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Isolated Familial Somatotropinomas: Clinical Features and Analysis of the MEN1 Gene

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Abstract. **Isolated familial somatotropinomas (IFS) rarely occurs in the absence of multiple endocrine neoplasia type I (MEN1) or the Carney complex. In the present study we report two Italian siblings affected by GH-secreting adenomas. There was no history of parental consanguinity. The sister presented at 18 years of age with secondary amenorrhea and acromegalic features and one of her two brothers presented with gigantism at the same age. Endocrinological investigations confirmed GH hypersecretion in both cases. Although a pituitary microadenoma was detected in both patients, transsphenoidal surgery was not successful. The sister received conventional radiotherapy and acromegaly is now considered controlled; the brother is being treated with octreotide LAR 30 mg monthly and the disease is considered clinically active. Patients, their parents and the unaffected brother underwent extensive evaluation, and no features of MEN1 or Carney complex were found. Analysis of polymorphic microsatellite markers from chromosome 11q13 (D11S599, D11S4945, D11S4939, D11S4938 and D11S987) showed that the acromegalic siblings had inherited different maternal chromosomes and shared the paternal chromosome. No pathogenic *MEN1* sequence changes were detected by sequencing or dideoxy fingerprinting of the coding sequence (exons 2-10) and exon/intron junctions. Although mutations in the promoter, introns or untranslated regions of the *MEN1* gene cannot be excluded, germline mutations within the coding region of this gene do not appear responsible for IFS in this family.**

Key Words. **acromegaly, pituitary tumor, MEN1, 11q13, linkage analysis**

Abbreviations. **GH, growth hormone, PRL, prolactin, MEN1, multiple endocrine neoplasia type I, IFS, isolated familial somatotropinomas, ACTH, adrenocorticotropic hormone, LH, luteinizing hormone, FSH, follicle stimulating hormone, TSH, thyroid stimulating hormone, IGF-I, insulin-like growth factor 1, PTH, parathyroid hormone.**

Introduction

Most GH-secreting pituitary tumors are sporadic but a few cases occur with a familial aggregation either as a component of a multiple endocrine neoplasia complex (MEN1 or Carney complex) or as isolated familial somatotropinomas or acromegaly [1]. As GH-secreting tumors in prepubertal patients may result in gigantism, the term "isolated familial somatotropinomas" (IFS) is a more appropriate definition than familial acromegaly to describe the inherited predisposition to develop these pituitary tumors. The mode of transmission and the responsible gene(s) of this very rare disorder are still unknown. In particular, genomic mutations in the coding region of *MEN1* have not been identified [2–5]. We report a new family with two siblings affected by GH-secreting adenomas. The clinical features and analysis of the *MEN1* gene are compared with an updated review of the literature.

Subjects

The index case (III-1) (Fig. 1) was an 18 year-old otherwise healthy female referred for evaluation of secondary amenorrhea for one year. Her appearance was evidently acromegalic but her height was normal. Mean 4-hour GH levels were 28 ng/ml (normal <2 ng/ml) and failed to suppress during an oral glucose load. PRL levels were 8.9 ng/ml (normal 8–25 ng/ml). FSH, LH, cortisol and thyroid hormones were normal. Magnetic resonance imaging revealed a 10-mm intrasellar pituitary adenoma which was resected by transsphenoidal surgery. The adenoma was acidophilic; immunohistochemical staining was positive for GH and negative for PRL, ACTH, LH, FSH and TSH. Post-operative GH levels were 10 ng/ml and IGF-I levels were 618 ng/ml. The patient was treated with conventional external radiotherapy and subcutaneous octreotide (100 mcg t.i.d.). Octreotide was discontinued at 28 years of age for hormonal reassessment; mean basal GH was 1 ng/ml and

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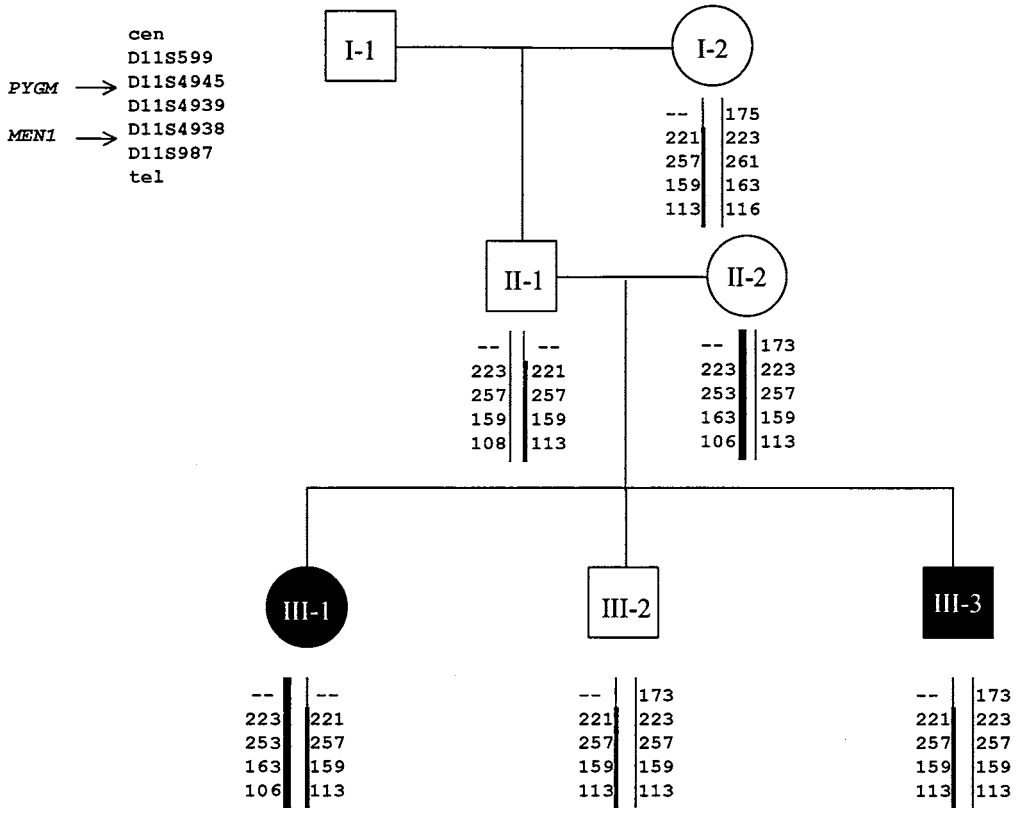

Fig. 1. *Analysis of the Italian IFS family for linkage to chromosomal region 11q13. The five microsatellite markers from 11q13 (top left) were analyzed in six individuals from this pedigree, and allele sizes are indicated. The affected siblings (III-1, III-3) inherited distinct haplotypes from their mother, II-2, but all three siblings share a paternal haplotype (thin bold). Symbols: (--) failed to amplify, (square) male, (circle) female, (filled symbol) affected.*

IGF-I was 147 ng/ml. Therefore, acromegaly was considered controlled.

The second case (III-3), one of her two brothers, was referred at age 18 for excessive linear growth (height 196 cm) and acromegalic features. Mean GH was 15 ng/ml (normal <2 ng/ml) and was not suppressed by oral glucose load (nadir 9.8 ng/ml); IGF-I levels were 1512 ng/ml (normal 180–380 ng/ml). Serum PRL, TSH, ACTH, LH and FSH were normal. An 8-mm pituitary adenoma was resected transsphenoidally. This tumor was acidophilic and strongly positive only for GH. The disease was still active after surgery, with a mean basal GH of 7.0 ng/ml and IGF-I of 664 ng/ml, and treatment with octreotide was initiated. He is now 24 years old and with the current treatment (octreotide LAR 30 mg monthly) mean GH is 2.2 ng/ml and IGF-I 390 ng/ml.

The third sibling (III-2), age 25, has normal height, normal IGF-I and mean basal GH and PRL levels. The patients' father (II-1) is a 63-year old Italian whose medical history is negative apart from excision of a shoulder lipoma. The 64-year old Scottish mother has autoimmune hypothyroidism. Both parents do not have clinical features of acromegaly and their mean basal GH, IGF-I and PRL levels are normal.

All family members had normal serum concentrations of calcium, phosphate, intact PTH, calcitonin, insulin and C-peptide, glucagon, gastrin and urinary excretion of norepinephrine and epinephrine. Echography showed no lesions of the thyroid, parathyroids and adrenals and no myxomas were detected by echocardiography in affected siblings. Second-degree relatives (aunts, uncles and grandparents) and third-degree relatives (cousins) had normal medical history and laboratory investigations (GH and IGF-I levels).

Genetic Investigations

Molecular genetic analysis was performed using DNA derived from peripheral blood lymphocytes of siblings, parents and paternal grandmother (Fig. 1). Microsatellite polymorphic markers from chromosome 11q13 (D11S599, D11S4945, D11S4939, D11S4938, D11S987) were amplified using 50 ng lymphocyte DNAs, fluorescently labeled primers [6], 0.2 mM dNTPs (Gibco) and Expand High Fidelity PCR enzyme (Roche). Primers were a generous gift from Drs. Nicholas Katsanis and James Lupski (Baylor College of Medicine, Texas). The

Cedars-Sinai Medical Center Genotyping Core electrophoresed the labeled PCR products and provided allele size information.

The *MEN1* coding region [7,8] was screened by a combination of dideoxy fingerprinting and sequencing, using lymphocyte DNA of individual III-3 as described [9]. The two patients inherited different maternal alleles. The three siblings share paternal alleles for the region spanning D11S4945 to D11S987. No *MEN1* coding sequence changes were observed.

Discussion

IFS is defined as the occurrence of at least two cases of acromegaly or gigantism in a family that does not exhibit MEN1 syndrome or Carney complex [1]. Although familial cases of acromegaly have been recognized since 1926 [10], most previously described patients lacked adequate endocrinological investigation to exclude familial acromegaloidism or MEN1 syndrome. In 1974 Levin [11] was the first to use GH assay to confirm the diagnosis of familial gigantism. In recent years well-documented IFS families have been identified in Europe, Asia, America and Australia. A 1999 review [1] described 22 families with 55 patients [1–4,11–25]. Since then, another 6 families have been reported [5,26,27, this study], for a total of 28 families comprising 68 patients. Detailed study of pituitary function is available for 49 patients. Most cases show only GH excess, but serum PRL was increased in 18 patients and one case also co-secreted TSH and alpha-subunit [19].

The most striking feature of familial acromegaly is the early onset of the disease. This accounts for the high frequency of gigantism associated with IFS (25 of 65 patients). Most familial cases are diagnosed at about 20 years of age (Fig. 2) in contrast to sporadic acromegaly, with an average age at onset of 40–45 years [28]. Early onset is characteristic of the hereditary forms of common tumors, such as breast and colon cancers, and fits well with Knudson's two-hit hypothesis [29], in which both alleles of a tumor suppressor gene (tsg) must be inactivated. Sporadic tumors arise when two rare inactivating events occur, whereas patients who inherit a germline mutation in one allele only require a single somatic event to inactivate the wild type tsg allele, which is reflected by an earlier age of onset. In some IFS families, however, the peak age of onset is in the fifth and sixth decades (Fig. 2). Ten of 12 patients with late onset acromegaly belong to five families whose members were all diagnosed at a late age [2,14,17,27]. The similar age of onset in these families suggests a late-onset genetically distinct form of IFS, but MEN1 cannot be excluded in two families because PTH was increased [17], or detailed endocrine data were lacking [27].

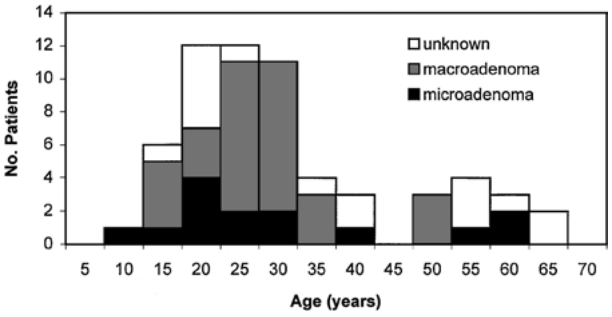

Fig. 2. *Age range at diagnosis and tumor size in patients with isolated familial somatotropinomas. The ages of onset of pituitary tumor symptoms and tumor sizes were tabulated from references [1–5,11–27] and the present family. The age distribution suggests that IFS primarily affects younger patients compared to sporadic acromegaly. Macroadenomas are reported twice as frequently as microadenomas: 31 vs. 14 cases. A multigenic mechanism for IFS is suggested by the bimodal distribution and the fact that 10 of 12 patients who developed pituitary tumors beyond age 45 belonged to 5 families. (Macroadenoma ≥1 cm, microadenoma <1 cm).*

In IFS, macroadenomas and invasive tumors are reported more than twice as frequently as microadenomas (31 cases versus 14, Fig. 2). This could be due to an intrinsic characteristic of the hereditary form, or because GH-secreting tumors tend to be more aggressive in younger people [28]. The reported results of surgical treatment are disappointing. Considering only the results of transsphenoidal surgery and adopting strict criteria of cure [30], only 2 of 8 microadenomas (25%) and 2 of 20 macroadenomas (10%) were cured by surgery, a success rate lower than for sporadic GH-secreting tumors (microadenomas ~80% cure, macroadenomas <50% cure [30]). Once again a peculiar characteristic of the disease, or the young age of patients, may account for these suboptimal results. The family we report exhibited somatotropinoma onset in late puberty, and clinical and endocrinological investigations excluded the presence of MEN1 syndrome or Carney complex. Patients displayed only GH hypersecretion and were not cured by surgery despite the fact that the tumors were microadenomas and operations were performed by a skilled neurosurgeon.

Linkage analysis in our family revealed that affected siblings share a paternal haplotype for the IFS candidate region of 11q13, spanning markers D11S4945 and D11S987 [31]. The gene(s) responsible for IFS are still unknown, but *MEN1* maps to this locus [7,8]. We did not detect mutations in the *MEN1* coding sequence or exon/intron junctions in patient III-3. Mutations in the promoter, introns or untranslated regions of this gene or hypermethylation of CpG islands are alternative explanations for *MEN1* inactivation that may have been undetected by our study. However, *MEN1* mutations were not observed in 12 other IFS kindreds [2–5], and *MEN1* was effectively transcribed in 3 tumors derived from 2 families, as assessed by RT-PCR [4,31]. This candidate region is also implicated in sporadic pituitary tumors, particularly somatotropinomas, which undergo loss of heterozygosity (LOH) of 11q13, but do not commonly

harbor mutations or abnormal expression of the *MEN1* gene [9]. In IFS, LOH of 11q13 occurred in seven tumors from three families [4,25]. Again, no *MEN1* germline mutations were identified and only one tumor had a somatic *MEN1* mutation [25]. Taken together, these results suggest the presence of a tumor suppressor gene distinct from *MEN1* in this region. Other loci may be involved in IFS. Mutation analyses excluded *GHRH-R* [4,5], *GNAS1* [5,20,26] and *GNAI2* [5] in some IFS families, but a potential second IFS locus at chromosomal region 2p16-12 was suggested by haplotype and allelotype analysis in a study proposing that IFS may be a digenic disease, requiring the inheritance of two disease genes [31].

We found that the paternal haplotype for 11q13 is shared with an unaffected sibling. This raises two possibilities: either the susceptibility gene is unlinked to 11q13 and the affected siblings share a haplotype by chance, or mutation of an 11q13 IFS gene is involved in this family's pituitary tumors, but the second hit did not occur in the unaffected sibling. Tumor DNAs were not available to screen for LOH, which would provide stronger evidence for involvement of this chromosomal region in pituitary tumorigenesis in this family. In either case, strict clinical and endocrinological follow-up of the unaffected sibling is warranted until the IFS mutation is identified in this family.

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