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A Kindred of Multiple Endocrine Neoplasia Type 2B

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Abstract. We describe familial cases of multiple endocrine neoplasia (MEN) 2B: A 48-year-old man is the proband. He had pheochromocytoma, medullary thyroid carcinomas (MTCs), parathyroid hyperplasia, mucosal neuromas, eversion of eyelids and Marfanoid appearance, and then underwent adrenalectomy and total thyroidectomy. Family screening revealed that his two daughters (10 and 8 years old) had mucosal neuromas and increased serum calcitonin (CT). Both of them had MTCs but no pheochromocytoma, and their MTCs were surgically removed. The father and his children have been in favorable condition since the operations. Southern blot analysis with 33 polymorphic DNA probes was done in MTCs obtained from two daughters. An *RBP3* (*10q11.2*) locus linked to a predisposing gene on chromosome 10 was uninformative in either patient because of constitutional homozygosity. Loss of heterozygosity at the *MYCL1* locus on chromosome *1p32* was observed in MTC from the younger sister, but no loss of heterozygosity was recognized in other loci examined. Deletion of the *1p32* locus may play a role in the development of MEN 2B.

Key words: Multiple endocrine neoplasia (MEN) 2B, Medullary thyroid carcinoma (MTC), Pheochromocytoma. (*Endocrinol Japon* **39**: 25–30, 1992)

MEDULLARY thyroid carcinoma (MTC) and pheochromocytoma are the principal components of multiple endocrine neoplasia (MEN) 2B [1, 2, 3]. MEN 2B disease was first reported by Williams and Pollock in 1966 [4] and is an inherited disorder characterized by mucosal neuromas, Marfanoid appearances, in addition to MTC and pheochromocytoma. Moreover, hyperparathyroidism is rarely a feature of MEN 2B [1, 2, 3]. Eighteen patients have been described with MEN 2B in Japan [2]. Although transmission of MEN 2B occurs in an autosomal dominant pattern, in approximately one half of the patients it is in a sporadic form and is believed to represent new mutations [5, 6]. Recent studies have demonstrated that the predisposing genes for MEN 1 and 2A are located on chromosome 11 [7] and chromosome 10 [8, 9, 10], respectively. Furthermore, DNA analysis with restriction fragment length polymorphisms (RFLPs) for multiple regions of the chromosome showed loss of heterozygosity on other chromosomes in a tumor derived from MEN syndrome [2, 11, 12, 13]. However, there has not been enough information on oncogenesis in MEN 2B.

This paper describes the first MEN 2B pedigree confirmed in Japan in a 48-year-old male (proband) and his daughters (10 and 8 years old). We also report on DNA analysis with MTCs obtained from daughters.

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Case Reports

Case 1

A forty-eight-year-old male (proband) who was referred to our clinic because of paroxysmal hypertension and headache in 1982. He had recognized headache and palpitation since 1970. In 1972, he was diagnosed as having diabetes mellitus, and an oral antihyperglycemic agent (sulfonylurea) was administered. At this time, his systolic blood pressure was 130–150 mmHg. In 1982, hypertension was pointed out (158/104 mmHg). This coexisted with a headache and he was hospitalized. On evaluation, he appeared to be of a Marfanoid build which was asthenic habitus with a decreased upper to lower body segment ratio and increased arm span; height. 172 cm, weight. 48 kg. Blood pressure was labile and changed from 160/70 to 208/108 and then to 124/68 mmHg in a few minutes. Thickened lips, small nodules (mucosal neuromas) on the tongue margin, eversion of the eyelids and bilateral thyroid nodules were observed. Biochemical examination revealed increased serum and urinary catecholamines; serum adrenaline was 10.1 ng/m*l* (normal 0.12>), serum noradrenaline 10.8 ng/m*l* (0.1–0.41), urinary adrenaline 2428.7 μg/day (2–30) and urinary noradrenaline 1517.9 μg/day (25–120). Serum calcitonin (CT) and carcinoembryonic antigen (CEA) were 4991 pg/m*l* (100>) and 189 ng/m*l* (2.5>), respectively. Serum parathyroid hormone (PTH) was 1.9 ng/m*l* (1.3>). Serum calcium and phosphorus were 9.5 mg/d*l* and 4.3 mg/d*l*, respectively. Computer tomography and echogram revealed bilateral adrenal tumors (rt. 40×33×25 mm, lt. 90×45×33 mm) and bilateral thyroid tumors (rt. 20×20 mm, lt. 15×15 mm). A $^{99m}TcO_4$ scintigram showed an uptake defect in the thyroid tumors. Barium enema revealed that he had megacolon. As a result of the above examinations, he was diagnosed as MEN 2B. He underwent partial adrenalectomy (left) and total adrenalectomy (right), and then total thyroidectomy with lymphadenorectomy. Histological examinations revealed that the right adrenal tumor was a benign pheochromocytoma, but the left adrenal tumor was not a pheochromocytoma and it consisted of fatty tissue. The thyroid tumors were MTCs, and the parathyroid glands were slightly enlarged but histologically normal. Examining the various kinds of humoral factors in the tissue of MTC, CT, somatostatin, vasoactive intestinal peptide (VIP) and gastrin releasing peptide (GRP) were detected (data not shown). After the operation, he was in favorable condition, and no recurrence was observed until 1990, but serum CT and CEA levels have tended to increase moderately for the last 6 months (CT: 180 pg/m*l*, CEA: 4.20 ng/m*l*).

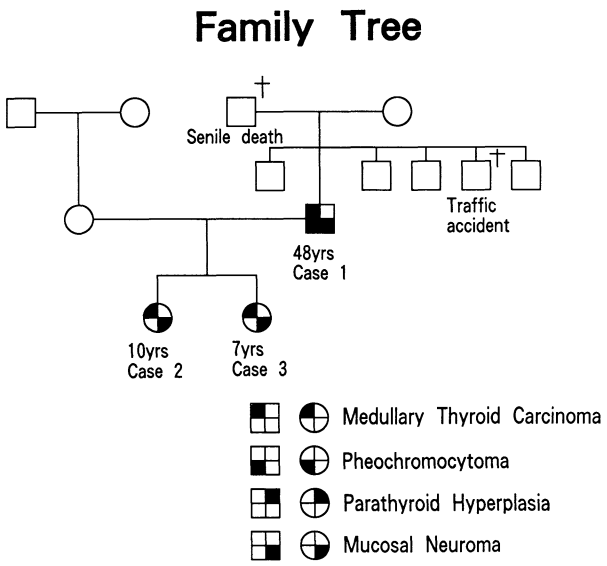

Fig. 1. Pedigree of the patients. A 48 year-old man is the proband. He and his daughters are found to be MEN type 2B. No patients with a similar disease are found on the paternal side.

Case 2

The ten-year-old elder daughter was admitted to our hospital because of thyroid enlargement. At her birth thickened lips were recognized. At the age of 6 years, oral mucosal neuromas on her tongue appeared. When she was 9 years old in 1989, serum CEA and CT levels were found to be high (CEA 24.2 ng/m*l*, CT 1285 pg/m*l*). However, her parents rejected further examination for MTC. In 1990 swelling of her right thyroid gland was noted and she was admitted to our hospital. On examination, she appeared to be of Marfanoid build; height: 138 cm; weight: 26 kg. Blood pressure was 102/64 mmHg. Mucosal neuromas on her tongue and lips, a nodule in the right thyroid gland and swelling of four submandibular lymphonodi (10×5 mm) were revealed. Laboratory examinations showed that serum CT and

CEA levels were 2010.0 pg/m*l* and 89.90 ng/m*l*, respectively. Urinary and serum catecholamines were normal. Serum PTH was also normal. The thyroid gland and its surrounding lymphonodi were surgically removed in 1990. Histological examinations revealed MTC with lymphonodus metastases. After the operation, serum CT and CEA became normal (CT: 57.0 pg/m*l*, CEA: 1.00> ng/m*l*).

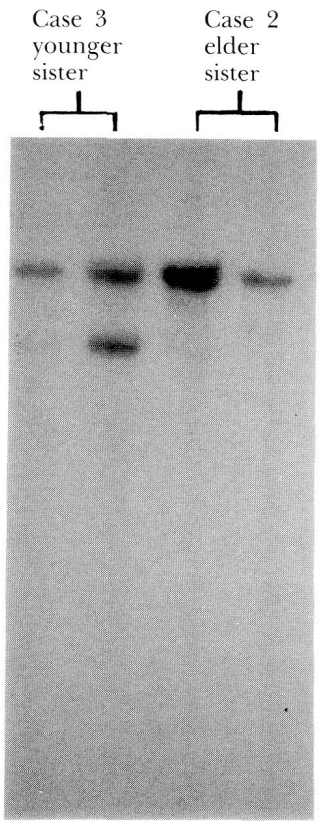

Fig. 2. Loss of constitutional heterozygosity at locus on chromosome 1 in medullary thyroid carcinoma (MTC) from the younger sister. DNAs of tumors (left lane) and normal thyroid tissue (right lane) from the two daughters with MEN type 2B were digested with *EcoR1*, separated by electrophoresis in 0.7% agarose gel, and transferred to a nylon membrane. Southern blots were hybridized to ^{32}P-labeled 1.8-kilobase *SmaI-EcoRI* fragment for locus on *1p32* chromosome 1 (*MYCL1*). The younger sister had the deleted on *1p32* chromosome 1 in MTC tissue, but DNA in normal thyroid tissue from the elder sister was homozygous and therefore uninformative.

Case 3

The eight-year-old younger daughter was admitted to our hospital with her elder sister because she also had thyroid enlargement. Thickening of the lips was noticed at her birth. At the age of 5 years, mucosal neuromas on her tongue were noted. Her serum CT level was 887.0 pg/m*l* at the age of 6 in 1989, but the serum CEA level was normal (2.5 ng/m*l*). In 1990, a nodule was observed in her right thyroid gland. She also appeared to be of Marfanoid build; height: 128 cm; weight: 26 kg. Serum CT and CEA levels were 1639 pg/m*l* and 5.60 ng/m*l*, respectively. Total thyroidectomy was performed and histological finding of the thyroid nodule was MTC, however, no metastasis was observed in the regional lymphonodi, and the parathyroid glands were normal. After the operation, serum CT and CEA levels became normal (CT: 62.0 pg/m*l*, CEA: 1.00 ng/m*l*).

Genetic Analysis

DNA analysis with polymorphic DNA probes was done in the respective MTCs and normal thyroid glands obtained from the two daughters. DNAs were digested with appropriate restriction endonucleases and Southern blot analyses were done with 33 probes to detect RFLPs on chromosomal loci (Table 1) [11]. They were uninformative on chromosome *10q11.2* (*RBP3* locus) because of constitutional homozygosity, and loss of heterozygosity was not observed at *D10S1* locus on chromosome *10q22–10q23*. However, on digestion with *EcoR1* and hybridization with probe for 1.8–Kilobase *SmaI-EcoRI* fragment of *MYCL1* gene, a loss of heterozygosity at locus on *1p32* (*MYCL1*) was observed in MTC from the younger sister, but DNA analysis in elder sister was uninformative because of homozygosity at that locus in normal thyroid tissue. No loss of heterozygosity was recognized in other loci we examined.

Discussion

It has been reported that there were only 18 patients (6 males 12 females) with MEN 2B for 21 years from 1968 to 1988 in Japan [2]. This is the

Table 1.

Map location	Locus symbol	Case 2 elder sister	Case 3 younger sister
1p32	MYCL1	—	1
1p35–p33	D1S7	1,2	1,2
1p36.3	D1Z2	1,2	1,2
1pter	D1S57	1,2	1,2
1p	D1S60	—	—
1p	D1S73	1,2	1,2
1q23–q25.1	AT3	1,2	1,2
1q42–q43	D1S8	1,2	1,2
2p13	TGFA	1,2	1,2
2p24	MYCN	—	1,2
3p14	D3S3	—	1,2
5pter–p15	D5S2	—	—
5q34–qter	D5S4	-	—
6q22–q23	MYB	—	—
7p13–p12	EGFR	1,2	1,2
7q31	MET	1,2	1,2
10q11.2	RBP3	—	—
10q21	D10S5	—	—
10q22–q23	D10S1	1,2	1,2
10q22–q23	D10S4	—	—
11p15.5	D11S12	1,2	1,2
11q23–q24	APOA1	—	—
11q13	INT2	1,2	1,2
12p12.1	KRAS2	—	—
14q32	D14S16	—	—
15q11–q12	D15S18	1,2	1,2
16q13.13–p13.11	D16S79	1,2	1,2
17p13.1–p11.2	D17S31	1,2	1,2
18q11.2–q12.1	PALB	1,2	1,2
20p12	D20S5	1,2	1,2
20q13.2	D20S4	—	—
21q11.2–q21	D21S11	1,2	1,2
22q11.1–q11.2	D22S9	—	1,2

–, Uninformative due to homozygosity.
1, 2, Keep of constitutional heterozygosity no. 1 is large and no. 2 is small.
1 or 2, A loss of constitutional heterozygosity (numbers denote the remaining bands).

first familial cases confirmed in Japan. Mucosal neuromas, MTC and pheochromocytoma occurred in 100%, 95% and 53% of cases, respectively, and hyperparathyroidism represents only 5% of cases[14]. In addition, Marfanoid habitus appeared in 74%, megacolon in 63%, and diverticula and polyps in gastrointestinal ducts in 37% of cases[14].

Among our cases, a 48-year-old man (case 1) is the proband. He had mucosal neuromas, MTCs and pheochromocytoma. His two daughters (cases 2 and 3) had mucosal neuromas and MTCs, but not pheochromocytoma. Thus, our familial cases were compatible with the diagnosis of MEN 2B. No affected member was observed in the family on the paternal side.

Genetically, the MEN syndrome is inherited in an autosomal dominant pattern and is fully penetrant with variable expressions [2, 3]. MEN 2B may have diseases in target organs at risk even though manifestation of the diseases is not expressed clinically. Previous studies [15] indicated that screening of family members by measurement of serum CT should be initiated when the tumor is still at a clinically occult stage. MTC arises from the parafollicular cells (C-cells) of the thyroid gland. It is recognized that C-cell hyperplasia is a premalignant stage, which progresses through microinvasive disease before developing into macroscopical MTC [16]. According to Knudson's two-hit model of carcinogenesis [17], inherited cancer syndromes such as MEN are the result of two pathogenic events. The first event is an inherited mutation that renders all cells of the body highly susceptible to tumor formation. The second is another mutation that induce a somatic cell change, namely C-cells leading to MTCs. The predisposing gene of MEN 2A was located on chromosome *10q* linked to the *RBP3* gene and *D10S5* [8, 9, 10]. Norum *et al.* reported recently that the genes for MEN 2A and 2B were located close to each other [18]. However, previous studies indicated that no loss of heterozygosity on chromosome 10 was noted in MEN 2A [19, 20, 21] and 2B [19]. The abnormalities on chromosome 10 in MEN 2A and 2B may be too small to be detected by Southern blot analysis with currently available probes. Furthermore, DNA analysis in tumor cells has also shown deletion of other chromosome loci which are thought to represent the tumor suppressor genes. In our analysis of RFLPs for multiple regions of chromosome in both daughter's MTCs, the *IRBP* locus on chromosome *10q11.2* was uninformative because of constitutional homozygosity and no loss of heterozygosity was observed at locus *D10S1*. On the other hand, loss of heterozygosity at the locus on *1p32* (*MYCL1* locus) was observed in MTC obtained from the younger sister, however, DNA in the elder sister was uninformative because of homozygosity at that locus. There are a few reports on DNA analysis of MEN 2B [12, 20], and Mathew *et al.* [12] reported loss of heterozygosity on chromosome *1p* in MTC of MEN 2B. Thus, it might be possible that deletion of chromosome *1p* plays a role in the development of MTC in MEN

2B. A high frequency of abnormalities on chromosome *1p* is observed in MEN 2A, and familial and sporadic MTC [12, 13, 19]. However, those MTCs are a genetically heterozygous disease and clinical manifestations of MEN 2A and 2B are different. Okazaki *et al.* [20] also reported allele loss on chromosome 22 in MEN 2A. DNA abnormalities responsible for MEN may be located separately on other chromosomes in addition to abnormalities on chromosomes 1 and 10. Further study is required to elucidate the pathogenesis of MEN.

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