RET gene mutations and polymorphisms in medullary thyroid carcinomas in Indian patients

BP Sharma and D Saranath*

Molecular Medicine, Reliance Life Sciences Pvt Ltd, Dhirubhai Ambani Life Sciences Center, Navi Mumbai 400 701, India

**Corresponding author (Fax, 91-022-4067 8099; Email, saranathd@aol.com)*

Germline mutations of *RET* gene are pathognomonic of multiple endocrine neoplasia (MEN; MEN 2A/MEN 2B) and familial medullary thyroid carcinoma (FMTC), constituting 25% of medullary thyroid carcinomas (MTCs). We investigated *RET* gene mutations and polymorphisms at exons 10, 11, 13, 14, 15 and 16 in 140 samples, comprising 51 clinically diagnosed MTC patients, 39 family members of patients and 50 normal individuals. The method of choice was PCR and direct nucleotide sequencing of the PCR products. *RET* gene mutations were detected in 15 (29.4%) patients, with MEN 2A/FMTC in 13 patients and MEN 2B in 2 patients. Further, 39 family members of seven index cases were analysed, wherein four of the seven index cases showed identical mutations, in 13 of 25 family members. We also examined single nucleotide polymorphisms (SNPs) in *RET* gene exons in 101 unrelated samples. Significant differences in the allelic frequencies of SNPs at codons 691, 769, 836 and 904 between patient and control groups were not observed. However, SNP frequencies were significantly different in the Indian group as compared with other European groups. We identified two novel, rare and unique SNPs separately in single patients. Our study demonstrated presence of MEN 2A/MEN 2B/FMTC-associated mutations in accordance with the reported literature. Thus, *RET* gene mutations in exons 10, 11, 13, 14, 15 and 16 constitute a rapid test to confirm diagnosis and assess risk of the disease in familial MEN 2A/MEN 2B/FMTC.

[Sharma BP and Saranath D 2011 *RET* gene mutations and polymorphisms in medullary thyroid carcinomas in Indian patients. *J. Biosci.* **36** 603–611] **DOI** 10.1007/s12038-011-9095-0

1. Introduction

Medullary thyroid carcinoma (MTC) constitutes 5–10% of all thyroid malignancies, with 75% sporadic MTCs and 25% hereditary MTCs transmitted in an autosomal-dominant pattern (Eng *et al.* 1996). The inherited MTCs present as multiple endocrine neoplasia (MEN) syndromes, MEN 2A, MEN 2B and familial medullary thyroid carcinoma (FMTC), with MEN 2A accounting for about 90% of all MEN 2 cases, and is characterized by hyperplasia or adenoma of parathyroid glands and multifocal pheochromocytoma (Eng 1999; Ponder 1999). The most aggressive variant of MTC is MEN 2B, which appears in conjunction with marfanoid habitus, intestinal ganglioneuromatosis, myelinated corneal nerves, buccal neuromas and pheochromocytoma (Eng 1996). The molecular pathology of inherited MTCs is constitutive activation of proto-oncogene *RET* (REarranged during Transfection, MIM 164761). The *RET* gene is located on chromosome 10q11.2, consisting of 21 exons (Eng 1996). The *RET* gene encodes a putative receptor tyrosine kinase with a cysteine-rich extracellular domain, a transmembrane domain and an intracellular tyrosine kinase domain (Eng 1999; Ponder 1999). The *RET* gene plays a critical role in cell signalling, and mutations in the gene leads to disruption of cell proliferation and differentiation of tissues derived from neural crest cells, such as C-cells of the thyroid gland, adrenal medulla, parathyroid gland and enteric autonomous nerve plexus. Germline mutations in the *RET* gene result in a spontaneous, ligand-independent receptor dimerization, autophosphorylation of tyrosine residues and consequent cell proliferation and tumour development (Eng 1999; Ponder 1999). Several independent mutations in the *RET* gene in the extracellular and tyrosine kinase domains, at exons 10, 11, 13, 14, 15 and 16, have been unequivocally

Keywords. Indian patients; MEN 2A, MEN 2B, FMTC; PCR nucleotide sequencing; RET gene mutations; *RET* gene polymorphisms

established as pathognomonic of MEN 2A, MEN 2B and FMTC (Eng *et al.* 1996).

In the more common MEN syndromes, MEN 2A and FMTC, 88–98% of the patients demonstrate primarily germline mutations, encoding cysteine codons 609, 611, 618, 620 and 634, with rare insertions/deletions reported in MEN 2A and FMTC. Mutations at exon 16 codon 918 are observed in 95% MEN 2B patients, with 5% mutations at codon 922 (Mulligan *et al.* 1995; Eng *et al.* 1996). The germline *RET* mutations are of diagnostic significance, and valuable in the treatment of MTC patients, as well as risk of multiple endocrine neoplasias in first-degree relatives of the patients. Thus, in patients with germinal mutations with or without macroscopic changes, total thyroidectomy is necessary, as partial thyroidectomy may result in bad prognosis. A prophylactic total thyroidectomy is favoured in children with *RET* gene mutation at an early age, resulting in markedly reduced morbidity and mortality (Sakorafas *et al.* 2008).

In several Western countries including North America and Europe, *RET* gene mutations are tested in medullary thyroid cancer patients and family members of the index cases with mutations (Sánchez *et al.* 1999; Klein *et al.* 2001; Jindrichová *et al.* 2004; Bergant *et al.* 2006; Strahm and Malkin 2006; Patocs *et al.* 2006; Sakorafas *et al.* 2008; *http://www.cancer.gov/*). However, in India, *RET* gene mutations are not tested routinely as per the study conducted on 234 cases at one of the premier cancer referral hospitals (Desai *et al.* 2005). There is paucity of data on *RET* gene mutations in Indian patients. A single study on 28 MTC patients has reported MEN 2A mutations in 4 patients (Menon and Simha 2005). Hence, the focus of our study was to determine the *RET* gene mutations at high-risk exons 10, 11, 13, 14, 15 and 16 in MTC patients and family members primarily at-risk first-degree relatives, using PCR and direct nucleotide sequencing of the PCR products. We also examined *RET* gene exon polymorphisms in the patients and unrelated control healthy individuals.

2. Materials and methods

2.1 *Samples*

The study group comprised 140 samples with 51 MTC patients, 39 first-degree relatives of 13 index cases with family history (1 to 7 members per family) and 50 healthy unrelated controls, for *RET* gene mutation and single nucleotide polymorphism (SNP) analysis. Patients presented with thyroid nodules or enlarged lymph nodes, neck pain, hoarseness of voice, macroglossia due to amyloid deposition, and elevated plasma calcitonin. Histopathology of biopsy/FNAC was used to confirm the diagnosis of MTC.

The patients constituted 24 males and 27 females, age 15 months to 60 years, mean age 37.5 years and median age 38.5 years. Ethnically, the patients were 51% (26/51) Dravidians and 49% (25/51) Aryans. A majority, i.e. 45/51 patients, were Hindus, 4 Muslim and 2 Christians, with an equivalent distribution in both the ethnic groups. The 39 first-degree relatives from 13 index cases comprised 18 males and 21 females, age 4.5 to 65 years, mean age 21.5 years and median age 18 years. *RET* mutations were detected in 7 index cases, whereas 6 index cases were without mutations.

The control samples consisted of 50 normal healthy individuals with no family history of MTC, and were investigated for SNPs at the critical exons in the Indian group. These normal controls comprised 32 males and 18 females, age 19 to 60 years. The ethnicity of the control group was similar to the patient group comprising 50% Dravidians and 50% Aryans, primarily Hindus, with a single individual of Muslim religion.

Peripheral blood samples (2–5 ml) in EDTA vacutainers (Becton & Dickinson, New Jersey, USA) were collected after obtaining informed consent from the patients, relatives and controls. The samples were investigated for *RET* gene mutation and SNP analysis. As the sample size was rather small, the frequency distribution was considered as 'proof-of-principle' to be confirmed in large sample sizes.

The project was approved by the independent Ethics Committee of Reliance Life Sciences, Navi Mumbai.

2.2 *Genomic DNA extraction*

Genomic DNA was isolated from peripheral blood using Qiagen DNA extraction kit as per the manufacturer's instructions (Qiagen, Hilden, Germany). The DNA was quantitated on Spectrophotometer ND-1000 (NanoDrop, Delaware, USA), and the 260/280 nm absorbance noted.

2.3 *PCR assay*

DNA (50 ng) was subject to PCR amplification for *RET* gene exons 10, 11, 13, 14, 15 and 16 as detailed in table 1. Positive controls containing specific mutations in the exons, negative controls with no *RET* gene mutations and reagent blank controls with no DNA were used in the assay. The PCR master mix contained 1× PCR buffer (100 mM NaCl, 50 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol and 1% Triton X-100), 200 µM dNTP (Promega, Wisconsin, USA), 10 pmol forward and reverse primers (Sigma-Genosys, Bangalore, India), 1.5 U Taq DNA Polymerase (Promega, Wisconsin, USA) and 1.5–2.0 mM $MgCl_2$. Standard primer sequences for individual *RET* gene exons were used (Jindrichová *et al.* 2004). Thermal cycling profile of the amplifications constituted initial denaturation

at 95°C for 5 min, followed by 40 cycles with denaturation at 95°C for 30 s, annealing at 60−68°C for 30 s dependent on the primer sequences, extension at 72°C for 1 min, with a final extension at 72°C for 10 min (table 1).

The PCR products were electrophoresed on 2% agarose gel containing ethidium bromide, visualized and recorded on Gel-Documentation system (Amersham Pharmacia Biotech, Uppsala, Sweden). A molecular weight marker, a 100 bp ladder (Fermantas, California, USA), was used to size the specific fragments. The amplimers were confirmed for the presence or absence of mutations by nucleotide sequencing.

2.4 *Nucleotide sequencing*

The PCR products of the *RET* gene exons were subjected to direct nucleotide sequencing to identify the mutations, using the forward and reverse primers in independent sequencing reactions for each exon. The exon PCR products and sequencing PCR products were purified by Montage™ SEQ_{96} clean-up kits as per the manufacturer's instructions (Millipore, Montana, USA). The automated nucleotide sequencer, ABI 3100 Genetic Analyzer and Big Dye Terminator v3.1 Cycle Sequencing Kit® were used as per the manufacturer's instructions (Applied Biosystems, California, USA). The sequences were compared with the reference *RET* gene sequence (AJ243297.1) using web-based tool ClustalW (*http://www.ebi.ac.uk/clustalW*), and Bioedit software for multiple alignment, to detect *RET* mutations and SNPs.

2.5 *Statistical analysis*

Allelic frequencies of SNPs in the index cases and control groups, and the compiled meta-analysis data on patient groups were compared using standard chi-square analysis. $p<0.05$ was considered statistically significant.

3. Results

3.1 *RET gene mutations in MTC patients*

Genomic DNA from the samples demonstrated specific amplification of *RET* exons as observed by the fragment sizes on agarose gel electrophoresis, indicating specific amplification of the exons (figure 1). The nucleotide sequences of the exons identified the PCR >99% products as *RET* exons 10, 11, 13, 14, 15 and 16, with 100% homology to the exons, with exception of the mutated sequences. A representative electropherogram of exon 11 codon 634 demonstrating the normal sequences and the mutation TGC to CGC is given in figure 2. We observed *RET* gene mutations in 15 MTC patients (29.4%), indicated in table 2, diagnostic of MEN 2A, FMTC or MEN 2B. Thus, mutations at exon 11 codon 634 were observed in 9 of 15 (60%) patients; 3 MTC patients demonstrated mutations in exon 10 codon 609/618, whereas 2 patients demonstrated mutations in exon 16 codon 918, and a single patient showed mutation in exon 14 codon 804 (table 2).

3.2 *RET gene mutations in family members*

Mutations were detected in 15 MTC patients (index cases), and were not observed in the other 36 patients clinically diagnosed with MTC. This group of patients with normal *RET* alleles were considered as sporadic MTC cases, also indicated by absence of MTC history in first-degree relatives of the patients. Samples from family

Table 1. Primers and PCR conditions for amplification of *RET* gene exons

Exon	Primers (5′– 3′)	$MgCl_2$ (mM)	Annealing temperature (°C)	Amplicon (bp)
10	10 F: GGG CCT ATG CTT GCG ACA CCA 10 R: CCA GAG GGA GGG AGG GAA GTT T	2.0	60	373
11	11 F: GGT CTA GGA GGG GGC AGT AAA TGG 11 R: CAG CGT TGG CAG CCC CTC ACA G	1.5	60	561
13	13 F: AGA AGC CTC AAG CAG CAT CGT C 13 R: AGG AGC AGT AGG GAA AGG GAG AAA	1.5	60	346
14	14 F: CAC GAG CAG CAG GAG GCA GAG A 14 R: GAG TGT GGC ATG GTG GGG GAG TGG	1.5	68	548
15	15 F: CCC CCG GCC CAG GTC TC 15 R: GCT CCA CTA ATC TTC GGT ATC TTT	2.0	66	354
16	16 F: GGC CTT CTC CTT TAC CCC TCC TT 16 R: CAG CCA TTT GCC TCA CGA ACC C	2.0	60	336

Initial denaturation: 95°C, 5 min; 40 cycles; denaturation: 95°C, 30 s; annealing: 30 s; extension: 72°C, 1 min; final extension: 72°C, 10 min.

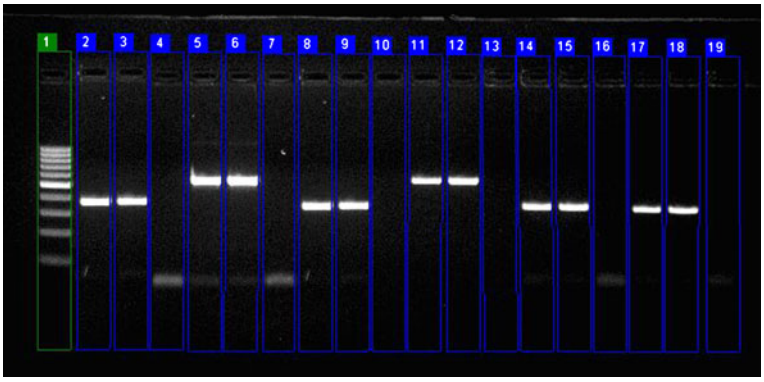

Figure 1. 2% Agarose gel electrophoresis of PCR products of *RET* exon 10 (373 bp, lanes 2, 3); exon 11 (561 bp, lanes 5, 6); exon 13 (346 bp, lanes 8, 9); exon 14 (548 bp, lanes 11, 12); exon 15 (354 bp, lanes 14, 15); exon 16 (336 bp, lanes 17, 18); 100 bp ladder (lane1); negative control (lane nos. 4, 7, 10, 13, 16, 19).

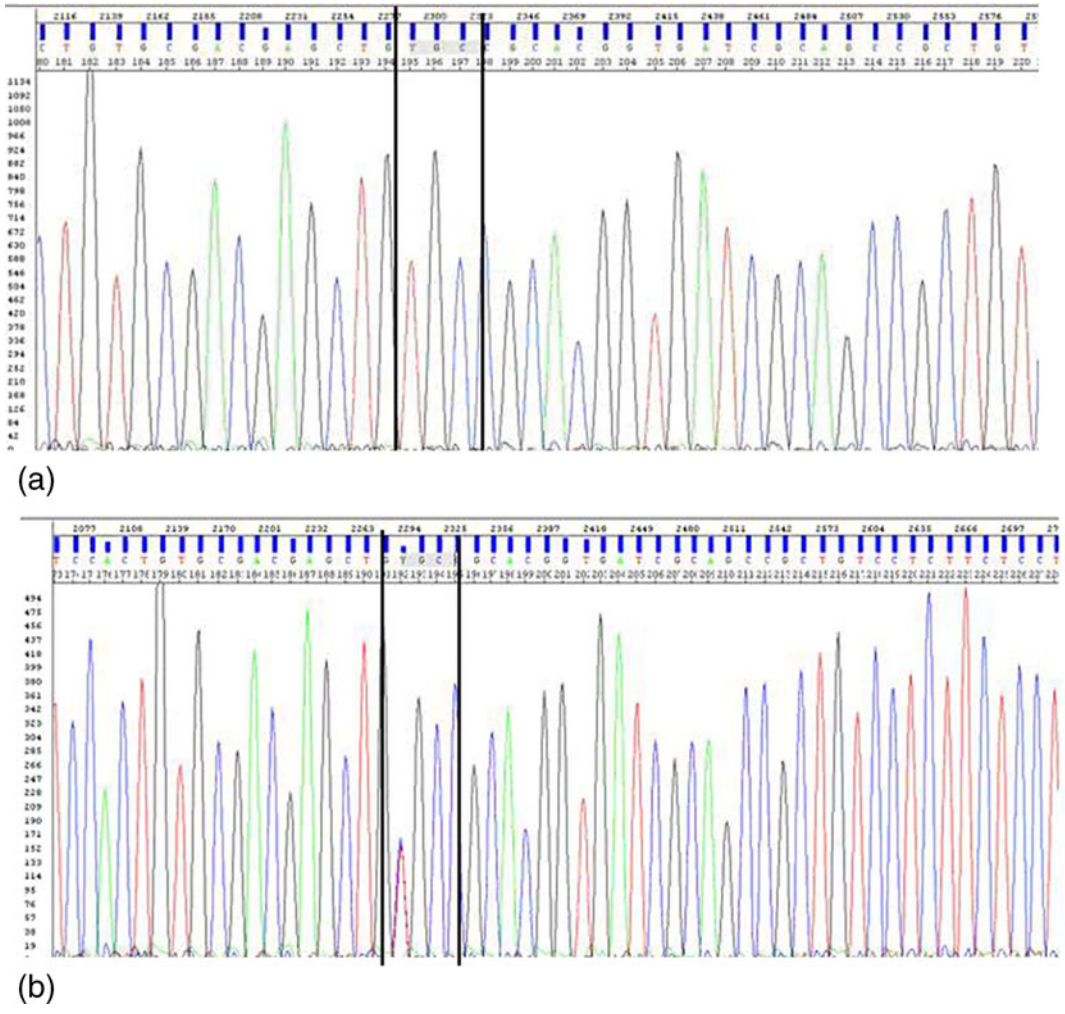

Figure 2. Sequencing electropherogram of *RET* gene, exon 11, codon 634, demonstrating individual patient with normal sequence TGC (**a**) and mutation TGC→ CGC (**b**). The codon sequences are shaded.

Table 2. *RET* gene mutations in hereditary MTC patients

Diagnosis	Exon	Alteration			Patients with mutations (n=51)
		Codon	Mutations	Amino acid substitution	
MEN 2A/FMTC	10	609	TGC → CGC	Cys → Arg	2
MEN 2A/FMTC	10	618	TGC → GGC	Cys → Gly	1
MEN 2A	11	634	TGC → CGC	Cys → Arg	4
MEN 2A	11	634	TGC → TAC	Cys → Tyr	4
MEN 2A	11	634	TGC → GGC	Cys → Gly	1
FMTC	14	804	GTG → ATG	Val → Met	1
MEN 2B	16	918	ATG → ACG	Met → Thr	2
Total					15 (29.4%)

The mutations are diagnostic of MEN 2A/FMTC/MEN 2B in the patients as indicated.

members (1–7 per family) of 13 index cases were available for *RET* gene mutation analysis. In this group, 6 index cases did not show characteristic *RET* gene mutations, and consequently, their family members (n=14) also did not show *RET* gene mutations. The additional 7 index cases demonstrated presence of *RET* gene mutations. We detected codon 634 mutations in 11 family members of 3 index cases, the mutations being identical in both the index cases and corresponding family members; 2 family members of a single index case showed identical mutation as the index case at codon 609, whereas 8 family members of 3 index cases with mutations, did not demonstrate the mutations (table 3). Representative pedigree charts of 7 MTC index cases with *RET* gene mutations are shown in figure 3.

3.3 *Mutation status in normal healthy controls*

Germline *RET* gene mutations were not observed in 50 healthy individuals with no family history of the disease.

3.4 *Polymorphisms in RET gene exons*

SNPs in the critical *RET* gene exons 10, 11, 13, 14, 15 and 16 were investigated in 101 samples comprising 51 MTC patients and 50 unrelated healthy individuals. Reference *RET* gene nucleotide sequence (AJ243297.1) was used for sequence alignment and identification of the SNPs. We observed polymorphisms at exon 11 codon 691, exon 13 codon 769, exon 14 codon 836 and exon 15 codon 904 (table 4). Genetic variants were not observed in exon 10. Rare variants were observed in exon 11, codon 682, and exon 16, codon 931, in a single patient each. The allele frequencies of the SNPs in MTC patients, healthy controls and total samples are indicated (table 4).

4. Discussion

The confirmation of *RET* gene mutations as diagnostic of MEN 2A, FMTC and MEN 2B in clinically diagnosed MTC patients, and screening of first-degree relatives for the

Table 3. *RET* gene mutations in family members of MEN 2A/FMTC/MEN 2B patients

Index case	Exon	Codon	Nucleotide	Amino acid substitution	Family members with *RET* mutation/total family members (n=25)
A.	11	634	TGC → CGC	Cys → Arg	4/5
B.	11	634	TGC → CGC	Cys → Arg	4/7
C.	11	634	TGC → TAC	Cys → Tyr	0/3
D.	11	634	TGC → CGC	Cys → Arg	3/3
E.	10	618	TGC → GGC	Cys → Gly	0/1
F.	10	609	TGC → CGC	Cys → Arg	2/2
G.	16	918	ATG → ACG	Met → Thr	0/4

Mutations in family members were identical to respective index case. 52% (13 of 25) of the family members inherited the mutations.

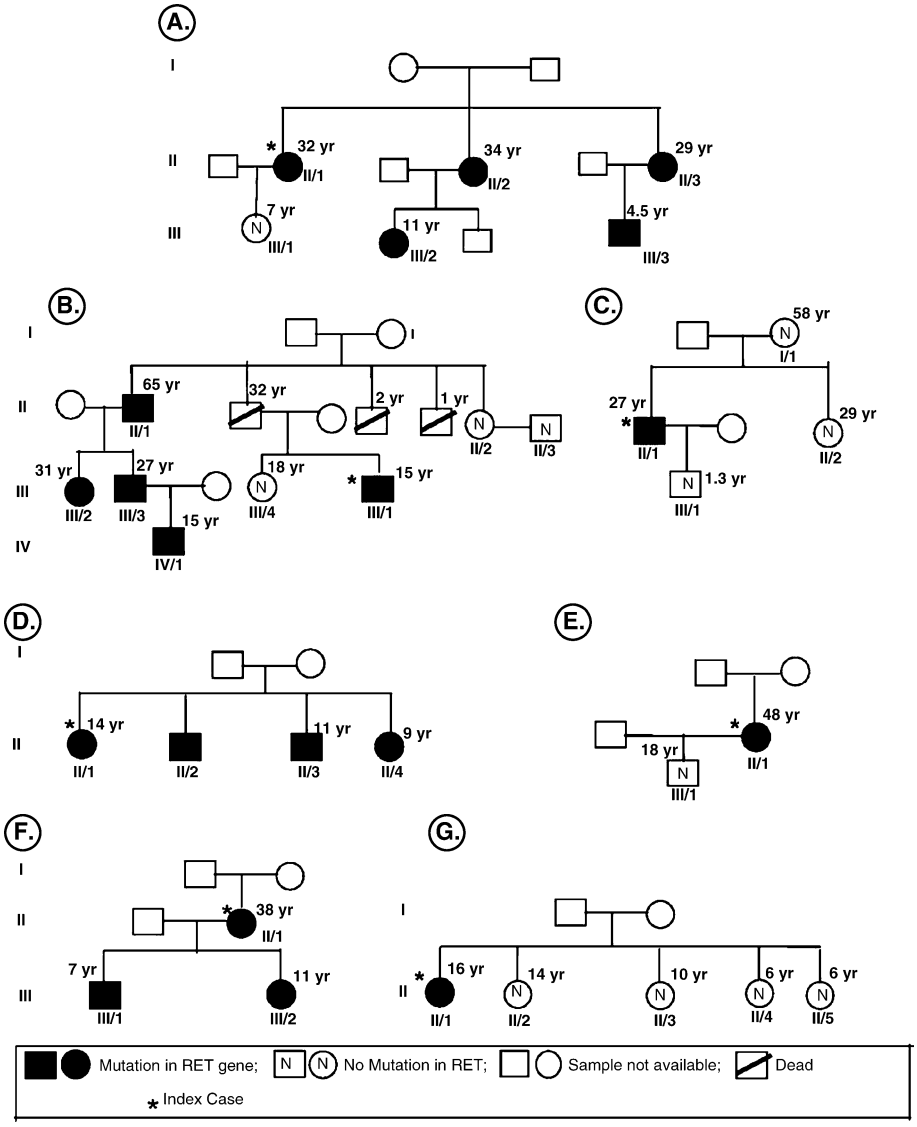

Figure 3. Pedigree charts of seven index cases and the family members.

Table 4. *RET* polymorphisms In Indian group

Exon	Codon	SNP	MTC patients Allelic frequency (*n*)	Healthy controls Allelic frequency (*n*)	Total allelic frequency (*n*)
11	691	**G**GT → **A**GT	0.49 (25/51)	0.48 ^{NS} (24/50)	0.48 (49/101)
13	769	CT**T** → CT**G**	0.45 (23/51)	0.58 ^{NS} (29/50)	0.51 (52/101)
14	836	AG**C** → AG**T**	0.25 (13/51)	0.22 ^{NS} (11/50)	0.23 (24/101)
15	904	TC**C** → TC**G**	0.37 (19/51)	0.28 ^{NS} (14/50)	0.33 (33/101)
11	682	**G**CC→ **T**CC*	0.019 (1/51)	0	0.009 (1/101)
16	931	AC**C**→ AC**G***	0.010 (1/51)	0	0.009 (1/101)

RET SNPs and allelic frequencies in patients and controls in codons 691, 769, 836, 904.

NS – No significant difference between MTC patients and controls. $p>0.05$ significant.

* Unique, novel polymorphism observed in single patients.

n = No. of patient samples/ control samples/total samples with SNPs/total samples tested.

mutations indicative of high risk of neoplasias in the family members, mandates analysis of *RET* gene mutations in MTC. The *RET* gene mutations are a critical factor in patient management, e.g. to decide on total or partial thyroidectomy or the age at which the child should be subject to surgery (de Groot *et al.* 2006). A large amount of data has been generated to unequivocally establish the value of *RET* gene mutations in diagnosis and management of inherited MTC patients, as also to predict risk in family members. However, there is paucity of data on *RET* gene mutations in patients from India. A single study on 28 MTC patients for detection of *RET* gene mutations has been reported (Menon and Simha 2005).

We investigated *RET* gene mutations in MTC patients and observed 29.4% (15/51) clinically diagnosed MTC patients, 13/25 (52%) family members with mutations identical to the index cases, and absence of mutations in 50 normal healthy control samples with no history of spontaneous or hereditary MTC. The most frequently observed mutation was exon 11 codon 634 (60% patients), followed by mutations in exon 10 (20%), exon 16 (13.3%) and exon 14 (6.6%). Thus, our data indicates codon 634 as the 'hot-spot codon' in MEN 2A and FMTC in our group of patients. A similar report from the International *RET* Mutation Consortium states that codon 634 is commonly mutated in 85% MEN 2A patients, with specific mutations TGC→CGC in 52% patients, TGC→TAC in 26% patients, and TGC→AGC in 18% patients (Mulligan *et al.* 1995; Eng *et al.* 1996). We observed pathognomonic mutations in exons 10, 11, 14 and 16 in the patient group, inherited in 52% of family members with mutations (table 3; figure 3), indicative of high risk of MEN. Besides, germline mutations were observed in exon 10 at codon 609:TGC→CGC and codon 618:TGC→GGC. Menon and Simha (2005) in their study on Indian patients have reported *RET* mutations at exons 10, 11 and 16 in 4 MEN 2A patients and 18 sporadic MTC. Further, multiple exon mutations in individual patients were also reported by the authors. The presence of multiple mutations, and high percentage of mutations in spontaneous MTCs, has not been frequently observed (Frilling *et al.* 1995; Sánchez *et al.* 1999; Klein *et al.* 2001; Jindrichová *et al.* 2004; Kameyama *et al.* 2004; Bergant *et al.* 2006). We also did not observe multiple mutations and mutations in sporadic MTC patients in our study, in accordance with earlier studies on various European sample groups.

In our study, first-degree relatives of MEN 2A/MEN 2B/FMTC showed *RET* gene mutations identical to the index cases in 52% of the family members. Our data is in concordance with 38–52% of the reports on inheritance of *RET* gene mutations in MEN 2A, FMTC and MEN 2B family members (Frilling *et al.* 1995; Bergant *et al.* 2006).

SNPs at the critical *RET* gene exons were investigated in our study, and we observed SNPs primarily at codons 691: GGT→AGT, 769:CTT→CTG, 836:AGC→AGT and 904: TCC→TCG in the patient group and healthy controls. Significant differences in the frequency of the SNP alleles were not observed in the patient and control groups (table 4), in concordance with several reports (Baumgartner-Parzer *et al.* 2005; Costa *et al.* 2005; Weinhaeusel *et al.* 2008). However, the prevalence of the SNPs in the Indian group was significantly different from the data on European groups including Germans, Italians, French, Spanish and Hungarians (Griseri *et al* 2000; Bounacer *et al.* 2002; Magalhães *et al.* 2004; Baumgartner-Parzer *et al.* 2005; Costa *et al.* 2005; Bergant *et al.* 2006; Fernandez *et al.* 2006; Guerrero *et al.* 2006; Gursoy *et al.* 2006; Weinhaeusel *et al.* 2008). The SNP in codon 691 has been demonstrated in a collision tumour of the thyroid in an Indian patient (Rekhi *et al.* 2007). A meta-analysis of SNPs at the mentioned codons demonstrated differences of significantly increased percentages of the SNPs in our group (table 5) (Griseri *et al* 2000; Bounacer *et al.* 2002; Magalhães *et al.* 2004; Baumgartner-Parzer *et al.* 2005; Costa *et al.* 2005; Bergant *et al.* 2006; Fernandez *et al.* 2006; Guerrero *et al.* 2006; Gursoy *et al.* 2006; Weinhaeusel *et al.* 2008).

Table 5. Meta-analysis of *RET* gene SNPs in the Indian group with European groups

	Meta analysis study *		
	Total samples analysed (*n*)	Samples with SNP *n* (%)	Current study (*n* = 101) SNP *n* (%)
Codon 691 (**G**GT → **A**GT)	3249	666 (20.5)	49 (48.5) $^{p > 0.001}$
Codon 769 (CC**T** → CT**G**)	2991	695 (23.25)	52 (51.5) $^{p > 0.001}$
Codon 836 (AG**C** → AG**T**)	2722	170 (6.2)	24 (23.8) $^{p > 0.001}$
Codon 904 (TC**C** → TC**G**)	1797	499 (27.8)	33 (32.7) $^{p = 0.002}$

Chi-square analysis; $p > 0.05$ significant.

The prevalence of the SNPs in the Indian group was significantly different from that of European groups.

* Griseri *et al.* 2000; Bounacer *et al.* 2002; Magalhães *et al.* 2004; Baumgartner-Parzer *et al.* 2005; Costa *et al.* 2005; Bergant *et al.* 2006; Fernandez *et al.* 2006; Guerrero *et al.* 2006; Gursoy *et al.* 2006; Weinhaeusel *et al.* 2008.

We did not observe co-segregation of alleles at codons 691 A allele and 904 G allele, and codons 836 T allele and 769 G allele, as reported by Weinhaeusel *et al.* (2008). Additionally, two unique and rare polymorphisms at codon 682:GCC→TCC and codon 904:TCC→TCG, not reported in the other studies cited in the meta-analysis data, were observed in two of our MTC patients. It cannot be ruled out that these two unique polymorphisms, observed in two patients, could be rare mutations with a low level of influence, acting as genetic modifiers and associated with a small increased relative risk for development of the disease. The *RET* gene SNPs in our study do not indicate a role in predisposition to multiple endocrine neoplasias, and show no significant difference in the allelic frequencies in the patient and control groups. A study with a larger number of patient samples and controls will be needed to confirm the same.

In conclusion, our studies confirm the constitutive mutations in peripheral blood of patients with MEN 2A/MEN 2B/FMTC at exons 10, 11, 13, 14, 15 and 16, and the common SNPs reported in several studies on European patient samples. The results emphasize the necessity of molecular genetic tests to detect and identify *RET* gene mutations associated with MEN. The first-degree relatives of index cases with *RET* gene mutations should be tested for MEN risk assessment by *RET* gene mutation testing.

Acknowledgements

The authors gratefully acknowledge Dr Vinay Deshmane, Consultant Oncosurgeon, Hinduja Hospital and Research Centre, Mumbai, the patients, relatives and volunteers who participated in the study, and the encouragement and support of Reliance Life Sciences Pvt Ltd, Navi Mumbai.

References

Baumgartner-Parzer SM, Lang R, Wagner L, Heinze G, Niederle B, Kaserer K, Waldhausl W and Vierhapper H 2005 Polymorphisms in Exon 13 and Intron 14 of the *RET* protooncogene: Genetic modifiers of medullary thyroid carcinoma. *J. Clin. Endocrinol. Metabol.* **90** 6232–6236

Bergant D, Hocevar M, Besic N, Glavac D, Korosec B and Caserman S 2006 Hereditary medullary thyroid cancer in Slovenia-genotype-phenotype correlations. *Middle Eur. J. Med.* **118** 411–416

Bounacer A, Villard JAD, Wicker R, Caillou B, Schlumberger M, Sarasin A and Suárez HG 2002 Association of *RET* codon 691 polymorphism in radiation-induced human thyroid tumours with C-cell hyperplasia in peritumoural tissue. *Brit. J. Cancer* **86** 929–1936

Costa P, Domingues R, Sobrinho L and Bugalho MJ 2005 *RET* polymorphisms and sporadic medullary thyroid carcinoma in a Portuguese population. *Endocrine* **27** 239–243

de Groot JWB, Links TP, Plukker JTM, Lips CJM and Hofstra RMW 2006 *RET* as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors. *Endocrinol. Rev.* **27** 535 –560

Desai S, Sarkar S and Borges A 2005 A study of histopathological features of medullary carcinoma of the of the thyroid: Cases from a single institute in India. *Indian J. Cancer* **42** 21–25

Eng C 1996 The RET proto-oncogene in Multiple endocrine neoplasia type 2 and Hirschsprung's disease. *New Engl. J. Med.* **335** 943 –951

Eng C 1999 *RET* proto-oncogene in the development of human cancer. *J. Clin. Oncol.* **17** 380 –393

Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, Amstel HKP, Lips CJM, Nishisho I, *et al.* 1996 The relationship between specific *RET* proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2 International *RET* mutation consortium analysis. *J. Am. Med. Association* **276** 1575–1579

Fernandez RM, Navarro E, Antinolo G, Ruiz-Ferrer M and Borrego S 2006 Evaluation of the role of *RET* polymorphisms/haplotypes as modifier loci for MEN 2, and analysis of the correlation with the type of *RET* mutation in a series of Spanish patients. *Int. J. Mol. Med.* **17** 575–581

Frilling A, Dralle H, Eng C, Raue F and Broelsch CE 1995 Presymptomatic DNA Screening in families with multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma. *Surgery* **118** 1099–1104

Griseri P, Sancandi M, Patrone G, Bocciardi R, Hofstra R, Ravazzolo R, Devoto M, Romeo G and Ceccherini I 2000 A single-nucleotide polymorphic variant of the *RET* proto-oncogene is underrepresented in sporadic Hirschsprung disease *Eur. J. Human Genet.* **8** 721–724

Guerrero IM, Pessoa CHC, Olmedo DB, Pontes ERP, Matos LC, Tilli TM, Barcinski MA and Gimba ERP 2006 Analysis of inherited genetic variants in *ret* proto-oncogene of Brazilian patients with apparently sporadic medullary thyroid carcinoma. *Thyroid* **16** 9 –12

Gursoy A, Erdogan MF and Erdogan G 2006 Significance of the *RET* proto-oncogene polymorphisms in Turkish sporadic medullary thyroid carcinoma patients. *J. Endocrinol. Invest.* **29** 858 –862

Jindrichová S, Vcelák J, Vlcek P, Neradilová M, Neˇmec J and Bendlová B 2004 Screening of six risk exons of the *RET* proto-oncogene in families with medullary thyroid carcinoma in the Czech Republic. *J. Endocrinol.* **183** 257 –265

Kameyama K, Okinaga H and Takami H 2004 *RET* oncogene mutations in 75 cases of familial medullary thyroid carcinoma in Japan. *Biomed. Pharmacotherapy* **58** 345 –347

Klein I, Ésik O, Homolya V, F Szeri and Váradi A 2001 Molecular genetic diagnostic program of multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma syndromes in Hungary. *J. Endocrinol.* **170** 661–666

Magalhães KRP, Castro M, Elias LLK, Soares EG and Maciel LM Z 2004 Polymorphisms in the *RET* proto-oncogene and the phenotypic presentation of familial medullary thyroid carcinoma. *Thyroid* **14** 848 –852

Menon M and Simha MR 2005 *RET* mutation status in medullary thyroid cancer (MTC) patients and the significance of genetic

screening for mutations in their immediate relatives–a preliminary report. *Indian J. Pathol. Microbiol.* ***48*** 161–165

Mulligan LM, Marsh DJ, Robinson BG, Schuffenecker I, Zedenius J, Lips CJ, Gagel RF, Takai SI, Noll WW, Fink M, *et al.* 1995 Genotype phenotype correlation in multiple endocrine neoplasia type 2: report of the international *RET* mutation consortium. *J. Intern. Med.* **238** 343 –346

Patocs A, Klein I, Szilvasi A, Gergics P, Toth M, Valkusz Z, Forizs E, Igaz P, Al-Farhat Y, Tordai A, Varadi A, Racz K and Esik O 2006 Genotype-phenotype correlations in Hungarian patients with hereditary medullary thyroid cancer. *Wien Klin Wochenschr.* **118** 417–21

Ponder BAJ 1999 The phenotypes associated with *ret* mutations in the multiple endocrine neoplasia type 2 syndrome. *Cancer Res.* **59** 1736 –1742

Rekhi B, Badhe RR, Desouza MA, Chaukar D, D'Cruz AK, Arya S and Kane SV 2007 A Unique *RET* exon 11 (G691S) polymorphism in an Indian patient with a collision tumor of the thyroid. *Diagnostic Pathol.* **2** doi:10.1186/1746-1596-2-39

Sakorafas G, Friess H and Peros G 2008 The genetic basis of hereditary medullary thyroid cancer: clinical implications for the surgeon, with a particular emphasis on the role of prophylactic thyroidectomy. *Endocrine-Related Cancer* **15** 871–884

Sánchez B, Robledo M, Biarnes J, Sáez ME, Volpini V, Benítez J, Navarro E, Ruiz A, Antiñolo G and Borrego S 1999 High prevalence of the C634Y mutation in the *RET* proto-oncogene in MEN 2A families in Spain. *J. Med. Genet.* **36** 68–70

Strahm B and Malkin D 2006 Hereditary cancer predisposition in children: Genetic basis and clinical implications. *Int. J. Cancer* **119** 2001–2006

Weinhaeusel A, Scheuba C, Lauss M, Kriegner A, Kaserer K, Vierlinger K, Haas O and Niederle B 2008 The influence of gender, age, and *RET* polymorphisms on C-cell hyperplasia and medullary thyroid carcinoma. *Thyroid* **18** 1269–1276

MS received 13 January 2011; accepted 17 May 2011

*e*Publication: 16 August 2011

Corresponding editor: PARTHA P MAJUMDER