CLINICAL STUDY

Presentation of a kindred with familial medullary thyroid carcinoma and Cys611Phe mutation of the *RET* proto-oncogene demonstrating low grade malignancy

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Abstract

Objective: Both multiple endocrine neoplasia type 2A (MEN 2A) and familial medullary thyroid carcinoma (FMTc) are caused by germline mutations of the *RET* proto-oncogene. A broad spectrum of malignancy within and between families has been described with no clear genotype–phenotype correlation due to a scarcity of available data of large kindreds.

Design: Here we present the only known family with a germline mutation of codon 611 TGC to TTC (exon 10) in the *RET* proto-oncogene leading to a replacement of cysteine by phenylalanine (Cys611Phe or C611F).

Results: Twenty family members of this large kindred are gene carriers (GCs) and seven (5–13 years old) are potential carriers but have yet to be analysed. The clinical course of medullary thyroid carcinoma (MTC) in this family is characterized by a very slow evolution and progression of the tumour with no MTC-related death to date. Of 11 patients (30–69 years old) having undergone thyroidectomy six were classified as pT₁, four as pT₂ and one as C-cell hyperplasia according to the TNM system of the International Union Against Cancer. Due to cervical and mediastinal lymph node metastasis one patient (44 years old) had to be operated on a second time. The seven non-operated GCs of the fourth and fifth generation (17–26 years old) are yearly monitored with pentagastrin stimulation tests; one non-operated GC (43 years old) has refused any further investigations. Screening for primary hyperparathyroidism and pheochromocytoma was negative in all cases.

Conclusion: We suggest from these experiences that the general advice for thyroidectomy in early childhood should be modified in certain families, depending on genotype.

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Introduction

Multiple endocrine neoplasia type 2A (MEN 2A) is a hereditary syndrome characterized by development of medullary thyroid carcinoma (MTC), pheochromocytoma and hyperparathyroidism. Familial MTC (FMTc) presents with MTC only (1). Both MEN 2A and FMTc, are caused by germline mutations of the *RET* proto-oncogene and show autosomal dominant inheritance (2, 3). Genetic identification of asymptomatic gene carriers now allows the accurate diagnosis of MEN-2 at an early stage of the disease (4–6). Medullary thyroid carcinoma develops in virtually all patients with MEN 2A or FMTc (7). It is the most important manifestation of the syndrome and the most frequent cause of mortality. Cure by operation can only be achieved if the tumour is limited to the thyroid gland and spreading to the local lymph nodes has not

occurred. In addition, preoperative calcitonin levels do not distinguish reliably in all cases between C-cell-hyperplasia and carcinoma (7, 8). Microcarcinoma despite normal pentagastrin test has been observed in children 4–9 years old (8, 9). Lymph node metastasis may occur very early in the course of the disease and has been observed at the age of 14 years (10) or even earlier (11). Therefore, prophylactic thyroidectomy before the development of microcarcinoma is usually recommended (8–12).

However, in some studies variable biological activity of MTC was shown (13, 14). The MTC that occurs in FMTc seems to develop later in life and to grow more slowly compared with MTC in patients with MEN 2A. Indeed, specific mutations of the *RET* proto-oncogene considerably influence the phenotype, but data on the relationship between *RET* mutations and the clinical outcome in MEN-2A are scarce. It has been

Figure 1 Pedigree of the FMTC kindred with a Cys611Phe mutation of the *RET* proto-oncogene. Members of each generation are numbered consecutively (grey numbers above the symbols). The numbers below the symbols represent the present age of the kindred member.

demonstrated by clinical and functional studies that the non-634 cysteine codon mutations are of lower penetrance and the further away from the membrane these codons are, the lower the penetrance (3, 15, 16).

In this study we present the largest kindred described to date with germline codon 611 TGC to TTC mutation (exon 10) in the *RET* proto-oncogene. In affected family members MTC developed relatively late in life and never caused death in any of the hosts.

Subjects and methods

Patient population

A German FMTC kindred of 80 individuals spanning three generations (III to V, Fig. 1) was evaluated for disease expression. Initial screening of the third generation included medical history, physical examination and laboratory tests, which included the pentagastrin test, *RET* gene mutation analysis and thyroid sonography. Members of the fourth and fifth generation were examined similarly if they had an affected parent.

Genetic screening

Extraction of genomic DNA and amplification of exons 10, 11 and 16 from the *RET* proto-oncogene were performed as previously described (17). Genomic DNA was isolated using the QIAMP blood kit (Qiagen, Hilden, Germany). PCR amplifications were carried out with the oligonucleotide primer ret19S (5'-GCAG-CATTGTTGGGGGACA-3') and ret10Rb (5'-GTC-CCGGCCACCCACT-3') for exon 10 (size of amplified

fragment 140 bp). A total of 100 ng DNA were amplified in a Hybaid Omnigene apparatus (Hybaid, Teddington, UK) in a volume of 25 µl containing 1 µmol/l of each oligonucleotide primer, 1 mmol/l dNTP, 10 mmol/l Tris-HCl (pH 8.3), 2.5 mmol/l MgCl₂, and 1 U *Taq* polymerase (Appligen, Heidelberg, Germany). The PCR was started with 1 min of denaturation at 95 °C, and, finally 5 min at 72 °C. The amplified DNA was analysed on a 2% agarose gel and purified with a Quickspin kit (Qiagen). PCR-amplified DNA was sequenced using fluorescently labelled dideoxy terminators (Prism Ready Reaction, Applied Biosystems kit, Foster City, CA, USA). After identification of the MTC-causing mutation, the amplified DNA-fragments from other members of the family were analysed by restriction enzyme analysis (MboI, New England Biolabs, Frankfurt, Germany) only. Restriction enzyme digests were carried out according to the instructions of the supplier. Analysis of the DNA fragments was performed by PAGE with subsequent silver staining.

Biochemical testing

Family members positive for the *RET* mutation underwent biochemical analysis for MTC by radioimmunoassay measurement of plasma calcitonin levels following intravenous pentagastrin (0.5 µg/kg body weight/10 s). The patients were studied in a fasting state. Blood samples were collected before and at 2 and 5 min after the intravenous injection of pentagastrin (Pentagastrin BP, Cambridge Laboratories, Newcastle upon Tyne, UK). We used two different calcitonin

assays. Until 1997 the test used (dsl, Diagnostics Systems Laboratories, Webster, TX, USA) was regarded positive when detecting elevated (>100 pg/ml) levels of plasma calcitonin (CT) either in the basal or stimulated state (intra-assay variation 4.5%, inter-assay variation 7.5%). Using a more sensitive assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) the normal plasma CT levels were less than or equal to 4.6 pg/ml in females and less than or equal to 11.5 pg/ml in males (intra-assay variation 3.8%, inter-assay variation 8.6%).

Primary hyperparathyroidism (pHPT)

To assess parathyroid function, total calcium was determined using routine diagnostic testing. Serum parathyroid hormone (PTH1–84) was determined by immunoradiometric assay with carboxy-terminal specific antisera (DPC Biermann, Bad Nauheim, Germany). Testing for pHPT was repeated every year in patients positive for the *RET* proto-oncogene.

Phaeochromocytoma

Evaluation for phaeochromocytoma included determination of 24 h urinary excretion of adrenaline, noradrenaline and vanillylmandelic acid. Testing for phaeochromocytoma was repeated every year in patients positive for the *RET* mutation.

Surgery

Medullary thyroid carcinoma was treated by total thyroidectomy with resection of the lymph nodes in the central zone of the neck. The TNM system of the International Union Against Cancer (1997) was used for tumour classification. Postoperatively, L-thyroxine was given as replacement therapy.

Postoperative evaluation

All affected members have been screened annually by physical examination, determination of serum calcium, urinary catecholamine, basal and stimulated plasma CT levels and an ultrasound examination of the thyroid compartment and the neck.

Evaluation of gene carriers

Operated and non-operated gene carriers (GCs) are followed up at yearly intervals.

Results

Evaluation of GCs

The index case is a female now 74 years old (Fig. 1, III14). In 1982 at the age of 56 she had a

thyroidectomy followed by radiation therapy. In 1986 she underwent further surgery due to tumour recurrence and lymph node metastasis. Ever since, calcitonin levels have been elevated due to mediastinal lymph node and suspected bone metastasis. Until now she has not had any symptoms attributable to the tumour.

We were able to test eight other members of the same generation and found six gene carriers (GCs) (Fig. 1). Of these subjects five underwent total thyroidectomy with neck dissection between 1992 and 1997. When classified three (aged 68–72 years) had $pT_1N_0M_0$ (two continued to have a positive pentagastrin stimulation test postoperatively), and two (aged 59 and 67 years) had $pT_2N_0M_0$ (Table 1). A 67-year-old male with positive pentagastrin stimulation test could not be operated on because of concomitant diseases. Four members of this generation refused investigation of themselves or their families.

Among the 23 members of the fourth generation, 17 were tested and nine were found to be GCs (Fig. 1). Six of these had total thyroidectomy with neck dissection between 1989 and 1997. When classified three (aged 45–51 years) had $pT_1N_0M_0$, and one (aged 34 years) had hyperplastic C-cells (Table 1). All had negative pentagastrin stimulation tests postoperatively. One (aged 50 years) had $pT_2N_1M_0$ (two operations) and postoperatively continues to show raised CT levels. The three non-operated GCs (aged 23–43 years) have had negative pentagastrin stimulation tests until now and refuse thyroid surgery. One member of this generation (IV17) has refused further testing so far.

The fifth generation has 34 known members. We tested all but six of those with a GC parent. Of nine tested subjects four (aged 17–26 years) were GCs (Fig. 1). All had negative pentagastrin stimulation tests and none have undergone thyroidectomy to this point. The six members not tested to date, children under 13 years old, are planned to have gene analysis as soon as possible.

All GCs repeatedly tested negative for pHPT and phaeochromocytoma.

Evaluation of operated patients

At the time of the initial pentagastrin testing all patients who subsequently underwent surgery had established or advanced disease. The youngest operated patient (30 years old) showed only C-cell hyperplasia. The youngest patient with histologically established disease was 34 years old. Only three patients showed persistent disease postoperatively according to pentagastrin testing; one is the index patient (III14, Table 1) with advanced disease but with slow progression in the control visits. The second, a 69-year-old man at time of operation (III9, Table 1), showed only local tumour and postoperatively normal basal calcitonin but stimulated levels remain above normal. The third, a 44-year-old woman (IV4, Table 1) with lymph node metastasis,

Table 1 Clinical characteristics of affected family members operated on by thyroidectomy. Calcitonin (CT) levels measured by: ¹²⁵I-Nichols assay (normal values: <4.6 females, <11.5 males), ¹²⁵I-dsl diagnostics (normal values: <100).

Patient generation (Fig. 1)	Sex	Date of total thyroidectomy (month/year)	Age at operation (years)	Tumour stage (pTNM)	Basal CT preoperatively (pg/ml) ^d	Stimulated CT preoperatively (at 2 min, pg/ml) ^d	Basal CT postoperatively (pg/ml)	Stimulated CT postoperatively (at 2 min, pg/ml)	Normal CT values postoperatively (pg/ml)
III 9	m	5/97	69	pT ₁ N ₀ M ₀	212	4538	19 ^d	183 ^d	<100
III 5	f	9/96	65	pT ₁ N ₀ M ₀	310	nd	2 ^d	108 ^d	<100
III 10	m	1/96	63	pT _{2b} N ₀ M ₀	832	18 800	14 ^d	10 ^d	<100
III 16	m	8/92	60	pT ₁ N ₀ M ₀	137	1924	<0.7 ^d	<0.7 ^d	<100
III 3	m	4/97	56	pT _{2a} N ₀ M ₀	405	11 185	<0.7 ⁿ	3.5 ⁿ	<11.5
III 14	f	2/82	56	pT ₁ N ₁ M _x	nd	nd	1830 ⁿ	nd	<4.6
IV 4	f	11/96	44	pT _{2b} N _{1b} M ₀	970	810	72 ⁿ	nd	<4.6
IV 5	f	2/97	42	pT ₁ N ₀ M ₀	211	1123	16 ^d	19 ^d	<100
IV 15	f	8/89	40	pT ₁ N ₀ M ₀	287	12 084	<0.7 ⁿ	<0.7 ⁿ	<4.6
IV 8	m	12/96	39	pT _{2a} N ₀ M ₀	2447	22 692	15 ^d	53 ^d	<100
IV 5	f	8/89	34	pT ₁ N ₀ M ₀	32	578	<0.7 ⁿ	<0.7 ⁿ	<4.6
IV 18	f	6/96	30	CCH	36	127	<0.7 ⁿ	nd	<4.6

CCH, C-cell hyperplasia; nd, not determined.

presented the most aggressive form of the tumour yet observed under our surveillance. Therefore, all three patients with persistent disease were more than 40 years old and, with one exception, had tumours >T₁N₀. The main difference between the 'cured' and the 'persistent' disease groups was the more advanced age in the persistent disease group. All other patients who underwent surgery and whose tumours were classified between CCH and pT_{2b}N₀M₀ were cured.

Discussion

In this paper, we present a large kindred with germline codon 611 TGC to TTC mutation (exon 10) in the *RET* proto-oncogene which results in the substitution of phenylalanine for cysteine (C611F). The MTC developed relatively late in life, progressed slowly and never caused death in any of the hosts. The presence of hyperparathyroidism and phaeochromocytoma was excluded by extensive testing.

To date 13 different mutations at codon 611 in Exon 10 have been described (3); six of them were characterized according to their phenotype (17–21), leading to both the MEN 2A (*n* = 4) and FMTC (*n* = 2) phenotype (Table 2). Due to small family size or incomplete screening information, the other seven mutations were not further classified (3). So far this is the largest reported family with a codon 611 mutation and the only family with a phenylalanine substitution at this codon. A C611G kindred in the north of Spain with nine members, three of them with FMTC, showed a rather benign disease progression (21). Furthermore, Wells *et al.* (8) reported on a C611T kindred with eleven members, three of them with proven clinical disease, and six at risk of inheritance but with no data on the clinical course of the disease. In other families with a mutation of codon 611 (Table 2) a genotype to phenotype correlation has not been established because of the small number of kindred members. Cysteine mutations of codon 611, as well as codons 609, 618 and 620, are most likely to be associated with FMTC, are less frequent in families with MEN 2A and show weaker *RET* activity (22–24). In this context a large kindred of 150 members with a C618S mutation in exon 10 and clinically or biochemically proven MTC in 44 patients is of great scientific interest. The C618S mutation shows a later age of conversion of the pentagastrin testing (>18 versus >6 years), higher cure rates and a life expectancy of 60 years compared with 48 years in families with C634 mutation. This demonstrates a distinct clinical course of MTC with the C618S mutation (25), similar to the findings in our C611F kindred.

In MEN 2A patients the clinical course of MTC is highly variable and these experiences have led to the recommendation of prophylactic thyroidectomy in MEN 2A patients as young as 5 years old (9–11, 14) and strict yearly provocation test screening from 1 year old

Table 2 Correlation of different amino acid substitutions at codon 611 of the *RET* proto-oncogene with the phenotype (from The Human Gene Mutation Database Cardiff (HGMD)).

Codon	Nucleotide	Amino acid	Phenotype	Reference
611	TGC→TAC	Cys→Tyr	MEN 2A	Landsvater <i>et al.</i> 1996
611	TGC→TTC	Cys→Phe	FMTC ^a	Frank-Raue <i>et al.</i> 1996 ^b
611	TGC→TGG	Cys→Trp	MEN 2A	Donis-Keller <i>et al.</i> 1993
611	cTGC→AGC	Cys→Ser	MEN 2A	Kambouris <i>et al.</i> 1996
611	cTGC→CGC	Cys→Arg	MEN 2A	Kambouris <i>et al.</i> 1996
611	cTGC→GGC	Cys→Gly	FMTC	Oriola <i>et al.</i> 1998

^aIncorrectly referred to as MEN 2A in the database. ^bTwo members of our kindred were published within the German MTC Study Group.

onwards. The rationale for this approach is based on the assumption that earlier diagnosis should allow curative surgery in nearly all cases. However, it is of interest to recall that Lips *et al.* (7) emphasized that the risk of complications of surgery (e.g. recurrent nerve paralysis and hypoparathyroidism) in young children is not outweighed by the benefit of prevention of MTC (7). They pointed out that the majority of their patients who were operated on, based on the conversion of the plasma calcitonin stimulation test, had no recurrence of the disease later on. Therefore, they suggested postponement of surgery either until results of the stimulation test become positive or until the age of 12 or 13 years, provided regular follow up examinations are conducted (7). However, in a later publication the same author agreed with the above-described more aggressive management approach (9). Even with regard to the previously mentioned MEN 2A family with codon C618S and a very benign clinical course, no exception from the rule was proposed (25). More recently, in genotype–phenotype studies of patients with MEN 2A in Japan the authors were able to correlate variation in tumour size and clinical course of MTC to mutations 618, 634 and 918 (26). The authors suggested modification of the timing of operative procedures in patients with MEN 2A according to genotype. In their opinion, prophylactic thyroidectomy should be performed at an early age for the C634Y or M918T genotype carriers, whereas it could be withheld in the C618S or C634R carriers until the conventional biochemical or imaging examinations become positive (26).

In addition to these genotype–phenotype studies, two different mutations in codon 804 in exon 14 were described which also lead to a mild phenotype of FMTC. The replacement of a Val by a Leu (27) in one large family with 12 GCs in three generations revealed MTC after the age of 50 years in three GCs and foci of C-cell hyperplasia but no medullary thyroid carcinoma in a GC aged 30 years. Two GCs had a positive pentagastrin stimulation test but had yet to undergo surgery. Six GCs with negative stimulation tests were 29 to 41 years old and had not yet undergone surgery (27). The replacement of a Val with a Met residue (28) also revealed a mild phenotype. Interestingly, in the two families

described the subsequent generations showed an earlier and more aggressive phenotype with MTC occurring at the age of 16 years in one GC. Three young GCs (7–12 years old) had not yet undergone surgery due to negative pentagastrin stimulation tests (28). Recently, Feldman *et al.* (29) reported on two additional FMTC families with the V804M mutation in exon 14. Some GCs over the age of 70 years had no overt clinical evidence of MTC. However, because of active MTC and metastases in younger members (30 and 32 years) of these families, the authors continued to advocate thyroid surgery in mutation-positive individuals (29).

Here we have presented evidence that the mutation C611F in exon 10 causes FMTC and shows an indolent clinical course of the disease in the affected kindred. All patients described in Table 1 were operated on because of visible tumour and/or pathological pentagastrin testing. So far we have only observed conversion of the normal pentagastrin testing in one patient at the age of 25 years. It is planned to operate on this patient. Bearing this in mind we question whether the general recommendations for early prophylactic thyroidectomy should be followed without modification. In our opinion it seems more appropriate to use serial pentagastrin testing rather than a fixed age in order to decide the time of operation. Because of the low aggressiveness of the MTC in the presented kindred with a C611F germline mutation in exon 10 we adopted the following strategy for screening and follow up. We screen for the mutation between the age of 6 and 10 years. Pentagastrin-stimulated calcitonin testing is started at the age of 10 years and repeated every 2 years until the age of 18 years and then every year thereafter. Thyroidectomy is recommended as soon as the test becomes positive, using the very rigid criteria of the French study group (30) who regarded a stimulated concentration of calcitonin at any level above normal as pathological. We feel that a careful follow-up of the not yet operated GCs is necessary and we would not hesitate to abandon the conservative follow-up approach in case of appearance of a more aggressive course in a younger patient.

In summary, the spectrum of clinical appearance of MEN 2A appears to be so broad that, depending on the genotype, therapeutic recommendations should be

more individualized. To obtain reliable data allowing for such an individualization more work on genotype–phenotype correlation is needed. In this context FMTC, although not thought to be a distinctive pathological entity by some authors (25), appears to remain a clinically useful concept in respect to prognosis and therapy.

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References

- Schimke RN. Genetic aspects of multiple endocrine neoplasia. *Annual Review of Medicine* 1984 **35** 25–31.
- Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E *et al.* Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 1993 **363** 458–460.
- Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF *et al.* The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. *Journal of the American Medical Association* 1996 **276** 1575–1579.
- McMahon R, Mulligan LM, Healey CS, Payne SJ, Ponder M, Ferguson SM *et al.* Direct, non-radioactive detection of mutations in multiple endocrine neoplasia type 2A families. *Human Molecular Genetics* 1994 **3** 643–646.
- Marsh DJ, Robinson BG, Andrew S, Richardson AL, Pojer R, Schnitzler M *et al.* A rapid screening method for the detection of mutations in the RET proto-oncogene in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma families. *Genomics* 1994 **23** 477–479.
- Feldman GL, Kambouris M, Talpos GB, Mulligan LM, Ponder BA & Jackson CE. Clinical value of direct DNA analysis of the RET proto-oncogene in families with multiple endocrine neoplasia type 2A. *Surgery* 1994 **116** 1042–1047.
- Lips CJ, Landsvater RM, Hoppener JW, Geerdink RA, Blijham G, van Veen JM *et al.* Clinical screening as compared with DNA analysis in families with multiple endocrine neoplasia type 2A [see comments]. *New England Journal of Medicine* 1994 **331** 828–835.
- Wells SJ, Chi DD, Toshima K, Dehner LP, Coffin CM, Dowton SB *et al.* Predictive DNA testing and prophylactic thyroidectomy in patients at risk for multiple endocrine neoplasia type 2A. *Annals of Surgery* 1994 **220** 237–247.
- Lips CJ, Landsvater RM, Hoppener JW, Geerdink RA, Blijham GH, Jansen-Schillhorn van Veen JM *et al.* From medical history and biochemical tests to presymptomatic treatment in a large MEN 2A family. *Journal of Internal Medicine* 1995 **238** 347–356.
- Dralle H, Gimm O, Simon D, Frank RK, Gortz G, Niederle B *et al.* Prophylactic thyroidectomy in 75 children and adolescents with hereditary medullary thyroid carcinoma: German and Austrian experience. *World Journal of Surgery* 1998 **22** 744–750.
- Gill JR, Reyes MM, Iyengar S, Kidd KK, Touloukian RJ, Smith C *et al.* Early presentation of metastatic medullary carcinoma in multiple endocrine neoplasia, type IIA: implications for therapy. *Journal of Pediatrics* 1996 **129** 459–464.
- Wells SJ & Skinner MA. Prophylactic thyroidectomy, based on direct genetic testing, in patients at risk for the multiple endocrine neoplasia type 2 syndromes. *Experimental and Clinical Endocrinology and Diabetes* 1998 **106** 29–34.
- Norton JA, Froome LC, Farrell RE & Wells SJ. Multiple endocrine neoplasia type IIb: the most aggressive form of medullary thyroid carcinoma. *Surgical Clinics of North America* 1979 **59** 109–118.
- Wells SJ & Skinner MA. Prophylactic thyroidectomy, based on direct genetic testing, in patients at risk for the multiple endocrine neoplasia type 2 syndromes. *Experimental and Clinical Endocrinology and Diabetes* 1998 **106** 29–34.
- Ponder BA. The phenotypes associated with ret mutations in the multiple endocrine neoplasia type 2 syndrome. *Cancer Research* 1999 **S1736**–**S1741**; discussion **S1742**.
- Iwashita T, Kato M, Murakami H, Asai N, Ishiguro Y, Ito S *et al.* Biological and biochemical properties of Ret with kinase domain mutations identified in multiple endocrine neoplasia type 2B and familial medullary thyroid carcinoma. *Oncogene* 1999 **18** 3919–3922.
- Frank-Raue K, Hoppner W, Frilling A, Kotzerke J, Dralle H, Haase R *et al.* Mutations of the ret protooncogene in German multiple endocrine neoplasia families: relation between genotype and phenotype. German Medullary Thyroid Carcinoma Study Group. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 1780–1783.
- Kambouris M, Jackson CE & Feldman GL. Diagnosis of multiple endocrine neoplasia [MEN] 2A, 2B and familial medullary thyroid cancer [FMTC] by multiplex PCR and heteroduplex analyses of RET proto-oncogene mutations. *Human Mutation* 1996 **8** 64–70.
- Landsvater RM, Jansen RP, Hofstra RM, Buys CH, Lips CJ & Ploos van Amstel HK. Mutation analysis of the RET proto-oncogene in Dutch families with MEN 2A, MEN 2B and FMTC: two novel mutations and one de novo mutation for MEN 2A. *Human Genetics* 1996 **97** 11–14.
- Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC *et al.* Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. *Human Molecular Genetics* 1993 **2** 851–856.
- Oriola J, Paramo C, Halperin I, Garcia MR & Rivera FE. Novel point mutation in exon 10 of the RET proto-oncogene in a family with medullary thyroid carcinoma. *American Journal of Medical Genetics* 1998 **78** 271–273.
- Carlomagno F, Melillo RM, Visconti R, Salvatore G, De Vita G, Lupoli G *et al.* Glial cell line-derived neurotrophic factor differentially stimulates ret mutants associated with the multiple endocrine neoplasia type 2 syndromes and Hirschsprung's disease. *Endocrinology* 1998 **139** 3613–3619.
- Carlomagno F, Salvatore G, Cirafo AM, De Vita G, Melillo RM, de Fransciscis V *et al.* The different RET-activating capability of mutations of cysteine 620 or cysteine 634 correlates with the multiple endocrine neoplasia type 2 disease phenotype. *Cancer Research* 1997 **57** 391–395.
- Chappuis-Flament S, Pasini A, De Vita G, Segouffin-Cariou C, Fusco A, Attie T *et al.* Dual effect on the RET receptor of MEN 2 mutations affecting specific extracytoplasmic cysteines. *Oncogene* 1998 **17** 2851–2861.
- Moers AM, Landsvater RM, Schaap C, Jansen-Schillhorn van Veen JM, de Valk IA, Blijham GH *et al.* Familial medullary thyroid carcinoma: not a distinct entity? Genotype–phenotype correlation in a large family. *American Journal of Medicine* 1996 **101** 635–641.
- Egawa S, Futami H, Takasaki K, Iihara M, Okamoto T, Kanbe M *et al.* Genotype–phenotype correlation of patients with multiple endocrine neoplasia type 2 in Japan [see comments]. *Japanese Journal of Clinical Oncology* 1998 **28** 590–596.
- Bolino A, Schuffenecker I, Luo Y, Seri M, Silengo M, Tocco T *et al.* RET mutations in exons 13 and 14 of FMTC patients. *Oncogene* 1995 **10** 2415–2419.
- Fattoruso O, Quadro L, Libroia A, Verga U, Lupoli G, Cascone E *et al.* A GTG to ATG novel point mutation at codon 804 in exon 14 of the RET proto-oncogene in two families affected by familial medullary thyroid carcinoma. *Human Mutation* 1998 **S167**–**S171**.
- Feldman GL, Edmonds MW, Ainsworth PJ, Schuffenecker I, Lenoir GM, Saxe AW *et al.* Variable expressivity of familial

- medullary thyroid carcinoma (FMTC) due to a RET V804M (GTG→ATG) mutation. *Surgery* 2000 **128** 93–98.
- 30 Niccoli-Sire P, Murat A, Baudin E, Henry J-F, Proye C, Bigorgne J-C *et al.* Early of prophylactic thyroidectomy in MEN 2/FMTC gene carriers: results in 71 thyroidectomized patients. *European Journal of Endocrinology* 1999 **141** 468–474.
- 31 Melzer A, Siggelkow H, Höppner W & Hübner M. Presentation of a large kindred with familial medullary thyroid carcinoma demonstrating low grade malignancy. *Experimental and Clinical Endocrinology and Diabetes* 1999 **107** S70.
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