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Familial medullary carcinoma prevention, risk evaluation, and *RET* in children of families with MEN2

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Abstract
The ability to predict the risk of MEN2 and medullary thyroid carcinoma (MTC) by genetic *RET* proto-oncogene analysis has provided an essential tool in identifying patients in whom thyroid cancer can be prevented by prophylactic thyroidectomy but emphasizes the need for clear policy guidelines. Children of families with *RET* cysteine mutations (exons 10, 11, 13, and 16) may develop early metastatic tumours and require prophylactic thyroidectomy. The 918 mutation associated with MEN2B is associated with early aggressive behaviour and distant metastatic spread. This has led to active screening of affected families underlining the need for specific intervention strategies.

Aim: To evaluate the risk to children of families with MEN2 and to assess the risk and determine the treatment.

Methods: Twenty-five patients from 10 families with MEN2 phenotypes were screened for *RET* mutations. Polymerase chain reaction amplification was performed on all 21 exons of the *RET* proto-oncogene, followed by heteroduplex single-strand conformation polymorphism (HEX-SSCP) analysis. Polymerase chain reaction products demonstrating variation in the HEX-SSCP gels were subjected to automated DNA sequencing analysis.

Results: Eleven significant *RET* mutations were detected in affected families. Eight index cases received initial thyroidectomy for established MTC (plus 2 advised). In the family members screened, 3 prophylactic thyroidectomies (2 with early MTC) were performed and a further 2 recommended. An exon 10 C620W missense mutation (the "Janus" gene) was detected in a patient with Hirschsprung's disease plus 1 family member.

Conclusion: *RET* analysis of MEN has revolutionized the management of children of families with MEN2 and allowed surgical prediction and prophylaxis to take place. The presence of an exon 10 C620W mutation in association with Hirschsprung's disease was difficult to assess. We suggest possible guidelines for management of families with MTC and the role of genetic testing in their evaluation.

The REarranged during Transfection (*RET*) proto-oncogene (located at 10q22), which encodes a transmembrane tyrosine kinase receptor, appears to be the major etiologic factor in inherited medullary thyroid carcinoma (MTC) and is strongly associated with several clinical conditions such

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as multiple endocrine neoplasia types 2A and 2B (MEN2A and MEN2B), familial MTC (FMTC), Hirschsprung's disease (HSCR), papillary thyroid carcinoma, and possibly other thyroid conditions. Genetic screening is extremely sensitive in MEN2 syndromes, and children of families with MTC who are carriers of mutant genes are at risk and may be identified by means of detecting the germline mutation in the *RET* proto-oncogene. These are particularly related to the *RET* cysteine mutations in exons 10, 11, 13, and 16, but other *RET* variations also appear to carry risk.

Children of families with MTC and MEN2 syndromes may develop early metastatic tumours often before the age of 5 years and may develop C-cell hyperplasia (CCH) even earlier [1]. The risk to family members appears to be particularly high with certain genetic mutations (including variants at amino acid positions 634 and 918) when compared to others and with cases of inherited MTC being reported under the age of 5 years [2] occurring as early as 17 months of age in cases of the 918 mutation associated with MEN2B [3], where tumours may develop early aggressive behaviour and distant metastatic spread. A major benefit of genetic testing therefore is the early preclinical identification of carriers of the *RET* mutation and timely prophylactic thyroidectomy, thus removing the target organ.

There are currently a number of major areas of debate in the management of children of families with MEN. The first is the question of timing and extent of genetic screening; secondly, the significance of *RET*-related specific genetic variations; and, thirdly, the question of codon-directed timing for prophylactic thyroid surgery. A final debate would probably include the nature of follow-up of patients and the subsequent risk of other MEN-related tumours. What is emerging is an element of surgical decision making which affects the childhood years, although many of the tumours occur later in life.

The aim of this study was to evaluate the risk to children of families with MEN2 based on our experience and to assess the risk and determine the treatment protocols for their management.

1. Materials and methods

The study was approved by the Ethics Review Committee of the University of Stellenbosch (ref: 2001/C019).

1.1. Subjects

The study cohort included 25 patients from 10 families with MEN2 phenotypes.

1.2. DNA analysis

Blood-extracted DNA of these patients was screened for *RET* mutations [4]. Polymerase chain reaction (PCR) amplification of the 21 exons of the *RET* proto-oncogene was performed using intronic primers [5]. The PCR products were subjected to heteroduplex single-strand conformation polymorphism (HEX-SSCP) analysis [6] and resolved on a 12% polyacrylamide gel with 7.5% urea at 4°C (350 V) for 18 hours. After electrophoresis, the gel was stained with ethidium bromide and the DNA visualized by ultraviolet light transillumination. DNA sequencing was performed on PCR products demonstrating mobility or conformational variants in the polyacrylamide gels, using an ABI 3100 PRISM automated sequencer (Applied Biosystems, Foster City, CA). An additional 56 blood-derived DNA samples were included as controls.

1.3. Statistical analysis

Allele and genotype frequencies were estimated by allele counting, and differences between patient and control groups were tested for significance by the Fisher exact test and/or χ^2 analysis, where applicable. A probability value smaller than .05 was regarded as statistically significant.

Table 1 Potential disease-causing mutations identified in the *RET* proto-oncogene in MTC

Family no.	Exon/intron	Mutation	Nucleotide change	Effect on coding sequence	Sex/ethnic group[a]	Diagnosis
12	19	D1017N[b]	GAC→AAC	Missense	CM	MTC
12A	20	IVS19-37G/C[b]		Splice acceptor	CM	MTC carrier
102	13	D771N	GAC→AAC	Missense	WF	FMTC
102A	18	M1009V	ATG→GTG	Missense	WF	FMTC
106	10	C620W	TGC→TGG	Missense	CM/CF	HSCR[c]
112	11	C634S[d]	TGC→AGC	Missense	BF/BF/BF	MEN 2A
112A	19	V1041G[d]	GTG→GGG	Missense	BF	MEN 2A
130	4	IVS4-18G/A		Splice acceptor	WM	MEN 2
146	11	C634Y[e]	TGC→TAC	Missense	WF	MEN 2A
146	12	A750P[e]	GCA→CCA	Missense	CF/CF	MEN 2A
148	11	C634R	TGC→CGC	Missense	CF/CF	MEN 2A

[a] Ethnic grouping: W (M/F) indicates white; C (M/F), mixed ancestry (coloured); B (M/F), African (Black).
[b] Variants identified in the same individuals.
[c] Mother of index patient diagnosed with MEN 2A.
[d] Variants identified in the same individuals.
[e] Variants identified in the same individuals.