Tandem Germline *RET* Mutations in a Family Pathogenetic for Multiple Endocrine Neoplasia 2B, Confirmed by a Natural Experiment

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What Is Known about This Topic?

- To date, there is only one report of a double *RET* mutation, i.e. V804M/Y806C, in a patient with the MEN 2B phenotype.
- Previous in vitro studies have suggested that these double mutations are on the same allele in a tandem fashion.
- The pathogenetic role of this double mutation has not been clarified.

What Does This Case Report Add?

- The tandem V804M/Y806C mutations were inherited by the daughter of the proband, reproducing the MEN 2B phenotype observed in the proband and thus confirming the results of the in vitro studies as a natural experiment.
- These tandem double *RET* mutations are pathogenetic for MEN 2B.

Key Words

RET · Tandem mutations · Multiple endocrine neoplasia 2B ·
De novo mutation · Medullary thyroid carcinoma

the offspring as a natural experiment. Thus, the tandem double *RET* mutations are pathogenetic for MEN 2B.

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Abstract

A family with germline tandem V804M/Y806C mutations in the *RET* proto-oncogene was reported. The in vitro study results showing that these mutations were on the same allele and that *RET* with these mutations had a moderate transforming activity were confirmed by the clinical features of

Introduction

Medullary thyroid carcinoma (MTC) presents as a sporadic or hereditary variant [1]. Hereditary MTC arises as a component of multiple endocrine neoplasia (MEN) type 2A or 2B or as familial MTC (FMTC). These are genetic

Fig. 1. Bumpy lips and multiple mucosal neuromas on the tongues of the proband and her daughter (picture taken recently).

tumor syndromes caused by germline mutations in the *RET* proto-oncogene and are transmitted in an autosomal dominant pattern. MEN 2B is characterized by the presence of MTC, adrenal pheochromocytoma, mucosal neuromatosis, and a marfanoid habitus. About 95% of patients with MEN 2B carry a germline M918T mutation in the *RET* proto-oncogene, while a few carry an A883F mutation [1]. Although all of the reported *RET* mutations in patients with MEN 2A, 2B, or FMTC were single missense mutations, we reported the presence of double *RET* mutations, i.e. V804M and Y806C, for the first time in a female patient with the MEN 2B phenotype [2]. In vitro studies showed that these 2 missense mutations were on the same allele in a tandem fashion, that Y806C was inherited from the patient's father and V804M was a de novo mutation, and that the transforming activity of *RET* with the V804M/Y806C mutations was moderately high [2, 3]. Following our report, several other cases of double mutations of *RET* were reported in a small number of patients with MEN 2 [2, 4–12]. Here we report that the same tandem mutations were inherited to our former patient's daughter, causing the MEN 2B phenotype and thus confirming the results of the in vitro studies as a natural experiment.

Patients

Patient 1

We previously reported a female patient showing the MEN 2B phenotype with tandem *RET* mutations (V804M/Y806C) [2]. In brief, she was 23 years old at the time of diagnosis of bilateral mul-

Fig. 2. Patient 1 at diagnosis. **a** Abdominal CT scan showing a dilated colon. The adrenal glands are thick without nodules. **b** Thickening of the corneal nerves on ophthalmological examination.

Fig. 3. Sequence analysis in exon 14 of *RET*. **a** The proband (patient 1) exhibited double mutations, i.e. V804M and Y806C. **b** Her father and brother carried Y806C only. **c** Her daughter (patient 2) had V804M and Y806C double mutations (the same as those of her mother, i.e. patient 1).

multiple MTC (T3N0M0, stage II). She had a marfanoid habitus, bumpy lips, and multiple mucosal neuromas on her tongue (fig. 1); she had had severe constipation since childhood. Her urinary epinephrine and metanephrine levels were close to the upper limits of the normal ranges, and computed tomography showed thickening of her adrenal glands, suggesting adrenal medullary hyperplasia. She had a dilated colon (fig. 2a) with many diverticula and thickening of the corneal nerves (fig. 2b). Based on these findings, she was diagnosed with MEN 2B. There was no family history of endocrine diseases.

We performed a total thyroidectomy with central and bilateral modified radical neck dissection, and histological examinations confirmed bilateral multiple foci of medullary carcinoma with a lymph node metastasis. After surgery, the calcium-loading test revealed no response in the patient's serum calcitonin level, suggesting the biochemical cure of her MTC. Germline mutation analysis of the *RET* gene revealed 2 heterozygous missense mutations, i.e. V804M and Y806C (fig. 3a). Her father and brother carried only the Y806C mutation (fig. 3b), and her mother had the wild-type *RET*. Her father and brother had normal serum calcitonin levels, and the pathogenetic meaning of the Y806C mutation was not clear. An in vitro study suggested that the V804M mutation was on the same allele as the Y806C mutation, thus confirming that the V804M mutation was a de novo mutation occurring on the gene transmitted from the patient's father.

Another in vitro study showed that the transforming activity of *RET* with the V804M/Y806C double mutations was approximately 8- to 13-fold higher than that of *RET* with a single V804M or Y806C mutation, almost the same as that of the C634R mutation or the A883F mutation, and about two thirds of the M918T mutation [3]. The C634R mutation is a representative mutation for MEN 2A, and the M918T and A883F mutations are pathogenetic for MEN 2B [12].

Patient 2

Patient 1 later gave birth to a daughter and 2 sons (fig. 4). We recommended she have her children screened for MEN 2B, including a germline gene analysis. She gave us informed consent for her 2 elder children, i.e. her daughter and older son. The daughter (patient 2) underwent screening at the age of 7 years. She also had bumpy lips, multiple nodules on her tongue similar to those of patient 1 (fig. 1), and the symptom of severe constipation after birth like her mother. There was no thyroid tumor on the ultrasonography (T0N0M0). Computed tomography showed a dilated colon but no adrenal tumor (fig. 5). Her urinary metanephrine and normetanephrine levels were within the normal ranges. Her serum calcitonin level was 69.0 pg/ml, and her carcinoembryonic antigen level was 1.3 ng/ml. The calcium-loading test showed a clear response in serum calcitonin levels, with the basal and peak values being 59 and 440 pg/ml, respectively, suggesting the presence of C-cell hyperplasia or micro-MTC.

Her genomic DNA was extracted from the peripheral blood and screened for mutations in exon 14 of the *RET* gene by polymerase chain reaction-direct sequencing analysis, as described [2]. The direct sequencing analysis detected 2 heterozygous missense mutations, i.e. V804M and Y806C in exon 14, the same as those of her mother (fig. 3c), confirming that both mutations were on one allele. If they had been on different alleles, only one of the mutations would have been transmitted. When patient 2 reached 8 years of age, we performed a total thyroidectomy with central neck dis-

Fig. 4. Pedigree of the present family. The arrow indicates the proband.

section as a prophylactic surgery. Histopathologic examination of the resected specimen revealed bilateral multiple foci of MTC less than 3 mm in size without lymph node metastasis (pT1pN0cM0). After surgery, a calcium-loading test revealed no response in the patient's serum calcitonin level, suggesting the biochemical cure of her MTC.

A germline *RET* analysis of the elder son showed no mutation, and his serum calcitonin level was normal. The younger son had not been examined at the time of reporting.

At the time of writing, the proband and her daughter had remained biochemically cured for 194 and 37 months postoperatively, respectively. However, a 1.8-cm nodule in the adrenal gland and marginally elevated urinary metanephrine excretions were identified in the proband.

Discussion

MEN 2 is a hereditary neoplasia syndrome with autosomal dominant inheritance caused by activating germline mutations in the *RET* proto-oncogene. MEN 2B is the rarest and most aggressive form of MEN 2. More than 95% of patients with MEN 2B carry an M918T *RET* mutation, and 2–3% of patients harbor an A883F *RET* mutation [1, 13]. All of the *RET* mutations in patients with MEN 2 or FMTC were single missense mutations until we reported the double *RET* mutations V804M and Y806C in patient 1 [2]. In vitro studies suggested that these mutations were on the same allele, and thus Y806C was in-

Fig. 5. Abdominal CT scan showing a dilated colon in patient 2. The adrenal glands are normal in size and shape.

herited from this patient's father and V804M was a de novo mutation occurring on the gene transmitted from her father [2]. Although MEN 2 and FMTC are basically hereditary diseases, the *RET* mutations in approximately 50% of the patients with MEN 2B are de novo mutations, suggesting that de novo mutations are not extremely rare [1, 13].

Following our report, 11 types of double mutations, including ours, were reported in patients with MEN 2 syndromes (table 1) [4–12]. The phenotypes in these cases were FMTC in 2 cases, MEN 2A in 4 cases, and MEN 2B in 5 cases. Familial occurrence was reported in 7 of the 11 cases. Very interestingly, 6 of the double mutations involved the V804M mutation. The transforming activity of the mutant *RET* gene was studied in only 2 cases,

Table 1. Reported double *RET* mutations in MEN type 2

Exon	Mutation	De novo mutation	Phenotype	Family history	PHEO/HPT	Transforming activity ^a	Reference
10, 13	C620F/Y791F	–	2A	+	+/-	not described	Dvorakova et al. [9]
11	C634R/A640G	C634R, A640G	2A	–	+/-	not described	Tessitore et al. [11]
11	C634S/A641S	–	2A	+	+/-	not described	Poturnajova et al. [10]
11, 13	C634Y/Y791F	–	2A	+	+/-	not described	Toledo et al. [8]
13, 14	V778I/V804M	–	FMTC	+	-/-	not described	Kasprzak et al. [6]
13, 14	Q781R/V804M	V804M	2B	–	-/-	not described	Nakao et al. [12]
13, 16	Y791F/M918T	–	2B	+	+/-	not described	Dvorakova et al. [9]
14	V804M/E805K	–	2B	–	+/-	5	Cranston et al. [5]
14	V804M/Y806C	V804M	2B	–	-/- → +/-	8–13	Miyauchi et al. [2]
14	V804M/R844L	–	FMTC	+	-/-	not described	Bartsch et al. [7]
14, 15	V804M/S904C	–	2B	+	-/-	not described	Menko et al. [4]

PHEO = Pheochromocytoma; HPT = hyperparathyroidism.

^a Of the *RET* gene with double mutations compared to a single mutation (fold higher).

including ours, and showed increased activities. Cranston et al. [5] reported that the transforming activity of V804M/E805K was about 5 times that of V804M. However, in these reports the functions or pathogenetic roles of the mutations were not clarified. We compared the transforming activities of the V804M/Y806C *RET* mutation with those of *RET* mutations common among individuals with MEN 2A or 2B as well as those of single V804M or Y806C mutations [3]. The V804M mutation was reported in patients with FMTC, while the Y806C mutation has not been reported in patients with MEN 2, FMTC, or Hirschsprung's disease, to our knowledge [1].

The transforming activity of *RET* with the tandem V804M/Y806C mutations was approximately 8- to 13-fold higher than that of *RET* with a single V804M or Y806C mutation, almost the same as that of the C634R mutation or the A883F mutation, and about two thirds of the M918T mutation [3]. The C634R mutation is a representative mutation for MEN 2A, and the M918T and A883F mutations are pathogenetic for MEN 2B [1]. Patient 2 had the MEN 2B phenotype and small MTC foci at the age of 8 years. These findings agree with the in vitro study results regarding the transforming activities of *RET* mutations. The diseases in patients with these tandem mutations might be less aggressive than those in typical MEN 2B with an M918T mutation, as suggested by the transforming activity studies and the clinical courses of the present patients.

A single V804M mutation has been reported in several families with FMTC or MEN 2A but not MEN 2B [1, 13]. We expect that a de novo Y806C mutation in family members with an inherited V804M mutation might cause an MEN 2B phenotype, as shown in the present report.

Disclosure Statement

The authors have no conflict of interests.

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