CASE REPORT

A meiotic recombination in a new isolated familial somatotropinoma kindred

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Abstract

We report here the genetic findings of a new isolated familial somatotropinoma (IFS) kindred in which the mother (subject I:2) and one daughter (subject II:2) are affected; their ages at diagnosis were 25 and 14 years respectively. Additionally, patient I:2 developed virilization due to an androgen-secreting adrenocortical mass, presenting clinical and molecular features of sporadic adrenal carcinoma. To genotype this family and to narrow down the candidate interval of the putative *IFS* gene at 11q13, we performed haplotyping on the DNA from all five members of the family and allelotyping of one available somatotropinoma using polymorphic microsatellite markers from chromosome region 11q12.1–11q13.5. Results indicated that the disease haplotype, between markers D11S956 and D11S527, was transmitted from subject I:2 only to subject II:2. A meiotic recombination event was detected in the fraternal twin sister of II:2 (subject II:1), but her disease status is unknown. Since she is only 18 years old this genetic event cannot yet narrow down the area involved in the pathogenesis of IFS. Allelotyping of the somatotropinoma from II:2 revealed loss of the chromosome carrying the wild-type copy of the putative *IFS* gene inherited from her father. These results support the involvement of a tumor suppressor gene at 11q13.1–q13.3 in the pathogenesis of IFS.

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Introduction

The majority of growth hormone (GH)-secreting pituitary adenomas are sporadic. Only a small number of somatotropinomas occur either as a component of multiple endocrine neoplasia type 1 (MEN-1) and Carney complex (CNC) syndromes, or as isolated familial somatotropinomas (IFS) (1–3). IFS is defined as the occurrence of at least two cases of acromegaly or gigantism in a family that does not exhibit MEN-1 or CNC. Since a reliable biochemical diagnosis of GH excess has become available, about 103 cases of familial acromegaly and/or gigantism have been reported (4–13), or mentioned to the authors, in 44 IFS families. To date, however, the gene responsible for IFS is still unknown. We have previously established linkage between IFS and chromosome region 11q13 and, through meiotic recombination analysis and allelotyping in an IFS family from Brazil, we have mapped the

IFS gene to approximately 8.6 Megabases (Mb) interval, flanked by microsatellite markers D11S1335 and INT2 (FGF3) (14). Recently, Kong *et al.* (15) published a high-resolution recombination map of the human genome based on 1257 meioses. Combination of this new genetic map and the UCSC database (www.genome.ucsc.edu, 07/09/2003 (16, 17)) using the completed human sequence has led to the recalculation of the interval with a resultant size of 10 Mb. Our results from genome-wide linkage studies of an IFS family also revealed a linked region of 31 Mb between markers D11S905 and D11S1314 at 11q13, encompassing the original 10 Mb interval (11). Loss of heterozygosity (LOH) at chromosome 11q13 has been reported in somatotropinomas from most IFS kindreds (5, 10, 18), providing evidence that the gene involved in the pathogenesis of IFS is a tumor suppressor gene (*TSG*). The first candidate gene was *MEN-1*, but its sequencing did not reveal any germline mutation (5, 6, 7, 9, 13, 19, 20)

and its mRNA was detectable by RT-PCR (5), indicating that the *IFS* gene is a *TSG* distinct from the *MEN-1* gene.

In the present study, we performed molecular analyses on a new IFS family, in which a mother and her daughter exhibited acromegaly. The mother also developed a virilizing adrenocortical carcinoma. We sought to: (i) perform haplotyping of 11q13 on the DNA from all five available family members and allelotyping of this region of the somatotropinoma available to further narrow down the *IFS* gene candidate interval; and (ii) perform allelotyping of the adrenal carcinoma.

Patients and methods

Clinical and laboratory studies

Family C, of Brazilian origin, is composed of parents with no history of consanguinity and three children (two fraternal twin girls and one boy) (Fig. 1). The mother (I:2) and one of the twins (II:2) are affected (Fig. 1). Individual I:2 (height 158 cm) presented at 38 years of age with virilization and secondary amenorrhea that had developed

over the previous 8 months. Thirteen years previously, the patient had been diagnosed with acromegaly and treated by transsphenoidal surgery and radiotherapy. The pathology report revealed a pituitary tumor without hyperplasia. Endocrine evaluation revealed an elevated serum total testosterone (538 ng/dl; normal 15–80) and dehydroepiandrosterone sulfate (DHEAS) (>10 000 ng/ml; normal 350–4300) levels. In addition, normal serum GH (0.4 ng/ml; normal < 5) and insulin-like growth factor-I (IGF-I) (402 ng/ml; normal 100–494) levels indicated that acromegaly had been controlled. An abdominal computed tomography scan confirmed the presence of a heterogeneous 9.0 × 8.9 cm mass replacing the right adrenal gland. Unilateral adrenalectomy was performed in October 1999 with removal of the tumor (478 g), which had invaded the organ capsule, vena cava and liver. Histological analysis and the tumor invasiveness confirmed the diagnosis of adrenocortical carcinoma. Two months postoperatively, the patient's menstrual periods resumed and serum total testosterone and DHEAS levels were normal. No biochemical or radiological evidence of recurrence has been noted (4 years after surgery). One of her 14-year-old twin daughters (II:2) subsequently presented with secondary amenorrhea. She exhibited a height of 167 cm, a weight of 58 kg and had mild features of acromegaly. Her laboratory results confirmed the diagnosis of acromegaly (GH 85.6 ng/ml, normal < 5; IGF-I 830 ng/ml, normal 203–831; prolactin 11.7 ng/ml, normal 2.5–14.5) and demonstrated normal values of adrenal steroids. Magnetic resonance imaging (MRI) revealed a large pituitary macroadenoma with suprasellar extension and para and infrasellar invasion (Fig. 2). Transcranial surgery (transcavernous approach) was performed in May 2000. Histopathologic analysis confirmed a GH-secreting adenoma without evidence of somatotroph hyperplasia. Subsequent clinical and laboratory screening of asymptomatic subjects II:1 and II:3 excluded acromegaly/gigantism. The past medical history, image studies and laboratory results provided no evidence for *MEN-1* or *CNC* in all affected and unaffected screened subjects. This family is unrelated to the Brazilian IFS family previously reported (5).

This study was approved by and completed according to the guidelines of the Ethical Committee of the Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro and the Hospital das Clínicas, Universidade de São Paulo. Informed consent was obtained from all subjects.

DNA isolation, haplotyping and allelotyping at 11q13

Peripheral blood samples were collected from all affected and unaffected individuals. DNA was extracted from leukocytes with the GFX genomic blood DNA purification kit (Amersham Pharmacia

Figure 1 Pedigree and haplotype analysis of the family. Generations are indicated by Roman numerals I and II (squares, males; circles, females) (index case, II:2). Vertical numbers denote relative allele sizes (1, largest; 4, smallest) for polymorphic markers, shown in centromeric to telomeric order. The solid bars represent the haplotype that co-segregates with the disease. The arrow indicates the meiotic recombination.

Figure 2 Gadolinium-enhanced, T1-weighted MRI of patient II:2. A sagittal view is shown in the upper panel and a coronal view in the lower panel. A macroadenoma is present with suprasellar extension and para and infrasellar invasion.

Biotech, Piscataway, NJ, USA). Adrenal carcinoma and pituitary adenoma samples were obtained from paraffin-embedded blocks from patients I:2 and II:2 respectively. Tumor DNA samples were extracted with the DNAeasy tissue kit (Qiagen Inc., Valencia, CA, USA). Thirteen polymorphic microsatellite markers from chromosome region 11q12.1–11q13.5 were used: centromere-D11S956, D11S4191, D11S1253, D11S480, D11S1883, PYGM (CAGA), D11S4941, D11S4908, D11S2072, D11S1889, D11S4095, INT2 (FGF3), D11S527-telomere. The sequences, chromosomal locations and genomic order of the primers were obtained from the UCSC database (www.genome.ucsc.edu, 07/09/2003 (16, 17)) and Kong *et al.* (15). PCR amplification was performed as previously described (14). The radioisotope-labeled PCR products were electrophoresed on 6% polyacrylamide–8.3 M urea gels and visualized by autoradiography. For allelotyping, LOH was defined as a greater than 50% loss of one of the alleles in tumor DNA compared with leukocyte DNA.

LOD scores

Two-point parametric logarithm of the odd (LOD) scores were calculated with the MLINK program in FASTLINK package version 4.1P (Genetic Analysis Software, National Center for Biotechnology Information, Bethesda, MD, USA) (21). In this family, IFS was modeled as a rare autosomal dominant disease with incomplete penetrance of 0.9 for both sexes and allele frequency of 0.0001. Genetic distances between microsatellite markers were obtained as described in the previous section. Marker allele frequencies were assumed to be equal due to the unavailability of reliable published data for the racial background of the family and its limited size. Multi-point parametric LOD scores were calculated with the program GENEHUNTER version 2.1 (Fred Hutchinson Cancer Research Center, Seattle, WA, USA) (22) using the same model as for the two-point analysis.

Incorporation of information from LOH analysis into the two-point LOD score calculations has been previously described (23–25). LOH data are treated as additional observations on disease phenotype and the main purpose is to enhance the power to detect linkage, especially for small families. A modified MLINK program (24) was used to calculate the LOD score using the LOH data.

MEN-1 sequencing

Leukocyte DNA from one affected subject (I:2) and two unaffected subjects (II:1 and II:3) were used for sequencing. Exons 2–10 (from the 90 bases 5' to the start codon in exon 2 to the 65 bases 3' to the stop codon in exon 10) and the exon–intron boundaries of the *MEN-1* gene were amplified by PCR in 30 µl reactions containing 100 ng DNA, 1 × PCR buffer (Applied Biosystems, Foster City, CA, USA), 1.5 mM MgCl₂ (Applied Biosystems), 0.2 mM dNTPs (Invitrogen Inc., Grand Island, NY, USA), 0.5 µM primer and 2.5 U AmpliTaq Gold (Applied Biosystems). Primers used for amplification and sequencing of the *MEN-1* were as described previously by Costa *et al.* (26) and Poncin *et al.* (27). Exon 2 was divided into five parts (H, A, B, J, C) (26). Enhancer solution (Invitrogen Inc.) was added for fragments H (1.5 ×) and J, and exon 10 (1 ×). PCR was performed under the following conditions: 95 °C for 10 min, and 40 cycles of 94 °C for 45 s, 52–67 °C for 45 s, and 72 °C for 50 s for exons 2–9 and for 3 min for exon 10, followed by a final extension at 72 °C for 7 min. The PCR products were purified with the Qiaquick PCR purification kit (Qiagen Inc.), sequenced by using the Dyanamic ET terminator cycle sequencing kit (Amersham Pharmacia Biotech) and analyzed in the ABI Prism 377 sequencer (Applied Biosystems).

Table 1 11q13 LOH status in the adrenocortical carcinoma (I:2) and somatotropinoma (II:2) from Family C.

Markers	Tumor	
	Adrenal (I:2)	Pituitary (II:2)
D11S956		●
D11S4191	●	●
D11S1253		●
D11S480	●	●
D11S1883	—	●
PYGM (CAGA)	●	●
D11S4941	●	●
D11S4908	●	
D11S2072	●	●
INT2 (FGF3)	●	●
D11S527	●	●

●, LOH; —, noninformative; blanks indicate information unavailable.

Results

Haplotyping and LOH at 11q13

Haplotypes of the five subjects were constructed with markers from chromosome 11q12.1–q13.5 (Fig. 1). The haplotype co-segregating with the disease, based on alleles shared by affected relatives, is shown in Fig. 1. The disease haplotype was shared at all 11q12.1–q13.5 markers between (including) D11S956 and D11S527 in the affected mother and affected daughter but not in the unaffected son (Fig. 1). Individual II:1 inherited a recombinant haplotype due to a meiotic recombination between markers D11S4908 and D11S2072 (Fig. 1, arrow). Allelotyping data across this region of chromosome 11q13 showed loss of the paternal alleles of all informative markers in the somatotropinoma from patient II:2 (Table 1). In addition, allelotyping of the adrenal tumor from patient I:2 demonstrated a similar pattern of LOH (Table 1).

Linkage analysis at 11q13

Two-point and multipoint LOD score calculations showed maximum LOD score of 0.52 for markers D11S956, D11S4191, D11S1253, D11S480, PYGM, D11S4941 and D11S4908 at recombination fraction (θ) = 0.0–2.0 (data not shown), when considering the disease status of subject II:1 as unknown. Incorporation of LOH data of subject II:2 increased the LOD score to 1.12 for markers D11S4191, D11S1253, PYGM and D11S4941 at θ = 0.0 (data not shown). In spite of the low LOD score, which is unavoidable because of the small sample size, the positive value of the LOD score obtained in this family is compatible with linkage of IFS to chromosome 11q13, as previously published (14).

MEN-1 mutations

Sequencing of PCR-amplified leukocyte DNA demonstrated no germline mutations within the entire

coding region and the exon–intron boundaries of the *MEN-1* gene.

Discussion

The present report describes a new family with acromegaly in which somatotropinomas were diagnosed at an early age (25 and 14 years old), as in most IFS cases. The tumor from patient II:2 was invasive, similar to our previously published IFS cases (5). In addition, at the age of 38 years patient I:2 had also developed amenorrhoea and hyperandrogenism due to a virilizing adrenocortical carcinoma. In none of the 44 IFS families reported to date has adrenocortical cancer been described.

Somatotropinomas and adrenal lesions have been detected in two familial syndromes: MEN-1 and CNC (28–30). Approximately 20–40% of patients with MEN-1 have adrenal adenomas or bilateral hyperplasia, the vast majority of which are non-functional, show retained heterozygosity at 11q13 markers and no *MEN-1* mutation (28, 29, 31, 32). All MEN-1 patients with adrenal involvement also had pancreatic endocrine tumors, which produced insulin, proinsulin or gastrin. In other words, adrenal tumors seem not to be due to the same pathogenetic mechanism as other MEN-1 neoplasms. In our study, patient I:2 presented only acromegaly (at 25 years of age) and a virilizing adrenocortical carcinoma. Allelotyping analysis of her adrenal tumor showed LOH for all informative markers at 11q13. Genetic analyses in sporadic adrenal carcinomas have shown that more than 60% exhibit LOH at 11q13 (28, 33–37), but these tumors rarely harbor *MEN-1* gene mutation (<7%) (33, 35–37) and they have normal *MEN-1* gene expression (33, 34), supporting the involvement of a TSG at 11q13, distinct from *MEN-1*, in the pathogenesis of adrenal carcinomas (36). Taken together, these data suggested that patient I:2 presented a sporadic adrenocortical carcinoma. Further, to our knowledge, adrenal cancer has been reported in a few patients with pituitary disease (31, 33, 38, 39), only two with clinical MEN-1 disease (33, 39). On the other hand, primary pigmented nodular adrenocortical disease occurs in approximately 25% of the patients with CNC (30). These nodules, which are small and brown colored, may cause Cushing's syndrome and the disease was mapped on two loci, one on chromosome 2p16 and the other on 17q22–24 (*PRKAR1A* gene) (30, 40). These considerations, together with our results, support the view that our patient (I:2) had an adrenal lesion distinct from those associated with MEN-1 and CNC syndromes. In addition, MEN-1 was excluded based on: (i) the absence of clinical and laboratory features of this syndrome in the family members; and (ii) no *MEN-1* germline mutation. CNC was also excluded based on the diagnostic criteria published recently (30, 40).

Figure 3 Chromosome 11q13 from subject II:1. The haplotype shared with affected members I:2 and II:2 is represented in black, showing one recombination event between markers D11S4908 and D11S2072. The candidate 10 Mb interval for the IFS locus (arrow), as determined separately from linkage analysis (14), is shown. Relative locations of 13 polymorphic DNA probes are shown (15). The map is not drawn to scale.

LOH at 11q13 was demonstrated in the somatotropinoma from subject II:2 and no germline mutation was found in *MEN-1*. As in other IFS families with LOH at 11q13 previously published (5, 13, 18), *MEN-1* sequencing was normal, supporting data that this is another entity. The haplotype analysis of this family demonstrated a meiotic recombination at 11q13, between markers D11S4908 and D11S2072, in the unaffected subject II:1. She is currently 18 years old and has been followed since the age of 15. Her disease status must therefore be characterized as unknown. If she does not develop acromegaly, it would not contribute to further narrowing down the candidate interval since she could be an obligate carrier. On the other hand, if she develops a somatotropinoma, D11S4908 and markers centromeric to it should be excluded as an IFS candidate region (Fig. 3). Therefore, strict clinical and endocrinological follow-up of individual II:1 is warranted. More IFS families and tumor deletion mapping of chromosome 11q13 in sporadic somatotropinomas are needed. In this context, we have recently

narrowed the location of the isolated acromegaly candidate gene to a 3.9 Mb region, beginning with D11D4908 and extending to marker INT2 by using a tumor deletion mapping approach in sporadic somatotropinomas (41). Marker D11S4908 was excluded due to retention of heterozygosity in one tumor showing one internal deletion between markers D11S2072 and D11S4136 (41).

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