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Familial Isolated Hyperparathyroidism as a Variant of Multiple Endocrine Neoplasia Type 1 in a Large Danish Pedigree*

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ABSTRACT

We report here our genetic findings of a family in which 14 members were affected with isolated primary hyperparathyroidism. Hyperparathyroidism is the main feature of multiple endocrine neoplasia type 1 (MEN1), making the recently cloned *MEN1* gene a prime candidate gene in this family. Significantly positive lod scores were achieved with D11S4946 (3.36) and D11S4940 (3.53), and by combining the results from these two markers, a maximum positive lod score of 4.12 at recombination fraction 0.00 was obtained. Mutation analysis of *MEN1* performed by full sequencing identified a missense mutation in exon 4, causing an amino acid change from glutamine to proline at codon 260. This mutation (Q260P) was present in all affected family members, and the inheritance of the mutation was in complete agreement with the disease-associated haplotype. In comparison with the recent functional studies of the menin protein interactions, this mutation is located in a region with little or no binding activity to JunD and activating protein-1 transcription factor. We conclude that some of the familial isolated primary hyperparathyroidism families constitute a milder variant of MEN 1, which is associated with a functionally milder missense mutation. (*J Clin Endocrinol Metab* **85:** 165–167, 2000)

FAMILIAL isolated hyperparathyroidism (FIHP) is defined as hereditary primary hyperparathyroidism without the association of other disease or tumors. FIHP has been proposed to occur as a distinct genetic entity (FIHP or HRPT1; OMIM 145000). However, it has also been suggested to be a variant of other familial tumor syndromes in which primary hyperparathyroidism is a main feature, *e.g.* multiple endocrine neoplasia type 1 (MEN 1; OMIM 131100) and the hyperparathyroidism-jaw tumor syndrome (HPT-JT or *HRPT2*; OMIM 145001). The FIHP diagnosis relies on the demonstration of hypercalcemia, inappropriately high PTH levels, and parathyroid adenomas plus the exclusion of MEN 1 and HPT-JT (1, 2).

In analyzing this disease group, which totals more than 100 families (3), two main histopathological entities are found. One is characterized by multiglandular disease or hyperplasia, and the other by solitary parathyroid adenoma occasionally associated with parathyroid carcinomas. Occasionally, mildly hyperplastic glands are found, which are associated with familial benign hypercalciuria hypercalcemia (4). To date, three large FIHP families have been shown to be linked to the *HRPT2* locus in chromosome 1q21-q32, therefore suggesting that they represent a variant of the HPT-JT syndrome (5, 6). The parathyroid tumors in these families were typically solitary adenomas with a cystic component, showing somatic loss of the wild-type 1q alleles and a reduced penetrance in women (5).

Yet another subset of FIHP families has been suggested to be a variant of MEN 1, an autosomal dominant disease characterized by tumors of the parathyroids, the endocrine pancreas and duodenum, and the anterior pituitary. Recently, this idea was supported by the demonstration of a novel missense mutation in the *MEN1* gene (7, 8) in an FIHP family with seven affected members (9). The affected family members developed multiglandular disease, with similar penetrance in women and men, and in the tumors somatic loss of the wild-type 11q13 alleles were regularly seen (9). Initially, the suggestion that FIHP could occur as a variant of MEN 1 came from genetic studies in a large Danish pedigree (10). In previous linkage analysis of this family, a maximum lod score of 2.12 at the recombination fraction 0.05 was obtained with the marker D11S97 located telomeric to the *MEN1* gene locus in 11q13 (10). Based on this finding we suggested that the disease gene involved was probably the *MEN1* gene, which had not been identified at that time. Here we have extended the linkage analysis in this family and in addition demonstrate that the disease is associated with a missense mutation in the *MEN1* gene.

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Materials and Methods

Subjects

The family has been followed clinically for more than 30 yr, and detailed clinical descriptions have been published previously (10). Extensive investigations, including hormonal markers (PRL, fasting gastrin, C peptide, and insulin) and radiology (abdominal ultrasound) were performed and failed to identify any MEN-related endocrinopathy other than hyperparathyroidism. No MEN 1-related dermal features (11) were identified. Consent was obtained from all participating family members, and the study was approved by the local ethics committee.

Linkage analysis

Genomic DNA was extracted from peripheral leukocytes and genotyped for four polymorphic microsatellite markers located close to *MEN1*: D11S1883-D11S4946-*MEN1*-D11S4940-D11S4937. DNA extraction and genotyping were performed using standard protocols.

Two-point lod scores were generated using the LINKAGE (version 5.1) package (12). For the linkage analysis, FIHP was modeled as a rare dominant disease (q = 0.0001) with a penetrance of 0.95, and the allele frequencies were based on those given by the Genome database (http://www.hgmp.mrc.ac.uk). A conservative approach was adopted in which all presently unaffected family members were scored as having an unknown disease status.

Mutation screening of the MEN1 gene

Mutation analysis was performed using single strand conformation analysis and direct sequencing. The 9 coding exons of the *MEN1* gene were amplified using 15 different fragments of 200–300 bp each, as previously described (8). In short, genomic DNA (50 ng) was amplified using standard PCR conditions and electrophoresed in 25% MDE (FMC, Rockport, ME) gels at room temperature for 12 h at 6–8 watts, after which the gels were dried, and autoradiography was performed.

The nine coding exons were sequenced in three affected family members. The DNA (50 ng) was amplified in a final volume of 15 μL containing 1.5 μL GeneAmp 10 $\times$ PCR buffer and 1 U AmpliTaq Gold DNA polymerase (Perkin-Elmer Corp., Foster City, CA), plus 1.5 mmol/L $MgCl_2$, 100 μmol/L of each deoy-NTP, and 100 nmol/L of each M13-labeled forward and reverse primer. Thermocycling conditions consisted of denaturing at 95 C for 10 min; 35 step cycles of 95 C for 30 s, 60 C for 30 s, and 72 C for 45 s; and a final extension for 5 min at 72 C. The PCR product was diluted and run in a cycle sequencing reaction using the BigDye Primer cycle sequencing kit (Perkin-Elmer Corp.) with thermocycling conditions as follows: denaturing at 95 C for 2 min; 20 cycles of 95 C for 30 s, 55 C for 30 s, and 70 C for 1 min; and 15 cycles of 95 C for 30 s and 70 C for 1 min. The product was then run on an ABI 377 automated sequencer (Perkin-Elmer Corp.).

Results

Figure 1 shows the family with 14 members affected by primary hyperparathyroidism in a pattern suggesting autosomal dominant transmission of the disease. Linkage analysis was performed using 4 polymorphic microsatellite markers close to *MEN1*. Significantly positive lod scores were achieved with D11S4946 (3.36) and D11S4940 (3.53), and by combining the results from these two markers a maximum positive lod score of 4.12 at recombination fraction 0.00 was obtained.

Mutation screening of *MEN1* by SSCP was negative, but direct sequencing in three affected members revealed a novel missense mutation in exon 4 (Q260P) causing an amino acid change from glutamine to proline. The presence of the Q260P mutation was further tested by an assay consisting of two subsequent PCR reactions followed by restriction cleavage. The first round PCR amplifies exon 4 as previously described (13), and this product then serves as a template in the second round PCR using a primer set (5′-GAGCTGGCTGTACCTGAAAGG-3′ and 5′-GGAGGTGAGGGCTGA GCC-3′) designed to introduce an *Msp*I restriction site in the presence of the Q260P mutation. The mutation was present in the 10 affected members and in 3 young presently unaffected members, aged 32 yr (patient IV:6), 5 yr (patient V:1), and 15 yr (patient V:4; Fig, 1). However, the mutation was absent in 2 unaffected members more than 80 yr of age, in 9 presently unaffected members, and in 7 spouses (Fig. 1). These results were completely in agreement with the inheritance of the haplotypes of the *MEN1* linked 11q13 markers. The mutation has never been reported before and was not present in 100 unrelated normal individuals tested. Furthermore, by comparison with the murine *Men1* sequence (14), it was shown to affect a conserved amino acid, further supporting its pathogenetic significance.

Discussion

The gene responsible for MEN 1 was cloned in 1997 (7, 8), after which over 200 *MEN1* germline mutations have been identified, but no genotype-phenotype correlation could be established (13, 15–18). We have very recently reported a *MEN1* missense mutation in exon 4 (E255K) in a FIHP family in which 7 members were affected with multiglandular disease (9). Taken together, the results from the 2 studies have several implications. First, a subset of FIHP families constitutes a variant of MEN 1, especially those with multiglandular disease. Second, in families characterized by multiglandular disease, clinical screening for MEN 1-related endocrinopathies and genetic analysis of *MEN1* should be a

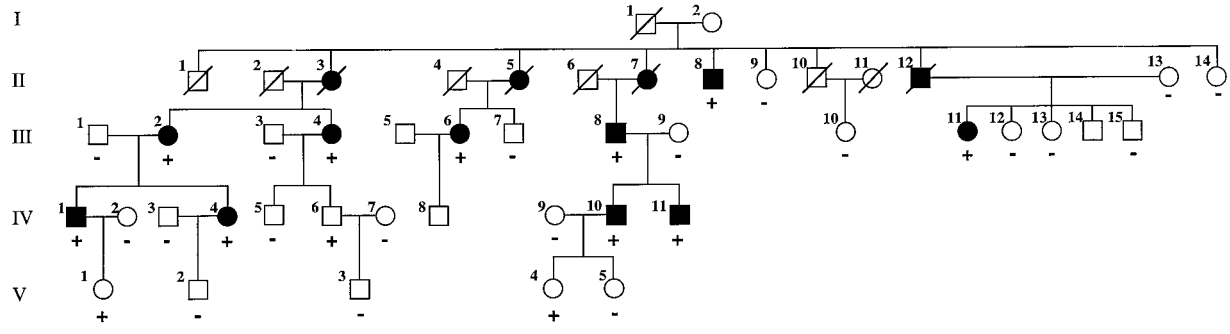

FIG. 1. Pedigree showing the family with autosomal, dominantly inherited, isolated hyperparathyroidism. *Filled symbols* indicate affected family members, and *empty symbols* indicate presently unaffected family members. Individuals carrying the Q260P mutation are marked by a +, and individuals without the mutation are indicated with a − *below* the pedigree symbol.

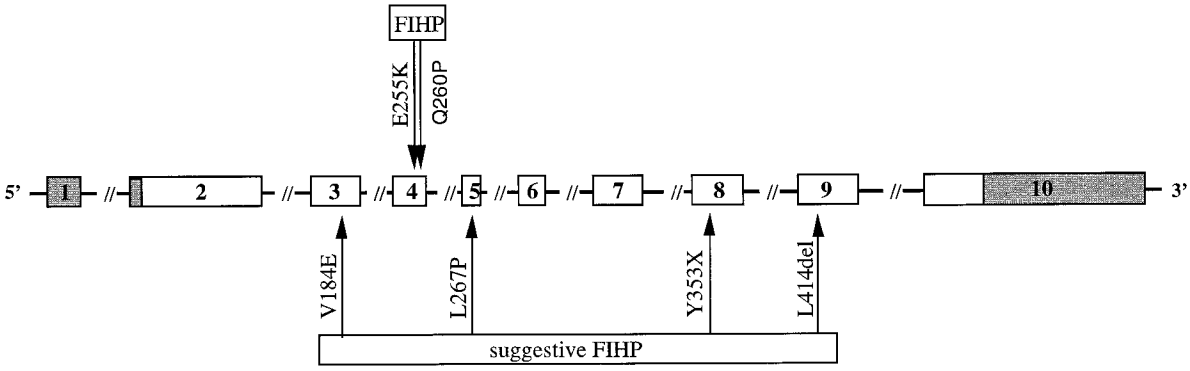

FIG. 2. Constitutional *MEN1* mutations reported in pedigrees with FIHP as well as with suggestive FIHP.

priority. On the other hand, in families characterized by parathyroid solitary adenoma and/or carcinoma, which more frequently present with profound hypercalcemia or hypercalcemic crisis, HPT-JT should be considered, and the *HRPT2* locus in 1q21-q32 investigated. Based on the 2 missense mutations found in close vicinity in exon 4 in both FIHP families, but not in MEN 1 families, it is tempting to speculate about a genotype-phenotype correlation (Fig. 2). It is even more interesting when considering the recent functional studies of menin protein interactions with the activating protein 1 transcription factor JunD (19). Two separate regions of menin, 139–242 and 323–428, were shown to separately bind JunD (19). Furthermore, 2 nuclear localization signals were identified at the C-terminal of menin (20). Interestingly, these FIHP mutations, E255K and Q260P, fall outside all of these regions, suggesting that these 2 mutations are unlikely to affect these functional activities of menin.

Hyperparathyroidism is the most frequent manifestation of MEN 1 and has an earlier age of onset than the other components. Therefore, some MEN 1 families will initially present with hyperparathyroidism and consequently be labeled suggestive FIHP, whereas classical MEN 1 is only diagnosed during follow-up (1). The 2 families described above with mutations in exon 4, have 7 and 14 affected members, respectively, thus representing FIHP. Recently, 4 smaller kindreds with familial hyperparathyroidism have been reported to be associated with *MEN1* mutations (21–24) (Fig. 2), whereas in other studies of similar pedigrees no mutations have been identified (13, 15, 17, 25). The limited clinical screening details and the relatively small number of affected cases in some of these families certainly warrant further careful follow-up to establish whether these mutations will result in MEN 1 or FIHP.

The function of the *MEN1* tumor suppressor gene has been only partly elucidated, but future functional studies of the *MEN1* mutations detected in FIHP families compared with those in classical MEN 1 families are likely to provide valuable information concerning its involvement in the tumorigenesis of parathyroid and other endocrine tumors.

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