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Familial Isolated Hyperparathyroidism Caused by Single Adenoma: A Distinct Entity Different from Multiple Endocrine Neoplasia

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Abstract. Familial hyperparathyroidism (FHPT) is a hereditary disease where hyperparathyroidism (HPT) is transmitted in an autosomal dominant fashion. FHPT consists of a variety of diseases such as multiple endocrine neoplasia type1 (MEN 1) and type2 (MEN 2), familial isolated hyperparathyroidism (FIHPT) with single adenoma and with multiple adenomas (or hyperplasia), and FHPT with jaw-tumor (FHPT-JT). Isolation of the genes responsible for MEN 1, and 2, i.e. *MEN1* and *RET*, respectively, makes it possible to examine the relations among disorders constituting FHPT. We studied germ-line mutations in these 2 genes in a family of FHPT with single parathyroid adenoma. The disorder in this family was proved to be an entity different from MEN 1 because no germ-line mutations in *MEN1* gene were found in the affected members. The loss of heterozygosity (LOH) at *MEN1 gene* and PYGM were not found in the abnormal parathyroid in this family, supporting the above conclusion. No mutations in exons 10, and 11 of *RET* proto-oncogene was found in germ-line DNA of the affected member of the family, suggesting no relation to MEN 2A. Linkage study excluded the possibility of FHPT-JT syndrome. PRAD1 was not overexpressed in the parathyroid tumors in this family. The relation of this disorder to FIHPT with multiple enlarged parathyroid glands remains to be clarified. A search for the gene(s) predisposing to FIHPT is needed.

Key words: Familial isolated hyperparathyroidism, MEN 1, MEN 2, HPT-JT

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THERE are several hereditary diseases in which hyperparathyroidism (HPT) is transmitted in an autosomal dominant fashion, e.g. multiple endocrine neoplasia type 1 (MEN 1) or type 2A (MEN 2A). These diseases are generally referred to as familial hyperparathyroidism (FHPT) [1–4]. FHPT without any other endocrinopathies (except for coincidental thyroid disease) is also known, and is called familial isolated hyperparathyroidism (FIHPT) to emphasize the absence of other endocrine diseases [5–7]. In FIHPT, HPT is usually caused by enlargement of multiple parathyroid glands, but some families have been reported where only one parathyroid is enlarged. Wassif *et al.* excluded linkage between FIHPT and the MEN 1 and MEN 2A [6]. Kassem *et al.*, however, reported that FIHPT might be caused by mutation of *MEN1* gene [7]. The relation between these diseases remains to be clarified. The gene responsible for MEN 1 (*MEN1*) was isolated in 1997 [8], and that

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for MEN 2 (*RET* proto-oncogene) in 1993 [9]. Isolation of these genes makes it possible to examine directly whether FIHPT is a variant of MEN syndromes or not. In order to clarify this issue, we examined germ-line mutation of *MEN1* gene and *RET* proto-oncogene in one family with FIHPT caused by a single parathyroid adenoma. The clonality of the enlarged parathyroid gland and the relation to the familial HPT with jaw tumor (FHPT-JT) [10–12] were also studied.

Patients and Methods

Patients

We have operated on 4 patients in 2 generations of a family.

Case 1 (III-4 in the Fig. 1)

In 1989, a 30-year-old male with macroscopic hematuria and pain in the lower abdomen was admitted to Toranomon Hospital. His serum Ca was 10.8 mg/dl, and midportion PTH (mPTH) was 990 pg/ml (the standard value of this assay: 180–560). Ultrasonography and CT scan of his neck revealed a mass behind the upper pole of the left lobe of the thyroid. An enlarged left upper parathyroid with a maximum diameter of 1.2 cm, and 800 mg in weight, was successfully removed. The remaining 3 parathyroid glands were normal or atrophic in size. His serum Ca level returned to the normal range on the first postoperative day, and remained within the normal range for more than 8 years. The latest Ca and intact PTH values were 8.8 mg/dl, and 38 pg/ml (the standard value: 20–53), respectively.

Case 2 (III-2 in Fig. 1)

The family history of Case 1 revealed that one of his cousins had been operated on for HPT at 27 years of age in 1986 in a different hospital. Pathological fracture of her scapula led to the diagnosis of primary HPT. Her serum Ca was 11.2 mg/dl, and the enlarged left lower parathyroid gland with a maximum diameter of 3.5 cm was removed. Her serum Ca levels remained normal thereafter for more than 9 years.

Case 3 (II-2 in Fig. 1)

Cases 1 and 2 prompted us to examine the 60-year-old mother of Case 1 for HPT. In a study after getting informed consent, hypercalcemia without any subjective symptoms was demonstrated. Her serum Ca was 10.8 mg/dl, and mPTH was 2,814 pg/ml. An enlarged left lower parathyroid gland (the maximum diameter: 1.5 cm) was removed, resulting in normalization of the serum Ca.

Case 4 (III-1 in Fig. 1)

In 1994, the elder sister of Case 2 (36 years old) presented with a mass in her right anterior neck. Serum Ca 11.0 mg/dl, and mPTH 8,320 pg/ml confirmed the diagnosis of HPT. The right lower parathyroid gland (the largest diameter: 3.1 cm) was removed, and her serum Ca and PTH returned to the normal range. Examination 2 years after surgery revealed serum Ca 7.6 mg/dl, and mPTH 768 pg/ml, indicating hypoparathyroidism with partial compensation.

In none of them have any other endocrinopathies been found. After obtaining informed consent, determinations of serum Ca and PTH were made in I-2, II-3, and III-3 (Fig. 1). All the results were within the normal range.

Methods

Clonal analysis of the enlarged parathyroid gland

Clonality of the enlarged parathyroid gland was analyzed by the method based on random inactivation of X-chromosomes [13]. The method for PCR amplification of phosphoglycerate kinase (PGK) gene and detection of its polymorphism, as well as the preparation of sections for DNA extraction, were the same as in the literature [13, 14]. The method for PCR-based clonality assay with androgen receptor (AR) gene was the same as described in the literature [15–17].

Analysis of MEN1 gene

MEN1 gene was amplified by PCR with primer sets described in the literature [18], and PCR products were sequenced (by Genetic analyzer 310, Perkin Elmer). Two separate determinations of the

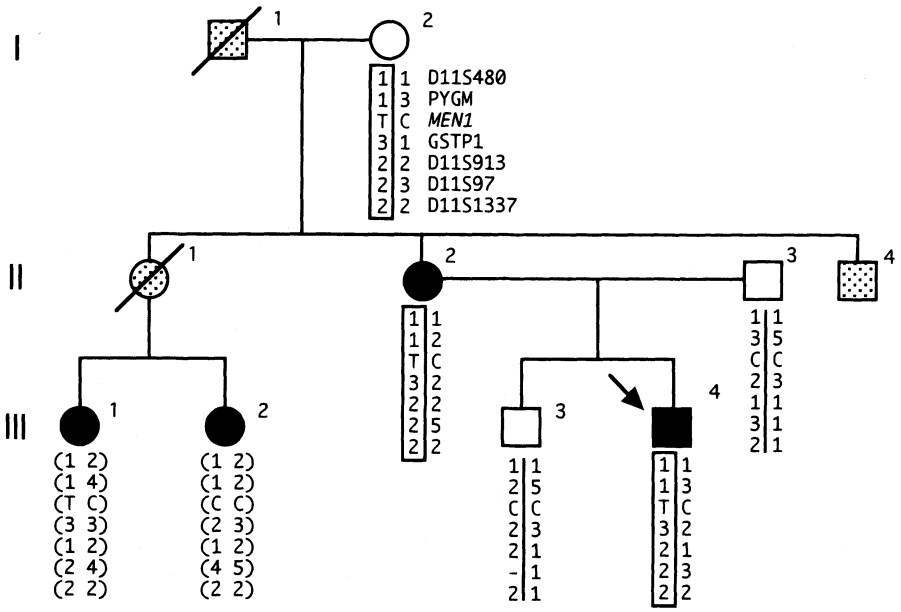

Fig. 1. Pedigree of the family, polymorphism of *MEN1* gene, and haplotypes for 6 markers flanking *MEN1* gene. Arrow indicates the proband. Numbers separated by a vertical line are the haplotype of each member for 6 flanking markers in the order from centromere to telomere. C and T show polymorphism of the *MEN1* gene. For III-1 and III-2, six pairs of numbers and a pair of letters of the alphabet (C and/or T) are shown without vertical lines. These are their genotypes for the above-mentioned genetic markers. All genetic markers are analyzed by a PCR-based method with primer pairs suitable for each locus: i.e.

D11S480 5′-TTTGAGGTAGGCTTCGTATAGT-3′ and
5′-CCCTCTGCCTCTCTTCAAATG-3′;
PYGM 5′-CAGGCCTAGCAGAGTCCACCTACTG-3′ and
5′-GAGGAGGCTGTCAGGTAGCAACTGAC-3′;
GSTP1 5′-GTTGCAGTGAGCCGCCGAGAT-3′ and
5′-AAACAGACAGCAGGAAGAGGACCG-3′;
D11S913 5′-GCCCATTTGGGAAATCCAGAAGA-3′ and
5′-CCTAGGTGTCTTATTTTTTGTTGCTTC-3′;
D11S97 5′-GATCAGCGAACTTCCTCTCCGGCTC-3′ and
5′-TCCACATTGAGGACTGTGGGAAGC-3′;
D11S1337 5′-AAGGTGTGAGGATCACTGGAGCC-3′ and
5′-AGCTCATGGGGGCTATTTCAGGC-3′;
MEN1 5′-GGGTGAGTAAGAGACTGATCTGTGC-3′ and
5′-TGTAGTGCCCAGACCTCTGTG-3′.

Squares, males; and circles, females. open symbols, unaffected with HPT; closed symbols, affected; stippled symbols, not examined; slashes, deceased.

sequence, i.e. one with the sense and one with the antisense strand, were performed.

Haplotype analyses of the family members, and LOH studies of the tumor by using polymorphic microsatellite markers on chromosome 1 and 11

For chromosome 1, microsatellite markers D1S196, D1S242 and D1S191 were used to study the relation to FHPT-JT [16, 17].

For chromosome 11, the microsatellite regions D11S480, PYGM, GSTP1, D11S913, D11S1337 and D11S97 were used for analysis of the family members, and for LOH study of the abnormal parathyroid gland (Fig. 1) [19–22].

Analysis of RET proto-oncogene

DNA was extracted from leukocytes of in Case 4. The exon 10, and 11 of the *RET* proto-oncogene were amplified by PCR with the following oligonucleotide primers:

(for Exon 10) 5′-TAGGAATTCGCTGAGTGGGCT ACGTCT-3′ and 5′-CTCAAGCTTACCCACTCACCC TGGATG-3′ ;
(for Exon 11) 5′-TAGGAATTCCTCTGCGGTGCC AAGC-3′ and 5′-CTCAAGCTTCACCGGAAGAG GAGTAGC-3′

The PCR products were screened for mutation by RNase protection analysis as described in the literature [23].

Expression of PRAD1

Abnormal parathyroid glands from Cases 2 and 4 were studied for expression of PRAD1. A labelled streptavidin biotin kit (DAKO) was used for immunohistochemical staining with anti-cycline D1/Bcl-1 monoclonal antibody (HBL Hitechlone) as the primary antibody. MCF 7 cells [24] were used as the positive control, and normal parathyroid glands resected at surgery for thyroid carcinoma were used as the negative control.

Results

Clonality of the enlarged parathyroid gland

The results of clonality analysis of the enlarged parathyroid gland in Case 4 are shown in Fig. 2. The patient was a heterozygote as to the polymorphism at BstX I site in the PGK gene, but only one band (530 bp) was seen after digestion of the DNA from the enlarged parathyroid with Hpa II, indicating that the enlarged parathyroid gland of Case 4 was monoclonal.

The enlarged parathyroid from Case 2 was analyzed by AR-PCR clonality assay because of homozygosity in the PGK gene. Again the monoclonality of the enlarged parathyroid was demonstrated (Data not shown).

Germ-line mutation of MEN1 gene

No mutations were found in any region of the *MEN1* gene examined in the affected individuals in this family. Benign polymorphism, D418D (GAC/GAT), was found in individuals I-2, II-2, III-1 and III-4 (Fig. 1). This is one of the benign polymorphisms already reported by Agarwal *et al.*

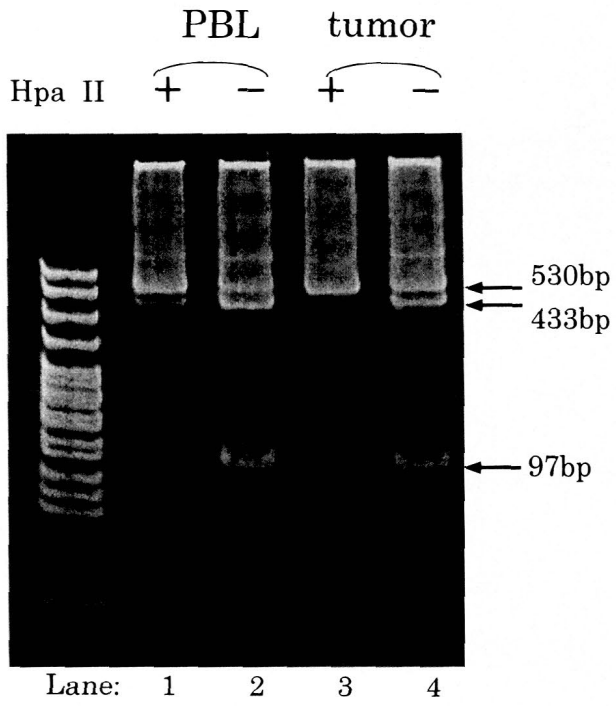

Fig. 2. Clonality of the pathologic parathyroid of Case 4. Lanes 1 & 2: DNA from peripheral blood leukocytes (PBL). Lanes 3 & 4: DNA from the parathyroid tumor of Case 4. Lane: − DNA amplified without *Hpa* II precutting; + DNA amplified after *Hpa* II precutting. Two bands (530 bp and 433 bp) are seen in lane 1, indicating that this patient is a heterozygote for PGK gene. The 433 bp-band has disappeared after *Hpa* II precutting in lane 3, suggesting that this tumor is monoclonal.

[25]. These individuals were heterozygous for this polymorphism, and all of them except individual I-2 were affected. On the other hand, III-2 and III-3 were homozygotes with 2 wild-type alleles (GAC/GAC), and III-2 was affected but III-3 was not affected (Figs. 1 and 3).

Haplotype analysis of the family members with genetic markers on chromosome 11

As shown in Fig. 1, the haplotype (113222) of 6 flanking markers of *MEN1* on 11q13 was cosegregated with the parathyroid disease in the right half branch of this family but individual I-2 sharing the same haplotype was not affected. Regarding the left half branch of the family (III-1 and III-2, i.e. Cases 4 and 2), haplotypes were not inducible because of the lack of information on I-1 and II-2.

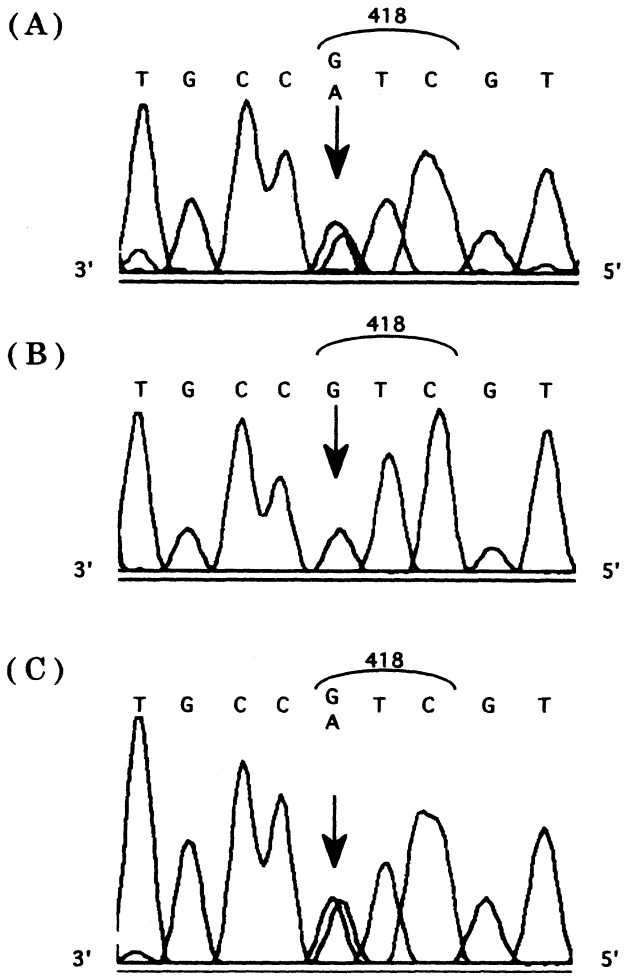

Fig. 3. Benign polymorphism of the *MEN1* gene observed in some members of this family. Both sense and antisense strands were sequenced. Here, the results for the antisense strand are shown. (A) Part of the sequence around codon 418. Arrow: Two peaks for both G and A (in the antisense strand) are seen in the germ-line of Case 4 (III-1), indicating that she is a heterozygote (GAC/GAT) for *MEN1* gene. (B) The sequence of the Case 2 (III-2). Arrow: Only a G peak is seen at the third nucleotide in the codon 418 of the antisense strand of the germ-line, indicating a homozygote (GAC/GAC). (C) Sequence of DNA extracted from the parathyroid tumor of Case 4 around codon 418. Arrow: Both G and A peaks are present in the antisense strand, demonstrating retention of both alleles in the tumor.

No loss of heterozygosity for the markers on chromosome 11 in the pathologic parathyroid gland of Case 4

The DNA extracted from the enlarged parathyroid gland was examined for LOH with 4 flanking markers of *MEN1*. No LOH was demonstrated at the 2 loci (PYGM, and D11S1337) where alleles were constitutionally heterozygous, and the patient was an homozygote at the remaining 2 loci. The retention of both alles of *MEN1* gene in the abnormal parathyroid gland was demonstrated in the Case 4 by using a benign polymorphism of this gene (D418D) (Fig. 3-C).

Analysis of RET proto-oncogene

No germ-line mutations were found in exons 10 and 11 of the *RET* proto-oncogene in any affected member.

Haplotype analysis of the family members with genetic markers on chromosomes 1

The haplotype (231) of 3 flanking markers of the gene for FHPT-JT syndrome on 1q21-q31 was seen in I-2, II-2, III-3 and III-4 in this family (Fig. 4). An unaffected member (III-3) also had the same haplotype.

Expression of PRAD1

No excessive expression of PRAD1 was observed in the enlarged parathyroid of Cases 2 and 4.

Discussion

Since Goldman *et al.* [1] reported HPT in siblings, more than 50 such families (FHPT) have been reported in Europe and in the USA [2, 5, 6, 11, 25, 26]. Recently Huang *et al.* reported the clinical features of FHPT without MEN [27]. They analyzed their own 16 patients from 14 families, together with a review of 51 reported cases. Twelve of their patients (75%) and 23 of the reported cases (45%) had multiple abnormal parathyroid glands. They also pointed out a tendency to profound hypercalcemia in patients with FHPT [27].

In Japan, 47 patients from 18 families (including the present cases) were reported up to 1996 (Table 1) [28–45]. Among the 18 reported families in Japan, at least 2 kindreds (#7, and #14 in Table 1) had affected members with jaw tumors, and 9 families (#6, 8, 9, 10, 11, 13, 14, 17, and 18 in Table 1) had patients with enlargement of multiple parathyroid glands. Three families (#5, 7 and 15) contained at

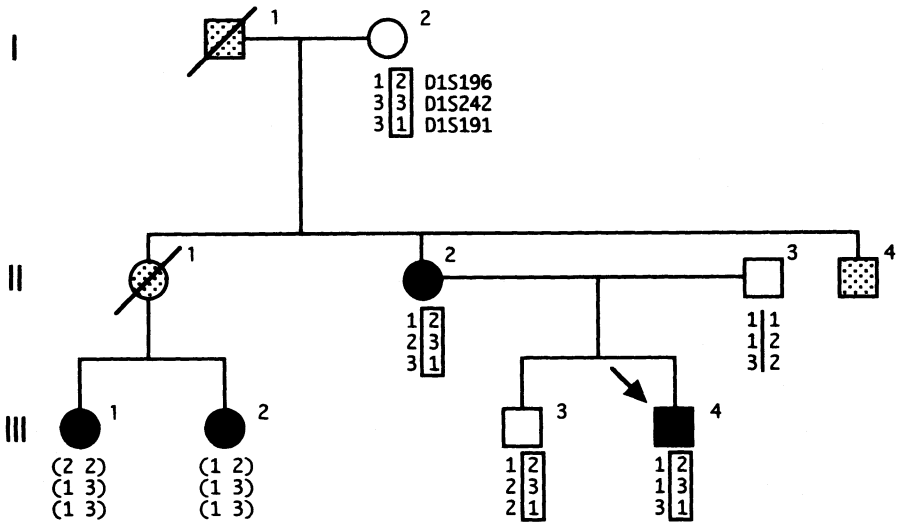

Fig. 4. Haplotypes for 3 markers flanking the FHPT-JT gene. Arrow indicates the proband. The haplotype of each member is shown at 3 loci (D1S196, D1S242, and D1S191) near the putative FHPT-JT gene. The haplotype (231) in II-2, III-3, and III-4 must have been transmitted from I-2. For III-1 and III-2, only their genotypes for the 3 markers are shown. The primer pairs used for this analysis are:

D1S196 5′-AGCTCCAACCAGCAGCCAACATATGG-3′ and
5′-CTTTACATTCTCACAGACTGTCAGTA-3′;
D1S242 5′-ACCACTCCAGTTTGAGCAAC-3′ and
5′-AATGCCATGAGGTGTTTCTC-3′;
D1S191 5′-ATGCATTTGCTTACAAATATCC-3′ and
5′-CCACTGTTCTGCTTGAAGGT-3′.

least one patient suffering from parathyroid carcinoma, so that FHPT consists of some heterologous entities.

It is also well known that MEN 1 patients are usually suffering from HPT due to hyperplasia of all parathyroid glands [46]. Patients with MEN 2A sometimes (in about 20%) had enlargement of one or more parathyroid gland(s) [47]. We propose the provisional classification of FHPT shown in Table 2. Apparently our patients had HPT due to a single gland disease (B-2 in Table 2). The monoclonality of enlarged parathyroid glands in Cases 2 and 4 suggested the neoplastic nature of the disease in our kindred [48].

Regarding the relation of FHPT to MEN 1, Agarwal *et al.* reported no germ-line mutations of *MEN1* gene in 5 kindreds of FHPT, suggesting that mutation in another gene or genes may cause FHPT [25]. All of their patients, however, had multiple parathyroid tumors, i.e. they all belonged to the category B-1 in Table 2.

Therefore it is worthwhile to examine germ-line mutations in FIHPT caused by single parathyroid adenoma (B-2 in Table 2). Our results showed no mutation of *MEN1* gene in the germ-line of the affected members. This strongly suggested that FIHPT due to single parathyroid adenoma is a distinct entity different from MEN 1, because heterozygous germ-line mutation of *MEN1* was recognized in 97% of familial MEN 1 [25], and no LOH at *MEN1* gene or PYGM was demonstrated in a parathyroid tumor in one member of our family. Since *MEN1* gene is considered to be a tumor suppressor gene [8, 25], lack of LOH at *MEN1* gene and PYGM supported the above argument. The presence of the haplotype (113222) at 6 loci on chromosome 11 in an unaffected member (I-2) also suggests no relation to MEN 1 syndrome. From these lines of evidences we would like to conclude that FIHPT due to single parathyroid adenoma is a different entity from MEN 1.

For MEN 2, an operational classification has been used according to the disease phenotype [49], that is MEN 2 is divided into 4 groups: MEN 2A, MEN 2B, Familial MTC (FMTC) and "other". MEN 2A

Table 1. Patients with familial isolated hyperparathyroidism treated in Japan

Year	Kindred	Age	Sex	Relation	No. of gland involved	Pathology	Complication	Reference
1972	#1	40	F	Proband	1	adenoma		[28] [29]
		38	F	Sister	1	adenoma		
		58	M	Brother	1	adenoma		
1974	#2		M	Proband		adenoma		[30]
		41	F	Sister		adenoma		
1975	#3	70	F	Proband		unknown		[31]
		37	F	Daughter	1	adenoma		
		29	M	Son	1	adenoma		
1985	#4	52	F	Proband	1	hyperplasia		[32]
		24	F	Daughter	1	adenoma		
1986	#5	57	M	Proband		carcinoma		[33]
		24	F	Daughter		adenoma		
1991	#6	58	F	Proband	2	hyperplasia		[34] [35]
		55	M	Brother		unknown		
		40	M	Brother	2	hyperplasia		
1991	#7	60	F	Proband	1	adenoma	jaw tumor	[36] [37]
		56	F	Sister	1	adenoma	Wilms	
		38	F	Sister		carcinoma		
1992	#8	16	F	Proband	2	adenoma		[38]
		14	F	Sister		adenoma		
	#9		F	Proband	1	adenoma		do.
			F	Sister	4	hyperplasia		
	#10		F	Proband	3	hyperplasia		do.
			F	Daughter	1	adenoma		
	#11		F	Proband		unknown		do.
			M	Son	4	hyperplasia		
1994	#12	30	M	Proband	1	adenoma		[39]
		60	F	Mother	1	adenoma		
		27	F	Cousin	1	adenoma		
		36	F	Cousin	1	adenoma		
1995	#13	48	F	Proband	4	hyperplasia		[40]
		63	F	Aunt	2	adenoma		
		38	F	Sister	1	unknown		
1995	#14	29	F	Proband	4	hyperplasia	jaw tumor	[41] [42]
			F	Sister	2	unknown		
1995	#15	34	F	Proband		carcinoma		[43]
			M	Brother		carcinoma		
		36	F	Sister		adenoma		
		29	F	Cousin		adenoma		
1996	#16	19	F	Proband	1	adenoma		[44]
		23	F	Sister	1	adenoma		
1996	#17	78	F	Proband	6	hyperplasia		[45]
		53	M	Brother		unknown		
		52	M	Brother		unknown		
	#18	33	F	Proband	3	hyperplasia		do.
		68	F	Mother		unknown		
		29	F	Niece		unknown		

Table 2. Proposed classification of FHPT

A. FHPT in MEN syndromes
A-1) FHPT in MEN 1
A-2) FHPT in MEN 2A
B. FHPT without other endocrinopathy
B-1) FIHPT with enlargement of multiple parathyroid glands
B-2) FIHPT with enlargement of single parathyroid gland
B-3) FHPT with jaw-tumor

FHPT, familial hyperparathyroidism; MEN, multiple endocrine neoplasia.

is further subdivided into MEN 2A(1), 2A(2) and 2A(3). The MEN 2 family having at least 1 patient with HPT is classified as MEN 2A(1) or MEN 2A(3). The germ-line mutation of *RET* has been observed in 104 out of 107 families with MEN 2A(1) or 2A(3) at one of the cysteins in exon 10 or 11 [49]. Mutations at exon 13, 14 or 16 have never been detected in these 2 subgroups of MEN 2. Therefore no germ-line mutation in exons 10 and 11 of *RET* proto-oncogene in this family is sufficient to exclude any relation to MEN 2. The absence of any other endocrinopathy also supports the speculation that the FIHPT in this family is not a component of MEN 2A (with 97% certainty) [49].

The disease in this family seems to be a different entity from FHPT-JT syndrome, because none of the affected members has jaw tumor. The haplotype analysis of the markers on chromosome 1 also supported this conclusion.

Since there was a report of overexpression of PRAD1/Cyclin D gene in sporadic parathyroid adenomas [50], we studied the expression of PRAD1 in the specimens from Cases 2 and 4, but no abnormal expression was demonstrated.

Finally the relation of this entity (B-2 in Table 2) to FIHP with multiple abnormal parathyroid glands (B-1) remains to be clarified. These two diseases might be caused by mutation of different genes, but we cannot exclude the possibility that these diseases may be caused by different mutations of an unidentified gene, just as in MEN 2A, FMTC and MEN 2B. Linkage analysis of many large families is mandatory to disclose the gene(s) responsible for these two diseases.

Nine years have passed since the first patient from this family was diagnosed, but neither endocrine lesion other than parathyroid adenopathy nor enlargement of the second parathyroid gland has been detected as yet. This is interesting because multiple tumor formation is usually seen in familial tumor syndromes. In some families the development of adenoma in a second parathyroid gland or the occurrence of parathyroid cancer has been reported during long-term observation [6, 10, 46]. We need further observation of the affected members of this family for the second tumor.

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