Endocrine Journal 1993, **40** (6), 649–657

Relatively Good Prognosis of Multiple Endocrine Neoplasia Type 2B in Japanese: Review of Cases in Japan and Analysis of Genetic Changes in Tumors

KATSUHIKO YOSHIMOTO, HIROYUKI IWAHANA, AND MITSUO ITAKURA

Otsuka Department of Clinical and Molecular Nutrition, School of Medicine, The University of Tokushima, Tokushima 770, Japan

Abstract. Since 1968, a total of 23 patients with multiple endocrine neoplasia type 2B (MEN 2B) have been identified in Japan. The mean age at diagnosis was 20.3 years (range, 6 to 39 years). All patients had neuromas and bumpy lips. All patients underwent thyroidectomy for medullary thyroid carcinoma (MTC). Ten of 23 patients had pheochromocytomas. One patient died of cerebral bleeding at the age of 43, 2 patients died of MTC at the age of 35 and 12. Five-, 10-, and 15-year survival rates were 100%, 92%, and 88%, respectively. The clinical course of MTC in MEN 2B in Japanese is not so aggressive when compared with about a 50% 10-year survival reported for Caucasians. Genetic analysis of 4 MTC and 1 pheochromocytoma from 4 patients with MEN 2B revealed no common changes in regard to loss of heterozygosity on 21 chromosomes or point mutations of the *ras*, Gsα, or p53 genes.

Key words: MEN 2B, Prognosis, Loss of heterozygosity. (*Endocrine Journal* **40**: 649–657, 1993)

MULTIPLE ENDOCRINE NEOPLASIA (MEN) 2B is characterized by the combined occurrence of medullary thyroid carcinoma (MTC), pheochromocytoma, Marfanoid habitus, and mucosal neuroma. MTC in MEN 2A or that of sporadic type is characterized by a relatively benign clinical course. A more aggressive behavior of MTC in patients with MEN 2B was suggested by Norton *et al.* in 1979 [1]. On the other hand, Vasen *et al.* recently reported that the clinical course of MTC in MEN 2B in Europe was not always as aggressive as was generally thought [2].

Few studies have addressed the natural clinical history of MEN 2B in Japan. In 1984, Takai *et al.* reported that six patients with MEN 2B in Japan had a better prognosis than those in Caucasians [3]. The present study was undertaken to characterize the natural clinical course including age at onset, clinical manifestations, the length of the survival of patients and the pathognomonic features of MEN 2B in Japan to facilitate their prompt detection at an early age.

The responsible gene for MEN 2B has been shown to map near the centromere of chromosome 10 as MEN 2A [4–6]. However, it is not known whether the gene predisposing to MEN 2B is the same as that for MEN 2A. Deletion or loss of heterozygosity (LOH) in the region of the predisposing locus on chromosome 10 was not found in 2 MTCs associated with MEN 2B [7]. We previously reported LOH of *MYCL* (1p32) out of 33 examined loci in one MTC associated with familial MEN 2B [8]. In this study, which extends our previous study, we report the results of analyses of 3 other tumors associated with MEN 2B in regard to LOH or mutation of *ras*, p53, or Gsα gene.

Received: June 22, 1993
Accepted: September 17, 1993
Correspondence to: Dr. Mitsuo ITAKURA, Otsuka Department of Clinical and Molecular Nutrition, School of Medicine, The University of Tokushima, 3–18–15 Kuramoto-cho, Tokushima-city 770, Japan.

Materials and Methods

Tissue samples

Four MTCs and 1 pheochromocytoma from 4 MEN 2B patients were examined. They are MTC and pheochromocytoma in Case 5 and MTC in Cases 21, 22, and 23. Primary tumors together with the adjacent noncancerous tissue, or peripheral blood leukocytes were obtained at the time of surgery.

DNA extraction

High molecular weight DNA was prepared from the tissues by proteinase K digestion and phenol/chloroform extraction as previously described [9].

Southern blot analysis

DNAs (5 μg) were digested with appropriate restriction endonucleases, and the resulting fragments were separated by electrophoresis in 0.7% agarose gel and transferred to nylon membrane (Hybond-N, Amersham). Radiolabelling of DNA probes, hybridization and washing conditions were described previously [9].

PCR-SSCP analysis and PCR-PIRA analysis

The method for polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) for screening mutations of *ras* or p53 genes was described previously [10, 11]. To screen mutations of codon 201 or 227 in the Gsα gene, we developed the method of PCR-primer-introduced restriction analysis (PIRA) [12]. Briefly, we amplified exons 8 and 9 of the Gsα gene, *i.e.*, the region encompassing codon 201 and 227, by PCR with specifically designed primers. Cleavage of PCR products with specific restriction endonucleases was detected by polyacrylamide gel electrophoresis following restriction enzyme digestion.

Japanese MEN 2B patients reported in literature

Information regarding the patients' tumors, original clinical status, and subsequent clinical course were obtained partly from the case reports in the literature and partly by correspondence with the patients' doctors. Survival time was calculated from the date of diagnosis to the date of the last follow-up or death.

Results

Clinical features

1. Incidence and clinical features

The confirmed number of reported patients with MEN 2B in Japan is at least 23. It is difficult to assess the precise number because of inadequate information in some cases and multiple reporting in others. Table 1 is a list of 23 patients and their general clinical features. There were 8 males and 15 females with a male/female ratio of 0.53. The age of patients at diagnosis of MEN 2B ranged from 6 to 39 years. The mean ages were 24.4 for male and 18.1 for female patients with an overall mean of 20.3 years. The peak incidence was seen in the second and third decade for female and male patients, respectively. There were three familial cases (cases 11, 21, and 22) in one pedigree. Evidence for the familial type based on clinical history and/or biochemical data with associated tumors was a prerequisite for classifying a patient as a familial type. In Case 13, his elder brother had the same facial features and neuromas in the tongue, but no information about development of MTC or pheochromocytoma was obtained. He was therefore classified as a sporadic type. Table 2 summarizes the incidence of clinical features in 23 patients with MEN 2B. An enlarged thyroid gland or mass(es) was noted in all patients. Episodes of paroxysmal hypertension, palpitation, headache, and sweating occurred in all patients with pheochromocytoma. Table 3 shows the incidence of combinations of various tumors in 23 patients. Forty-three percent of patients had the complete syndrome consisting of neuroma, MTC, and pheochromocytoma. The remaining 57% of patients had a combination of neuroma and MTC, and no patient had a combination of neuroma and pheochromocytoma.

2. Age at diagnosis and surgery for MTC and pheochromocytoma

The clinical onset of MTC was marked by the observation of a nodule in the neck, while the onset of pheochromocytoma was characterized by palpitation, hypertensive attacks, or recurrent headache. MTC was diagnosed and resected at a

Table 1. A list of MEN 2B cases reported in Japan

No.	Age	Sex	MTC	Type of surgery for MTC	Lymph nodes metastasis	Pheochromocytoma	Mucosal neuroma	Gastrointestinal manifestations	Marfanoid habitus	Parathyroid tumor	Order of diagnosis and/or surgery (yrs)	Family history	Outcome (present age)	Recurrence of MTC	Postoperative Follow-up (years)	Reference No.
1.	26	M	bilateral	total	+	bilateral	+	megacolon diverticulum	+	?	Pheo, MTC (0.5)	—	died at 43 yr	liver metastasis	17	23, 24, 25
2.	16	M	+	?	?	?	+	diverticulum	+	?	?	?	?	?	15a	26, 27
3.	28	F	bilateral	total	+	bilateral	+	diverticulum	+	—	Pheo, MTC (0.1)	—	alive (47)	lung metastasis	19	28
4.	23	F	bilateral	total	—	bilateral	+	diverticulum	+	—	MTC, Pheo (12)	—b	alive (42)	liver metastasis	20	29, 30, 31
5.	18	F	bilateral	total (L to R) (25 yr)	+	bilateral	+	megacolon	—	—	MTC, Pheo (10)	—	died at 35 yr	bone metastasis	17	32, 33
6.	14	F	bilateral	total	+	bilateral	+	megacolon	+	—	MTC, Pheo (7)	—	alive (29)	free	16	34, 35
7.	27	F	bilateral	total	+	—	+	megacolon	+	—		—	alive (44)	free	17	36
8.	6	M	bilateral	left	+	—	+	megacolon diverticulum	+	?		—	died at 12 yr	lung, mediasinum metastasis	6	37
9.	22	F	bilateral	total	?	bilateral (R to L)	+	megacolon	—	—	Pheo, MTC, Pheo (0.1, 3)	—	alive (34)	free	12	38, 39, 40
10.	29	F	bilateral	total	+	bilateral	+	megacolon	+	—	Pheo, MTC (0.1)	—	alive (41)	free	12	41, 42, 43
11.	39	M	bilateral	total	+	right	+	megacolon	+	—	Pheo, MTC (0)	+	alive (50)	free	11	8
12.	25	F	bilateral	total	—	left	+	megacolon	—	?	Pheo, MTC (0.2)	—	alive (34)	?	9	44, 45
13.	25	M	bilateral	total	+	—	+	megacolon diverticulum	—	?		?c	alive (35)	lymphnode metastasis	10	46, 47, 48
14.	19	F	bilateral	total	+	—	+	megacolon	—	?		—	alive (27)	?	8	49, 45
15.	35	M	bilateral	total (L to R)	+	bilateral	+	megacolon	+	—	Pheo, MTC (0.3)	—	alive (43)	free	8	50
16.	10	F	bilateral	total	—	—	+	megacolon	+	—		—	alive (21)	increased plasma CT	11	51
17.	17	F	?	total	+	—	+	megacolon	?	?		—	alive (22)	?	5	52
18.	30	M	bilateral	inoperable	+	—	+	megacolon	—	?		—	alive (35)	liver metastasis	5	53
19.	13	F	bilateral	total	+	—	+	megacolon	—	—		—	alive (20)	increased plasma CT	7	45
20.	12	F	bilateral	total	+	—	+	—	+	—		—	alive (17)	increased plasma CT	5	54
21.	10	F	unilateral	total	+	—	+	(unexamined)	+	—		+	alive (13)	increased plasma CT	3	8
22.	8	F	unilateral	total	—	—	+	(unexamined)	+	—		+	alive (10)	free	3	8
23.	18	M	bilateral	total	+	—	+	megacolon diverticulum	—	—		—	alive (22)	free	4	55

a, He was alive at least in 1990, but his present status is unknown; b, Her two siblings were reported to be hypercalcitoninemic on screening her family in 1982, but they have not developed MTC according to Dr. Masaaki Fukase; c, His elder brother had neuromas in the tongue, but no information on development of MTC or pheochromocytoma was not obtained. ?denotes uniformativeness.

Table 2. Summarized incidence of clinical features of 23 patients with MEN 2B

Clinical features	Number of patients with findings		Number of patients with inadequate information
	Positive	Negative	
Family history	3	19	1
Neuromas	23		
MTC	23		
Pheochromocytomas	10		
Bilateral	8		
Unilateral	2		
Marfanoid habitus	14	8	1
Parathyroid tumor	0	15	8

Table 3. Incidence of combinations of tumors in 23 patients with MEN 2B

Combination	Number of patients (%)
Neuroma, MTC, Pheochromocytoma	10 (43%)
Neuroma, MTC	13 (57%)
Neuroma, Pheochromocytoma	0 (0%)

mean age of 20.3 years, and pheochromocytoma at a mean age of 29.1 years. Age at diagnoses and surgery for MTC, was 24.4 years for male patients and 18.1 years for female patients. As for pheochromocytoma, the age was 33.3 years for male patients and 27.2 years for female patients. The age at surgery for MTC was 9 years earlier than that of pheochromocytoma overall. The age at diagnosis and surgery for both of MTC and pheochromocytoma was lower in female than in male patients. The length of time during which clinical symptoms and signs were evident prior to the diagnosis of tumor(s) varied from less than a month to 14 years, with a mean of 2.1 years for MTC and 4.1 years for pheochromocytomas.

3. Order of diagnosis and surgery for MTC and pheochromocytoma

In 7 cases (70%), MTC and pheochromocytomas were simultaneously diagnosed and resected at a mean age of 29.1 years. In one of them (Case 9), right pheochromocytoma and MTC were simultaneously resected, and 3 years later another contralateral pheochromocytoma was resected. In 3 other cases, MTC was diagnosed first and resected at a mean age of 18.3 years, and pheochromocytoma was resected later, after a mean interval of 9.7 years.

4. Tumor position and characteristics

a) MTC: All 23 cases had histologically proven MTCs. In cases 21 and 22, MTC was diagnosed by screening, because the disease had developed in their father (Case 11). The earliest age at which a thyroid tumor was palpated was 6 years (Case 8). In Cases 5 and 8, resected tumors were first histologically diagnosed as an anaplastic carcinoma and a carcinoid tumor, respectively. All informative cases except Cases 21 and 22 had bilateral and multiple MTCs. There were 3 exceptional cases including Cases 5, 8 and 18; One patient (Case 5) had left hemithyroidectomy followed by radiotherapy and chemotherapy at the age of 18 years. At the age of 24 years, enlarged cervical lymph nodes appeared. MTC was diagnosed and right hemithyroidectomy was performed at the age of 25 years. One patient (Case 8) had initial resection of a left thyroid tumor followed by left hemithyroidectomy 15 months later. Because total thyroidectomy was impossible, he was treated with irradiation to both the neck and anterior mediastinum resulting in transient clinical remission. Another patient (Case 18) had both locally advanced inoperable carcinoma and multiple liver metastases.

At primary surgery for MTC, 17 of 21 (81%) informative patients had cervical lymph node metastases, whereas distant metastasis was present in 5% (1/21). Metastatic MTC occurred as early as 6 years of age (Case 8). Three patients (Cases 3, 5, and 10) underwent surgical re-exploration for clinically or radiologically suggested recurrence. Five patients developed distant metastases postoperatively in multiple sites, including the anterior mediastinum, lung, liver, pancreas and bone in Case 1, lung in Case 3, liver in Case 4, bone in Case 5 and mediastinum and lung in Case 8.

b) Pheochromocytoma: Bilateral pheochromocyto-

mas were found in 8 (80%) of 10 cases. In Case 9, right adrenalectomy was initially performed for pheochromocytoma. After an additional 3 years, pheochromocytoma was found on the left side, and she underwent left adrenalectomy. There were 2 (20%) cases of unilateral adrenalectomy (Cases 11 and 12). These patients did not develop contralateral pheochromocytomas even after 11 (Case 11) and 7 (Case 12) years, respectively. The recurrence of pheochromocytomas was seen in cases 1 and 3. In Case 1, recurrence of pheochromocytoma with symptoms in the upper portion of the right kidney was observed. In Case 3, recurrent asymptomatic pheochromocytoma, which was accidentally found at resection of myoma uteri, was resected 12 years after the primary surgery for pheochromocytomas.

c) Mucosal neuromas, Marfanoid habitus: All patients had neuromas and bumpy lips, and 15 of 22 informative patients had a Marfanoid habitus. Neuromas and gastrointestinal abnormalities were found as the first sign in 18 and 2 patients, respectively. Six patients (Cases 9, 10, 12, 13, 14 and 19) visited dentists, plastic surgeons, or dermatologists complaining of malocclusion of the mouth, nodules on the tongue, or thick lips, and then were diagnosed histologically as having neuromas before developing MTC and/or pheochromocytomas. Constipation was noted in 2 patients (Cases 4 and 5) with megacolon.

d) Parathyroid lesions: Neither symptoms of hyperparathyroidism nor the presence of parathyroid tumors have been reported as shown in Table 2.

5. Outcome

The outcome of 23 cases with MEN 2B has been reported. Although follow-up information on Case 16 is not complete, recent inquiry of doctors at Cancer Research Institute Hospital, Tokyo confirmed that he was alive at least in 1990, 15 years after resection of MTC. Mean overall follow-up was 10.5 years (range, 3 to 20 years). The oldest survivor with MEN 2B is Case 11 at the age of 50 years. Three patients (13%) died of the syndrome at the age of between 12 and 43 years (mean 30 ± 13.1 years). The causes of death were MTC (2 death) and cerebral bleeding (1 death). They died at the age of 43 (Case 1), 35 (Case 5) and 12 (Case 8), respectively, 16, 17 and 6 years after the first surgery for MTC. One died of cerebral bleeding probably due to recurrence of pheochromocytoma, and obstruction of the airway by MTC was the cause of death in 2 other patients.

At the conclusion of the study, 9 (56%) of 16 informative patients were living with no apparent metastatic MTC or pheochromocytoma. Four patients had high levels of plasma CT and two patients have metastases to the liver. Overall 5-, 10- and 15-years survival rates were 100, 92 and 88%, respectively.

Genetic changes in tumors associated MEN 2B

1. Loss of heterozygosity

The following 53 loci were examined. *D1S57* (1p35-p32), *D1Z2* (1p36.3), *D1S80* (1p36-p35), *D1S7* (1p35-p33), *GLUT1* (1p35-p31.3), *MYCL* (1p32), *D1S60* (1p), *D1S73* (1p21-cen), *D1S64* (1p22.1-p13), *AMY* (1p21), *AT3* (1q23-q25.1), *D1S8* (1q42-q43), *MYCN* (2p24.1), *TGFA* (2p13), *THRB* (3p24.1-p22), *D3S3* (3p14.2), *D5S4* (5pter-p15.1), *D5S2* (5q34-qter), *MYB* (6q22-q23), *EGFR* (7p13-p12), *MET* (7q31), *MOS* (8q11), *ASSP3* (9q11-q22), *D10S24* (10p13-p12.2), *RBP3* (10q11.2), *D10S5* (10q21.1), *D10S1* (10q22-q23), *D10S4* (10q22-q23), *PLAU* (10q24-qter), *D11S12* (11p15.5), *WT1* (11p13), *INT2* (11q13), *INT2* (11q13), *APOA1* (11q23-q24), *KRAS2* (12p12.1), *D12S4* (12cen-q14), *RB1* (13q14.2), *D13S2* (13q22), *D14S16* (14q32.32-q32.33), *D15S18* (15q11-q12), *D16S79* (16p13.13-p13.11), *D17S31* (17p13.1-p11.2), *TP53* (17p13.1), *D17S4* (17q23-q25.3), *TTR* (18q11.2-q12.1), *DCC* (18q), *C3* (19p13.3-p13.2), *D19S7* (19q12), *D20S5* (20p12), *D20S6* (20p12), *D20S4* (20q13.2), *D21S11* (21q21), *D22S1* (22q11.2-q12), *D22S9* (22q11.1-q11.2). As for two MTCs from Cases 21 and 22, 20 new loci examined in this study showed no LOH. LOH was not detected in 2MTCs or 1 pheochromocytoma in any of the informative loci.

2. Point mutations in the *ras*, Gsα, and p53 genes

No point mutations of exons 1 and 2 of the H-, K-, N-*ras* genes and exons 5–10 of the p53 gene were detected by PCR-SSCP. PCR-PIRA of 4 MTCs and 1 pheochromocytoma showed no mutations at codon 201 or 227 of the Gsα gene.

Discussion

MEN 2B is characterized by a triad of MTC, pheochromocytoma, and mucosal neuroma. The frequency of appearance of each tumor in Japan,

summarized in Tables 2 and 3, was in accordance with the reported frequency [13]. Familial cases were reported at a frequency of 31 to 50% in the United States [13, 14]. In this study, a positive family history of MEN 2B was obtained only in 13% of all cases. Inspection of Table 1 shows no obvious differences between the familial and the non-familial group in any characteristics. The small number of familial cases might be due to excluding some patients of the familial type as those of the sporadic group.

In contrast to MEN 2A and sporadic MTC, most patients with MEN 2B were regarded as associated with a more malignant MTC with a tendency to early metastasis to the lung, liver or bone [1]. Although a high incidence (81%) of local metastasis was observed in our study, a low incidence (5%) of distant metastasis at primary surgery for MTC is different from their results. Carney *et al.* reported that the survival rates in 89 patients with MEN 2B were 80% at 5 years and 50% at 10 years, respectively [15]. They also reported a 5-year disease-free survival rate of less than 35% in patients with MEN 2B. According to Raue and Zink, the survival time for MEN 2B was similar to that for sporadic MTC (5 years: 78%, 10 years: 59%), while MEN 2A had a much better prognosis with 5-year and 10-year survival rates of 86 and 72%, respectively [16].

In contrast, Samaan *et al.* reported that the survival rates for patients in the MEN 2A and 2B groups did not differ, and patients with MEN 2A and 2B had a significantly higher survival rate than patients with sporadic MTC [17]. Takai *et al.* reported that all six Japanese patients with MEN 2B were alive at the time of survey in 1984. Recently, Vasen *et al.* reported that the natural clinical course of MTC in MEN 2B was comparable to that seen in MEN 2A, by investigating 18 patients with MEN 2B in Germany and the Netherlands [2]. The important factors that influence prognosis are the age and sex of the patients, size and stage of the disease, and the type of treatment. Although the reason for the difference between prognosis of 50 to 59% in Caucasians *vs.* 92% in Japanese in our study in regard to 10-year survival rates remains unclear, these results suggest that the difference between the genetic background of Caucasians and that of Japanese contributes to the outcome of the disease.

The diagnosis of MEN 2B is often missed or delayed because of the various manifestations of the condition, particularly when the cases are sporadic. These patients are often seen by numerous health care practitioners, including pediatricians, dentists, and plastic surgeons, for the associated signs and symptoms. The connection between the obvious facial signs (bumpy tongue and thick lips) and the more serious life-threatening problems (MTC and pheochromocytomas) must be made, because early diagnosis is the key to curing MEN 2B patients.

No LOH on chromosome 10 has been detected in tumors associated with MEN 2B in this study and others [7, 8]. Thus it is unlikely that the predisposing gene in the centromeric region on chromosome 10 belongs to the category of tumor suppressor genes which are causative for retinoblastoma, Wilms tumor or MEN 1. A more likely, but unproven, mechanism is a mutation of the MEN 2B gene which causes C cell hyperplasia and adrenomedullary hyperplasia. If this model is correct, one would expect to find other genetic alterations in MTCs and pheochromocytomas that may contribute to tumor formation and/or progression. As for other possible genetic changes, other groups have noted LOH on 1p in MEN 2B tumors. Mathew *et al.* noted LOH with a single 1p probe, *D1S7* (1p35-p33) in one MTC [18]. Khosla *et al.* also detected LOH on chromosome 1p in 33% of hereditary pheochromocytomas, but no information on LOH of pheochromocytomas associated with MEN 2B was mentioned [19]. Moley *et al.* reported LOH on chromosome 1p in 0% (0/5) of MTC and 100% (2/2) of pheochromocytomas [20]. However, we did not detect LOH on 1p32 in one pheochromocytoma.

In familial polyposis coli, colon cancer arises as an accumulation of inherited (*APC* gene) and/or somatic mutations such as the *ras*, p53 genes [21]. In addition, recent studies have shown that a mutation in the subunit of the stimulating GTP binding protein (Gs) is involved in the development of endocrine tumors [22]. However, we detected no mutations in the *ras*, p53, or Gsα genes in 5 tumors from 4 patients with MEN 2B. Therefore, genes other than the *ras*, p53, or Gsα genes are assumed to be associated with tumorigenesis in MEN 2B. Genetic studies are now under way to ultimately characterize and clone the *MEN 2B* gene and identify the molecular basis of

this disease.

In this study, we reported the characteristics and especially the relatively good prognosis of MEN 2B in Japan and the absence of common genetic abnormalities in MEN 2B-associated tumors in regard to LOH and oncogenes or a suppressor gene.

Comment in Proof: Germ-line mutations of the *RET* proto-oncogene were found in 20 of 23 MEN 2A families, but not in MEN 2B patients [56].

Acknowledgements

The authors thank Drs. Atsuhiko Sakamoto, Naoki Hirabayashi, Seiji Fujiwara, Eiji Itagaki, Masaaki Fukase, Masaru Tsuyuguchi, Syunichi Yagi, Hiroshi Hongo, Noriaki Sekita, Yuhichi Kaneko, Ichiro Komiya, Kazumasa Sugihara, Isao Morimoto, Masamichi Ohishi, Kyoko Hirai, Nozomu Kurozumi, Hiroshi Takami, Hiroomi Funahashi, Kiyoshi Kikuchi, Chiaki Sakaguchi, Takako Sonomura, Kyohji Akaishi, Sayoko Ohno, Tsuyoshi Sato, Hitoshi Kohno, Taijiro Okabe, and Tsunehiro Ishida for referring the patients. We also thank Drs. Masaru Tsuyuguchi, Isao Morimoto, Sumiya Eto, and Yasuo Koike for providing tumors. We appreciate Professor Shiro Saito for continuous support. This work was supported in part by a Grant-in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and by a grant from Otsuka Pharmaceutical Factory, Inc., for Otsuka Department of Clinical and Molecular Nutrition, School of Medicine, The University of Tokushima.

References

1. Norton JA, Froome LC, Farrell RE, Wells SA Jr (1979) Multiple Endocrine Neoplasia Type IIb: The most aggressive form of medullary thyroid carcinoma. *Surg Clin North Am* 59: 109–118.
2. Vasen HFA, va der Feltz M, Raue F, Kruseman AN, Koppeschaar HPE, Pieters G, Seif FJ, Blum WF, Lips CJM (1992) The natural course of multiple endocrine neoplasia type IIb: A study of 18 cases. *Arch Intern Med* 152: 1250–1252.
3. Takai S, Miyauchi A, Matsumoto H, Ikeuchi T, Miki T, Kuma K, Kumahara Y (1984) Multiple endocrine neoplasia type 2 syndromes in Japan. *Henry Ford Hosp Med J* 32: 246–250.
4. Norum RA, Lafreniere RG, O'Neal LW, Nikolai TF, Delaney JP, Sisson JC, Sobol H, Lenoir GM, Ponder BAJ, Willard HF, Jackson CE (1990) Linkage of the multiple endocrine neoplasia type 2B gene (MEN2B) to chromosome 10 markers linked to MEN2A. *Genomics* 8: 313–317.
5. Lairmore TC, Howe JR, Korte JA, Dilley WG, Aine E, Wells SA, Donis-Keller H (1991) Familial medullary thyroid carcinoma and multiple endocrine neoplasia type 2B map to the same region of chromosome 10 as multiple endocrine neoplasia type 2A. *Genomics* 9: 181–192.
6. Gardner E, Papi L, Easton DF, Cummings T, Jackson CE, Kaplan M, Love DR, Mole SE, Moore JK, Mulligan LM, Norum RA, Ponder MA, Reichlin S, Stall G, Telenius H, Telenius-Berg M, Tunnacliffe A, Ponder BAJ (1993) Genetic linkage studies map the multiple endocrine neoplasia type 2 loci to a small interval on chromosome 10q11.2. *Hum Mol Genet* 2: 241–246.
7. Nelkin BD, Nakamura Y, White RW, de Bustros ACV, Hgerman J, Wells SA Jr, Baylin SB (1989) Low incidence of loss of chromosome 10 in sporadic and hereditary human medullary thyroid carcinoma. *Cancer Res* 49: 4114–4119.
8. Yamamoto S, Morimoto I, Fujihira T, Watanabe K, Zeki K, Yoshimoto K, Zeze F, Eto S (1992) A kindred of multiple endocrine neoplasia type 2B. *Endocrinol Japon* 39: 25–30.
9. Yoshimoto K, Iizuka M, Iwahana H, Yamasaki R, Saito H, Saito S, Sekiya T (1989) Loss of same alleles of HRAS1 and D11S151 in two independent pancreatic cancers from a patient with multiple endocrine neoplasia type 1. *Cancer Res* 49: 2716–2721.
10. Yoshimoto K, Iwahana H, Fukuda A, Sano T, Saito S, Itakura M (1992) Role of p53 mutations in endocrine tumorigenesis: Mutation detection by polymerase chain reaction-single strand conformation polymorphism. *Cancer Res* 52: 5061–5064.
11. Yoshimoto K, Iwahana H, Fukuda A, Sano T, Katsuragi K, Kinoshita M, Saito S, Itakura M (1992) Ras mutations in endocrine tumors: Mutation detection by polymerase chain reaction-single strand conformation polymorphism. *Jpn J Cancer Res* 83: 1057–1062.
12. Yoshimoto K, Iwahana H, Fukuda A, Sano T, Itakura M (1993) Rare mutations of the Gs alpha gene in human endocrine tumors: Mutation detection by polymerase chain reaction-primer-introduced restriction analysis. *Cancer* 72: 1386–1393.
13. Khairi MRA, Dexter RN, Burzynski NJ, Johnston

CC (1975) Mucosal neuroma, pheochromocytoma, and medullary thyroid carcinoma: multiple endocrine neoplasia type 3. *Medicine* 54: 89–112.
14. Carney JA, Sizemore GW, Hayles AB (1978) Multiple endocrine neoplasia, type 2b. *Pathobiol Ann* 8: 105–153.
15. Carney JA, Sizemore GW, Hayles AB (1979) C-cell disease of the thyroid gland in multiple endocrine neoplasia, type 2b. *Cancer* 44: 2173–2183.
16. Raue F, Zink A (1992) Clinical features of multiple endocrine neoplasia type 1 and type 2. *Horm Res* 38 (suppl 2): 31–35.
17. Samaan NA, Schultz PN, Hickey RC (1988) Medullary thyroid carcinoma: Prognosis of familial *versus* sporadic disease and the role of radiotherapy. *J Clin Endocrinol Metab* 67: 801–805.
18. Mathew CGP, Smith BA, Thorpe K, Wong Z, Royle NJ, Jeffreys AJ, Ponder BAJ (1987) Deletion of genes on chromosome 1 in endocrine neoplasia. *Nature* 328: 524–526.
19. Khosia S, Patel VM, Hay ID, Scaid DJ, Grant CS, van Heerden JA, Thibodeau SN (1991) Loss of heterozygosity suggests multiple genetic alterations in pheochromocytomas and medullary thyroid carcinomas. *J Clin Invest* 87: 1691–1699.
20. Moley JF, Brother MB, Fong C-T, White PS, Baylin SB, Nelkin B, Wells SA Jr, Brodeur GM (1992) Consistent association of 1p loss of heterozygosity with pheochromocytomas from patients with multiple endocrine neoplasia type 2 syndromes. *Cancer Res* 52: 770–774.
21. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. *Cell* 61: 759–767.
22. Lyons J, Landis CA, Harsh G, Vallar L, Grunewald K, Feichtinger H, Ouan-Yang D, Clark OH (1990) Two G protein oncogenes in human endocrine tumors. *Science* 249: 655–659.
23. Watanabe I, Takushoku K, Matsuura K, Tanaka T, Gitoh Y, Yamagata Y, Ohnuma H (1968) A case of thyroid carcinoma associated with bilateral adrenal pheochromocytoma and Marfan's syndrome. *Nippon Naibunpitsu Gakkai Zasshi (Folia Endocrinol Japon)* 44: 281–282 (Abstract)(In Japanese).
24. Watanabe I, Kimishima I, Marutani H, Andoh H, Endoh T, Ishikawa H, Wakasa H (1983) A case of MEN type 2B: Findings of autopsy. *Kohjohsen Geka Kentokai Yokoshu* (the 16th) p106 (Abstract) (In Japanese).
25. Takeuchi K, Ohtani I, Aikawa T, Sato M (1989) Temporal bone findings in multiple endocrine neoplasia type 2B associated with Marfanoid habitus. *Jibika Rinsho (Prac Otologia)* Suppl 32: 97–99 (In Japanese).
26. Inazuki H, Adachi I, Abe K, Ogata E (1975) A case of medullary thyroid carcinoma associated with multiple mucosal neuroma. *Nippon Naibunpitsu Gakkai Zasshi (Folia Endocrinol Japan)* 51: 438 (Abstract) (In Japanese).
27. Sakamoto A, Kinoh I, Sakamoto M, Kawahara Y, Nakamura K, Sugano H (1974) Histopathological examination of medullary thyroid carcinoma. *Nippon Byori Gakkai Zasshi (Jpn J Pathol)* 63: 300–301 (Abstract) (In Japanese).
28. Nakano H, Mori K, Fukushige M, Nihira H, Hayashi Y, Kunita T, Matsuura H, Fukunaga Y, Chohshi K, Nagata N (1977) A syndrome of multiple mucosal neuroma, medullary thyroid carcinoma and pheochromocytoma: report of a case. *Nippon Hinyokika Gakkai Zasshi (Jpn J Urol)* 63: 294–306 (In Japanese).
29. Kato M, Nagatsu M, Urakawa K, Kawaguchi K, Takao K, Takiguchi Y, Boku S, Mitsuno T, Okada S, Akematsu T (1978) A case with medullary thyroid carcinoma, multiple mucosal neuroma, and megacolon: Multiple endocrine neoplasia type 3. *Syujyutsu (Operation)* 32: 443–449 (In Japanese).
30. Imai Y, Fukase M, Tsutsumi M, Fukami T, Sakaguchi K, Furumoto M, Yoshimoto Y, Matsukura S, Fujita T (1982) A case of multiple endocrine neoplasia type II b: Endocrinological evaluation and family screening. *Endocrinol Japon* 29: 49–56.
31. Fukase M (1991) MEN Type IIb. In: Fujita T (ed) Shourei ni yoru Naikagaku (Internal Medicine learned from Patients). Medicalview, Tokyo, p172–175 (In Japanese).
32. Tsuyuguchi M, Kakuta E, Saito K, Harada K, Inoue G, Saito H, Ohshima I, Saito S (1980) A case of medullary thyroid carcinoma (MEN, type 3) producing somatostatin and calcitonin. *Gann no Rinsho (Jpn J Cancer Clin)* 26: 280–286 (In Japanese).
33. Shimada Y, Tsuyuguchi M, Morimoto T, Sogabe H, Harada K, Inoue G, Sano T, Hizawa K (1982) A case of bilateral adrenal pheochromocytoma (multiple endocrine neoplasia, type 3). *Gann no Rinsho (Jpn J Cancer Clin)* 28: 1765–1770 (In Japanese).
34. Morimoto S, Takai S, Ogihara T, Okada Y, Onishi T, Miyai K, Miyauchi A, Hayashi K, Furukawa S, Kumahara Y (1980) Multiple endocrine neoplasia type 2b. (I) Report of a case. *Jpn. J Clin Oncol* 10: 147–156.
35. Ohji M, Higashi M, Shimizu Y, Kinoshita S (1985) Thickening of corneal nerves in multiple endocrine neoplasia type IIB. *Rinsho Ganka (Jpn J Clin Ophthal)* 39: 404–405 (In Japanese).
36. Yagi S (1983) A case of multiple endocrine neoplasia (type 3). *Horumon to Rinsho, Naibunpitsu Kyomi aru Shorei (Clinical Endocrinology Case Presentation)* 31: 190–192 (In Japanese).
37. Tsukuda K, Nomura S, Harada E, Tanaka K, Nakayasu K, Ofuji K, Kondo N, Hongo H, Hirayu H, Kamei T, Nakamura H (1982) Medullary thyroid carcinoma, multiple mucosal neuroma and Marfanoid habitus in a boy aged 6 years. *Nippon*

Shoni Geka Gakkai Zasshi (Jap J Ped Surg) 18: 1181–1188 (In Japanese).

38. Hamao T, Inoue T, Osada T, Kuroki K, Yoshio M, Kuroda S, Yamagoe M, Nakano M, Yukimoto Y (1983) Multiple endocrine neoplasia, type 2b: report of a case. *Rinsho Hinyokika (Jap J Clin Urol)* 37: 733–736 (In Japanese).
39. Kaneko Y, Kusumi A, Kumagai N, Hagino Y (1983) A case of multiple endocrine neoplasia type III. *Nippon Keisei Geka Gakkai Kaishi (J Jap Soc Plast Reconst Surg)* 3: 930–936 (In Japanese).
40. Miyamoto T, Komiya I, Otsuka H, Yamauchi K, Takasu N, Yamada T (1985) A case of pheochromocytoma associated with medullary hyperplasia in the same adrenal gland. *Nippon Naibunpitsu Gakkai Zasshi (Folia Endocrinol Japon)* 61: 1077 (Abstract) (In Japanese).
41. Ohkubo T, Hamada N, Bakune R, Yamakado K, Baba K, Kaneko Y, Aikou T, Nomura H, Nishi M (1983) A case of multiple endocrine neoplasia, type 2b. *Rinsho Geka (J Clin Surg)* 38: 711–716 (In Japanese).
42. Koreeda H, Kanzaki K, Matsuzaki I, Maeda Y, Yamashita J, Yamakado K, Sugihara K, Tanaka H (1983) A case of multiple endocrine neoplasia, type 2B. *Naika (Internal Medicine)* 52: 1187–1190 (In Japanese).
43. Sugihara K, Fujisaki M, Nagata H, Ishigami T, Yamashita N, Yamashita S (1988) A case of MEN 2B. *Nippon Koukuu Kagaku Gakkai Zasshi (J Jap Stomatol Soc)* 37: 836 (Abstract) (In Japanese).
44. Fujio N, Ohashi M, Yanase T, Nawata H, Wasada T, Kato K, Ibayashi H (1986) A case of multiple endocrine neoplasia (MEN) type IIb. *Nippon Naika Gakkai Zasshi (J Jap Soc Int Med)* 75: 79–84 (In Japanese).
45. Ohishi M, Ishi T, Shiratsuchi Y, Tashiro H (1990) Multiple endocrine neoplasia type 3: Three cases with mucosal neuromata. *Br J Oral Maxifacial Surg* 28: 317–321.
46. Kurozumi N, Takino Y, Ojime H, Ishikawa S, Isogai N, Utsugi R, Kamiishi H, Tezuka T (1983) A case of multiple endocrine adenomatosis type IIb. *Nippon Keisei Geka Gakkai Kaishi (J Jap Soc Plast Reconst Surg)* 3: 937–944 (In Japanese).
47. Esa A, Sugiyama T, Park YC, Kataoka K, Kaneko S, Kohri K, Kurita T (1985) Multiple endocrine neoplasia, type I and type IIb: Report of two cases. *Hinyokika Kiyou (Acta Urol Jap)* 31: 1593–1599 (In Japanese).
48. Saeki Y, Yamato M, Hohjo T, Nakano K, Kubo R, Okuno K, Ogawa M, Iwasa Y, Yasutomi M (1986) A case of multiple endocrine adenomatosis type IIb. *Horumon to Rinsho, Naibunpitsu Kyomi aru Shorei (Clinical Endocrinology Case Presentation)* 34: 228–230 (In Japanese).
49. Ohashi M, Fujio N, Kato K, Ibayashi H (1985) Two cases of multiple endocrine neoplasia type 2B: Secretion pattern of calcitonin. *Gann no Rinsho (Jpn J Cancer Clin)* 31: 453–454 (In Japanese).
50. Takami H, Ozaki O, Ito K, Shikata J, Hosoda Y, Mimura T, Abe O (1987) An operated case of multiple endocrine neoplasia type IIb and review of the Japanese literature. *Nippon Geka Gakkai Zasshi (J Jap Surg Soc)* 88: 1017–1023 (In Japanese).
51. Ueda M, Mizuno S, Takeuchi M, Imai T, Sato Y, Asano N, Yoshida N, Nozaki H, Kato N, Funahashi H, Takagi H. A case of multiple endocrine neoplasia type 2b. *Gann no Rinsho (Jpn J Cancer Clin)* 33: 1079–1085 (In Japanese).
52. Kikuchi K, Mimura T, Uematsu Y, Takada I, Kurihara E, Fujizaki M, Ohsaku M, Saotome K (1987) A case of multiple endocrine neoplasia type IIb. *Kohjohsen Geka Kentokai Yokoshu* (the 20th), p118 (Abstract) (In Japanese).
53. Satoh T, Ohuchi K, Urayama M, Tsukamoto M (1990) A case of multiple endocrine neoplasia type IIb (MENIIb). *Nippon Rinsho Gekai Gakkai Zasshi (J Jap Soc Clin Surg)* 51: 1932–1936 (In Japanese).
54. Sakaguchi C, Sonomura T, Shintaku K, Shimizu K, Okuno Y, Takahashi Y, Akaishi K, Ishii H, Tabuchi K, Ohno S (1992) A case of pediatric multiple endocrine neoplasia type IIb. *Shonika (Pediatrics Jap)* 33: 569–573 (In Japanese).
55. Sakamoto Y, Yoshimoto K, Yokoi K, Sano T, Saito S (1992) A case of MEN 2B: Inhibition of calcitonin secretion by octreotide acetate. *Horumon to Rinsho, Naibunpitsu Kyomi aru Shorei (Clinical Endocrinology Case Presentation)* 19: 87–90 (In Japanese).
56. Mulligan LM, Kwok JBJ, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L, Ponder MA, Telenius H, Tunacliffe A, Ponder BAJ (1993) Germ-line mutations of the *RET* proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 363: 458–460.