarkable, there is still much to learn.

Our understanding of advanced disease is based on cancer exome panels rather than whole exome or whole-genome sequencing, and the studies available so far have not integrated the multidimensional molecular data required to provide a deeper understanding of their biology. There is also scant information on the microevolution of metastatic thyroid cancer. The use of plasma free DNA assays to monitor response to therapy and acquisition of resistance in patients with metastatic thyroid cancer on systemic therapies is still in its infancy. Moreover, the clonal, transcriptional, and compositional heterogeneity of advanced disease poses challenges that will require unraveling mechanisms and pathways that may not necessarily be dependent directly on the genetic drivers of the disease.

Authors' Contributions

J.A.F. and Y.E.N. co-wrote and contributed equally to this review.

Author Disclosure Statement

Y.E.N. owns IP and receive royalties related to ThyroSeq from the University of Pittsburgh and serves as a consultant for Sonic Healthcare USA. J.A.F. reports no disclosures.

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References

1. Fialkow PJ, Jacobson RJ, Papayannopoulou T 1977 Chronic myelocytic leukemia: clonal origin in a stem cell common to the granulocyte, erythrocyte, platelet and monocyte/macrophage. Am J Med **63:**125–130.
2. Lyon MF 1963 Lyonisation of the X chromosome. Lancet **2:**1120–1121.
3. Vogelstein B, Fearon ER, Hamilton SR, Preisinger AC, Willard HF, Michelson AM, Riggs AD, Orkin SH 1987 Clonal analysis using recombinant DNA probes from the X-chromosome. Cancer Res **47:**4806–4813.
4. Namba H, Matsuo K, Fagin JA 1990 Clonal composition of benign and malignant human thyroid tumors. J Clin Invest **86:**120–125.
5. Hicks DG, LiVolsi VA, Neidich JA, Puck JM, Kant JA 1990 Clonal analysis of solitary follicular nodules in the thyroid. Am J Pathol **137:**553–562.
6. Fey MF, Peter HJ, Hinds HL, Zimmermann A, Liechti-Gallati S, Gerber H, Studer H, Tobler A 1992 Clonal

analysis of human tumors with M27 beta, a highly informative polymorphic X chromosomal probe. J Clin Invest **89:**1438–1444.
7. Jovanovic L, Delahunt B, McIver B, Eberhardt NL, Grebe SK 2003 Thyroid gland clonality revisited: the embryonal patch size of the normal human thyroid gland is very large, suggesting X-chromosome inactivation tumor clonality studies of thyroid tumors have to be interpreted with caution. J Clin Endocrinol Metab **88:** 3284–3291.
8. Moniz S, Catarino AL, Marques AR, Cavaco B, Sobrinho L, Leite V 2002 Clonal origin of non-medullary thyroid tumours assessed by non-random X-chromosome inactivation. Eur J Endocrinol **146:**27–33.
9. Jainchill JL, Aaronson SA, Todaro GJ 1969 Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells. J Virol **4:**549–553.
10. Santos E, Pulciani S, Barbacid M 1984 Characterization of a human transforming gene isolated from T24 bladder carcinoma cells. Fed Proc **43:**2280–2286.
11. Takahashi M, Ritz J, Cooper GM 1985 Activation of a novel human transforming gene, ret, by DNA rearrangement. Cell **42:**581–588.
12. Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della PG, Fusco A, Vecchio G 1990 PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell **60:**557–563.
13. Bongarzone I, Pierotti MA, Monzini N, Mondellini P, Manenti G, Donghi R, Pilotti S, Grieco M, Santoro M, Fusco A, Vecchio G, Porta GD 1989 High frequency of activation of tyrosine kinase oncogenes in human papillary thyroid carcinoma. Oncogene **4:**1457–1462.
14. Suarez HG, du Villard JA, Caillou B, Schlumberger M, Tubiana M, Parmentier C, Monier R 1988 Detection of activated ras oncogenes in human thyroid carcinomas. Oncogene **2:**403–406.
15. Lemoine NR, Mayall ES, Wyllie FS, Farr CJ, Hughes D, Padua RA, Thurston V, Williams ED, Wynford-Thomas D 1988 Activated ras oncogenes in human thyroid cancers. Cancer Res **48:**4459–4463.
16. Knudson AG, Jr. 1971 Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A **68:** 820–823.
17. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, Dryja TP 1986 A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature **323:**643–646.
18. Fearon ER, Vogelstein B 1990 A genetic model for colorectal tumorigenesis. [Review] [77 refs]. Cell **61:**759–767.
19. Cancer Genome Atlas Research Network 2014 Integrated genomic characterization of papillary thyroid carcinoma. Cell **159:**676–690.
20. Rall TW, Sutherland EW 1958 Formation of a cyclic adenine ribonucleotide by tissue particles. J Biol Chem **232:**1065–1076.
21. Wilson B, Raghupathy E, Tonoue T, Tong W 1968 TSH-like actions of dibutyryl-cAMP on isolated bovine thyroid cells. Endocrinology **83:**877–884.
22. Parmentier M, Libert F, Maenhaut C, Lefort A, Gerard C, Perret J, Van Sande J, Dumont JE, Vassart G 1989 Molecular cloning of the thyrotropin receptor. Science **246:**1620–1622.
23. Landis CA, Masters SB, Spada A, Pace AM, Bourne HR, Vallar L 1989 GTPase inhibiting mutations activate the alpha chain of G_s and stimulate adenylyl cyclase in human pituitary tumours. Nature **340:**692–696.
24. Lyons J, Landis CA, Harsh G, Vallar L, Grunewald K, Feichtinger H, Duh Q-Y, Clark OH, Kawasaki E, Bourne HR, McCormick F 1990 Two G protein oncogenes in human endocrine tumors. Science **249:**655–659.
25. Parma J, Duprez L, Van Sande J, Cochaux P, Gervy C, Mockel J, Dumont J, Vassart G 1993 Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature **365:**649–651.
26. Duprez L, Parma J, Van Sande J, Rodien P, Dumont JE, Vassart G, Abramowicz M 1998 TSH receptor mutations and thyroid disease. Trends Endocrinol Metab **9:**133–140.
27. Tirosh A, Jin DX, De Marco L, Laitman Y, Friedman E 2020 Activating genomic alterations in the Gs alpha gene (GNAS) in 274 694 tumors. Genes Chromosomes Cancer **59:**503–516.
28. Calebiro D, Grassi ES, Eszlinger M, Ronchi CL, Godbole A, Bathon K, Guizzardi F, de Filippis T, Krohn K, Jaeschke H, Schwarzmayr T, Bircan R, Gozu HI, Sancak S, Niedziela M, Strom TM, Fassnacht M, Persani L, Paschke R 2016 Recurrent EZH1 mutations are a second hit in autonomous thyroid adenomas. J Clin Invest **126:** 3383–3388.
29. Namba H, Rubin SA, Fagin JA 1990 Point mutations of ras oncogenes are an early event in thyroid tumorigenesis. Mol Endocrinol **4:**1474–1479.
30. Schark C, Fulton N, Jacoby RF, Westbrook CA, Straus FH, 2nd, Kaplan EL 1990 N-ras 61 oncogene mutations in Hürthle cell tumors. Surgery **108:**994–999; discussion 999–1000.
31. Ito T, Seyama T, Mizuno T, Tsuyama N, Hayashi T, Hayashi Y, Dohi K, Nakamura N, Akiyama M 1992 Unique association of p53 mutations with undifferentiated but not with differentiated carcinomas of the thyroid gland. Cancer Res **52:**1369–1371.
32. Nakamura T, Yana I, Kobayashi T, Shin E, Karakawa K, Fujita S, Miya A, Mori T, Nishisho I, Takai S 1992 p53 gene mutations associated with anaplastic transformation of human thyroid carcinomas. Jpn J Cancer Res **83:**1293–1298.
33. Fagin JA, Matsuo K, Karmakar A, Chen DL, Tang SH, Koeffler HP 1993 High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. J Clin Invest **91:**179–184.
34. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, *et al.* 1993 Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature **363:**458–460.
35. Eng C, Smith DP, Mulligan LM, Nagai MA, Healey CS, Ponder MA, Gardner E, Scheumann GF, Jackson CE, Tunnacliffe A, *et al.* 1994 Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours. Hum Mol Genet **3:**237–241.
36. Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, Pasini B, Hoppener JW, van Amstel HK, Romeo G, *et al.* 1994 A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature **367:**375–376.

37. American Thyroid Association Guidelines Task F, Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel MD, Schlumberger M, Wells SA, Jr. 2009 Medullary thyroid cancer: management guidelines of the American Thyroid Association. Thyroid **19:**565–612.
38. Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, Fletcher JA 2000 PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma [corrected]. Science **289:**1357–1360.
39. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW, 2nd, Tallini G, Kroll TG, Nikiforov YE 2003 RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab **88:**2318–2326.
40. Castro P, Rebocho AP, Soares RJ, Magalhaes J, Roque L, Trovisco V, Vieira de Castro I, Cardoso-de-Oliveira M, Fonseca E, Soares P, Sobrinho-Simoes M 2006 PAX8-PPARgamma rearrangement is frequently detected in the follicular variant of papillary thyroid carcinoma. J Clin Endocrinol Metab **91:**213–220.
41. Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA 2003 High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res **63:**1454–1457.
42. Cohen Y, Xing M, Mambo E, Guo Z, Wu G, Trink B, Beller U, Westra WH, Ladenson PW, Sidransky D 2003 BRAF mutation in papillary thyroid carcinoma. J Natl Cancer Inst **95:**625–627.
43. Namba H, Nakashima M, Hayashi T, Hayashida N, Maeda S, Rogounovitch TI, Ohtsuru A, Saenko VA, Kanematsu T, Yamashita S 2003 Clinical implication of hot spot BRAF mutation, V599E, in papillary thyroid cancers. J Clin Endocrinol Metab **88:**4393–4397.
44. Soares P, Trovisco V, Rocha AS, Lima J, Castro P, Preto A, Maximo V, Botelho T, Seruca R, Sobrinho-Simoes M 2003 BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Oncogene **22:**4578–4580.
45. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA 2002 Mutations of the BRAF gene in human cancer. Nature **417:**949–954.
46. Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knauf JA, Basolo F, Zhu Z, Giannini R, Salvatore G, Fusco A, Santoro M, Fagin JA, Nikiforov YE 2003 BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J Clin Endocrinol Metab **88:**5399–5404.
47. Adeniran AJ, Zhu Z, Gandhi M, Steward DL, Fidler JP, Giordano TJ, Biddinger PW, Nikiforov YE 2006 Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. Am J Surg Pathol **30:**216–222.
48. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, Kadel S, Moll I, Nagore E, Hemminki K, Schadendorf D, Kumar R 2013 TERT promoter mutations in familial and sporadic melanoma. Science **339:**959–961.
49. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA 2013 Highly recurrent TERT promoter mutations in human melanoma. Science **339:**957–959.
50. Liu X, Bishop J, Shan Y, Pai S, Liu D, Murugan AK, Sun H, el-Naggar AK, Xing M 2013 Highly prevalent TERT promoter mutations in aggressive thyroid cancers. Endocr Relat Cancer **20:**603–610.
51. Landa I, Ganly I, Chan TA, Mitsutake N, Matsuse M, Ibrahimpasic T, Ghossein RA, Fagin JA 2013 Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab **98:**E1562–E1566.
52. Vinagre J, Almeida A, Populo H, Batista R, Lyra J, Pinto V, Coelho R, Celestino R, Prazeres H, Lima L, Melo M, da Rocha AG, Preto A, Castro P, Castro L, Pardal F, Lopes JM, Santos LL, Reis RM, Cameselle-Teijeiro J, Sobrinho-Simoes M, Lima J, Maximo V, Soares P 2013 Frequency of TERT promoter mutations in human cancers. Nat Commun **4:**2185.
53. Liu T, Wang N, Cao J, Sofiadis A, Dinets A, Zedenius J, Larsson C, Xu D 2014 The age- and shorter telomere-dependent TERT promoter mutation in follicular thyroid cell-derived carcinomas. Oncogene **33:**4978–4984.
54. Xing MZ, Liu RY, Liu XL, Murugan AK, Zhu GW, Zeiger MA, Pai S, Bishop J 2014 BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. J Clin Oncol **32:**2718–2726.
55. Liu R, Bishop J, Zhu G, Zhang T, Ladenson PW, Xing M 2017 Mortality risk stratification by combining BRAF V600E and TERT promoter mutations in papillary thyroid cancer: genetic duet of BRAF and TERT promoter mutations in thyroid cancer mortality. JAMA Oncol **3:** 202–208.
56. Landa I, Ibrahimpasic T, Boucai L, Sinha R, Knauf JA, Shah RH, Dogan S, Ricarte-Filho JC, Krishnamoorthy GP, Xu B, Schultz N, Berger MF, Sander C, Taylor BS, Ghossein R, Ganly I, Fagin JA 2016 Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. J Clin Invest **126:**1052–1066.
57. Garcia-Rostan G, Costa AM, Pereira-Castro I, Salvatore G, Hernandez R, Hermsem MJ, Herrero A, Fusco A, Cameselle-Teijeiro J, Santoro M 2005 Mutation of the PIK3CA gene in anaplastic thyroid cancer. Cancer Res **65:**10199–10207.
58. Ricarte-Filho JC, Ryder M, Chitale DA, Rivera M, Heguy A, Ladanyi M, Janakiraman M, Solit D, Knauf JA, Tuttle RM, Ghossein RA, Fagin JA 2009 Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. Cancer Res **69:** 4885–4893.
59. Wu G, Mambo E, Guo Z, Hu S, Huang X, Gollin SM, Trink B, Ladenson PW, Sidransky D, Xing M 2005 Uncommon mutation, but common amplifications, of the PIK3CA gene in thyroid tumors. J Clin Endocrinol Metab **90:**4688–4693.

60. Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R 1997 Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet **16:**64–67.
61. Cameselle-Teijeiro JM, Mete O, Asa SL, LiVolsi V 2021 Inherited follicular epithelial-derived thyroid carcinomas: from molecular biology to histological correlates. Endocr Pathol **32:**77–101.
62. Pappa T, Ahmadi S, Marqusee E, Johnson HL, Nehs MA, Cho NL, Barletta JA, Lorch JH, Doherty GM, Lindeman NI, Alexander EK, Landa I 2021 Oncogenic mutations in PI3K/AKT/mTOR pathway effectors associate with worse prognosis in BRAF(V600E)-driven papillary thyroid cancer patients. Clin Cancer Res **27:**4256–4264.
63. Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacker K, LiVolsi V, Frankel W, Kloos RT, Eng C, Pellegata NS, de la Chapelle A 2001 Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. Proc Natl Acad Sci U S A **98:**15044–15049.
64. Barden CB, Shister KW, Zhu B, Guiter G, Greenblatt DY, Zeiger MA, Fahey TJ, 3rd 2003 Classification of follicular thyroid tumors by molecular signature: results of gene profiling. Clin Cancer Res **9:**1792–1800.
65. Finley DJ, Arora N, Zhu B, Gallagher L, Fahey TJ, 3rd 2004 Molecular profiling distinguishes papillary carcinoma from benign thyroid nodules. J Clin Endocrinol Metab **89:**3214–3223.
66. Chevillard S, Ugolin N, Vielh P, Ory K, Levalois C, Elliott D, Clayman GL, El-Naggar AK 2004 Gene expression profiling of differentiated thyroid neoplasms: diagnostic and clinical implications. Clin Cancer Res **10:** 6586–6597.
67. Frattini M, Ferrario C, Bressan P, Balestra D, De Cecco L, Mondellini P, Bongarzone I, Collini P, Gariboldi M, Pilotti S, Pierotti MA, Greco A 2004 Alternative mutations of BRAF, RET and NTRK1 are associated with similar but distinct gene expression patterns in papillary thyroid cancer. Oncogene **23:**7436–7440.
68. Giordano TJ, Kuick R, Thomas DG, Misek DE, Vinco M, Sanders D, Zhu Z, Ciampi R, Roh M, Shedden K, Gauger P, Doherty G, Thompson NW, Hanash S, Koenig RJ, Nikiforov YE 2005 Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. Oncogene **24:**6646–6656.
69. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM 2002 Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A **99:**15524–15529.
70. He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A 2005 The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci U S A **102:**19075–19080.
71. Visone R, Pallante P, Vecchione A, Cirombella R, Ferracin M, Ferraro A, Volinia S, Coluzzi S, Leone V, Borbone E, Liu CG, Petrocca F, Troncone G, Calin GA, Scarpa A, Colato C, Tallini G, Santoro M, Croce CM, Fusco A 2007 Specific microRNAs are downregulated in human thyroid anaplastic carcinomas. Oncogene **26:**7590–7595.
72. Weber F, Teresi RE, Broelsch CE, Frilling A, Eng C 2006 A limited set of human MicroRNA is deregulated in follicular thyroid carcinoma. J Clin Endocrinol Metab **91:** 3584–3591.
73. Nikiforova MN, Tseng GC, Steward D, Diorio D, Nikiforov YE 2008 MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metab **93:**1600–1608.
74. Sanger F, Nicklen S, Coulson AR 1977 DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A **74:**5463–5467.
75. Collins FS, Morgan M, Patrinos A 2003 The Human Genome Project: lessons from large-scale biology. Science **300:**286–290.
76. Lito P, Pratilas CA, Joseph EW, Tadi M, Halilovic E, Zubrowski M, Huang A, Wong WL, Callahan MK, Merghoub T, Wolchok JD, de SE, Chandarlapaty S, Poulikakos PI, Fagin JA, Rosen N 2012 Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. Cancer Cell **22:**668–682.
77. Maximo V, Soares P, Lima J, Cameselle-Teijeiro J, Sobrinho-Simoes M 2002 Mitochondrial DNA somatic mutations (point mutations and large deletions) and mitochondrial DNA variants in human thyroid pathology: a study with emphasis on Hurthle cell tumors. Am J Pathol **160:**1857–1865.
78. Gasparre G, Porcelli AM, Bonora E, Pennisi LF, Toller M, Iommarini L, Ghelli A, Moretti M, Betts CM, Martinelli GN, Ceroni AR, Curcio F, Carelli V, Rugolo M, Tallini G, Romeo G 2007 Disruptive mitochondrial DNA mutations in complex I subunits are markers of oncocytic phenotype in thyroid tumors. Proc Natl Acad Sci U S A **104:**9001–9006.
79. Corver WE, Ruano D, Weijers K, den Hartog WC, van Nieuwenhuizen MP, de Miranda N, van Eijk R, Middeldorp A, Jordanova ES, Oosting J, Kapiteijn E, Hovens G, Smit J, van Wezel T, Morreau H 2012 Genome haploidisation with chromosome 7 retention in oncocytic follicular thyroid carcinoma. PLoS One **7:**e38287.
80. Ganly I, Makarov V, Deraje S, Dong Y, Reznik E, Seshan V, Nanjangud G, Eng S, Bose P, Kuo F, Morris LGT, Landa I, Carrillo Albornoz PB, Riaz N, Nikiforov YE, Patel K, Umbricht C, Zeiger M, Kebebew E, Sherman E, Ghossein R, Fagin JA, Chan TA 2018 Integrated genomic analysis of Hurthle cell cancer reveals oncogenic drivers, recurrent mitochondrial mutations, and unique chromosomal landscapes. Cancer Cell **34:**256.e5–270.e5.
81. Gopal RK, Kübler K, Calvo SE, Polak P, Livitz D, Rosebrock D, Sadow PM, Campbell B, Donovan SE, Amin S, Gigliotti BJ, Grabarek Z, Hess JM, Stewart C, Braunstein LZ, Arndt PF, Mordecai S, Shih AR, Chaves F, Zhan T, Lubitz CC, Kim J, Iafrate AJ, Wirth L, Parangi S, Leshchiner I, Daniels GH, Mootha VK, Dias-Santagata D, Getz G, McFadden DG 2018 Widespread chromosomal losses and mitochondrial DNA alterations as genetic drivers in Hürthle cell carcinoma. Cancer Cell **34:**242.e5–255.e5.
82. Doerfler WR, Nikitski AV, Morariu EM, Ohori NP, Chiosea SI, Landau MS, Nikiforova MN, Nikiforov YE, Yip L, Manroa P 2021 Molecular alterations in Hurthle cell nodules and preoperative cancer risk. Endocr Relat Cancer **28:**301–309.

83. Kunstman JW, Juhlin CC, Goh G, Brown TC, Stenman A, Healy JM, Rubinstein JC, Choi M, Kiss N, Nelson-Williams C, Mane S, Rimm DL, Prasad ML, Hoog A, Zedenius J, Larsson C, Korah R, Lifton RP, Carling T 2015 Characterization of the mutational landscape of anaplastic thyroid cancer via whole-exome sequencing. Hum Mol Genet **24:**2318–2329.
84. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, Chandramohan R, Liu ZY, Won HH, Scott SN, Brannon AR, O'Reilly C, Sadowska J, Casanova J, Yannes A, Hechtman JF, Yao J, Song W, Ross DS, Oultache A, Dogan S, Borsu L, Hameed M, Nafa K, Arcila ME, Ladanyi M, Berger MF 2015 Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn **17:**251–264.
85. Garcia EP, Minkovsky A, Jia Y, Ducar MD, Shivdasani P, Gong X, Ligon AH, Sholl LM, Kuo FC, MacConaill LE, Lindeman NI, Dong F 2017 Validation of OncoPanel: a targeted next-generation sequencing assay for the detection of somatic variants in cancer. Arch Pathol Lab Med **141:**751–758.
86. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, Schnall-Levin M, White J, Sanford EM, An P, Sun J, Juhn F, Brennan K, Iwanik K, Maillet A, Buell J, White E, Zhao M, Balasubramanian S, Terzic S, Richards T, Banning V, Garcia L, Mahoney K, Zwirko Z, Donahue A, Beltran H, Mosquera JM, Rubin MA, Dogan S, Hedvat CV, Berger MF, Pusztai L, Lechner M, Boshoff C, Jarosz M, Vietz C, Parker A, Miller VA, Ross JS, Curran J, Cronin MT, Stephens PJ, Lipson D, Yelensky R 2013 Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol **31:**1023–1031.
87. Yoo SK, Song YS, Lee EK, Hwang J, Kim HH, Jung G, Kim YA, Kim SJ, Cho SW, Won JK, Chung EJ, Shin JY, Lee KE, Kim JI, Park YJ, Seo JS 2019 Integrative analysis of genomic and transcriptomic characteristics associated with progression of aggressive thyroid cancer. Nat Commun **10:**2764.
88. Pozdeyev N, Gay LM, Sokol ES, Hartmaier R, Deaver KE, Davis S, French JD, Borre PV, LaBarbera DV, Tan AC, Schweppe RE, Fishbein L, Ross JS, Haugen BR, Bowles DW 2018 Genetic analysis of 779 advanced differentiated and anaplastic thyroid cancers. Clin Cancer Res **24:**3059–3068.
89. Ibrahimpasic T, Xu B, Landa I, Dogan S, Middha S, Seshan V, Deraje S, Carlson DL, Migliacci J, Knauf JA, Untch B, Berger MF, Morris L, Tuttle RM, Chan T, Fagin JA, Ghossein R, Ganly I 2017 Genomic alterations in fatal forms of non-anaplastic thyroid cancer: identification of MED12 and RBM10 as novel thyroid cancer genes associated with tumor virulence. Clin Cancer Res **23:**5970–5980.
90. Wang Y, Gogol-Doring A, Hu H, Frohler S, Ma Y, Jens M, Maaskola J, Murakawa Y, Quedenau C, Landthaler M, Kalscheuer V, Wieczorek D, Wang Y, Hu Y, Chen W 2013 Integrative analysis revealed the molecular mechanism underlying RBM10-mediated splicing regulation. EMBO Mol Med **5:**1431–1442.
91. Krishnamoorthy GP, Davidson NR, Leach SD, Zhao Z, Lowe SW, Lee G, Landa I, Nagarajah J, Saqcena M, Singh K, Wendel HG, Dogan S, Tamarapu PP, Blenis J, Ghossein RA, Knauf JA, Ratsch G, Fagin JA 2019 EIF1AX and RAS mutations cooperate to drive thyroid tumorigenesis through ATF4 and c-MYC. Cancer Discov **9:**264–281.
92. Nguyen B, Fong C, Luthra A, Smith SA, DiNatale RG, Nandakumar S, Walch H, Chatila WK, Madupuri R, Kundra R, Bielski CM, Mastrogiacomo B, Donoghue MTA, Boire A, Chandarlapaty S, Ganesh K, Harding JJ, Iacobuzio-Donahue CA, Razavi P, Reznik E, Rudin CM, Zamarin D, Abida W, Abou-Alfa GK, Aghajanian C, Cercek A, Chi P, Feldman D, Ho AL, Iyer G, Janjigian YY, Morris M, Motzer RJ, O'Reilly EM, Postow MA, Raj NP, Riely GJ, Robson ME, Rosenberg JE, Safonov A, Shoushtari AN, Tap W, Teo MY, Varghese AM, Voss M, Yaeger R, Zauderer MG, Abu-Rustum N, Garcia-Aguilar J, Bochner B, Hakimi A, Jarnagin WR, Jones DR, Molena D, Morris L, Rios-Doria E, Russo P, Singer S, Strong VE, Chakravarty D, Ellenson LH, Gopalan A, Reis-Filho JS, Weigelt B, Ladanyi M, Gonen M, Shah SP, Massague J, Gao J, Zehir A, Berger MF, Solit DB, Bakhoum SF, Sanchez-Vega F, Schultz N 2022 Genomic characterization of metastatic patterns from prospective clinical sequencing of 25,000 patients. Cell **185:**563.e11–575.e11.
93. Saqcena M, Leandro-Garcia LJ, Maag JLV, Tchekmedyian V, Krishnamoorthy GP, Tamarapu PP, Tiedje V, Reuter V, Knauf JA, de Stanchina E, Xu B, Liao XH, Refetoff S, Ghossein R, Chi P, Ho AL, Koche RP, Fagin JA 2021 SWI/SNF complex mutations promote thyroid tumor progression and insensitivity to redifferentiation therapies. Cancer Discov **11:**1158–1175.
94. Garcia-Rendueles ME, Ricarte-Filho JC, Untch BR, Landa I, Knauf JA, Voza F, Smith VE, Ganly I, Taylor BS, Persaud Y, Oler G, Fang Y, Jhanwar SC, Viale A, Heguy A, Huberman KH, Giancotti F, Ghossein R, Fagin JA 2015 NF2 loss promotes oncogenic RAS-induced thyroid cancers via YAP-dependent transactivation of RAS proteins and sensitizes them to MEK inhibition. Cancer Discov **5:**1178–1193.
95. Garcia-Rendueles MER, Krishnamoorthy G, Saqcena M, Acuña-Ruiz A, Revilla G, de Stanchina E, Knauf JA, Lester R, Xu B, Ghossein RA, Fagin JA 2022 Yap governs a lineage-specific neuregulin1 pathway-driven adaptive resistance to RAF kinase inhibitors. Mol Cancer **21:**213.
96. Borrelli N, Panebianco F, Condello V, Barletta JA, Kaya C, Yip L, Nikiforova MN, Nikiforov YE 2019 Characterization of activating mutations of the MEK1 gene in papillary thyroid carcinomas. Thyroid **29:**1279–1285.
97. Ritterhouse LL, Wirth LJ, Randolph GW, Sadow PM, Ross DS, Liddy W, Lennerz JK 2016 ROS1 rearrangement in thyroid cancer. Thyroid **26:**794–797.
98. Nikiforova MN, Nikitski AV, Panebianco F, Kaya C, Yip L, Williams M, Chiosea SI, Seethala RR, Roy S, Condello V, Santana-Santos L, Wald AI, Carty SE, Ferris RL, El-Naggar AK, Nikiforov YE 2019 GLIS rearrangement is a genomic hallmark of hyalinizing trabecular tumor of the thyroid gland. Thyroid **29:**161–173.
99. de Kock L, Sabbaghian N, Soglio DB, Guillerman RP, Park BK, Chami R, Deal CL, Priest JR, Foulkes WD 2014 Exploring the association between DICER1 mutations and differentiated thyroid carcinoma. J Clin Endocrinol Metab **99:**E1072–E1077.
100. Wasserman JD, Sabbaghian N, Fahiminiya S, Chami R, Mete O, Acker M, Wu MK, Shlien A, de Kock L,

Foulkes WD 2018 DICER1 mutations are frequent in adolescent-onset papillary thyroid carcinoma. J Clin Endocrinol Metab **103:**2009–2015.
101. Chong AS, Nikiforov YE, Condello V, Wald AI, Nikiforova MN, Foulkes WD, Rivera B 2021 Prevalence and spectrum of DICER1 mutations in adult-onset thyroid nodules with indeterminate cytology. J Clin Endocrinol Metab **106:**968–977.
102. Chernock RD, Rivera B, Borrelli N, Hill DA, Fahiminiya S, Shah T, Chong AS, Aqil B, Mehrad M, Giordano TJ, Sheridan R, Rutter MM, Dehner LP, Foulkes WD, Nikiforov YE 2020 Poorly differentiated thyroid carcinoma of childhood and adolescence: a distinct entity characterized by DICER1 mutations. Mod Pathol **33:** 1264–1274.
103. Nose V 2011 Familial thyroid cancer: a review. Mod Pathol **24 Suppl 2:**S19–S33.
104. Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, Pottern LM, Schneider AB, Tucker MA, Boice JD, Jr. 1995 Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. Radiat Res **141:**259–277.
105. Winship T, Rosvoll RV 1970 Cancer of the thyroid in children. Proc Natl Cancer Conf **6:**677–681.
106. Kazakov VS, Demidchik EP, Astakhova LN 1992 Thyroid cancer after Chernobyl. Nature **359:**21.
107. Fugazzola L, Pilotti S, Pinchera A, Vorontsova TV, Mondellini P, Bongarzone I, Greco A, Astakhova L, Butti MG, Demidchik EP, *et al.* 1995 Oncogenic rearrangements of the RET proto-oncogene in papillary thyroid carcinomas from children exposed to the Chernobyl nuclear accident. Cancer Res **55:**5617–5620.
108. Ito T, Seyama T, Iwamoto KS, Mizuno T, Tronko ND, Komissarenko IV, Cherstovoy ED, Satow Y, Takeichi N, Dohi K, *et al.* 1994 Activated RET oncogene in thyroid cancers of children from areas contaminated by Chernobyl accident. Lancet **344:**259.
109. Klugbauer S, Lengfelder E, Demidchik EP, Rabes HM 1995 High prevalence of RET rearrangement in thyroid tumors of children from Belarus after the Chernobyl reactor accident. Oncogene **11:**2459–2467.
110. Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA 1997 Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Res **57:**1690–1694.
111. Rabes HM, Demidchik EP, Sidorow JD, Lengfelder E, Beimfohr C, Hoelzel D, Klugbauer S 2000 Pattern of radiation-induced RET and NTRK1 rearrangements in 191 post-chernobyl papillary thyroid carcinomas: biological, phenotypic, and clinical implications. Clin Cancer Res **6:**1093–1103.
112. Nikiforova MN, Ciampi R, Salvatore G, Santoro M, Gandhi M, Knauf JA, Thomas GA, Jeremiah S, Bogdanova TI, Tronko MD, Fagin JA, Nikiforov YE 2004 Low prevalence of BRAF mutations in radiation-induced thyroid tumors in contrast to sporadic papillary carcinomas. Cancer Lett **209:**1–6.
113. Ciampi R, Knauf JA, Kerler R, Gandhi M, Zhu Z, Nikiforova MN, Rabes HM, Fagin JA, Nikiforov YE 2005 Oncogenic AKAP9-BRAF fusion is a novel mechanism of MAPK pathway activation in thyroid cancer. J Clin Invest **115:**94–101.
114. Bounacer A, Wicker R, Caillou B, Cailleux AF, Sarasin A, Schlumberger M, Suarez HG 1997 High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumors from patients who had received external radiation. Oncogene **15:**1263–1273.
115. Mizuno T, Iwamoto KS, Kyoizumi S, Nagamura H, Shinohara T, Koyama K, Seyama T, Hamatani K 2000 Preferential induction of RET/PTC1 rearrangement by X-ray irradiation. Oncogene **19:**438–443.
116. Nikiforova MN, Stringer JR, Blough R, Medvedovic M, Fagin JA, Nikiforov YE 2000 Proximity of chromosomal loci that participate in radiation-induced rearrangements in human cells. Science **290:**138–141.
117. Ricarte-Filho JC, Li S, Garcia-Rendueles ME, Montero-Conde C, Voza F, Knauf JA, Heguy A, Viale A, Bogdanova T, Thomas GA, Mason CE, Fagin JA 2013 Identification of kinase fusion oncogenes in post-Chernobyl radiation-induced thyroid cancers. J Clin Invest **123:**4935–4944.
118. Efanov AA, Brenner AV, Bogdanova TI, Kelly LM, Liu P, Little MP, Wald AI, Hatch M, Zurnadzy LY, Nikiforova MN, Drozdovitch V, Mabuchi K, Tronko MD, Chanock SJ, Nikiforov YE 2017 Investigation of the relationship between radiation dose and gene mutations and fusions in post-Chernobyl thyroid cancer. J Natl Cancer Inst **110:**371–378.
119. Morton LM, Karyadi DM, Stewart C, Bogdanova TI, Dawson ET, Steinberg MK, Dai J, Hartley SW, Schonfeld SJ, Sampson JN, Maruvka YE, Kapoor V, Ramsden DA, Carvajal-Garcia J, Perou CM, Parker JS, Krznaric M, Yeager M, Boland JF, Hutchinson A, Hicks BD, Dagnall CL, Gastier-Foster JM, Bowen J, Lee O, Machiela MJ, Cahoon EK, Brenner AV, Mabuchi K, Drozdovitch V, Masiuk S, Chepurny M, Zurnadzhy LY, Hatch M, Berrington de Gonzalez A, Thomas GA, Tronko MD, Getz G, Chanock SJ 2021 Radiation-related genomic profile of papillary thyroid carcinoma after the Chernobyl accident. Science **372:**eabg2538.
120. Yeager M, Machiela MJ, Kothiyal P, Dean M, Bodelon C, Suman S, Wang M, Mirabello L, Nelson CW, Zhou W, Palmer C, Ballew B, Colli LM, Freedman ND, Dagnall C, Hutchinson A, Vij V, Maruvka Y, Hatch M, Illienko I, Belayev Y, Nakamura N, Chumak V, Bakhanova E, Belyi D, Kryuchkov V, Golovanov I, Gudzenko N, Cahoon EK, Albert P, Drozdovitch V, Little MP, Mabuchi K, Stewart C, Getz G, Bazyka D, Berrington de Gonzalez A, Chanock SJ 2021 Lack of transgenerational effects of ionizing radiation exposure from the Chernobyl accident. Science **372:**725–729.
121. Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, Joslyn G, Stevens J, Spirio L, Robertson M, *et al.* 1991 Identification and characterization of the familial adenomatous polyposis coli gene. Cell **66:**589–600.
122. Harach HR, Williams GT, Williams ED 1994 Familial adenomatous polyposis associated thyroid carcinoma: a distinct type of follicular cell neoplasm. Histopathology **25:**549–561.
123. Hobert JA, Eng C 2009 PTEN hamartoma tumor syndrome: an overview. Genet Med **11:**687–694.
124. Casey M, Vaughan CJ, He J, Hatcher CJ, Winter JM, Weremowicz S, Montgomery K, Kucherlapati R, Morton CC, Basson CT 2000 Mutations in the protein kinase A R1alpha regulatory subunit cause familial cardiac myxomas and Carney complex. J Clin Invest **106:** R31–R38.

125. Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA 2000 Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. Nat Genet **26:**89–92.
126. Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, Matthews S, Nakura J, Miki T, Ouais S, Martin GM, Mulligan J, Schellenberg GD 1996 Positional cloning of the Werner's syndrome gene. Science **272:**258–262.
127. Foulkes WD, Bahubeshi A, Hamel N, Pasini B, Asioli S, Baynam G, Choong CS, Charles A, Frieder RP, Dishop MK, Graf N, Ekim M, Bouron-Dal Soglio D, Arseneau J, Young RH, Sabbaghian N, Srivastava A, Tischkowitz MD, Priest JR 2011 Extending the phenotypes associated with DICER1 mutations. Hum Mutat **32:**1381–1384.
128. Hill DA, Ivanovich J, Priest JR, Gurnett CA, Dehner LP, Desruisseau D, Jarzembowski JA, Wikenheiser-Brokamp KA, Suarez BK, Whelan AJ, Williams G, Bracamontes D, Messinger Y, Goodfellow PJ 2009 DICER1 mutations in familial pleuropulmonary blastoma. Science **325:**965.
129. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L 2016 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid **26:**1–133.
130. Gharib H 2004 Changing trends in thyroid practice: understanding nodular thyroid disease. Endocr Pract **10:** 31–39.
131. Sciacchitano S, Paliotta DS, Nardi F, Sacchi A, Andreoli M, Pontecorvi A 1994 PCR amplification and analysis of ras oncogenes from thyroid cytologic smears. Diagn Mol Pathol **3:**114–121.
132. Nikiforov YE, Steward DL, Robinson-Smith TM, Haugen BR, Klopper JP, Zhu Z, Fagin JA, Falciglia M, Weber K, Nikiforova MN 2009 Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab **94:**2092–2098.
133. Cantara S, Capezzone M, Marchisotta S, Capuano S, Busonero G, Toti P, Di Santo A, Caruso G, Carli AF, Brilli L, Montanaro A, Pacini F 2010 Impact of proto-oncogene mutation detection in cytological specimens from thyroid nodules improves the diagnostic accuracy of cytology. J Clin Endocrinol Metab **95:**1365–1369.
134. Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, Friedman L, Kloos RT, LiVolsi VA, Mandel SJ, Raab SS, Rosai J, Steward DL, Walsh PS, Wilde JI, Zeiger MA, Lanman RB, Haugen BR 2012 Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med **367:**705–715.
135. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, Hodak SP, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN 2014 Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. Cancer **120:**3627–3634.
136. Nikiforov YE, Carty SE, Chiosea SI, Coyne C, Duvvuri U, Ferris RL, Gooding WE, LeBeau SO, Ohori NP, Seethala RR, Tublin ME, Yip L, Nikiforova MN 2015 Impact of the multi-gene ThyroSeq next-generation sequencing assay on cancer diagnosis in thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance cytology. Thyroid **25:**1217–1223.
137. Labourier E, Shifrin A, Busseniers AE, Lupo MA, Manganelli ML, Andruss B, Wylie D, Beaudenon-Huibregtse S 2015 Molecular testing for miRNA, mRNA, and DNA on fine-needle aspiration improves the preoperative diagnosis of thyroid nodules with indeterminate cytology. J Clin Endocrinol Metab **100:**2743–2750.
138. Lithwick-Yanai G, Dromi N, Shtabsky A, Morgenstern S, Strenov Y, Feinmesser M, Kravtsov V, Leon ME, Hajduch M, Ali SZ, VandenBussche CJ, Zhang X, Leider-Trejo L, Zubkov A, Vorobyov S, Kushnir M, Goren Y, Tabak S, Kadosh E, Benjamin H, Schnitzer-Perlman T, Marmor H, Motin M, Lebanony D, Kredo-Russo S, Mitchell H, Noller M, Smith A, Dattner O, Ashkenazi K, Sanden M, Berlin KA, Bar D, Meiri E 2017 Multicentre validation of a microRNA-based assay for diagnosing indeterminate thyroid nodules utilising fine needle aspirate smears. J Clin Pathol **70:**500–507.
139. Patel KN, Angell TE, Babiarz J, Barth NM, Blevins T, Duh QY, Ghossein RA, Harrell RM, Huang J, Kennedy GC, Kim SY, Kloos RT, LiVolsi VA, Randolph GW, Sadow PM, Shanik MH, Sosa JA, Traweek ST, Walsh PS, Whitney D, Yeh MW, Ladenson PW 2018 Performance of a genomic sequencing classifier for the preoperative diagnosis of cytologically indeterminate thyroid nodules. JAMA Surg **153:**817–824.
140. Steward DL, Carty SE, Sippel RS, Yang SP, Sosa JA, Sipos JA, Figge JJ, Mandel S, Haugen BR, Burman KD, Baloch ZW, Lloyd RV, Seethala RR, Gooding WE, Chiosea SI, Gomes-Lima C, Ferris RL, Folek JM, Khawaja RA, Kundra P, Loh KS, Marshall CB, Mayson S, McCoy KL, Nga ME, Ngiam KY, Nikiforova MN, Poehls JL, Ringel MD, Yang H, Yip L, Nikiforov YE 2019 Performance of a multigene genomic classifier in thyroid nodules with indeterminate cytology: a prospective blinded multicenter study. JAMA Oncol **5:**204–212.
141. Zhu CY, Donangelo I, Gupta D, Nguyen DT, Ochoa JE, Yeh MW, Livhits MJ 2021 Outcomes of indeterminate thyroid nodules managed nonoperatively after molecular testing. J Clin Endocrinol Metab **106:**e1240–e1247.
142. Schlumberger M, Tahara M, Wirth LJ, Robinson B, Brose MS, Elisei R, Habra MA, Newbold K, Shah MH, Hoff AO, Gianoukakis AG, Kiyota N, Taylor MH, Kim SB, Krzyzanowska MK, Dutcus CE, de las HB, Zhu J, Sherman SI 2015 Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. N Engl J Med **372:**621–630.
143. Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, de la Fouchardiere C, Pacini F, Paschke R, Shong YK, Sherman SI, Smit JW, Chung J, Kappeler C, Pena C, Molnar I, Schlumberger MJ; DECISION Investigators 2014 Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. Lancet **384:**319–328.
144. Wells SA, Jr., Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, Baudin E, Elisei R, Jarzab B, Vasselli JR, Read J, Langmuir P, Ryan AJ, Schlumberger MJ 2012 Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol **30:**134–141.

145. Sherman SI, Clary DO, Elisei R, Schlumberger MJ, Cohen EE, Schoffski P, Wirth LJ, Mangeshkar M, Aftab DT, Brose MS 2016 Correlative analyses of RET and RAS mutations in a phase 3 trial of cabozantinib in patients with progressive, metastatic medullary thyroid cancer. Cancer **122:**3856–3864.
146. Shonka DC, Jr., Ho A, Chintakuntlawar AV, Geiger JL, Park JC, Seetharamu N, Jasim S, Abdelhamid Ahmed AH, Bible KC, Brose MS, Cabanillas ME, Dabekaussen K, Davies L, Dias-Santagata D, Fagin JA, Faquin WC, Ghossein RA, Gopal RK, Miyauchi A, Nikiforov YE, Ringel MD, Robinson B, Ryder MM, Sherman EJ, Sadow PM, Shin JJ, Stack BC, Jr., Tuttle RM, Wirth LJ, Zafereo ME, Jr., Randolph GW 2022 American Head and Neck Society Endocrine Surgery Section and International Thyroid Oncology Group consensus statement on mutational testing in thyroid cancer: defining advanced thyroid cancer and its targeted treatment. Head Neck **44:**1277–1300.
147. Fagin JA, Wells SA Jr 2016 Biological and Clinical Perspectives on Thyroid Cancer. N Engl J Med **375:** 1054–1067.

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The Origin of Antithyroid Drugs

Clark T. Sawin† and David S. Cooper

Background: When the antithyroid drugs were discovered in the early 1940s, they were immediately recognized as a revolutionary new treatment for hyperthyroidism. Although much has been learned about their mechanism of action and clinical utility, they continue to be used today in much the same way as they have been since their introduction.
Summary: In 1995, Dr. Clark Sawin gave an address on the history of antithyroid drug development at the 11th International Thyroid Congress in Toronto, Ontario, Canada. In his review, Dr. Sawin recounted the original observations by Drs. Julia and Cosmo Mackenzie and Curt Richter at the Johns Hopkins University School of Medicine, and how their work ultimately led to Dr. Edwin (Ted) B. Astwood's seminal 1943 report on the use of thiourea and thiouracil in the Journal of the American Medical Association. He also described the development of propylthiouracil and methimazole as less toxic alternatives. He concluded his remarks by noting the often-serendipitous pathway of drug development and the role of pharmaceutical companies in the process.
Conclusions: Antithyroid drugs remain a cornerstone of thyroid therapeutics. It is informative to review the process by which they came into use, as this is a seminal part of the history of thyroid disease in the 20th century. This knowledge may also spark additional research leading to new pharmacotherapies for patients with hyperthyroidism.

Keywords: Graves' disease, antithyroid drugs, history

Introductory Note by David S. Cooper

Clark T. Sawin, MD (Fig. 1), was a Boston native, graduating from Brandeis University in 1954 and from Tufts University School of Medicine in 1958. He completed a fellowship in Endocrinology at the Tufts New England Medical Center Hospitals in Boston, under the direction of Edwin (Ted) Bennett Astwood, MD, the father of antithyroid drug therapy. Dr. Sawin was a Professor Medicine at Tufts University School of Medicine and Boston University School of Medicine. During his productive clinical research career, he made many important contributions to thyroidology, particularly in the areas of subclinical thyroid dysfunction and thyroid disease in older persons. In 1998, he moved from Boston to Washington, DC where he served in The Office of the Medical Inspector, Veterans Health Administration, Department of Veteran's Affairs, leading efforts to improve veterans' endocrine care, especially in the realm of diabetes.

However, medical history was his passion, with a special interest in the history of Endocrinology. He initiated the ''Historical Vignette'' lecture series at the annual meetings of the American Thyroid Association, a tradition that continues to this day. At the time of his death in 2004, Dr. Sawin was the President of the American Thyroid Association. After his death, to continue Dr. Sawin's work in Endocrinology history, his family established the Clark T. Sawin History Resource Center at the American Thyroid Association and the Clark T. Sawin Memorial Library and Resource Center at The Endocrine Society.

Dr. Sawin had been one of my mentors when I was a student at Tufts Medical School in the early 1970s, and we became good friends. He presented a lecture on the history

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†Dr. Sawin passed away on August 11, 2004.

of antithyroid drug development on September 13, 1995 at the 11th International Thyroid Congress in Toronto, Canada. At my request, he sent me a typed copy of his lecture in 1996, which I have had in my files for the last 26 years. The lecture, which follows, was written to be delivered orally by Dr. Sawin, and has never been published. Some of the content is based on a brief history of Dr. Astwood's work that Dr. Sawin published in 1993.[1] The address was meant to be heard rather than read, and I have edited the typescript to be more appropriate for print media, and I have added photos of the individuals discussed in the lecture and a few editorial comments. Dr. Sawin's original lecture notes did not include a bibliography, and I have taken the liberty of adding one based on my knowledge of the literature on antithyroid drugs. I hope that all of these efforts would meet with Dr. Sawin's approval.

The Origin of Antithyroid Drugs by Clark T. Sawin, MD

In 1944 the world was in turmoil. War was prevalent and caused many to leave their homes—and it was to get worse until it ended in 1945. But my story today is not of the second World War, but of another event that spanned the same years, 1941 to 1945. And that is the discovery and development of antithyroid drugs for the treatment of hyperthyroidism.

In 1941, Elmer V. McCollum had been the Chairman of biochemistry at Johns Hopkins' School of Hygiene and Public Health for more than 20 years. He was widely recognized as the foremost nutritional biochemist of his time. In his laboratory were two young married investigators, Julia Frances (nee Buzz) (Fig. 2) and Cosmo Glenn Mackenzie (Fig. 3).* Julia had a BA from Colorado College and a nursing degree from Johns Hopkins School of Nursing. Cosmo had a BA degree from Johns Hopkins, and both subsequently earned DSc degrees from Johns Hopkins. Both were Assistant Professors of biochemistry, and both were experts in vitamin E and the effects of its deficiency in rabbits. In mid-1941, in an attempt to make rats deficient in this vitamin as well, they administered a new sulfa drug, sulfaguanidine, to alter the bacterial flora in the gut. Not much happened to the rats' nutritional state but, to her great surprise, Julia found huge thyroid glands in the rats at autopsy. Rather than discard the rats or ignore the whole experiment as an anomaly, they set out to explain this peculiar finding.

The rats' thyroids were microscopically hyperplastic, so the Mackenzies took the histologic slides to Dr. Arnold Rich, the well-known pathologist at Johns Hopkins (e.g., acute interstitial pneumonia [the Hamman-Rich syndrome] was partly named after him), who immediately diagnosed hyperthyroidism (at the time, hyperplasia was often considered synonymous with hyperfunction and excessive hormonal secretion). The Mackenzies demurred because the rats showed no outward signs to suggest thyroid overactivity. Rich also told them that Curt Richter, a behavioral scientist in the Johns Hopkins' Psychiatry department, had recently shown him quite similar slides from rats who had been given phenylthiourea as part of taste preference experiments designed to develop more effective rat poisons. The Mackenzies met with Richter to discuss the problem. Richter later noted the phenylthiourea-induced goiter in passing in a short article on gray hair in rats in December,

*Dr. Mackenzie preferred that his family name be spelled ''Mackenzie'' rather than MacKenzie. The erroneous spelling arose due to an error in spelling his surname in Mackenzie et al[3] (Cosmo Mackenzie, pers. commun.).

FIG. 1. Clark T. Sawin, MD (May 23, 1934 to August 11, 2004) (with permission from Ms. Leslie Sawin).

FIG. 2. Julia B. Mackenzie, ScD (April 1911 to November 1, 1999) (provided by Dr. Julia Mackenzie).

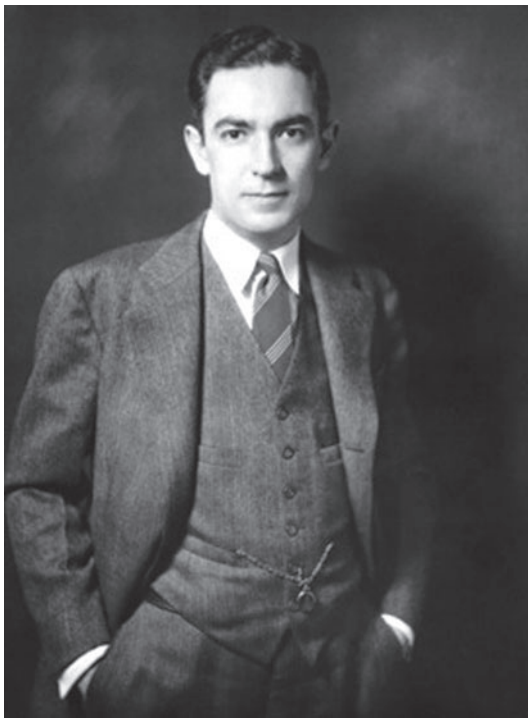


FIG. 3. Cosmo G. Mackenzie, ScD (May 22, 1907 to October 24, 1999) (provided by the Alan Mason Chesney Medical Archives of the Johns Hopkins Medical Institutions).

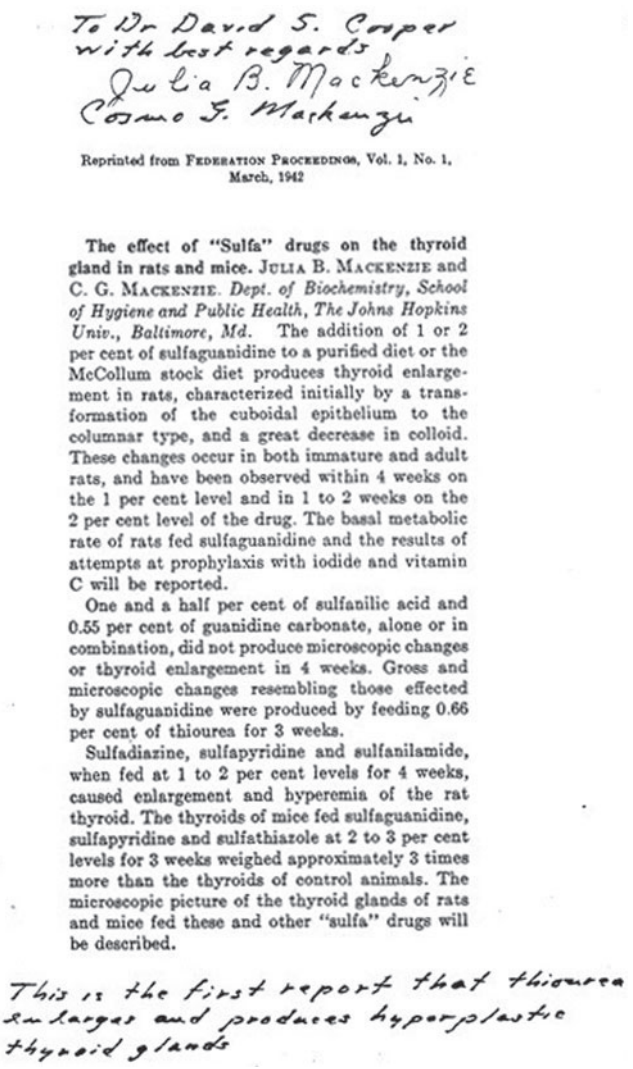
1941.[2] He did not pursue the problem, as his main interest was in rat behavior and, later, in how to kill rats roaming the streets of Baltimore.[†]

The Mackenzies published their initial findings in November, 1941,[3] and continued to seek an explanation for why their rats developed goiters. They recognized the decidedly unusual nature of the goiter—it was caused by a specific chemical and, in contrast to other experimental goiters, it occurred despite the presence of sufficient iodine in the rats' diets. Finally, they were concerned about the millions of patients already being treated with sulfa drugs.[‡]

By the end of December, 1941, they had written an abstract for the forthcoming April, 1942, American Federation for Clinical Research (AFCR) meeting in Boston, in which they showed that several sulfa drugs caused goiters in rats, as did thiourea itself (Fig. 4). By April, when Julia presented the abstract, they were able to show that these drugs caused the rats' basal metabolic rate (BMR) to decrease rather than increase, thus excluding hyperthyroidism. They also showed that the drugs did not block thyroxine's (T4s) effect on the BMR, thus excluding a peripheral effect. She proposed that the drugs probably decreased thyroid hormone formation and that this led to goiter by increasing pituitary function. At

[†]Curt Paul Richter (1894–1988) was a psychobiologist and geneticist who first identified the hypothalamus as a center that controlled sleep and wakefulness cycles in the rat.: Astwood also acknowledged the work of Kennedy and Purves showing that diets containing rape seed extracts also induced goiters (cited in Himsworth[10]).

[‡]Sulfa drugs were also an integral part of infection prevention in the military in WWII, and were part of all first-aid kits to prevent wound infections.



To Dr David S. Cooper
with best regards
Julia B. Mackenzie
Cosmo G. Mackenzie

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The effect of "Sulfa" drugs on the thyroid gland in rats and mice. JULIA B. MACKENZIE and C. G. MACKENZIE. *Dept. of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins Univ., Baltimore, Md.* The addition of 1 or 2 per cent of sulfaguanidine to a purified diet or the McCollum stock diet produces thyroid enlargement in rats, characterized initially by a transformation of the cuboidal epithelium to the columnar type, and a great decrease in colloid. These changes occur in both immature and adult rats, and have been observed within 4 weeks on the 1 per cent level and in 1 to 2 weeks on the 2 per cent level of the drug. The basal metabolic rate of rats fed sulfaguanidine and the results of attempts at prophylaxis with iodide and vitamin C will be reported.

One and a half per cent of sulfanilic acid and 0.55 per cent of guanidine carbonate, alone or in combination, did not produce microscopic changes or thyroid enlargement in 4 weeks. Gross and microscopic changes resembling those effected by sulfaguanidine were produced by feeding 0.66 per cent of thiourea for 3 weeks.

Sulfadiazine, sulfapyridine and sulfanilamide, when fed at 1 to 2 per cent levels for 4 weeks, caused enlargement and hyperemia of the rat thyroid. The thyroids of mice fed sulfaguanidine, sulfapyridine and sulfathiazole at 2 to 3 per cent levels for 3 weeks weighed approximately 3 times more than the thyroids of control animals. The microscopic picture of the thyroid glands of rats and mice fed these and other "sulfa" drugs will be described.

This is the first report that thiourea enlarges and produces hyperplastic thyroid glands

FIG. 4. Abstract from the 1942 American Federation for Clinical Research meeting, provided to the author (D.S.C) by Drs. Mackenzie in 1983.

FIG. 5. Edwin B. Astwood (December 29, 1909 to February 17, 1976) in 1954 when he received the Lasker Award (with permission of the Lasker Foundation).

the end of her talk, Edwin (Ted) B. Astwood commented that he had confirmed that sulfaguanidine caused goiter in rats, and agreed with the Mackenzies' findings.

Ted Astwood was a rising star in American Endocrinology (Fig. 5). He had trained at McGill (where he earned his MD degree), at Johns Hopkins (where he completed a gynecologic endocrinology fellowship studying hormonal control of mammary gland development), and at Harvard (earning a PhD in Pharmacology). Astwood had known the Mackenzies for several years, having met them in the mid-1930s during his fellowship at Johns Hopkins. In 1942 at age 32, he was on the staff at Boston's Peter Bent Brigham Hospital and an Assistant Professor of Pharmacotherapy at Harvard Medical School, where he was studying the effects of adrenocorticotropic hormone (ACTH) in hypophysectomized rats; he was also the Editor-in-Chief of the journal, *Endocrinology*.

Although he presented two articles on ACTH at that 1942 AFCR meeting, he immediately stopped most of his work on ACTH in December, 1941, after he learned of the Mackenzies' work at a Harvard Medical School seminar. Until that time he had never done any work related to the thyroid gland, but he subsequently shifted his efforts mainly to studying the thyroid; in fact, he later received permission to divert his National Research Council grant of $2500 from studying ACTH to explaining the drug-induced rat goiters that had been described by the Mackenzies. His rationale was twofold: first, he wanted to explain the underlying mechanism, and second, when it became fairly clear that the goiter was due to blockade of thyroid hormone synthesis, because it might be a therapy for hyperthyroidism. It is of interest that just after that AFCR meeting in April of 1942, Cosmo Mackenzie was asked by a science journalist if he would say that thiourea might be a treatment for hyperthyroidism. He declined, stating that there was no evidence for this in humans.

By October of 1942, both the Mackenzies and Astwood had concluded that sulfaguanidine did indeed most likely block thyroid hormone synthesis. Their articles were essentially contemporaneous and were published, back-to-back in February, 1943, in the same issue of *Endocrinology*, with the Mackenzies' article placed first (by Editor-in-Chief Astwood).[4,5] Publication was delayed somewhat because Astwood was waiting for the Mackenzies' article. He had been encouraging them to submit it for several months, but writing it was difficult for them because Cosmo, who had enlisted in 1941, was now a member of the United States Army Air Forces studying high altitude physiology.

Both articles took a truly modern approach; that is, they embraced the concept that the thyroid gland fed back on the pituitary to inhibit its secretion, an idea then not universally accepted. Both articles showed that these drugs caused goiter despite adequate amounts of dietary iodine, that they decreased the BMR, that they did not block the metabolic effects of T4, and that both thyroid hormone and hypophysectomy prevented the drugs from causing goiter, and thus, the pituitary was needed for the effect. Both concluded by inference, as none of the data gave direct proof, that the drugs probably acted directly on the thyroid gland to block thyroid function, and that the goiter occurred because of increased pituitary thyrotropin (TSH) secretion.

In March, 1942, Astwood had decided to study thiourea as a potential treatment for hyperthyroidism. First, he took one dose himself, without anything terrible happening; then, he gave it to a few patients without hyperthyroidism and to a few patients with hyperthyroidism. Absolutely nothing happened. Finally, in July 1942, he gave thiourea to one hyperthyroid patient for a long enough time, namely, more than for a few days, and the patient improved remarkably. At the same time he was also studying dozens of synthetic thiourea analogs in collaboration with Richard Roblin of the American Cyanamid/Lederle company. They found a less toxic drug which did not cause the terrible breath odor imparted by thiourea. Astwood gave large doses of this new drug, thiouracil, to two more hyperthyroid patients in October and December, 1942. The first patient receiving thiourea developed a rash, and one of the thiouracil-treated patients developed agranulocytosis, but all survived and all became euthyroid.

The report of these three patients, published in *JAMA* on May 8, 1943, was a landmark.[6,7] While investigators in New Zealand were on the same track and had proposed a clinical trial of thiourea in April, 1943 (summarized in Hercus[8]), Astwood had provided for the first time a consistently effective medical therapy for hyperthyroidism. The efficacy was rapidly confirmed both in Boston[9] and Europe.[10] Astwood also found that overtreatment could cause severe hypothyroidism and, because a few patients stopped the drug when they felt better, he serendipitously found that some patients remained in remission with no further treatment.[11]

Still, the toxic effects of thiouracil were worrisome. A trial with thiobarbital was disastrous; it turned out to be more toxic than any of the other compounds that had been tested.[12] In 1945, Astwood and Vanderlaan had moved from Harvard to Tufts University Medical School. By late 1945, their team had studied a total of 315 different compounds,[13] most made by the American Cynamid/Lederle company, and found among a series of thiouracil derivatives an effective compound which had fewer side effects. This compound, 6-n-propylthiouracil (PTU), remains to this day the major antithyroid drug used in the United States, although methimazole, also discovered by Stanley and Astwood,[14] and the methimazole prodrug, carbimazole, are also widely used.[§] Thiouracil was approved by the FDA in 1946 but was withdrawn from the market by the drug companies when PTU was approved by the FDA in 1947.

Concluding remarks

What I have given you is only a sketch of the whole story. Nevertheless, some points of interest are:

1. This successful therapy was devised without understanding the precise intracellular mechanism of the drugs' action, without knowing their pharmacokinetics, without knowing the etiology of hyperthyroidism, and without any hormonal assays in either the rats or the patients.
2. Still, physiologic understanding of the thyroid-pituitary feedback system was a major underpinning to the success of this work, even though it turned out that excessive TSH was not the cause of hyperthyroidism.
3. Nevertheless, as Astwood himself said in 1945: ''…the development was by no means a simple process but one marked by many unprofitable detours.'' It was ever thus in science and medicine.

[§]When this lecture was presented in 1995, PTU was the dominant antithyroid drug in the United States, but methimazole now predominates.

4. Note that the work was all done before the National Science Foundation existed and before the explosive growth of the NIH. Were it not for private contributions to the National Research Council and separate support from the Rockefeller Foundation, it is unlikely the Mackenzies or Astwood would have done what they did.
5. Further, this successful therapy would not have come when it did were it not for a close collaboration of the investigators with an interested pharmaceutical company, which in this case occurred without any patents or ownership in the company accruing to the investigators.

Finally, it is worth noting that Astwood always acknowledged the Mackenzies as having started him on his way.

Afterword by David S. Cooper

Astwood continued to make important observations related to antithyroid drug therapy (he coined the term ''antithyroid compound''[11]), including the seminal concept of remission after discontinuation of antithyroid drug therapy,[15] a phenomenon also observed earlier by Williams et al.[16] Astwood also documented the use of antithyroid drugs in pregnancy[17] and continued his work on these compounds into the 1960s.[18] His fervent hope was that drug therapy for Graves' disease would eventually replace radioiodine and surgery as treatments, and stated at the end of his Harvey Lecture in 1945: ''When means are found to prevent the occurrence of toxic reactions from antithyroid drugs, or when a compound is discovered which does not give rise to side effects, then it will be possible to treat all cases of hyperthyroidism by medical means''.[11]

In recognition of his work with antithyroid drugs, Astwood received the prestigious Lasker Award in 1954 and the Koch medal from the Endocrine Society in 1967. Astwood retired from his academic position at Tufts Medical School in 1972 and returned to his native Bermuda to practice general internal medicine until his death in 1976. An appreciation of Astwood's contributions and his philosophy of life can be found in a tribute by one of his most esteemed fellows, Dr. Jerome Hershman.[19]

Cosmo Mackenzie did not pursue thyroid research after World War II. In 1950, he became Professor of Biochemistry and Chairman of the Department of Biochemistry at the University of Colorado School of Medicine. He was elected a Fellow of the New York Academy of Sciences and of the American Association for the Advancement of Sciences. He served as editor for the *Journal of Nutrition* and the *Proceedings of the Society for Experimental Biology and Medicine*. He died in 1999 at the age of 92. Dr. Julia Mackenzie became Professor of Biochemistry and Professor of Anatomy at the University of Colorado School of Medicine and worked with her husband in a variety of areas, including vitamin E biochemistry and lipid metabolism in cultured mammalian cells. She also died in 1999.

Final thoughts

The thionamide antithyroid drugs are among a group of compounds, such as lidocaine, phenobarbital, thiazides, and

Box 1. Timeline of Important Discoveries and Clinical Uses Related to the Thionamide Antithyroid Drugs

I. Discovery
- 1920s: Webster and Chesney: cabbage goiter in rabbits[21]
- 1941: Kennedy and Purves: goiter in rabbits with *Brassica* seeds[22]
- 1941: Richter: goiter in rats with thiocarbamide[2]
- 1941: Mackenzies: goiter in rats with sulfaguanidine[3]
- 1942: Mackenzies and Astwood: thioureas disrupt thyroid hormone synthesis; increase in TSH causes goiter[4,5]
- 1943: Astwood's landmark article describing therapy of Graves' disease with thiourea and thiouracil[6]
- 1945: Astwood coins the term ''antithyroid drug''[11]
- 1949: Stanley and Astwood: development of methimazole[14]
- 1951: Lawson and Barry: development of the prodrug carbimazole[23]

II. Clinical use
- 1947: Williams et al: ''remissions'' after antithyroid therapy[16]
- 1940s–1950s: major and minor ATD side-effects described by many authors[24]
- 1953: Astwood: concept of remissions and concept of long-term ATD therapy[15]
- 1950s: Astwood and many others: use of ATDs to prepare for surgery and their use in pregnancy[17]

III. Mechanisms
- 1960s: Hershman and Van Middlesworth: PTU but not methimazole inhibits T4 metabolism[25]
- 1970s: Taurog and Dorris[26] and Maloof et al[27]: elucidation of the basic intrathyroidal mechanisms of ATD action to inhibit thyroid hormone synthesis
- 1973: Beck et al: decreased lymphocytic infiltration in thymus and thyroid of thyroidectomy patients prepared with ATDs[28]
- 1980: McGregor et al: decreased serum levels of TRAb in patients treated with carbimazole[29]
- 1983: Silva et al: two pathways of T4 deiodination, one PTU sensitive (type 1 deiodinase) and the other PTU insensitive (type 2 deiodinase)[30]

IV. Modern day issues
- 1990s–2000s: increasing recognition of methimazole embryopathy in pregnancy[31]
- 2009: Rivkees and Mattison and Bahn et al: PTU hepatotoxicity concerns lead to U.S. FDA Black Box warning for PTU[32,33]
- 2013: Andersen et al: recognition that PTU can cause birth defects[34]
- 1979–2015: studies suggesting that long-term ATD use is a viable alternative for some patients, and may be preferable to radioiodine ablation[35,36]
- 2013–2019: studies re-examining the idea that duration of ATD treatment has an effect to increase rates of remission[37,38]
- 2015–2016: studies on ATD side-effects showing that certain HLA types are associated with increased susceptibility to agranulocytosis[39,40]
- 2015: Neumann et al: studies on small molecule TSH receptor antagonists[41]
- 2020: Brix et al: pancreatitis is a rare complication of methimazole therapy[42]

This is not a complete list of references, and I regret that space limitations prevent an exhaustive bibliography.

ATD, antithyroid drug; PTU, 6-n-propylthiouracil; T4, thyroxine; TSH, thyrotropin.

penicillin, that are still important therapies today, despite having been developed 75–100 years ago. It is interesting and perhaps surprising that there is still much to learn about the antithyroid drugs, including the optimal duration of therapy, management of side effects, and their optimal use in pregnancy. The timeline in Box 1 reviews the story of the antithyroid drugs from the original observations in the early 1940s over the last 7 decades to new data from the 21st century.

Researchers are exploring new therapies for Graves' disease, which are directed at the underlying pathophysiology: an immune dysregulation leading to the presence of stimulating antibodies directed at the thyrocyte TSH receptor.[20] While these exciting potential treatments represent the future, it is hard to imagine a scenario in which the thionamide antithyroid drugs will not be used to treat thyrotoxicosis, at least as initial therapy, while awaiting the salutary effects of more specific treatments.

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Authors' Contributions

David S. Cooper is responsible for writing the commentary and background information regarding Dr. Sawin and his lecture, as well as the brief history of events occurring in the years after the development of antithyroid drugs. Clark T. Sawin wrote the text of the lecture that he delivered in 1995 to the International Thyroid Congress.

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References

1. Sawin CT. Edwin B. Astwood. Historical note. Endocrinologist 1993;3(4):239–241.
2. Richter CP, Clisby KH. Graying of hair produced by ingestion of phenylthiocarbamide. Proc Soc Exp Biol Med 1941:48(3):684–687; doi: 10.3181/00379727-48-13428
3. Mackenzie JB, Mackenzie CG, McCollum EV. The effect of sulfanilylguanidine on the thyroid of the rat. Science 1941;94(2448):518–519; doi: 10.1126/science.94.2448.518
4. Mackenzie CG, Mackenzie JB. Effect of sulfonamides and thioureas on the thyroid gland and basal metabolism. Endocrinology 1943;32(2):185–209; doi: 10.1210/endo-32-2-185
5. Astwood EB, Sullivan J, Bissell A, et al. Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat. Endocrinology 1943;32(2):210–225; doi: 10.1210/endo-32-2-210
6. Astwood EB. Treatment of hyperthyroidism with thiourea and thiouracil. JAMA 1943;122(2):78–81; doi: 10.1001/jama.1943.02840190008003
7. Landau R. Treatment of hyperthyroidism. JAMA 1984; 251(13):1747–1748; doi: 10.1001/jama.1984.03340370079037
8. Hercus C. Thyroid disease in New Zealand. Can Med Assoc J 1953;68(6):531–537
9. Williams RH. Antithyroid drugs, with particular reference to thiouracil. Arch Intern Med 1944;74(6):479–487; doi: 10.1001/archinte.1944.00210240069008
10. Himsworth HP. Thyrotoxicosis treated with thiourea. Lancet 1943;242;465–466; doi: 10.1016/S0140-6736(00)87485-1
11. Astwood EB. The Harvey Lectures is a annual compendium of lectures given at Rockefeller University. 1945;40: 195–245.
12. Astwood EB. Some observations on the use of thiobarbital as an antithyroid agent in the treatment of Graves' disease. J Clin Endocrinol 1945;5(8);345–352; doi: 10.1210/jcem-5-8-345
13. Astwood EB, Vanderlaan WP. Thiouracil derivatives of greater activity for the treatment of hyperthyroidism. J Clin Endocrinol Metab 1945;5:424–430; doi: 10.1210/jcem-5-10-424
14. Stanley MM, Astwood EB. 1-Methyl-2-mercaptoimidazole; an antithyroid compound highly active in man. Endocrinology 1949;44(6):588; doi: 10.1210/endo-44-6-588
15. Solomon DH, Beck JC, VanderLaan WP, et al. Prognosis of hyperthyroidism treated by antithyroid drugs. J Am Med Assoc 1953;152(3):201–205; doi: 10.1001/jama.1953.03690030001001
16. Williams RH, Asper SP, Rogers WF, et al. Persistence of remissions of thyrotoxicosis after cessation of thiouracil therapy. N Engl J Med 1947;236(20):737–741; doi: 10.1056/NEJM194705152362002
17. Astwood EB. The use of antithyroid drugs during pregnancy. J Clin Endocrinol Metab 1951;11(10):1045–1056; doi: 10.1210/jcem-11-10-1045
18. Hershman JM, Givens JR, Cassidy CE, et al. Long-term outcome of hyperthyroidism treated with antithyroid drugs. J Clin Endocrinol Metab 1966;26(8):803–807; doi: 10.1210/jcem-26-8-803
19. Hershman JM. Ted Astwood's intellectual legacy: Some personal viewpoints. Thyroid 1994;4(3):313–317; doi: 10.1089/thy.1994.4.313
20. Lane L, Cheetham TD, Perros P, et al. New therapeutic horizons for Graves' hyperthyroidism. Endocr Rev 2020; 41(6):873–884; doi:10.1210/endrev/bnaa022
21. Webster B, Chesney A. Endemic goiter in rabbits. I. Incidence and characteristics. Bull Johns Hopkins Hosp 1928;43(5):261–277.
22. Kennedy TH, Purves HD. Studies on experimental goitre. I: The effect of Brassica seed diets on rats. Br J Exp Path 1941;22(5):241–244.
23. Lawson A, Barry G. Treatment of thyrotoxicosis with 2-carbethoxythio-1-methylglyoxaline. Lancet 1951;2(6684): 621; doi: 10.1016/s0140-6736(51)92893-0
24. Cooper DS. The side effects of antithyroid drugs. Endocrinologist 1999;9(6):457–467.
25. Hershman JM, Van Middlesworth L. Effect of antithyroid compounds on the deiodination of thyroxine in the rat. Endocrinology 1962;71:94–100; doi: 10.1210/endo-71-1-94
26. Taurog A, Dorris ML. A reexamination of the proposed inactivation of thyroid peroxidase in the rat thyroid by

propylthiouracil. Endocrinology 1989;124(6):3038–3042; doi: 10.1210/endo-124-6-3038
27. Davidson B, Soodak M, Neary JT, et al. The irreversible inactivation of thyroid peroxidase by methylmercaptoimidazole, thiouracil, and propylthiouracil in vitro and its relationship to in vivo findings. Endocrinology 1978; 103(3):871–882; doi: 10.1210/endo-103-3-871
28. Beck JS, Young RJ, Simpson JG, et al. Lymphoid tissue in the thyroid gland and thymus of patients with primary thyrotoxicosis. Br J Surg 1973;60(10):769–771; doi: 10.1002/bjs.1800601005
29. McGregor AM, Petersen MM, McLachlan SM, et al. Carbimazole and the autoimmune response in Graves' disease. N Engl J Med 1980;303(6):302–307; doi: 10.1056/NEJM 198008073030603
30. Silva JE, Leonard JL, Crantz FR, et al. Evidence for two tissue-specific pathways for in vivo thyroxine 5'-deiodination in the rat. J Clin Invest 1982;69(5):1176–1184; doi: 10.1172/jci110554
31. Di Gianantonio E, Schaefer C, Mastroiacovo PP, et al. Adverse effects of prenatal methimazole exposure. Teratology 2001;64(5):262–266; doi: 10.1002/tera.1072
32. Rivkees SA, Mattison DR. Ending propylthiouracil-induced liver failure in children. N Engl J Med 2009; 360(15):1574–1575; doi: 10.1056/NEJMc0809750
33. Bahn RS, Burch HB, Cooper DS, et al. The role of propylthiouracil in the management of Graves' disease in adults: Report of a meeting jointly sponsored by the American Thyroid Association and the Food and Drug Administration. Thyroid 2009;19(7):673–674; doi:10.1089/thy.2009.0169
34. Andersen SL, Olsen J, Wu CS, et al. Birth defects after early pregnancy use of antithyroid drugs: A Danish nationwide study. J Clin Endocrinol Metab 2013;98(11): 4373–4381; doi:10.1210/jc.2013-2831
35. Azizi F, Ataie L, Hedayati M, et al. Effect of long-term continuous methimazole treatment of hyperthyroidism: Comparison with radioiodine. Eur J Endocrinol 2005; 152(5):695–701; doi:10.1530/eje.1.01904
36. Villagelin D, Romaldini JH, Santos RB, et al. Outcomes in relapsed Graves' disease patients following radioiodine or prolonged low dose of methimazole treatment. Thyroid 2015;25(12):1282–1290; doi:10.1089/thy.2015.0195
37. Leger J, Gelwane G, Kaguelidou F, et al. Positive impact of long-term antithyroid drug treatment on the outcome of children with Graves' disease: National long-term cohort study. J Clin Endocrinol Metab 2012;97(1):110–119; doi: 10.1210/jc.2011-1944
38. Azizi F, Amouzegar A, Tohidi M, et al. Increased remission rates after long-term methimazole therapy in patients with Graves' disease: Results of a randomized clinical trial. Thyroid 2019;29(9):1192–1200; doi:10.1089/thy.2019.0180
39. Chen PL, Shih SR, Wang PW, et al. Genetic determinants of antithyroid drug-induced agranulocytosis by human leukocyte antigen genotyping and genome-wide association study. Nat Commun 2015;6:7633; doi: 10.1038/ncomms 8633
40. Hallberg P, Eriksson N, Ibanez L, et al. Genetic variants associated with antithyroid drug-induced agranulocytosis: A genome-wide association study in a European population. Lancet Diabetes Endocrinol 2016;4(6):507–516; doi: 10.1016/s2213-8587(16)00113-3
41. Neumann S, Nir EA, Eliseeva E, et al. A selective TSH receptor antagonist inhibits stimulation of thyroid function in female mice. Endocrinology 2014;155(1):310–314; doi: 10.1210/en.2013-1835
42. Brix TH, Lund LC, Henriksen DP, et al. Methimazole and risk of acute pancreatitis. Lancet Diabetes Endocrinol 2020; 8(3):187–189; doi:10.1016/s2213-8587(20)30025-5

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