REVIEW

Multiple endocrine neoplasia type 2: achievements and current challenges

Andreas Machens, Henning Dralle

Martin-Luther-University Halle-Wittenberg, Department of General, Visceral and Vascular Surgery, Halle (Saale)/Germany.

Incremental advances in medical technology, such as the development of sensitive hormonal assays for routine clinical care, are the drivers of medical progress. This principle is exemplified by the creation of the concept of multiple endocrine neoplasia type 2, encompassing medullary thyroid cancer, pheochromocytoma, and primary hyperparathyroidism, which did not emerge before the early 1960s. This review sets out to highlight key achievements, such as joint biochemical and DNA-based screening of individuals at risk of developing multiple endocrine neoplasia type 2, before casting a spotlight on current challenges which include: (i) ill-defined upper limits of calcitonin assays for infants and young children, rendering it difficult to implement the biochemical part of the integrated DNA-based/biochemical concept; (ii) our increasingly mobile society in which different service providers are caring for one individual at various stages in the disease process. With familial relationships disintegrating as a result of geographic dispersion, information about the history of the origin family may become sketchy or just unavailable. This is when DNA-based gene tests come into play, confirming or excluding an individual's genetic predisposition to multiple endocrine neoplasia type 2 even before there is any biochemical or clinical evidence of the disease. However, the unrivaled molecular genetic progress in multiple endocrine neoplasia type 2 does not come without a price. Screening may uncover unknown gene sequence variants representing either harmless polymorphisms or pathogenic mutations. In this setting, functional characterization of mutant cells in vitro may generate helpful ancillary evidence with regard to the pathogenicity of gene variants in comparison with established mutations.

KEYWORDS: Medullary thyroid carcinoma; Pheochromocytoma; Primary hyperparathyroidism; RET proto-oncogene; Age-related progression; Calcitonin screening; DNA-based screening.

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E-mail: AndreasMachens@aol.com

Tel.: 49 345 5572314

INTRODUCTION

Incremental advances in medical technology, such as the development of sensitive hormonal assays for routine clinical care, are the drivers of medical progress. This principle is exemplified by the creation of the concept of multiple endocrine neoplasia type 2 (MEN2), which did not emerge before the early 1960s (1). This hereditable syndrome combines three distinct disease entities into one clinical syndrome: medullary thyroid cancer (MTC); pheochromocytoma; and primary hyperparathyroidism (2,3). MEN2B patients who do not have primary hyperparathyroidism also develop conjunctival, corneal, buccal, lip and tongue neuromas, intestinal ganglioneuromas (dubbed ''pseudo-Hirschsprung's disease''), and skeletal anomalies subsumed under the designation of ''Marfanoid habitus'' as they grow older (2–4).

Before this landmark event, individuals operated on for MTC used to be diagnosed with papillary, follicular, or undifferentiated thyroid cancer. After completing their fourth decade of life, some of these patients succumbed to metastatic MTC or to acute hypertensive crisis owing to adrenergic hormonal excess from a previously undetected pheochromocytoma. In the absence of a postmortem examination, these deaths were frequently ascribed to acute cardiovascular or cerebrovascular events. Without clinical–pathologic characterization of the disease, targeted interventions are difficult, if not impossible, to carry out at an early stage in the disease process.

BIOCHEMICAL SCREENING

The widespread availability of commercial calcitonin and catecholamine assays in the 1960s completely changed the scene, enabling biochemical screening of asymptomatic individuals from MEN2 families who were suspected to harbor C-cell disease and adrenal medullary disease (5). At the very beginning, calcitonin screening, augmented through stimulation with intravenous bolus injections of pentagastrin or calcium gluconate short infusions, was largely cross-sectional, including kindreds of various age groups at different stages of tumor development (5). With the increasing inclusion of asymptomatic infants and young

children at risk of developing MEN2 tumors, the focus of the screening programs shifted toward longitudinal observation of family members (6). This prompted biochemical screening of individuals at risk of developing MEN2 over many years who were slated for preemptive thyroidectomy as soon as basal serum calcitonin levels exceeded the upper normal limit of the assay. With the exception of a few false-positives from physiologic C-cell disease of the thyroid or calcitonin-secreting small cell lung cancer or pancreatic neuroendocrine cancer (7), this approach worked fairly well, reducing the number of patients newly diagnosed with advanced MTC (5,6). As a result, family members from the younger generations were found to have more localized disease (''anticipation phenomenon''), as opposed to the metastatic disease that used to haunt earlier generations.

This breakthrough in the management of MEN2 patients is beautifully illustrated in Figure 1 with the pedigree of an MEN2A family of Transylvanian Saxons (ethnic Germans whose ancestors colonized Sibiu (Hermannstadt) county in present-day Romania during the 13th century). The first two affected individuals, I-2 and II-3, developed huge neck lumps (presumably MTC) and severe hypertension (presumably due to pheochromocytoma), causing aphonia (presumably as a result of recurrent laryngeal nerve invasion) and hemiplegia at the age of 42 years (presumably following catecholamine excess) in individual II-3. Both

MTC and pheochromocytoma went unrecognized as such by the rural Communist healthcare system, so that the diagnosis of MEN2A was not made at the time (Figure 1, shaded area). After the fall of the Eastern bloc in 1989, the whole family migrated to Germany where individuals III-7 and IV-9 were found to harbor node-positive MTC and pheochromocytoma at the age of 36 and 14 years in 1994 and 1995, respectively (Figure 1). Unfortunately, several family members declined screening.

DNA-BASED SCREENING

The 1993 and 1994 discoveries of RET (*RE*arranged during *T*ransfection) as the susceptibility gene for MEN2 ushered in the molecular era (8–10). Owing to the need for genetic association studies for unequivocal cases of MEN type 2, higher risk mutations in codon 918 (the classic MEN2B mutation; American Thyroid Association/ATA D), codon 634 (the classic MEN2A mutation; ATA C) and codons 609, 611, 618, 620, and 630 (ATA B) were overrepresented in the International MEN2 Consortium (2). The subsequent scanning of the RET proto-oncogene for additional mutations identified more RET mutations in codons 768, 790, 791, 804, and 891 between 1995 and 1998 (11–14). These milder mutations were often associated with familial MTC only, an abortive form of MEN2.
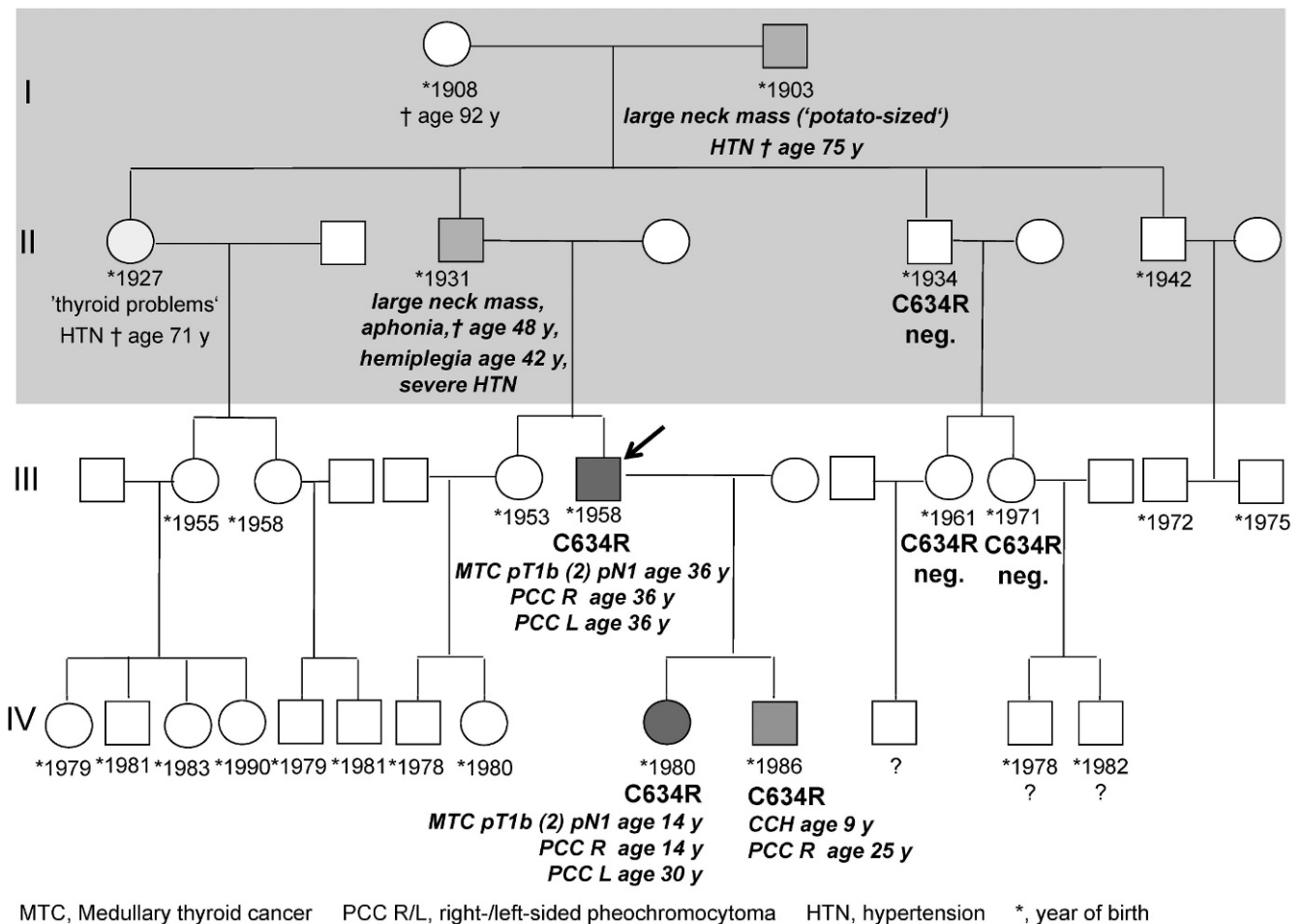

Figure 1 - Multigenerational Ethnic German C634R Family from Romania.

GENOTYPE–PHENOTYPE CORRELATIONS

Transformation from hyperplasia to neoplasia

The RET proto-oncogene encodes for a transmembrane tyrosine kinase receptor. The constitutive (i.e., genetically encoded) activation of the mutated RET receptor protein is believed to cause, in decreasing frequency, hyperplasia of the parafollicular C-cells, adrenal medullary cells, and parathyroid chief cells (3). A second mutation in one of these neuroendocrine cells ("second hit") is thought to cause MTC (C-cell cancer), pheochromocytoma, and primary hyperparathyroidism. Because the acquisition of somatic mutations by these cells reflects the play of chance, the development of the various MEN2 components can vary tremendously even among members of the same family, compromising predictions regarding the age by which the various MEN2 components will have developed. As a rule, the weaker the transforming activity of the inherited RET mutation, the more variable will be the clinical presentation (phenotype) of the various MEN2 components (3,15,16), with steep gradients from highest risk ATA-D to very high-risk ATA-C, high-risk ATA-B, and lowest risk ATA-A mutations (Table 1). Carriers of the last two groups of mutations may develop familial MTC only.

Youngest age of tumor development

A systematic review of the literature (2,15–28), focused on the youngest carrier age at which a given MEN2-related tumor was ever reported, yielded the following chart of earliest tumor development (Table 1): medullary thyroid cancer as early as age 2 months (carriers of mutations in codon 918), 10 months (carriers of mutations in codon 634), 12 months (carriers of mutations in codon 630), 4–7 years (carriers of mutations in codons 609, 611, 618 and 620) and 9–21 years (carriers of mutations in codons 533, 768, 790, 791, 804 and 891); pheochromocytoma as early as age 12 years (carriers of mutations in codon 918 and 634), 19–30 years (carriers of mutations in codons 609, 611, 618, and 620), and 28–59 years (carriers of mutations in codons 768, 790, 791, 804, and 891); primary hyperparathyroidism as early as 5 years (carriers of mutations in codon 634), 34–41 years (carriers of mutations in codons 609, 611, 618, and 620), and 38–54 years (carriers of mutations in codons 533, 768, 790, 791, 804, and 891), but for unknown reasons, never in MEN2B patients (carriers of mutations in codon 918).

With more data set to be forthcoming, the current ATA assignments of the rare RET mutations in codon 630 (currently ATA-B) and 883 (currently ATA D) may need to be revised.

Youngest age of node-positive MTC

Another literature review of the youngest age of carriers harboring lymph node metastases from MTC revealed a comparable pattern (16). Barring rare exceptions, lymph node metastases have not yet developed before age 2 years (carriers of mutations in codon 918), not before age 5 years (carriers of mutations in codon 634) and 15 years (carriers of mutations in codons 630) and not before age 20 years in the remaining RET carriers.

Window of opportunity for cure

The lead time provided by early screening of infants and young children from RET families defines a "window of opportunity", setting the time frame within which total thyroidectomy alone will eliminate all C-cell disease (3). If this time frame has been exceeded, systematic dissection of the central and lateral lymph nodes on either side of the neck is unavoidable. In this situation, the patient's prospects of cure fall from 95% with no lymph node metastases to 57–31% with 1–10 lymph node metastases and 0–4% with >10 lymph node metastases (29,30).

INTEGRATED DNA-BASED/BIOCHEMICAL CONCEPT OF EARLY THYROIDECTOMY

The impossibility of anticipating the development of the various MEN2 components has prompted both overtreatment

**Table 1 -** RET genotype–phenotype relationships and ATA recommendations for thyroidectomy.

ATA group	RET genotype		Relative frequency of genotype among RET families (%)		RET phenotype: youngest age at tissue diagnosis (years) (2,15–28)			ATA recommendations for thyroidectomy (31)	
	Exon	Codon	Germany (n = 140) (19)	Italy (n = 235) (22)	MTC	PCC	pHPT	DNA based	b+stCT based
D	16	918	15	8	0.2	12	–	ASAP (≤1 year)	if ↑
	15*	883*	0	0.4	39*	39*	N/A		
C	11	634	41	37	0.8	12	5	<5 years	if ↑
B	11†	630†	0.7	2	1†	–	32	<5 years (may delay if b+stCT WNL)	if ↑
	10	620	7	4	5	19	N/A		
	10	618	5	6	5	19	41		
	10	611	1	0.4	7	30	40		
	10	609	0.7	3	4	19	34		
A	8	533	0‡	0‡	21	35	N/A	May delay >5 years if b+stCT WNL	if ↑
	13	768	1	4	9	59	N/A		
	13	790	12	3	10	28	N/A		
	13	791	7	<1	15	38	38		
	14	804	6	22	6, 12	28–33	54		
	15	891	2	10	39	39	N/A		

ASAP = as soon as possible; ATA = American Thyroid Association; b+stCT = basal and stimulated serum calcitonin levels; MTC = medullary thyroid cancer; PCC = pheochromocytoma; pHPT = primary hyperparathyroidism; RET = REarranged during Transfection.
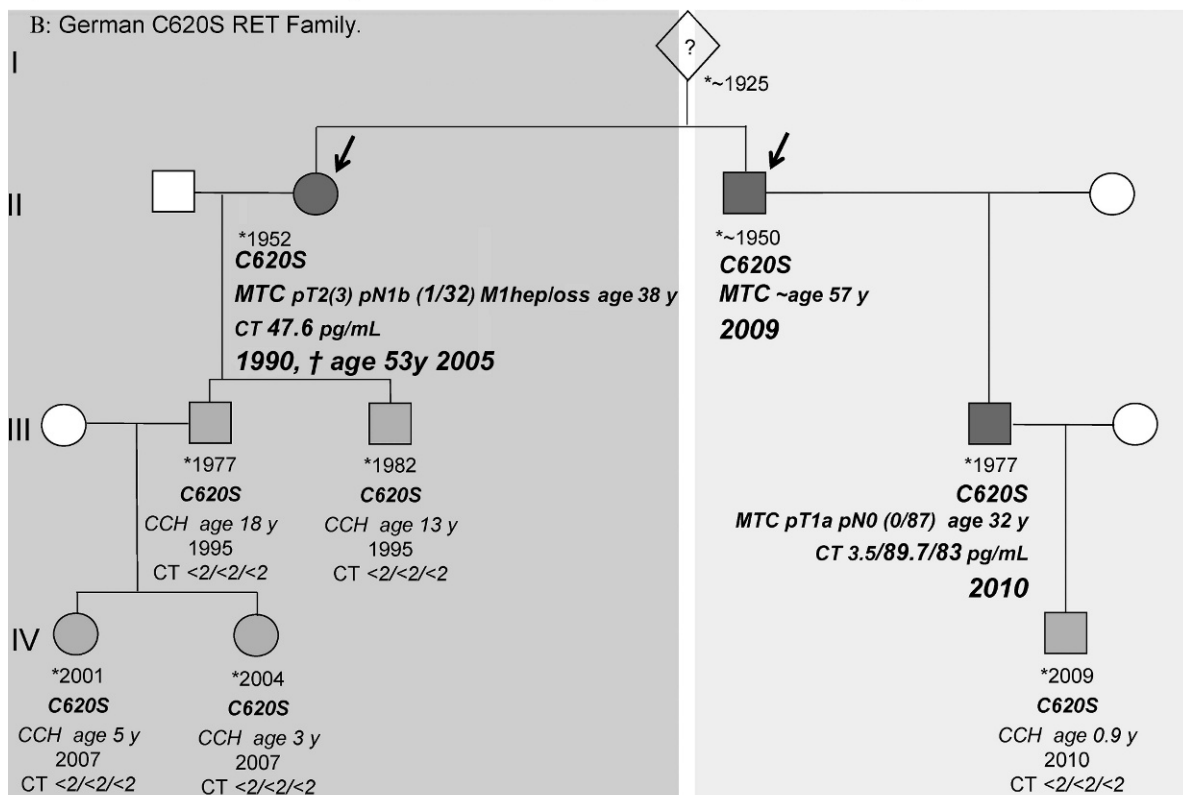*May need regrouping if confirmed in subsequent studies (ATA group A?).
†May need regrouping if confirmed in subsequent studies (ATA group C?).
‡May be more common in Brazilians of Catalan/Spanish ancestry (17) and the Greek population (20).
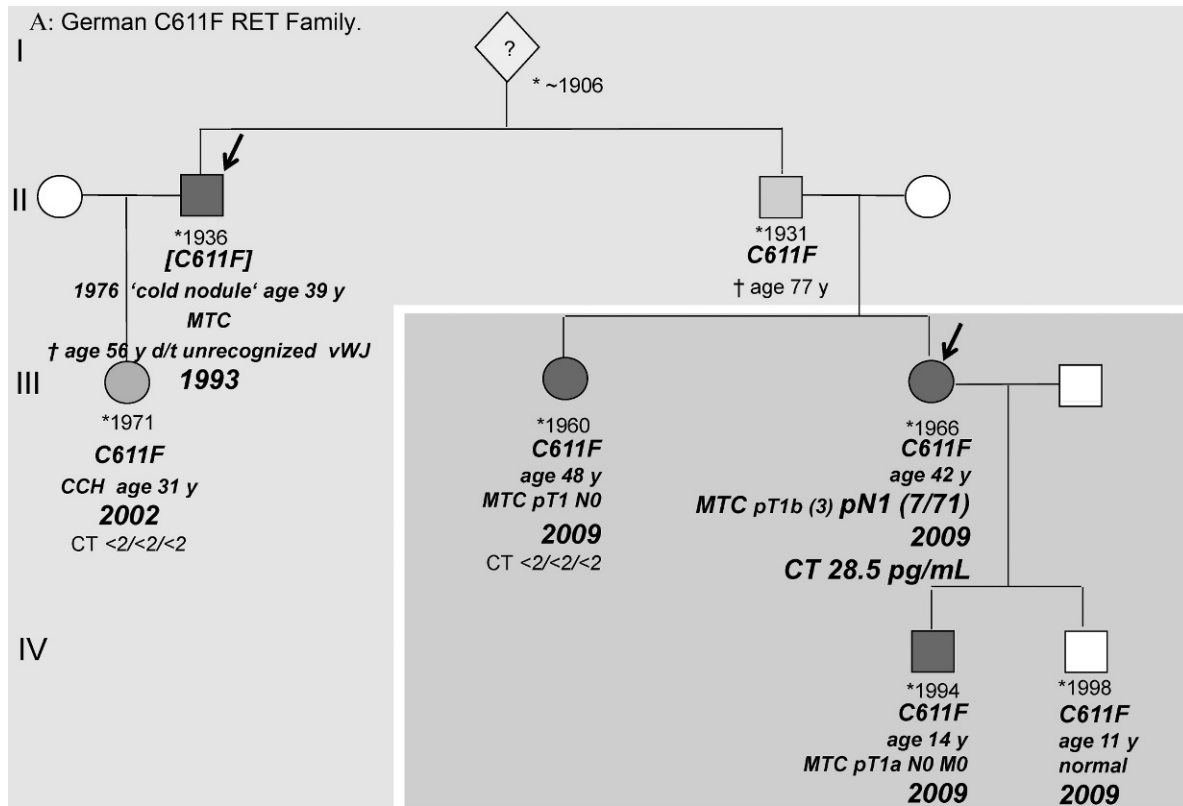
Figure 2 - German RET Families with 2 Index Patients.

(based on the above worst-case scenarios) and undertreatment (practicing the law of averages) of gene carriers. Although the respective RET mutation sets the stage for tumor development, defining a time corridor, this corridor often is too wide to be useful for individual decisions with regard to the timing of prophylactic thyroidectomy.

This imperfection of the DNA-based approach prompted researchers to build on the strengths of the DNA-based and biochemical screening programs, giving rise to the integrated DNA-based/biochemical concept of early thyroidectomy (16). Herein, biochemical information (basal serum calcitonin levels) is utilized to spot imminent or early malignant transformation from C-cell hyperplasia to MTC while it is still confined to the thyroid gland. There is good evidence to suggest that MTC has not developed as long as basal calcitonin levels remain within normal limits. No more than one-third of pediatric RET gene carriers with increased basal calcitonin levels are believed to harbor occult MTC (16). It may be interesting to note that lymph node metastases have not yet been reported with basal serum calcitonin levels ≤40 pg/ml (upper normal limit <10 pg/ml) (16,26,31).

What precisely constitutes a normal or elevated serum calcitonin level for infants and young children remains to be determined. As a rule, calcitonin levels tend to be higher in infants younger than 6 months but are thought to approximate to adult levels by the age of 3 years (32). In male and female children older than 6 months (i.e., aged 1–9 years), normal calcitonin values as high as 17 pg/ml and 8.5 pg/ml, respectively, have been noted, exceeding the manufacturer's upper normal limits of <11.5 pg/ml for male adults and <4.6 pg/ml for female adults (33). The application of adult reference data to young children for whom upper normal limits are rarely, if ever, available from the manufacturer of the calcitonin assay may yield incorrect information for children whose seemingly abnormal test results may actually be within the normal limits for that age group. This renders it challenging to implement the calcitonin-based part of the ATA guidelines (Table 1; right panel) for young children unless calcitonin serum levels are quite high.

CURRENT CHALLENGES

In our increasingly mobile society, clinical care for MEN2 has become fragmented, with different service providers caring for one individual at various stages in the disease process. Geographical dispersion of family members, some of whom may be living abroad, can add to a screening program's difficulty in reaching out to all relatives who may have inherited the trait running in the family. These issues frustrated screening efforts directed at all 86 members of a large French L790F RET family. Of these potential gene carriers, no more than 22 family members (26%) ended up being tested despite the authors' vigorous attempts at complete ascertainment (34). Moreover, not every newly identified gene carrier is prepared to share personal genetic information with his/her next of kin.

This attitude explains why there can be more than one index patient within an extended RET family whose members, living in silos as it were, underwent RET screening after developing MTC independently of one another. Figure 2 depicts the pedigrees of two unrelated RET families in whom a longstanding breakdown of

communication had occurred. This disruption delayed the diagnosis of hereditary MTC in the second index patient by 7 years in the C611F family (Figure 2a) and 19 years in the C620S family (Figure 2b). This significant delay may have contributed to our inability to biochemically cure the second index patient, giving the C-cell disease 7 and 19 additional years to spread beyond curability. There is a paucity of research into the reasons behind some gene carriers' unwillingness to share genetic risk with individuals at risk within their families. Hypothetical reasons include repression, feelings of guilt and resentment, emotional distress, and poor familial interaction.

This is when DNA-based screening, requiring only a venous blood draw, comes into play. Barring sample mixup, the RET gene test confirms or rules out an individual's genetic predisposition to MEN2 even before there is any biochemical or clinical evidence of disease. This is most relevant in the event of adopted children or broken families in whom information about the history of the origin family is sketchy or just unavailable.

However, this unrivaled molecular genetic progress does not come without a price. DNA-based screening may occasionally uncover previously unknown RET sequence variants dubbed ''variants of unknown significance'' (VUS), which may represent harmless polymorphisms or pathogenic mutations. This distinction is difficult, if not impossible, to make when one sole family member is clinically affected so that there is no demonstrable segregation of the trait with the disease. This scenario is common in small and younger families whose members conceivably may not have been given sufficient time to develop the disease. In this setting, functional characterization of RET mutant cells *in vitro* has been harnessed to generate ancillary evidence regarding the pathogenicity of unknown sequence variants in comparison with established RET mutations (35–38). This experimental approach, albeit promising, will need further validation before being considered for routine clinical use.

AUTHOR CONTRIBUTIONS

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