A Kindred of Familial Acromegaly without Evidence for Linkage to MEN-1 Locus


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Abstract. Familial acromegaly (FA) is a rare inherited disease characterized by clustering of somatotrophic adenomas and acromegaly within a family without other manifestations of multiple endocrine neoplasia-type 1 (MEN-1). The genetic basis of this pituitary-specific phenotype is largely unknown, and its relationship to the MEN-1 locus on chromosome 11q13 also remains unclear. To test the hypothesis that FA results from a germline mutation of the MEN-1 locus, we performed a linkage analysis in a Japanese family with 2 members showing manifestations of acromegaly due to somatotroph adenomas. We also examined the adenoma of one patient for loss of heterozygosity (LOH) at 11q13 locus and for the presence of mutations of codon 201 and 227 in the gene for Gsα. Our results provided no evidence that either germline alterations of the MEN-1 locus, LOH at 11q13, or somatic mutation of Gsα plays a causative role in the development of somatotroph adenomas in our FA family. Together with the previous reports, these results suggest that there are at least two distinct subgroups of FA: one that results from a mutation in MEN-1 locus and the other whose causative gene is located outside the 11q13 locus.

Key words: Acromegaly, Pituitary adenoma, Growth hormone, Multiple endocrine neoplasia, Menin


FAMILIAL clustering of pituitary somatotroph adenomas is an extremely rare subset of familial syndrome with pituitary tumors. It is presented either

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as a component of a multiple endocrine neoplasia complex, including the Wermer syndrome (multiple endocrine neoplasia type 1 [MEN-1] [1]) and Carney complex [2], or as an isolated acromegaly/gigantism which is designated as familial acromegaly/acrogigantism (FA) [3].

About 20 families have been reported in the literature since Levin et al. described the first FA family [3–22]. Although clinical evidence suggests that FA is distinct from MEN-1, it still remains possible that
FA is a variant form of MEN-1 with unique clinical expression. Recently the gene responsible for MEN-1 has been identified [23–25], allowing us to test the hypothesis that FA is a variant form of MEN-1 which is caused by a germline mutation in the MEN-1 gene located on human chromosome 11q13 (Table 1). Linkage to the MEN-1 locus has been reported in several FA families [17, 20, 26], but not in other FA families [14, 18]. Furthermore, no germline mutation has been identified in the MEN-1 gene [19–22].

Thirty-five to 40% of GH-secreting pituitary somatotroph adenomas harbor mutations at two specific sites of α subunit of stimulatory G (Gsa) protein, codon 201 or 227, which renders it constitutively active (gsp mutation) [27, 28].

We here report a Japanese family in which two members, a proband and his maternal grandmother, developed somatotroph adenomas without any other clinical manifestations of the MEN-1 syndrome. To investigate whether the MEN-1 locus and/or the gsp mutations underlie the pathogenesis of our case of FA, we performed linkage analysis and examined the occurrence of LOH with microsatellite markers on the MEN-1 locus as well as the presence of gsp mutations.

Subjects and Methods

Patients (Fig. 1)

The proband (III-2) was a 27-year-old male who visited our hospital because of progressive enlargement of his hands and feet at the age of 26. Physical examination revealed an acromegalic appearance of the patient with height of 179 cm and weight of 70.4 kg. His plasma levels of GH (21.2 ng/ml) and IGF-1 (1400 ng/ml) were elevated. Impaired glucose tolerance was noted, but there were no other abnormalities in pituitary hormones including prolactin, ACTH, TSH, LH, and FSH. Computed tomography (CT) revealed a poorly enhanced mass with diameter of approximately 1 cm in the pituitary body without extracellular extension. The adenoma was surgically removed via transphenoidal approach with clinically successful outcome.

His maternal grandmother (I-2) was 78 years old. She also visited our hospital because of pain and paresthesia of both hands in her 40s, and was diagnosed to have acromegaly due to a pituitary adenoma. Radiation therapy was performed with successful outcome; she has been free from symptoms except for acromegalic appearance (enlarged hands, etc.). Her recent levels of GH and IGF-1 levels were within normal range. His mother (II-3) was 54 years old, and had suffered from rheumatoid arthritis for 10 years. She had a past history of having a lipoma on the back, which was surgically removed at the age of 52. CT scan and endocrinological exams revealed no evidence of pituitary adenoma in the mother.

His father, sister, brother and three aunts (mother's siblings) were free from any endocrine diseases including hyperparathyroidism or acromegaly.

All the family members listed in Fig. 1 were examined by the same physician. All the following studies were performed after all subjects had given their

| Table 1 | Previous reports of familial acromegaly with reference to linkage to the MEN-1 locus |
|-----------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Matsumo et al. (1994) | 12 | 2/1 | N.D.** | N.D. | N.D. | N.D. | No (0/1)† |
| Benlian et al. (1995) | 14 | 2/1 | No (0/1) | N.D. | N.D. | N.D. | N.D. |
| Tanaka et al. (1998) | 17, 19 | 6/3 | Yes (1/1) | No (0/6) | Yes (2/2) | Yes (1/2) | N.D. |
| Teh et al. (1998) | 18, 20 | 18/8 | Yes (1/8) | No (0/18) | N.D. | N.D. | N.D. |
| Ackermann et al. (1999) | 22 | 2/1 | N.D. | N.D. | N.D. | No (0/2) | No (0/1) |
| Gadelha et al. (2000) | 21, 26 | 8/2 | Yes (2/2) | No (0/2) | Yes (5/5) | No (0/1) | N.D. |
| The present case | 2/1 | No (0/1) | N.D. | No (0/1) | N.D. | No (0/1) |

* LOH, loss of heterozygosity; ** N.D., not described; † numbers in parentheses, (number of families positive/number of families tested) in the column for linkage to MEN-1, (number of cases positive/number of cases tested) in other columns.
FIG. 1. Segregation analysis at the MEN-1 locus. Clinical findings are given by the following symbols. Empty symbol: asymptomatic; symbol filled in black: acromegaly. Symbol quarterly filled: lipoma. Symbol with a diagonal slash: dead. P: proband. The nomenclatures and order of the microsatellite markers are given in the box at the upper right. The bold letters denote the DNA haplotypes observed in the proband. The vertical letters in each column denote the allele sizes for each markers. Each letter represents unique size and the sizes decrease in alphabetic order; for example, A>B>C>D>E for D11S956. The ages at this study are shown below the symbols. N.T.: not tested.

informed consent for the study, including extracting, preserving, and analyzing their DNA, according to the ethical guidelines on researches of human genome and genetic analysis (Ministry of Health, Labour and Welfare; Ministry of Education, Culture, Sports, Science and Technology; Ministry of Economy, Trade and Industry) and the WHO guidelines on ethical issues in medical genetics and genetic services.

**Genetic linkage analysis**

DNA was isolated from peripheral blood leukocytes by the sodium iodine method with a DNA extraction kit (Wako, Osaka, Japan). Polymerase chain reaction (PCR) was used to analyze 4 polymorphic markers in the vicinity of the MEN-1 locus at 11q13: D11S956 [29], D11S480 [29], PYGM [29], D11S2072 [30]. PYGM locus is closely linked to the MEN-1 locus. D11S480 and D11S956 flank the MEN-1 locus on its centromeric side, while D11S2072 on its telomeric end. Fluorescent dUTP (R110; PE Applied Biosystems, Foster City, CA, USA) was added to the PCR reaction mixtures to visualize the amplified products, which were electrophoresed in polyacrylamide gels using a Model 373A PRISM DNA sequencer (PE Applied Biosystems). The ladder patterns of the 4 markers were compared among the family members, and chromosomal DNA haplotypes of the loci were assigned to each person. The lod scores (log10 of the odds ratio favoring linkage) were calculated as previously described [31], assuming that the proband’s mother, who had a lipoma, was affected.

**Analysis of LOH on MEN-1 locus**

DNA was extracted from the surgically excised pituitary adenoma with phenol/chloroform after digestion with 0.1 mg/ml of proteinase K. The tumor and leukocyte DNA were used as templates for PCR-based genotyping of polymorphic markers in the vicinity of the MEN-1 locus as described above. In the analysis of LOH, we used another marker, D11S987, which is located on the telomeric end of the MEN-1 locus. PCR was performed without fluorescent


\[ \text{dUTP,} \text{ and the products were electrophoresed in 4% (w/v) agarose gel (Nusieve™ BMA, Rockland, ME, USA).} \]

**Examination of \text{Gsa} mutations of the tumor**

Poly(A)\(^+\) RNA was extracted from the pituitary adenoma of the proband. Reverse-transcriptase PCR (RT-PCR) was performed to amplify a cDNA fragment spanning exon 7 to exon 10 using the following oligonucleotide primers: 5'-GTGATCAAG CAGGCTGACTATGTG-3' and 5'-CAGGCAGATTGTCTGGTT-3' [32]. The amplified PCR products (249 bp) were sequenced directly.

**Results**

**Genetic linkage analysis**

To determine whether familial clustering of acromegaly represents a variant form of \text{MEN-1}, family members were studied for genotypes of 4 polymorphic loci in the \text{MEN-1} region. Fig. 1 illustrates how linkage to the \text{MEN-1} gene was finally excluded based on the haplotype analysis. DNA haplotypes were tentatively assigned as shown in Fig. 1.

Only representative results of the haplotype analyses on \text{PYGM} and \text{D11S2072} are shown in Fig. 2, because these two loci were informative enough to preclude the possibility that the two affected members, I-2 and III-2, shared the same DNA haplotype. The haplotype [DHLO] of the proband was shared with his unaffected father (II-2), and the other haplotype [AFLO] was shared with his unaffected aunt (II-4). Furthermore, the haplotype [EHMP] of the affected grandmother was transmitted to the unaffected aunt (II-1), but not to the proband. Therefore, there was no evidence for the co-segregation of the observed phenotypes (acromegaly and/or lipoma) with the \text{MEN-1} locus in this pedigree. Meiotic recombination should have occurred between \text{D11S480} and either \text{D11S956} or \text{PYGM} in the members II-4 and II-6. Lod scores were calculated to be less that 2.5 at all of the four markers, indicating that it is quite unlikely that the endocrine disorders in this family are linked to the \text{MEN-1} locus.

![Fig. 2. Microsatellite analysis on \text{PYGM} and \text{D11S2072} loci. Genotypes of microsatellite markers are tentatively assigned as follows: I-M for \text{PYGM} and N-P to \text{D11S2072}. Three size markers are shown.](image-url)
Analysis of LOH on MEN-1 locus

Since leukocyte DNA of the proband was heterozygous for two polymorphic markers, D11S956 and D11S987, we used these markers to analyze LOH on MEN-1 locus. As shown in Fig. 3, the tumor DNA was heterozygous for both markers, indicating that the tumor did not harbor LOH on the MEN-1 locus.

gsp mutations

Neither of the two common point mutations in codons 201 and 227 of the Gsp gene were found in the cDNA sequence derived from the tumor of the proband.

Discussion

MEN-1 syndrome usually exhibits florid clinical presentations. Some 90–97% of the affected patients have primary hyperparathyroidism, 30–50% exhibit pancreatic tumors, and 15–50% express pituitary tumor. These expressions typically begin at ages between 20–40. Furthermore, the MEN-1 disease is highly penetrant, and as high as 80% of carriers express the disease by their fifth decade [33]. In the family described in this study, however, none of the family members examined had any of parathyroid or pancreatic disorders. Only two members were affected out of 11, suggesting incomplete penetrance of inheritance. These clinical considerations support the view that our family had FA clinically distinct from MEN-1.

In this context, it is noteworthy that the mother of the proband (II-2) developed a lipoma, because MEN-1 is frequently associated with lipomas (up to 10%). Association with lipomas has also been reported in several other families with FA [9, 14]. Interestingly, acromegalic subjects were free from lipomas in the reported families of FA in contrast to MEN-1, as we observed here. More detailed investigation of the phenotypic relationship between somatotroph adenomas and lipomas might disclose some intriguing facts about the genetic nature of the disease. Another characteristic feature of FA is that acromegaly, if present, appears more likely to be inherited in the maternal branch as was in our case. Genomic imprinting or mitochondrial disorder may be involved in the unique phenotypic expression of FA.

With regard to the genetic etiology of FA in relation to the MEN-1 locus, Benlian et al. and Stock et al. reported independently that acromegaly in their FA kindreds were not genetically related to the MEN-1 locus [14, 18] (Table 1). In support of this, no germline mutation has been found in the MEN-1 gene [19–21, 26]. On the other hand, Yamada et al. [17], Teh et al. [20] and Gadelha et al. [26] reported that identical sets of haplotype were shared between patients of their FA families. In our case, linkage of the somatotroph adenomas to the MEN-1 gene was clearly precluded based on the results from DNA haplotype analyses (Figs. 1 and 2). Therefore, it is unlikely that the germline mutations in the MEN-1 gene underlie the development of the somatotroph adenomas in our FA family.

Several investigators have reported loss of heterozygosity (LOH) of the markers for the MEN-1 locus in the adenomas from the FA families [17, 19, 21] (Table 1). These results indicate that somatotroph adenomas in FA may arise from the LOH of tumor suppressor genes that is closely associated with the MEN-1 locus. In this case, however, we did not find
LOH of this locus, indicating that some other genetic factors, quite distinct from 11q13 locus itself, may cause the somatotroph adenomas in this kindred.

Landis et al. showed that the gsp mutations were found in about 35-40% of somatotroph adenomas [27]. However, we found no mutations in both codons of Gsα, in our patient, which was in agreement with the observations of others [12, 22] (Table 1).

Although the precise etiology of FA is still unknown, neither germline mutations of the MEN-1 locus nor somatic mutation of Gsα was likely to play a causative role in the development of somatotroph adenomas at least in our FA family. Together with the previous observations, these results suggest that there are at least two distinct types of FA families: one which results from a mutation in the MEN-1 locus and the other whose causative gene is located outside the 11q13 locus. Further studies are needed to identify the respective genetic causes of this pathogenetically divergent syndrome.

References

sence of germ-line mutations of the multiple endoc-
ocrine neoplasia type I (MEN1) gene in familial pitui-


