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Long-term outcome after DNA-based prophylactic neck surgery in children at risk of hereditary medullary thyroid cancer

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Advances in sequencing technology, providing unprecedented insights into cancer progression, have shifted the treatment paradigm towards precision medicine for hereditary medullary thyroid cancer (MTC), away from the '*one-size-fits-all*' approach predicated on genetic risk alone.

The DNA-based/biochemical concept, factoring serum calcitonin into the benefit–risk equation, optimizes biochemical cure while minimizing extent of prophylactic surgery and operative morbidity in children at risk.

The transformative effect that has taking effect on medical practice has been impressive: Increasingly earlier molecular diagnosis and more limited prophylactic neck operations yielded excellent clinical outcomes at expert facilities 7–16 years postoperatively: biochemical cure rates approximating 100%; absence of residual structural disease or recurrence; and rarely any permanent operative morbidity.

These excellent results, contingent on proper health care funding and pediatric surgical specialization, make a case for early prophylactic thyroidectomy in experienced hands once calcitonin serum levels exceed the upper normal limit of the assay in young gene carriers.

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Introduction

Rapid adoption of novel technology has revolutionized the practice of medicine. Advances in sequencing technology and analysis are opening an unprecedented glimpse on cancer progression, informing new approaches to diagnosis and treatment across the disease pathway [1]. Hereditary medullary thyroid cancer (MTC), raising genetic, medical and ethical quandaries, is an excellent case in point to illustrate the breathtaking pace and magnitude of the progress made in the field of endocrine surgical oncology.

This incremental progress can be broken down into four successive phases (Table 1):

Morphological era (1860s–1950s): medullary thyroid cancer as tumor entity

In terms of thyroid morphology, '*medullary*' carcinoma of the thyroid was not unknown to pathologists and surgeons in the nineteenth century [2–5]. At the dawn of the twentieth century, Walter Burk reported what seemed to be the first detailed account of this neuroendocrine malignancy [6]. In his doctoral thesis presented in 1901 to the Medical Faculty of the University of Tübingen, Germany, Burk described a 12-year-old boy of '*slender frame*' with 'an '*acute infrasternal angle*' and '*bumpy lips*' who had died of a '*metastatic amyloid tumor of the thyroid gland*'. In hindsight, age, phenotype and extent of metastatic disease pointed to MTC, probably connected to multiple endocrine neoplasia type 2B (MEN 2B), a syndrome not appreciated at the time [7–9].

Another fifty years elapsed before MTC was contradistinguished from follicular cell-derived thyroid cancer, rendering it a tumor entity in its own right [10,11]. Before the description of MTC, patients used to be misdiagnosed with papillary, follicular or anaplastic thyroid cancer. In the absence of modern anatomic imaging modalities, many tumors were clinically diagnosed by digital palpation after having turned symptomatic.

Biochemical era (1960s–1980s): calcitonin screening for hereditary C cell disease

The discovery of the hormone calcitonin in 1962 [12], helping identify parafollicular C (-alcitonin) cells in the thyroid as intrinsic source of calcitonin production [13,14] and allowing the development of

Table 1
Milestones in medullary thyroid cancer research.

Historic timeline	Landmark event
1860s–1900s	Historic morphological era: - First morphologic descriptions of 'medullary' carcinoma of the thyroid - First account of what appears to be an instance of MTC in the context of MEN 2B
1950s	Modern morphological era: - Histopathological characterization of MTC as tumor entity in its own right - First description of MEN 2A
1960s–1980s	Biochemical era: - Biochemical characterization of MTC as a calcitonin-secreting malignancy - Development of the first radioimmunoassay to measure serum calcitonin - Systematic calcitonin screening and early thyroidectomy: proof of concept
1990s–2000s	Molecular-genetic era: - DNA-based screening and therapeutic neck surgery - Genotype-phenotype correlations (based on cross-sectional data)
2010s–date	Molecular-epidemiological era: - Early DNA-based screening and prophylactic thyroidectomy - Pathogenicity of unique RET sequence variants - Quest for precise risk estimates (based on longitudinal data)

MTC: medullary thyroid cancer.
MEN 2: multiple endocrine neoplasia type 2.

radioimmunoassays to measure serum calcitonin [15,16], was instrumental in positioning MTC as a calcitonin-secreting neuroendocrine malignancy.

Early selection of children at risk of hereditary MTC for prophylactic thyroidectomy in the 1970s from families harboring the trait by calcitonin screening reduced primary tumor size from 0.8 to 0.2 cm; decreased the percentage of bilateral neoplasms from 100% to 13%; and diminished the proportion of nodal metastases from 58% to 0% [17]. Because affected children remained free from MTC over a mean of 11 years after thyroidectomy, biochemical screening and early thyroidectomy were thought to have prevented metastasis and death from hereditary MTC [18].

Hereditary, unlike sporadic, MTC can be associated with pheochromocytoma and parathyroid hyperplasia or adenomas (primary hyperparathyroidism), all of which are subsumed under the umbrella term of multiple endocrine neoplasia type 2A (MEN 2A).

Molecular genetic era (1990s–2000s): DNA-based screening and therapeutic neck surgery

With increasing sophistication of molecular genetic technology, the *RET* (*RE*arranged during *T*ransfection) proto-oncogene was identified in 1993 on chromosome 10q11.2 as the susceptibility gene of hereditary MTC [19,20]. By the end of the twentieth century, the most common pathogenic RET sequence variants, hereinafter referred to as mutations, had been identified:

- p.Met918Thr, underlying MEN 2B,
- p.Cys634X, underlying almost always MEN 2A,
- p.Cys630X, p.Cys620X, p.Cys618X, p.Cys611X, and p.Cys609X, underlying hereditary MTC and infrequently MEN 2A, and
- p.Glu768Asp, p.Leu790Phe, p.Val804Leu, p.Val804Met, and p.Ser891Ala, underlying hereditary MTC

Using readily available cross-sectional data, first genotype-phenotype correlations were established based on admixtures of index and non-index patients subject to selection bias. Inclusion of index cases, however, invariably results in upwards bias of penetrance estimates. More common RET sequence variants, including p.G691S, p.L769L, p.S836S and p.S904S which affect >1% of the general population, were assigned polymorphism status from inception [21].

These seminal discoveries paved the way for prophylactic surgery based on a positive *RET* gene test alone, with the intent of eliminating the risk of MTC once and for all by removing the thyroid gland [22–26]. Conventionally, the term “prophylactic” is used to denote thyroidectomy before MTC develops or while it is clinically unapparent and confined to the thyroid gland [27].

Molecular epidemiological era (2010s–date): the quest for precise risk estimates

Genetic association studies typically are enriched with advanced disease, reflecting the researchers’ need for unambiguous cases with the clinical disease of interest [28]. As a result, more penetrant mutations were overrepresented in earlier clinical research, exaggerating the risk of hereditary MTC. These inflated risk estimates precipitated advocacy of aggressive surgical interventions for asymptomatic non-index patients. Previous research was also compounded by the adoption of inconsistent genetic terminology [29]. Misguided in retrospect was the classification of RET sequence variants as pathogenic mutations. According to one common, though confusing definition, sequence variants are defined as polymorphisms just because they involve $\geq$1% of the general population [29]. This resulted, until recently, in misclassification of three RET sequence variants as causative mutations, p.S649L, p.Y791F, and p.I852M, which affect 0.7% [30], 0.31% to 0.6–0.9% [30,31], and 0.015% [32] of the general population. In good keeping, none of these RET sequence variants exhibited the age-dependent progression typical of hereditary MTC [33].

Precision medicine is predicated on the principle that the intervention must be commensurate with the disease or risk at hand, with minimum ill consequences and maximum efficacy [34]. Unbiased and precise estimates of genetic risk, which are not easy to obtain and need to be interpreted in light of the individual calcitonin serum level, are at the heart of personalized medicine. In the genomic era, there is

a dire need for high-quality outcome data, deriving from prospective observation of cohorts of carriers over decades (longitudinal data) for each unique RET sequence variant.

The present review was undertaken to deduce more precise risk estimates from the body of published molecular and clinical evidence as a basis for personalized medicine. Particular emphasis will be laid on long-term surgical and oncologic outcome after DNA-based prophylactic neck surgery, a concept introduced into clinical practice some 25 years ago.

Methods/evidence synthesis

Literature search criteria

An electronic Medline search of the international literature was performed with a cutoff of March 31, 2019. Key words included pathogenic RET sequence variants (henceforth referred to as RET mutations) in codons 533, 609, 611, 618, 620, 630, 634, 768, 790, 804, 883, 891, and 918, 'medullary thyroid cancer/carcinoma', 'thyroidectomy', 'multiple endocrine neoplasia type 2' or 'MEN 2', MEN 2A, or 'MEN 2B'. A diagnosis of MTC typically requires evidence of tumor extension beyond the basement membrane, demonstration of lymphatic or vascular invasion or both on histopathological examination.

To determine the clinical relevance ('pathogenicity') of other unique RET sequence variants, the web-based ARUP MEN2 database (http://www.arup.utah.edu/database/MEN2/MEN2_display.php) was questioned [21]. To qualify as mutations, unique RET sequence variants needed to have documented proof of at least two affected kindreds with MTC from the same family. This conventional genetic requirement removed three RET sequence variants, p.S649L, p.Y791F, and p.I852M, affecting fewer than 1% of the general population, from the list of RET mutations [30–33].

The terms of this literature search were identified in the title, abstract, or medical subject heading. Not considered for the avoidance of duplication were earlier reports from groups that updated previously published information in a subsequent, more comprehensive report. Pertinent publications were reviewed further, and data elements were extracted in structured format as appropriate.

Objectives of the review

The present review was devised to characterize unique RET sequence variants regarding:

- Relative frequency in the general population (with differences in frequencies evaluated with the two-tailed Fisher's exact test as appropriate),
- Pathogenicity with a focus on age-dependent progression to MTC (biological risk), and
- Long-term clinical outcome, taking into account the child's age, serum calcitonin level, and the extent of neck surgery (surgical and oncologic benefit and risk)

Results

Targeting pathogenic unique RET sequence variants

An estimated 25–30% of patients with MTC reveal germline mutations in the *RET* proto-oncogene. Table 2 [33,35–38] gives an overview of established pathogenic RET sequence variants [21] running in Italy, France and Germany, including relative mutational frequencies and evidence of at least two patients with MTC in the same family.

Based on 1190 RET carriers, the most common RET mutations in Western Europe are, in descending order:

- p.Val804Met (10.0–20.9%),
- p.Cys634Arg (12.4–15.8%),

Table 2
Relative frequency of pathogenic RET sequence variants[a] in Western Europe.

ATA risk category	Unique RET sequence variant		Relative frequency, n (%)		
	Protein change	Genotype (cDNA)	Italy (234 carriers)	France (426 carriers)	Germany (530 carriers)
Highest (ATA HST)	p.Met918Thr	c.2753T > C	17 (7.3)	30 (7.0)	39 (7.4)
	p.Ala883Phe[b]	c.2647_2648delGCinsTT	0	2 (0.5)	0
High (ATA H)	p.Cys634Arg	c.1900T > C	34 (14.5)	53 (12.4)	84 (15.8)
	p.Cys634Gly	c.1900T > G	6 (2.6)	7 (1.6)	10 (1.9)
	p.Cys634Lys	c.1901_1902delinsTG	0	2 (0.5)	0
	p.Cys634Phe	c.1901G > T	7 (3.0)	9 (2.1)	22 (4.2)
	p.Cys634Ser	c.1900T > A	6 (2.6)	12 (2.8)	0
	p.Cys634Ser	c.1901G > C		3 (0.7)	15 (2.8)
	p.Cys634Trp	c.1902C > G	4 (1.7)	12 (2.8)	1 (0.2)
	p.Cys634Tyr	c.1901G > A	28 (12.0)	46 (10.8)	52 (9.8)
Moderate (ATA MOD); extracellular	p.Cys515Ser	c.1544_1545delinsCT	1 (0.4)	0	0
	p.Gly533Cys	c.1597G > T	0	3 (0.7)	0
	p.Cys609Arg	c.1825T > C	1 (0.4)	0	0
	p.Cys609Gly	c.1825T > G	1 (0.4)	0	7 (1.3)
	p.Cys609Phe	c.1826G > T	1 (0.4)	1 (0.2)	0
	p.Cys609Ser	c.1825T > A	1 (0.4)	0	0
	p.Cys609Trp	c.1827C > G	0	1 (0.2)	0
	p.Cys609Tyr	c.1826G > A	2 (0.9)	3 (0.7)	0
	p.Cys611Gly	c.1831T > G	1 (0.4)	2 (0.5)	0
	p.Cys611Phe	c.1832G > T	0	0	17 (3.2)
	p.Cys611Phe	c.1832_1833delinsTT	0	0	6 (1.1)
	p.Cys611Trp	c.1833C > G	0	3 (0.7)	0
	p.Cys611Tyr	c.1832G > A	0	7 (1.6)	19 (3.6)
	p.Cys618Arg	c.1852T > C	5 (2.1)	6 (1.4)	6 (1.1)
	p.Cys618Gly	c.1852T > G	2 (0.9)	0	7 (1.3)
	p.Cys618Phe	c.1853G > T	0	1 (0.2)	10 (1.9)
	p.Cys618Ser	c.1852T > A	6 (2.6)	12 (2.8)	22 (4.2)
	p.Cys618Ser	c.1853G > C		8 (1.9)	6 (1.1)
	p.Cys618Trp	c.1854C > G	0	1 (0.2)	0
	p.Cys618Tyr	c.1853G > A	2 (0.9)	1 (0.2)	2 (0.4)
	p.Cys620Arg	c.1858T > C	4 (1.7)	10 (2.3)	18 (3.4)
	p.Cys620Gly	c.1858T > G	1 (0.4)	5 (1.2)	0
	p.Cys620Phe	c.1859G > T	1 (0.4)	3 (0.7)	5 (0.9)
	p.Cys620Ser	c.1858T > A	3 (1.3)	0	7 (1.3)
	p.Cys620Ser	c.1859G > C		1 (0.2)	2 (0.4)
	p.Cys620Trp	c.1860C > G	0	4 (0.9)	0

(*continued on next page*)

Table 2 (*continued*)

ATA risk category	Unique RET sequence variant		Relative frequency, n (%)		
	Protein change	Genotype (cDNA)	Italy (234 carriers)	France (426 carriers)	Germany (530 carriers)
Moderate (ATA MOD); intracellular	p.Cys620Tyr	c.1859G > A	0	8 (1.9)	6 (1.1)
	p.Cys630Arg	c.1888T > C	1 (0.4)	1 (0.2)	4 (0.8)
	p.Cys630Tyr	c.1889G > A	3 (1.3)	0	0
	p.Lys666Glu	c.1996A > G	0	1 (0.2)	0
	p.Glu768Asp	c.2304G > C	9 (3.8)	10 (2.3)	10 (1.9)
	p.Leu790Phe	c.2370G > C	8 (3.4) [$P_{\text{vs. Germany}} \leq 0.002$]	5 (1.2)	9 (1.7)
	p.Leu790Phe	c.2370G > T		38 (8.9) [$P_{\text{vs. Italy}} \leq 0.010$]	52 (9.8)
	p.Val804Leu	c.2410G > T	3 (1.3)	9 (2.1)	14 (2.6)
	p.Val804Met	c.2410G > A	49 (20.9) [$P_{\text{vs. Germany}} < 0.001$]	83 (19.5) [$P_{\text{vs. Germany}} < 0.001$]	53 (10.0)
	p.Ala883Thr[c]	c.2647G > A	1 (0.4)	1 (0.2)	0
	p.Ser891Ala	c.2671T > G	23 (9.8) [$P_{\text{vs. Germany}} = 0.009$]	20 (4.7) [$P_{\text{vs. Italy}} = 0.013$]	25 (4.7)
	p.Ser904Phe	c.2711C > T	1 (0.4)	0	0
	p.Arg912Pro	c.2735G > C	0	1 (0.2)	0
	p.Met918Val	c.2752A > G	2 (0.9)	1 (0.2)	0

ATA, American Thyroid Association [27]; RET, *RE*arranged during *T*ransfection; *P* values are based on a two-tailed Fisher's exact test.
Owing to rounding, not all percentages add up.

[a] Pathogenicity of unique sequence variants listed on the ARUP MEN2 database with evidence of at least 2 patients with medullary thyroid cancer in the same family (database accessible at: http://www.arup.utah.edu/database/MEN2/MEN2_display.php) [21].

[b] p.Ala883Phe may warrant re-classification into the high risk (ATA H) category [37].

[c] p.Ala883Thr may be pathogenic only in homozygous condition [38].

- p.Cys634Tyr (9.8–12.0%),
- p.Leu790Phe (3.4–11.5%), and
- p.Met918Thr (7.0–7.4%)

All other *RET* germline mutations, accounting for <5% each, are infrequent.

Italy, France and Germany have comprehensive DNA-based screening programs for MTC in place [35,36,39]. This is why the Italian, French and German data agree surprisingly well with one other.

Intriguingly, the p.Val804Met mutation (20.9% in Italy and 19.5% in France versus 10.0% in Germany; both $P < 0.001$; Table 2) has been linked to Roman ancestral heritage, whereas p.Leu790Phe (c. 2370G > T) (≤3.4% in Italy vs. 8.9% in France and 9.8% in Germany; $P \leq 0.010$ and $P \leq 0.002$) may be related to Germanic ancestral heritage [40]. More frequent in Italy is p.Ser891Ala (9.8% vs. 4.7% in both France and Germany; $P = 0.013$ and $P = 0.009$, respectively; Table 2). This finding is apparently due to a founder effect in an isolated mountainous region in Northern Italy [41].

It is also worthwhile to note that p.Cys634Arg was 1.2-fold (34 vs. 28 carriers and 53 vs. 46 carriers) and 1.6-fold (84 vs. 52 carriers) more prevalent that p.Cys634Tyr in the Italian, French, and German data (Table 2). The converse (26 vs. 173 carriers, and 74 vs. 166 carriers) has been reported from the Spanish Online National Database [42] and the Brazilian BrasMEN Study [43].

Barring rare spontaneous mutations [44], many RET mutations are being transmitted to future generations. This is why these causative RET mutations, unlike RET polymorphisms such as p.G691S, p.L769L, p.S836S and p.S904S, have become the target of DNA-based screening programs for hereditary C cell disease.

A common presumption of the benefit of screening programs is that early detection of disease, before it produces complaints, leads to better clinical outcomes because therapy for asymptomatic disease is more effective than therapy for symptomatic disease. To be eligible for screening, the diagnostic test should be accurate and easy to interpret (*RET* gene analysis is exact and straightforward); the disease should be serious and highly penetrant (such as MTC), and therapy initiated before complaints emerge (such as prophylactic thyroidectomy) should diminish morbidity or mortality more than therapy started after complaints have appeared. The implications of not spotting disease in an asymptomatic stage must be so grave as to warrant the effort and burden of screening. The expenditure associated with screening must be in proportion to the anticipated benefit of eliminating the disease (e.g., by prophylactic thyroidectomy) and/or mitigating disease-related health risks (e.g., maintenance of loco-regional control if the disease cannot be surgically cured).

Genetically encoded progression of medullary thyroid cancer

The constitutive activation of the mutated RET receptor protein ('*primary hit*') is believed to produce neoplastic hyperplasia of the neuroendocrine C cells embedded in the thyroid parenchyma [45]. Secondary somatic mutations ('*second hits*') are thought to give rise to hereditary MTC. Acquisition of these secondary mutational events may reflect the play of chance, so that the development of MTC is variable even within the same family [28].

The age-dependent progression from neoplastic C cell hyperplasia to node-negative MTC, and ultimately to node-positive MTC can be grouped according to genetic risk, as detailed in Table 3. Remarkably, the speed of MTC development and progression differs significantly across and within highest risk (ATA category HST) mutations; high risk (ATA category H) mutations; and moderate risk (ATA category MOD) mutations [27]. To better reflect the clinical situation (Table 3), the moderate risk group (ATA category MOD) has been subdivided into moderate–high (MOD–H) and low–moderate (L–MOD) risk groups in their own right [33].

There are additional, more subtle codon-specific differences among moderate risk mutations, subject to the mutational position on the *RET* receptor: faster progression to MTC when the mutation resides in the extracellular cysteine-rich domain, in particular closer to the cell membrane [46,47]; slower progression to MTC when the mutation lodges in one of the two the intracellular tyrosine kinase domains [45,48]. These genotype-phenotype associations lay the groundwork for precision medicine in infants and children harboring the trait.

Table 3
ATA risk category-specific and codon-specific progression of medullary thyroid cancer[a].

Mutational risk		No of carriers	Carrier age at thyroidectomy, yr, mean [95% CI], median (range)[b]		
ATA risk category	RET protein change(s)[c]		Normal and C cell hyperplasia (CCH)	MTC, node-negative (MTCN0)	MTC, node-positive (MTCN1)
Highest (ATA HST)[b]	p.Met918Thr	39 (1/13/25)	**0.5**	**6.2 [0.7;11.7] (0.5–31)**	**16.2 [13.1;19.3] (5–31)**
High (ATA H)[b]	p.Cys634X	184 (42/88/54)	**4.9 [3.9;5.8] (1–14)**	**19.0 [15.8;22.2] (1–69)**	**31.1 [27.8;34.4] (9–63)**
Moderate (ATA MOD)	p.Cys611X	42 (14/17/11)	15.1 [7.6; 22.7] (3–47)	40.1 [33.5; 46.7] (14–63)	50.0 [41.0; 59.0] (31–75)
	p.Cys618X	53 (14/15/24)	11.1 [5.0; 17.2] (4–45)	26.5 [16.2; 36.9] (5–67)	40.5 [34.1; 46.9] (17–70)
	p.Cys620X	38 (18/10/10)	8.1 [4.7; 11.5] (0.9–29)	33.1 [25.6; 40.6] (18–54)	41.1 [29.8; 52.4] (21–66)
	Moderate–High (extracellular)[b]	144 (49/47/48)	**10.7 [7.8;13.6] (0.9–47)**	**33.0 [28.2;37.9] (1–67)**	**41.8 [37.3;46.3] (15–75)**
	p.Glu768Asp	10 (4/4/2)	13.5 [0; 36.6] (3–35)	48.0 [24.9; 71.1] (29–64)	71.0 [32.9; 109.1] (68–74)
	p.Leu790Phe	61 (19/20/22)	21.2 [13.7; 28.8] (5–53)	45.2 [36.6; 53.7] (12–75)	54.0 [46.6; 61.4] (10–81)
	p.Val804Leu/Met	67 (30/22/15)	27.7 [21.8; 33.6] (6–66)	44.4 [38.4; 50.3] (21–66)	51.9 [44.1; 59.7] (23–75)
	p.Ser891Ala	25 (10/9/6)	14.4 [7.4; 21.4] (4–34)	39.8 [27.2; 52.4] (17–68)	62.5 [54.1; 70.9] (55–78)
	Low–Moderate (intracellular)[b]	163 (63/55/45)	**22.7 [18.8;26.7] (3–66)**	**44.2 [39.9;48.4] (12–75)**	**55.2 [50.7;59.7] (10–81)**

ATA: American Thyroid Association [27]; RET: *RE*arranged during *T*ransfection.
[a] Based on Machens et al. 2018 [33].
[b] All differences between bolded age groups are statistically significant, both within ($P \leq 0.023$) and across ($P \leq 0.001$) columns.
[c] Minimum of 10 carriers per group.

Personalizing prediction of malignant transformation

DNA-based screening discloses a person's genetic susceptibility to hereditary MTC before neoplastic C cell disease comes into being. The close genotype–phenotype relationship provides for a strong molecular structure within which C cell hyperplasia transforms into early MTC [33,49,50]. Molecular information alone cannot predict when precisely this malignant transformation is going to take place at the individual level. Biochemical screening, being superior to neck ultrasonography [51], is ideally positioned to fill this void, narrowing down an operative '*window of opportunity*'. When basal calcitonin serum levels remain within normal limits, MTC is not present [49,52]. When these levels climb above the upper normal limit of the calcitonin assay, typically <10 pg/ml for children aged 2 years and older [53], malignant transformation to MTC is imminent or may just have occurred. This biochemical threshold marks the last opportunity for prophylactic thyroidectomy without need for concomitant central node dissection.

Hereditary MTC is estimated to grow annually by 0.4–0.5 mm (node-negative MTC) and 1.2–2.6 mm (node-positive MTC) [54]. This slow tumor growth affords some leeway in children whose basal calcitonin levels remain within normal limits. As long as basal calcitonin serum levels remain ≤30 pg/ml, node metastases are absent [55]. This allows one, navigating between these biochemical landmarks, to delay, but not avoid, prophylactic thyroidectomy for some time.

Long-term clinical outcome after DNA-based neck surgery

Surgery remains the one and only curative treatment for MTC. To keep surgical morbidity to a minimum, the inferior parathyroid glands and recurrent laryngeal nerves are identified and preserved in situ before dissecting the thyroid gland off its bed, keeping the line of dissection as close as possible to the thyroid capsule. Very useful in this endeavor are optical magnification and bipolar forceps coagulation [56], nerve monitoring devices [57], and in situ preservation of parathyroid glands or autografting of parathyroid glands that have become completely devascularized. Continuous intraoperative nerve monitoring heralds impending nerve injury, typically due to traction on the nerve, prompting the surgeon to initiate corrective action by reversing the causative maneuver [57].

Before devising surgical treatment plans, the oncologic benefits to be expected from the operation need to be weighed against the operative risks. The net benefit of DNA-based prophylactic neck surgery for hereditary C cell disease is a continuous function of (i) the risk of oncologic morbidity if left untreated (which is hard to predict); (ii) the treatment's relative risk reduction (surgical cure, likely to be high); and (iii) the treatment's risk of harm (operative morbidity, likely to be low in experienced hands). The clinical outcome of any operation for MTC hinges on the completeness of neck surgery, specifically on '*what has been left behind*'. A neck operation for MTC generally is considered successful when raised preoperative calcitonin levels become unmeasurable postoperatively [58]. In infants and young children who have normal preoperative calcitonin serum levels at the time of prophylactic thyroidectomy, the adequacy of the operation is more difficult to evaluate. From a societal perspective, the one-time cost of reaching definitive cure and the cost of daily thyroxine supplementation may be smaller than the need for continual biochemical follow-up and imaging studies, some of which may prompt further operations at additional cost.

The advent of the molecular era some 25 years ago has augmented tumor lead time, which is defined as the length of time between tumor detection by screening and the usual clinical manifestation of the disease. DNA-based screening enabled truly prophylactic thyroidectomies in infants and young children who still have normal thyroid glands or neoplastic C cell hyperplasia only (Table 4) [26,50,59]. The goal of prophylactic neck surgery is not so much to prevent malignancy from occurring in the first place but to take out the thyroid gland before the disease can spread beyond the thyroid capsule, making it more difficult to clear.

Total thyroidectomy once and for all removes the risk of malignant transformation inherited by each thyroid C cell while sparing the child the excess morbidity from central node dissection, in particular postoperative hypoparathyroidism [50,60]. In experienced hands [50,59,60], children aged 3 years or younger, despite having more delicate anatomical structures in a shorter neck, have no greater operative morbidity than children older than 3 years of age (Table 5) [50,59–61]. In infants and young

Table 4
Age-related penetrance of medullary thyroid cancer in children who underwent prophylactic surgery[a] (literature review).

ATA risk category	Protein change(s)	Reference	Children with medullary thyroid cancer, n (%)					Biochemical cure, n (%)
			Age at thyroidectomy, y					
			≤3	4–6	7–12	13–18	Total	Total
Highest (ATA HST)	p.M918T	Machens et al. 2018 [50]	5 of 6 (83)	–	–	–	5 of 6 (83)	5 of 6 (83)
High (ATA H)	p.C634X	Skinner et al. 2005 [26]	–	6 of 9 (66)	8 of 8 (100)	1 of 1 (100)	15 of 18 (83)	16 of 18 (89)
		Machens et al. 2018 [50]	6 of 20 (30)	16 of 36 (44)	11 of 16 (69)	0 of 1	33 of 73 (45)	73 of 73 (100)
	p.C634Y	Febrero et al. 2019 [59]	–	4 of 17 (24)	12 of 20 (60)	5 of 6 (83)	21 of 43 (49)	43 of 43 (100)
Moderate (ATA MOD)	[b]	Skinner et al. 2005 [26]	0 of 1 (0)	1 of 7 (14)	4 of 8 (50)	10 of 13 (77)	13 of 23 (57)	26 of 29 (90)
		Machens et al. 2018 [50]	1 of 9 (11)	1 of 26 (4)	3 of 26 (12)	7 of 16 (44)	12 of 77 (16)	77 of 77 (100)

ATA: American Thyroid Association [27]; RET: *RE*arranged during *T*ransfection.
[a] Absence of clinical and radiological disease.
[b] p.C609X, p.C611X, p.C618X, p.C620X p.C630X, p.Glu768Asp, p.Leu790Phe, p.Val804Leu, p.Val804Met, or p.Ser891Ala.

Table 5
Age-related incidence of complications after thyroidectomy in children (literature review).

Complication	Central node dissection	Reference	Children with complication after total thyroidectomy, n (%)				
			Age at thyroidectomy, y				
			<3 [61] ≤3 [50,59]	3–6 [61] 4–6 [50,59]	7–12	13–18	Total
Transient RLN palsy	Not performed	Kluijfhout et al. 2015 [61]	1 of 9 (11)	0 of 15 (0)	N/A	N/A	1 of 20 (5)
	Not performed	Machens et al. 2018 [50]	0 of 25 (0)	0 of 48 (0)	0 of 26 (0)	0 of 10 (0)	0 of 109 (0)
	Performed	Machens et al. 2018 [50]	1 of 10 (10)	0 of 16 (0)	1 of 23 (4)	1 of 9 (11)	3 of 58 (5)
Permanent RLN palsy	Not performed	Kluijfhout et al. 2015 [61]	1 of 9 (11)	0 of 15 (0)	N/A	N/A	0 of 20 (0)
	Not performed	Machens et al. 2018 [50]	0 of 25 (0)	0 of 48 (0)	0 of 26 (0)	0 of 10 (0)	0 of 109 (0)
	Not performed	Febrero et al. 2019 [59]	–	0 of 15 (0)	0 of 12 (0)	–	0 of 27 (0)
	Performed	Machens et al. 2018 [50]	0 of 10 (0)	0 of 16 (0)	0 of 23 (0)	0 of 9 (11)	0 of 58 (0)
	Performed	Febrero et al. 2019 [59]	–	0 of 2 (0)	0 of 8 (0)	0 of 6 (0)	0 of 16 (0)
Transient hypoparathyroidism	Not performed	Kluijfhout et al. 2015 [61]	6 of 9 (67)	7 of 15 (47)	N/A	N/A	8 of 20 (40)
	Not performed	Machens et al. 2018 [50]	1 of 25 (4)	10 of 48 (21)	5 of 26 (19)	3 of 10 (30)	19 of 109 (17.4)
	Performed	Machens et al. 2018 [50]	0 of 10 (0)	1 of 16 (6)	8 of 23 (35)	3 of 9 (33)	12 of 58 (21)
Permanent hypoparathyroidism	Not performed	Kluijfhout et al. 2015 [61]	2 of 9 (22)	2 of 15 (13)	N/A	N/A	5 of 20 (25)
	Not performed	Machens et al. 2018 [50]	0 of 25 (0)	0 of 48 (0)	0 of 26 (0)	0 of 10 (0)	0 of 109 (0)
	Not performed	Febrero et al. 2019 [59]	–	0 of 15 (0)	0 of 12 (0)	–	0 of 27 (0)
	Performed	Machens et al. 2018 [50]	0 of 10 (0)	0 of 16 (0)	0 of 23 (0)	0 of 9 (0)	0 of 98 (0)
	Performed	Febrero et al. 2019 [59]	–	0 of 2 (0)	0 of 8 (0)	0 of 6 (0)	0 of 16 (0)

N/A: not available.
RLN: recurrent laryngeal nerve.

children, parathyroid glands are small, translucent, and hard to distinguish from adjacent soft tissues, thymus, and neck nodes. Besides, the thymus of infants and young children can take the size of a normal thyroid gland, diminishing surgical exposure further [50]. When children were older at prophylactic thyroidectomy (10 years versus 6.1 years), only 44 (88%) of 50 children [26], as compared to 114 (99.1%) of 115 children [50], were biochemically cured. The low rates of transient and permanent recurrent laryngeal nerve palsies and postoperative hypoparathyroidism in experienced hands (Table 5) and the absence of residual structural disease or recurrence 7 years (mean; range 1 month–21 years) [50] and 16 years (mean ± 6 years; range 1–24 years) [59] after prophylactic neck surgery make a case for early prophylactic thyroidectomy in affected infants and small children once calcitonin serum levels exceed the upper normal limit of the calcitonin assay.

Time to calcitonin normalization depends a great deal on the level of serum calcitonin before the operation, in particular in patients with node-negative MTC [62]. In node-positive patients, time to calcitonin normalization is also determined by the number of node metastases because calcitonin-enriched lymphatic fluid needs to proceed towards the loco-regional venous system before emptying into the systemic circulation. Postoperative serum calcitonin typically normalizes within one week; and within a fortnight in patients with node-positive MTC and preoperative serum calcitonin levels of 500–1000 pg/ml [62].

Discussion

The future direction of healthcare in the genomic era is clear. At one end, there is an emphasis on cost-effective improvements to population health and, at the other, increasing personalization of diagnosis and treatment [1]. Less obvious is the transformative effect that has been, and currently is, taking effect on medical practice, surgical decision-making and patient outcomes.

Rare diseases pose specific challenges in providing the medical community with evidence-based treatment guidelines based on high quality research with long-term follow-up. An important consideration in judging the benefits-to-harms tradeoff for a surgical intervention is that many of the harms are front-loaded, occurring much earlier than the benefits, which are delayed by years. This raises the question of how long one must wait to realize the benefits to counter-balance the harm.

The present review found that early genetic diagnosis and early prophylactic neck surgery for hereditary C cell disease in experienced hands were associated with excellent results 7–16 years after prophylactic neck surgery [50,59]: biochemical cure rates approximating 100%; absence of residual structural disease or recurrence; and rarely, if ever, any permanent operative morbidity.

Because most of the research was undertaken at tertiary referral centers in Western Europe, it is not immediately apparent if, and to what extent, the present findings (distribution of pathogenic RET sequence variants and clinical outcomes) may apply to other populations.

Prevalence of pathogenic RET sequence variants across geographies

Molecular epidemiology, examining the contribution of genetic factors to the etiology, distribution, and prevention of disease, relies on population-based molecular, histopathological and clinical outcome data. No population-wide national cohort satisfies all these requirements. Even under quasi-ideal conditions, subjects carrying RET mutations with low transforming activity are unlikely to be captured. Many national health care systems do not routinely cover DNA-based testing of patients upon diagnosis of MTC in the absence of a pertinent family history. As a natural consequence, less penetrant RET mutations, specifically p.Glu768Asp, p.Leu790Phe, p.Val804Leu, p.Val804Met, and p.Ser891Ala, are under-recognized in many parts of the world [39].

Coming close to the ideal state is the TenGen network affiliated with the French Endocrine Society [36]. Another valuable resource is the German center of expertise cohort, covering at an estimated one-half of the German RET population [33,39]. Perhaps the most reliable population-based estimates of age-standardized incidence (0.06 per 100 000 per year) and prevalence (1.3 per 100 000 for hereditary MTC) derive from the Danish RET cohort constructed with the support of the Danish Civil Registration

System and the Danish National Archives, the Danish Thyroid Cancer Database, the Danish Cancer Registry and the Danish Pathology Register [63].

Recently, Loveday et al., drawing on a convenience sample of 51 000 patients with complex disease other than cancer from the Exome Aggregation Consortium (ExAC) database, estimated the penetrance of the pathogenic RET sequence variant p.Val804Met at 46% [64,65]. This estimate matched up remarkably well with the corresponding estimate of 51% from the German center of expertise cohort, in which 27 of 53 carriers thyroidectomized had developed MTC [33,66]. This complementary research illustrates the importance of approaching complex genetic association topics from various angles, particularly by tapping into large genomic databases.

The almost perfect agreement of the Italian, French and German RET data (Table 2) is best explained with the Hardy–Weinberg principle, which states that allele frequencies remain constant from one generation to the next within sufficiently large populations unless the equilibrium is disturbed by migration, genetic mutations, or selection.

Some pathogenic RET sequence variants, by means of pedigree linkage, were shown to have been transmitted for centuries to offspring, dating back to at least:

- 1896 in Sweden: p.Cys618Ser (c.1853G > C) [67].
- 1876 in Germany: p.Cys611Phe (c.1832G > T) [68].
- 1865 in Germany: p.Cys634Trp (c.1902C > G) [69].
- 1493 in Italy: p.Ser891Ala (c.2671T > G) [41].

De novo germline mutations affect >90% of all p.Met918Thr carriers [70–72], so that the Italian, French and German data may approximate the spontaneous mutational rate.

Recent ethnographic fieldwork and haplotype analysis unearthed rare '*founder*' mutations:

- p.Gly533Cys (c.1597G > T), clustering in the southern part of central Greece (Fokis and around Lake Trichonis; 10 families) and in the Peleponnese (Laconia, mount Parnon region, and Arcadia; 11 families) [73].
- p.Gly533Cys (c.1597G > T), which migrated from Spain (Catalunia region) to Southeastern Brazil in the late 1800s and is thought to have originated from Greece [74].
- p.Cys611Tyr (c.1832G > A), clustering in Denmark [75], possibly inflating Danish RET incidence and prevalence estimates [76].
- p.Cys618Arg (c.1852T > C), clustering in Cyprus (5 of 9 families were from the same village in the Northwestern part of the province of Limassol) [77].
- p.Ser891Ala (c.2671T > G), clustering in Northern Italy (28 genetically related families), namely in Castione della Presolana in the mountainous Seriana valley, province of Bergamo, and the plain of Breccia; migration from the former to the latter has been documented since 1630 [41].
- p.Met918Val (c.2752A > G), which migrated from Portugal (city of Braga) to the state of Ceará in Northeastern Brazil (currently 9 families) in the 1600s [78,79].

Such random samples of RET alleles from geographic isolates or newly formed colonies, reflecting disparate colonization patterns, can grossly misrepresent the original populations.

Altogether, the Italian, French and German RET data (Tables 2–4), in all likelihood, represent the European population fairly well. More research is needed to confirm the purported generalizability of the European genetic data to non-European populations or populations with complex recent ancestral histories.

Effectiveness of DNA-based screening and prophylactic thyroidectomy

In Germany where biochemical and DNA-based screening and prophylactic thyroidectomy have been implemented free of charge to patients from the very beginning, the therapeutic effectiveness of DNA-based screening for control of MTC at the population level has been amazing [80]:

For high-risk mutations (ATA category H; 'classic' MEN2A), a decline since 1963 in the proportion of:

- Index patients among all carriers from 50 to 16%,
- MTC among non-index patients from 100 to 28%,
- Node-positive MTC from 100 to 0%,

whereas biochemical cure increased from 0 to 100%.

For moderate risk mutations (ATA category MOD; 'incomplete' MEN2A), a fall since 1995 in the proportion of:

- Index patients among all carriers from 100% to 29–31%,
- MTC among non-index patients from 48–81% to 19–60%,
- Node-positive MTC from 67 to 33% (cysteine codons 609–630) and 11 to 10% (non-cysteine codons 768–891),

whereas biochemical cure increased from 71 to 78% (cysteine codons 609–630) and 95–100% (non-cysteine codons 768–891).

Based on data from the French MEN2 registry [44], RET mutations can arise *de novo* in the parental germline, replenishing the pool of index patients that screening programs seek to deplete. These opposing effects may make it unfeasible to diminish the percentage of index patients below the spontaneous mutation rate of 5.6–9.1%.

Overcoming barriers in clinical practice

In our mobile society, different service providers attend to the same person at various stages in the disease process so that medical information has become fragmented. After patients have moved from the area, their former caregivers may not know their current whereabouts. Disintegration of familial relationships is another issue. Patients may also not be prepared to disclose confidential genetic information to their next of kin. Such refusal can delay recognition of a hereditable condition in other branches of the family for some time [81]. Potential reasons include repression, feelings of guilt and resentment, emotional distress, and poor familial interaction [82,83]. Other hurdles to overcome are covert social and ethical barriers to genetic screening for MTC, which include literacy, education, income, culture/religion, social/family relationships, inadequate decision-making capacity, and the perceived stigma of genetic testing [84,85].

Data privacy and confidentiality rules also raise significant barriers to the release of genetic information, which almost always has implications for close relatives who may share genes. The *RET* gene test definitely confirms, or rules out, a patient's genetic predisposition to hereditary MTC, unless samples have been mixed up. This is particularly relevant to adopted children or broken families in whom information about the history of the origin family is sketchy or unavailable.

Geographic dispersion can frustrate screening efforts in a patient's next of kin: in one instance, only 22 (26%) of 86 members of a large French p.Leu790Phe (c.2370G > T) family consented to screening [86]. Equally poor was utilization of DNA-based testing in a Kaiser Permanente Medical Group outreach project to previously untested patients with MTC, with no more than 8 (25%) of 32 eligible patients showing for their RET test appointment [87]. Performing *RET* gene analysis at the time of diagnosis thus may be more effective than attempting to contact patients after they have completed diagnostic work-up and treatment. Outreach to underprivileged patients who live in rural areas and cannot afford the cost of traveling across large geographic distances, as in the state of Ceará in Northwestern Brazil [79], can be even more of a challenge.

Precision medicine at times can leave caretakers and patients at a loss for how to deal with infrequent RET sequence variants of unknown significance, which do not segregate with the disease. Under such circumstances, balancing respect for individual autonomy and parental rights with the best interest of the child, expectant observation with continual monitoring of serum calcitonin levels can be a viable option for physicians, children and their parents.

Summary

The treatment paradigm for MTC has moved away from a '*one-size-fits-all*' approach predicated on genetic risk alone towards precision medicine. The DNA-based/biochemical concept strikes a perfect balance between oncologic and surgical benefit and risk in experienced hands. Factoring mutational class, age and serum calcitonin level into the benefit—risk equation optimizes a child's prospects of biochemical cure while minimizing extent of prophylactic surgery and operative morbidity.

DNA-based screening and early prophylactic thyroidectomy in RET carriers hold the hope that death and major morbidity from hereditary MTC can be eliminated in our lifetime. To accomplish that mission, proper funding of national health care systems will be essential to cover DNA-based screening and prophylactic thyroidectomy for hereditary C cell disease in additional countries.

The current trend towards earlier prophylactic thyroidectomy is likely to gather further momentum with the implementation of genomic sequencing in neonates for monogenic disorders, resulting in increased speed and access and lower costs of testing. To fully reap the fruits of these scientific advances, there is a need for more tertiary pediatric surgical centers to assure the safe deliverability of DNA-based prophylactic thyroidectomy on otherwise healthy infants and young children.

Practice Points

- Germline mutations in the *RET* proto-oncogene, with an estimated age-standardized incidence of 0.06 per 100 000 per year and prevalence of 1.3 per 100 000, predispose to hereditary medullary thyroid cancer (MTC) and multiple endocrine neoplasia type 2 (MEN 2).
- DNA-based screening discloses a person's genetic susceptibility to hereditary MTC before it has a chance to develop, providing a *window of opportunity* for prophylactic thyroidectomy.
- The close genotype—phenotype relationship forms a molecular structure within which neoplastic C cell hyperplasia transforms into early MTC. Molecular information alone cannot predict when precisely this malignant transformation is going to take place at the individual level.
- Biochemical screening narrows down that point of transition within the molecular framework (*DNA-based/biochemical concept*). As long as calcitonin serum levels are normal, MTC is not present.
- When calcitonin serum levels exceed the upper normal limit of the calcitonin assay, progression to MTC is imminent or may just have occurred. This biochemical threshold marks the last opportunity for prophylactic thyroidectomy without addition of central node dissection.
- Increasingly earlier molecular diagnosis and more limited prophylactic neck surgery yielded excellent clinical outcomes 7—16 years after prophylactic neck surgery at expert facilities: biochemical cure rates approximating 100%; absence of residual structural disease or recurrence; and rarely, if ever, permanent operative morbidity.

Research Agenda

- Tapping into large genomic databases to obtain precise risk estimates for less penetrant RET sequence variants remains an important research topic, complementing classic genetic association studies.
- The generalizability of genetic association studies from Europe to non-European populations or populations with complex recent ancestral histories should be studied in more depth.
- The excellent clinical outcomes reported from tertiary surgical centers dedicated to pediatric neck surgery in the Western hemisphere require replication in other healthcare settings.

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References

[1] Hill S. Introducing genomics into cancer care. Br J Surg 2018;105:e14–5.
[2] Gurlt E. Bericht über die Leistungen und Fortschritte auf dem Gebiet der Chirurgie im Jahre 1859. Arch Klin Chir 1861;1:184–7 [German].
[3] Billroth T. Chirurgische Erfahrungen. Zürich 1860 – 1867. Arch Klin Chir 1869;10:158–71 [German].
[4] Wölfer A. Über die Entwicklung und den Bau des Kropfes. Arch Klin Chir 1883;29:1–97. and 754–866 [German].
[5] Stoffel E. Lokales Amyloid der Schilddrüse. Virchows Arch 1910;201:245–52 [German].
[6] Burk W. Über einen Amyloidtumor mit Metastasen. Tübingen: Inaugural-Dissertation; 1901 [German].
[7] Sipple JH. The association of pheochromocytoma with carcinoma of the thyroid gland. Am J Med 1961;31:163–6.
[8] Manning PC, Molnar GD, Black BM, et al. Pheochromocytoma, hyperparathyroidism and thyroid carcinoma occurring coincidentally – report of a case. N Engl J Med 1963;268:68–72.
[9] Williams ED. A review of 17 cases of carcinoma of the thyroid and phaeochromocytoma. J Clin Pathol 1965;18:288–92.
[10] Horn RC. Carcinoma of the thyroid. Description of a distinctive morphological variant and report of seven cases. Cancer 1951;4:697–707.
[11] Hazard JB, Hawk WA, Crile G. Medullary (solid) carcinoma of the thyroid, a clinicopathologic entity. J Clin Endocrinol Metab 1959;19:152–61.
[12] Copp DH. Evidence for calcitonin: new hormone from parathyroid that lowers blood calcium. Endocrinology 1962;70:638–49.
[13] Foster GV, McIntyre I, Pearse AGE. Thyroid origin of calcitonin. Nature 1964;202:1303–5.
[14] Pearse AGE, Polak JM. Cytochemical evidence for the neural crest origin of mammalian ultimobranchial C-cells. Histochem Cell Biol 1971;27:96–102.
[15] Tashjian Jr AH, Melvin EW. Medullary carcinoma of the thyroid gland. Studies of thyrocalcitonin in plasma and tumor extracts. N Engl J Med 1968;279:279–83.
[16] Tashjian Jr AH, Howland BG, Melvin KEW, et al. Immunoassay of human calcitonin: clinical measurement, relation to serum calcium and studies in patients with medullary thyroid carcinoma. N Engl J Med 1970;283:890–5.
[17] Graze K, Spiler IJ, Tashjian Jr AH, et al. Natural history of familial medullary thyroid carcinoma: effect of a program for early diagnosis. N Engl J Med 1978;299:980–5.
[18] Gagel RF, Tashjian Jr AH, Cummings T, et al. The clinical outcome of prospective screening for multiple endocrine neoplasia type 2a. An 18-year experience. N Engl J Med 1988;318:478–84.
[19] 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;2:851–6.
[20] Mulligan LM, Kwok JB, Healey CS, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 1993;363:458–60.
[21] Margraf RL, Crockett DK, Krautscheid PM, et al. Multiple endocrine neoplasia type 2 RET protooncogene database: repository of MEN2-associated RET sequence variation and reference for genotype/phenotype correlations. Hum Mutat 2009;30:548–56.
[22] Lips CJ, Landsvater RM, Höppener JW, et al. Clinical screening as compared with DNA analysis in families with multiple endocrine neoplasia type 2A. N Engl J Med 1994;331:828–35.
[23] 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;220:237–47. discussion 247–250.
[24] Dralle H, Gimm O, Simon D, et al. Prophylactic thyroidectomy in 75 children and adolescents with hereditary medullary thyroid carcinoma: German and Austrian experience. World J Surg 1998;22:744–50. discussion 750–751.
[25] Machens A, Niccoli-Sire P, Hoegel J, et al. European Multiple Endocrine Neoplasia (EUROMEN) Study Group. Early malignant progression of hereditary medullary thyroid cancer. N Engl J Med 2003;349:1517–25.
[26] Skinner MA, Moley JA, Dilley WG, et al. Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A. N Engl J Med 2005;353:1105–13.
*[27] Wells Jr SA, Asa SL, Dralle H, et al. American Thyroid Association Guidelines Task Force on Medullary Thyroid Carcinoma. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid 2015;25:567–610.
[28] Machens A, Dralle H. Advances in risk-oriented surgery for multiple endocrine neoplasia type 2. Endocr Relat Cancer 2018;25:T41–52.
[29] den Dunnen JT, Dalgleish R, Maglott DR, et al. HGVS recommendations for the description of sequence variants: 2016 update. Hum Mutat 2016;37:564–9.
[30] Erlic Z, Hoffmann MM, Sullivan M, et al. Pathogenicity of DNA variants and double mutations in multiple endocrine neoplasia type 2 and von Hippel-Lindau syndrome. J Clin Endocrinol Metab 2010;95:308–13.

[31] Toledo RA, Hatakana R, Lourenço Jr DM, et al. Comprehensive assessment of the disputed RET Y791F variant shows no association with medullary thyroid carcinoma susceptibility. Endocr Relat Cancer 2015;22:65–76.
[32] Mathiesen JS, van Overeem Hansen T, Rasmussen ÅK, et al. Novel somatic RET mutation questioning the causality of the RET I852M germline sequence variant in multiple endocrine neoplasia 2A. Thyroid 2017;27:1103–4.
*[33] Machens A, Lorenz K, Weber F, et al. Genotype-specific progression of hereditary medullary thyroid cancer. Hum Mutat 2018;39:860–9.
[34] Mirnezami R, Nicholson J, Darzi A. Preparing for precision medicine. N Engl J Med 2012;366:489–91.
*[35] Romei C, Mariotti S, Fugazzola L, et al., ItaMEN network. Multiple endocrine neoplasia type 2 syndromes (MEN 2): results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes. Eur J Endocrinol 2010;163:301–8.
*[36] Lebeault M, Pinson S, Guillaud-Bataille M, et al. Nationwide French study of RET variants detected from 2003 to 2013 suggests a possible influence of polymorphisms as modifiers. Thyroid 2017;27:1511–22.
[37] Mathiesen JS, Habra MA, Bassett JHD, et al. Risk profile of the RET A883F germline mutation: an international collaborative study. J Clin Endocrinol Metab 2017;102:2069–74.
[38] Elisei R, Cosci B, Romei C, et al. Identification of a novel point mutation in the RET gene (Ala883Thr), which is associated with medullary thyroid carcinoma phenotype only in homozygous condition. J Clin Endocrinol Metab 2004;89:5823–7.
*[39] Machens A, Lorenz K, Sekulla C, et al. Molecular epidemiology of multiple endocrine neoplasia 2: implications for RET screening in the new millennium. Eur J Endocrinol 2013;168:307–14.
[40] Machens A, Lorenz K, Weber F, et al. Geographic epidemiology of medullary thyroid cancer families: unearthing European ancestral heritage. Endocr Relat Cancer 2018;25:L27–30.
[41] Giacché M, Panarotto A, Tacchetti MC, et al. Ser891Ala RET gene mutation in medullary thyroid cancer: phenotypical and genealogical characterization of 28 apparently unrelated kindreds and founder effect uncovering in Northern Italy. Hum Mutat 2019;40:926–37.
[42] Valdés N, Navarro E, Mesa J, et al. RET Cys634Arg mutation confers a more aggressive multiple endocrine neoplasia type 2A phenotype than Cys634Tyr mutation. Eur J Endocrinol 2015;172:301–7.
[43] Maciel RMB, Camacho CP, Assumpcao LV, et al. Genotype and phenotype landscape of MEN2 in 554 medullary thyroid cancer patients: the BrasMEN study. Endocr Connect 2019;8:289–98.
[44] Schuffenecker I, Ginet N, Goldgar D, et al. Prevalence and parental origin of de novo RET mutations in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma. Le Groupe d'Etude des Tumeurs a Calcitonine. Am J Hum Genet 1997;60:233–7.
[45] Abi-Raad R, Virk RK, Dinauer CA, et al. C-cell neoplasia in asymptomatic carriers of RET mutation in extracellular cysteine-rich and intracellular tyrosine kinase domain. Hum Pathol 2015;46:1121–8.
[46] Machens A, Hauptmann S, Dralle H. Modification of multiple endocrine neoplasia 2A phenotype by cell membrane proximity of RET mutations in exon 10. Endocr Relat Cancer 2009;16:171–7.
[47] Frank-Raue K, Rybicki LA, Erlic Z, et al., International RET Exon 10 Consortium. Risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germline RET mutations located in exon 10. Hum Mutat 2011;32:51–8.
[48] Rich TA, Feng L, Busaidy N, et al. Prevalence by age and predictors of medullary thyroid cancer in patients with lower risk germline RET proto-oncogene mutations. Thyroid 2014;24:1096–106.
*[49] Machens A, Lorenz K, Dralle H. Individualization of lymph node dissection in RET (rearranged during transfection) carriers at risk for medullary thyroid cancer: value of pretherapeutic calcitonin levels. Ann Surg 2009;250:305–10.
*[50] Machens A, Elwerr M, Lorenz K, et al. Long-term outcome of prophylactic thyroidectomy in children carrying RET germline mutations. Br J Surg 2018;105:e150–7.
[51] Morris LF, Waguespack SG, Edeiken-Monroe BS, et al. Ultrasonography should not guide the timing of thyroidectomy in pediatric patients diagnosed with multiple endocrine neoplasia syndrome 2A through genetic screening. Ann Surg Oncol 2013;20:53–9.
*[52] 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;97:426–35.
[53] Castagna MG, Fugazzola L, Maino F, et al. Reference range of serum calcitonin in pediatric population. J Clin Endocrinol Metab 2015;100:1780–4.
[54] Machens A, Lorenz K, Dralle H. Progression of medullary thyroid cancer in RET carriers of ATA class A and C mutations. J Clin Endocrinol Metab 2014;99:E286–92.
[55] Rohmer V, Vidal-Trecan G, Bourdelot A, et al. Groupe Français des Tumeurs Endocrines. Prognostic factors of disease-free survival after thyroidectomy in 170 young patients with a RET germline mutation: a multicenter study of the Groupe Francais d'Etude des Tumeurs Endocrines. J Clin Endocrinol Metab 2011;96:E509–18.
[56] Dralle H. Lymph node dissection and medullary thyroid carcinoma. Br J Surg 2002;89:1073–5.
[57] Schneider R, Machens A, Sekulla C, et al. Twenty-year experience of paediatric thyroid surgery using intraoperative nerve monitoring. Br J Surg 2018;105:996–1005.
[58] Machens A, Dralle H. Benefit-risk balance of reoperation for persistent medullary thyroid cancer. Ann Surg 2013;257:751–7.
*[59] Febrero B, Rodríguez JM, Ríos A, et al. Prophylactic thyroidectomy in multiple endocrine neoplasia 2 (MEN2) patients with the C634Y mutation: a long-term follow-up in a large single-center cohort. Eur J Surg Oncol 2019;45:625–30.
[60] Prete FP, Abdel-Aziz T, Morkane C, et al. MEN2 in Children UK Collaborative Group. Prophylactic thyroidectomy in children with multiple endocrine neoplasia type 2. Br J Surg 2018;105:1319–27.
[61] Kluijfhout WP, van Beek DJ, Verrijn Stuart AA, et al. Postoperative complications after prophylactic thyroidectomy for very young patients with multiple endocrine neoplasia type 2: retrospective cohort analysis. Medicine 2015;94:e1108.
[62] Machens A, Lorenz K, Dralle H. Time to calcitonin normalization after compartment-oriented surgery for node-negative and node-positive medullary thyroid cancer. Br J Surg 2019;106:412–8.
[63] Mathiesen JS, Kroustrup JP, Vestergaard P, et al. Danish Thyroid Cancer Group (DATHYRCA). Incidence and prevalence of sporadic and hereditary MTC in Denmark 1960-2014: a nationwide study. Endocr Connect 2018;7:829–39.

[64] Loveday C, Josephs K, Chubb D, et al. p.Val804Met, the most frequent pathogenic mutation in RET, confers a very low lifetime risk of medullary thyroid cancer. J Clin Endocrinol Metab 2018;103:4275–82.
[65] Turnbull C, Loveday C, Izatt L, et al. Response to Letter to the Editor: "p.Val804Met, the most frequent pathogenic mutation in RET, confers a very low lifetime risk of medullary thyroid cancer". J Clin Endocrinol Metab 2018;103:3518–9.
[66] Machens A, Dralle H. Letter to the Editor: "p.Val804Met, the most frequent pathogenic mutation in RET, confers a very low lifetime risk of medullary thyroid cancer". J Clin Endocrinol Metab 2018;103:3516–7.
[67] Lindskog S, Nilsson O, Jansson S, et al. Phenotypic expression of a family with multiple endocrine neoplasia type 2A due to a RET mutation at codon 618. Br J Surg 2004;91:713–8.
[68] Machens A, Dralle H. Familial prevalence and age of RET germline mutations: implications for screening. Clin Endocrinol 2008;69:81–7.
[69] Neumann HP, Vortmeyer A, Schmidt D, et al. Evidence of MEN-2 in the original description of classic pheochromocytoma. N Engl J Med 2007;357:1311–5.
[70] Brauckhoff M, Machens A, Lorenz K, et al. Surgical curability of medullary thyroid cancer in multiple endocrine neoplasia 2B: a changing perspective. Ann Surg 2014;259:800–6.
[71] Raue F, Dralle H, Machens A, et al. Long-term survivorship in multiple endocrine neoplasia type 2B diagnosed before and in the new millennium. J Clin Endocrinol Metab 2018;103:235–43.
[72] Castinetti F, Waguespack SG, Machens A, et al. Natural history, treatment, and long-term follow up of patients with multiple endocrine neoplasia type 2B: an international, multicentre, retrospective study. Lancet Diabetes Endocrinol 2019;7:213–20.
[73] Saltiki K, Anagnostou E, Simeakis G, et al. Familial MTC with RET exon 8 Gly533Cys mutation: origin and prevalence of second malignancy. Endocr Connect 2017;6:676–84.
[74] Cunha LL, Lindsey SC, França MI, et al. Evidence for the founder effect of RET533 as the common Greek and Brazilian ancestor spreading multiple endocrine neoplasia 2A. Eur J Endocrinol 2017;176:515–9.
[75] Mathiesen JS, Kroustrup JP, Vestergaard P, et al. Founder effect of the RET^{C611Y} mutation in multiple endocrine neoplasia 2A in Denmark: a nationwide study. Thyroid 2017;27:1505–10.
[76] Mathiesen JS, Kroustrup JP, Vestergaard P, et al. Incidence and prevalence of multiple endocrine neoplasia 2A in Denmark 1901–2014: a nationwide study. Clin Epidemiol 2018;10:1479–87.
[77] Fanis P, Skordis N, Frangos S, et al. Multiple endocrine neoplasia 2 in Cyprus: evidence for a founder effect. J Endocrinol Investig 2018;41:1149–57.
[78] Martins-Costa MC, Cunha LL, Lindsey SC, et al. M918V RET mutation causes familial medullary thyroid carcinoma: study of 8 affected kindreds. Endocr Relat Cancer 2016;23:909–20.
[79] Martins-Costa MC, Lindsey SC, Cunha LL, et al. A pioneering RET genetic screening study in the State of Ceará, Brazil, evaluating patients with medullary thyroid cancer and at-risk relatives: experience with 247 individuals. Arch Endocrinol Metab 2018;62:623–35.
*[80] Machens A, Dralle H. Therapeutic effectiveness of screening for multiple endocrine neoplasia type 2A. J Clin Endocrinol Metab 2015;100:2539–45.
[81] Machens A, Dralle H. Multiple endocrine neoplasia type 2: achievements and current challenges. Clinics 2012;67(Suppl 1):113–8.
[82] Grosfeld FJ, Lips CJ, Ten Kroode HF, et al. Psychosocial consequences of DNA analysis for MEN type 2. Oncology 1996;10:141–6. discussion 146, 152, 157.
[83] Freyer G, Dazord A, Schlumberger M, et al. Psychosocial impact of genetic testing in familial medullary-thyroid carcinoma: a multicentric pilot-evaluation. Ann Oncol 1999;10:87–95.
[84] Shuman AG, Shaha AR, Tuttle RM, et al. Medullary thyroid carcinoma: ethical issues for the surgeon. Ann Surg Oncol 2012;19:2102–7.
[85] Nozhat Z, Hedayati M. Medullary thyroid carcinoma: a review on ethical considerations in treatment of children. J Pediatr Endocrinol Metab 2016;29:633–9.
[86] Bihan H, Baudin E, Meas T, et al., French Group of Endocrine Tumors. Role of prophylactic thyroidectomy in RET 790 familial medullary thyroid carcinoma. Head Neck 2012;34:493–8.
[87] Parkhurst E, Calonico E, Abboy S. Utilization of genetic testing for RET mutations in patients with medullary thyroid carcinoma: a single-center experience. J Genet Couns 2018;27:1411–6.