# Letters to the Editor

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## A Family with Isolated Hyperparathyroidism Segregating a Missense *MEN1* Mutation and Showing Loss of the Wild-Type Alleles in the Parathyroid Tumors

*To the Editor:*

Familial isolated primary hyperparathyroidism (FIHP, or HRPT1; MIM 145000) is characterized by hypercalcemia, elevated parathyroid hormone (PTH) levels, and uniglandular or multiglandular parathyroid tumors. The diagnosis involves the exclusion of other familial disorders characterized by primary hyperparathyroidism, mainly multiple endocrine neoplasia type 1 (MEN1) and hyperparathyroidism–jaw tumor syndrome (HPT-JT, or HRPT2). To date, >70 FIHP families have been reported (Huang et al. 1997), and FIHP has been proposed to be either a distinct genetic entity or a variant of MEN1 or of HPT-JT. MEN1 is characterized by tumors of the parathyroids, the endocrine pancreas and duodenum, and the anterior pituitary. Other associated features include adrenocortical tumors, lipomas, and carcinoids. The *MEN1* gene has been mapped to 11q13 (Larsson et al. 1988) and was recently cloned (Chandrasekharappa et al. 1997; The European Consortium on MEN1 1997). Frequent loss of heterozygosity (LOH) in MEN1-related tumors (Friedman et al. 1994) and the inactivating mutations found in patients and tumors (Agarwal et al. 1997; Heppner et al. 1997) suggest that *MEN1* is a tumor-suppressor gene. HPT-JT is characterized by solitary parathyroid adenomas/carcinomas and fibro-osseous jaw tumors and occasionally by renal lesions, namely, Wilm tumors, polycystic kidney disease, and renal hamartomas (Szabo et al. 1995; Teh et al. 1996*a*). The *HRPT2* gene, which has been mapped to 1q21-q32 but which has not yet been cloned, is also considered to be a tumor-suppressor gene (Teh et al. 1996*a*).

We recently have found that, in two FIHP families characterized by solitary adenomas, the disease was linked to 1q21-q32, suggesting that a subset of FIHP forms a variant of HPT-JT (Teh et al. 1998*b*). On the other hand, linkage to *MEN1* has also been implicated in one FIHP family, but without conclusive evidence (Kassem et al. 1994). The recent cloning of the *MEN1* gene has allowed mutation analysis of FIHP kindreds, but, to date, no *MEN1* mutation has been found in the nine small families analyzed (Agarwal et al. 1997; Teh et al. 1998*a*). We report a large family in which seven members are affected with primary hyperparathyroidism without association of other tumors, and we present genetic data to demonstrate that this is a MEN1 variant.

The family is of Caucasian origin and resides in England. Seven family members from two generations were found to have primary hyperparathyroidism (fig. 1 and table 1). The present age and age at diagnosis of parathyroid disease is detailed for each family member in table 1. Five family members have had parathyroid glands surgically removed, whereas two declined surgery. The index case (II-3) had three enlarged parathyroid glands removed at the first operation, and, subsequently, a mediastinal parathyroid tumor was removed, because of persistent disease. Subject II-2 had four enlarged glands removed, and subjects III-4, III-5, and III-8 each had three or three and a half enlarged glands removed. Histopathologically, the parathyroid glands were classified as hyperplastic and did not demonstrate any evidence of cysts or malignancy. Patients II-4 and III-9 declined surgery and were diagnosed as affected, on the basis of borderline hypercalcemia (2.6 mmol/liter each; reference range 2.20–2.60 mmol/liter) in combination with repeated increased PTH levels (113 pg/ml and 99 pg/ml, respectively; normal range 10–50 pg/ml). Subject I-1, who died from myocardial infarction at the age of 77 years, was known to have renal calculi, suggesting that he was also affected. None of the patients have clinical or biochemical evidence of MEN1 or MEN2. The family has been followed, at the Department of Medicine at King's College, with annual hormonal profiles, determined since 1994. Fasting serum gut-hormone profiles (insulin, pancreatic polypeptide, vasoactive intestinal polypeptide, gastrin, glucagon, somatostatin, and neurotensin) are all within normal ranges. Pentagastrin-stimulated serum calcitonin levels are all undetectable. Twenty-four–hour urinary catecholamine metabolites are within the normal ranges. Computed tomography and magnetic-resonance imaging of the abdomen did not detect any tumor of the pancreas and adrenal glands. No patients have evidence of a pituitary

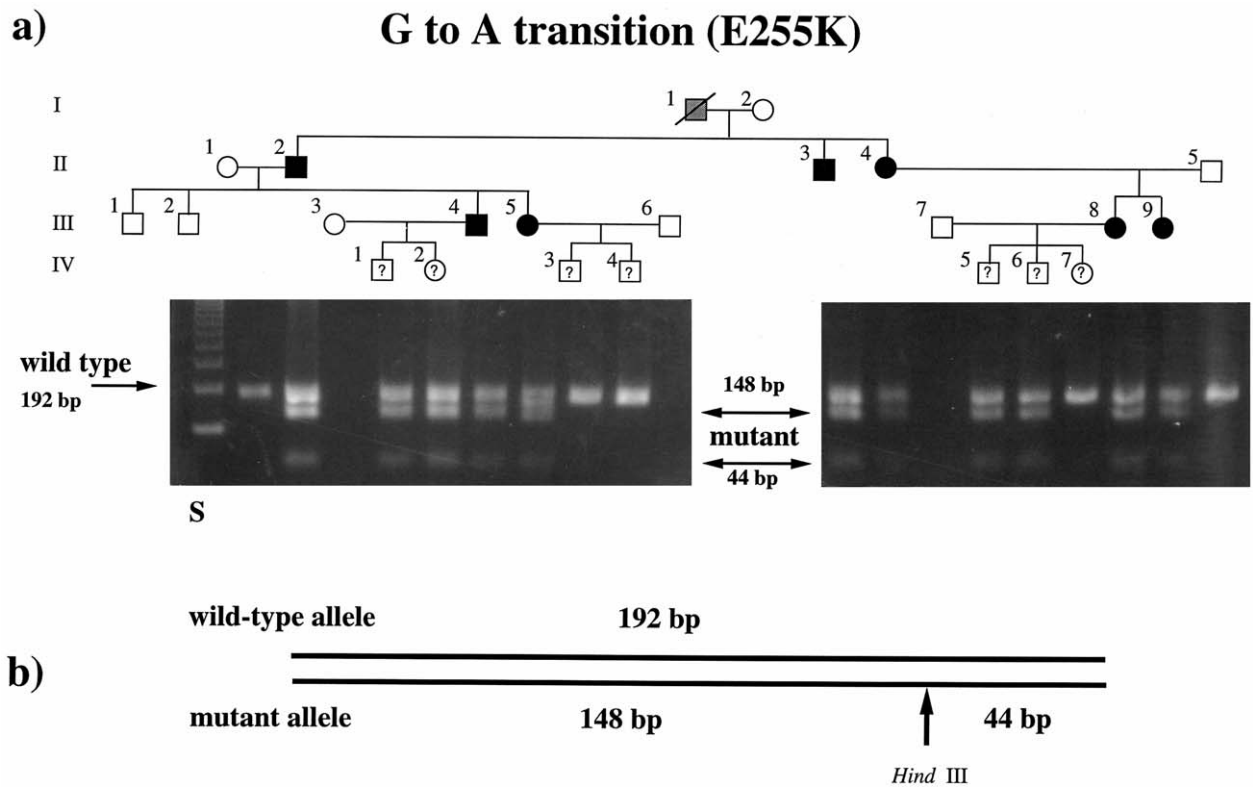


**Figure 1** *a,* Pedigree showing the family with autosomal dominantly inherited isolated hyperparathyroidism. Blackened symbols indicate affected family members, and unblackened symbols indicate unaffected family members. The gray-shaded symbol indicates that the individual (I-1) probably is affected, and the symbols with a question mark (?), in generation IV, indicate that the individuals are still at risk. The results from mutation analysis using restriction cleavage are shown below the pedigree. *b,* Illustration showing the *Hin*dIII site created by the E255K mutation.

tumor, either on skull radiography or on magnetic-resonance imaging, and their pituitary hormonal profiles, including prolactin, growth hormone, and adrenocorticotropic hormone, are within the normal range. In addition, no family member has any clinical evidence of HPT-JT. Orthopentography of the jaw was carried out on all affected family members, but no case of jaw tumor was found.

Consent was obtained from the participating family members, and the study was approved by the local ethics committee. Genomic DNA was extracted from peripheral leukocytes of 17 individuals and from fresh frozen tumor tissues as well as tissue blocks from the parathyroid operations of four affected individuals (table 2). Constitutional DNA was genotyped for the polymorphic microsatellite markers D11S956, *PYGM,* D11S787, and *INT2,* within the *MEN1* region at 11q13 (The European Consortium on MEN1 1996), and D1S218, D1S222, D1S428, D1S412, D1S413, and D1S510, from the *HRPT2* region in 1q21-q32 (Teh et al. 1996*a*). Paired constitutional and tumor DNA samples were analyzed for LOH by use of D11S956, *PYGM, INT2,* D11S787, D11S419, *HBB,* D11S1378, and *TYR.*

Linkage to the *HRPT2* locus in 1q21-q32 was excluded by significantly negative LOD scores ($<-2$) and by haplotyping (data not shown). We thus focused on the other candidate region, that is, the *MEN1* locus in chromosome 11q13. The seven affected members all shared the disease-associated haplotype constructed for four markers flanking the *MEN1* gene (not shown). LOH was identified with the same markers, in 5 of 11 tumors analyzed (table 2 and fig. 2). For all informative cases, these 5 tumors also showed LOH for the six additional chromosome 11 loci tested, suggesting loss of one entire chromosome 11 homologue (fig. 2). Combined analyses of the constitutional and tumor genotypes revealed that the losses invariably involved the wild-type alleles derived from the unaffected parent. Two-point linkage calculations were then performed by incorporation of the results from constitutional genotyping and LOH analysis, by use of a modification of the LINKAGE program (Cottingham et al. 1993; Rohde et al. 1995).

For D11S956 and *PYGM*, the maximum LOD scores of 2.48 and 1.99, respectively, were obtained at a recombination fraction of .00. Taken together, the results indicate the involvement of a tumor-suppressor gene in 11q13, presumably the *MEN1* gene, in this family.

The *MEN1* gene was screened for mutations by use of single-strand conformation analysis (SSCA) and sequencing, as described elsewhere (The European Consortium on MEN1 1997). By SSCA an aberrant shift was detected in the exon 4 fragment (192 bp). This shift was present in all seven affected cases and in four at-risk individuals in generation IV (aged 14, 15, 16, and 28 years; table 1). However, the shift was not detected in unrelated spouses in the family or in 150 unrelated individuals. Direct sequencing revealed a missense mutation in codon 255 (GAG→AAG) of exon 4, causing an amino acid change from glutamic acid to lysine (E255K or c.763G→A). This G→A transition also gave rise to a *Hin*dIII restriction-cleavage site (AAGCTT) for the mutant allele. As the result of the enzyme cleavage, two bands of 144 bp and 44 bp were obtained that were consistent with the SSCA results (fig. 1). The results from the mutation analysis were completely in agreement with those obtained by haplotyping of the 11q13 markers (table 1).

It is now established that mutations in some familial cancer genes can give rise to similar but distinct clinical variants. For example, specific mutations of the *RET* proto-oncogene are associated with each of the three variants of multiple endocrine neoplasia type 2 (MEN2), that is, MEN2A, MEN2B, and familial medullary carcinoma of the thyroid (Eng 1996). For MEN1, many researchers have tried to determine clinically distinct variants. Reports of FIHP and familial pituitary tumors, for example, are abundant, but, to date, there is no conclu-

**Table 2**

**Results from LOH Studies of the Parathyroid Tumors, Using Microsatellite Markers within the *MEN1* Region at 11q13**

| Patient and Gland Number | D11S956 | *PYGM* | D11S787 | *INT2* | Allele Lost |
|---|---|---|---|---|---|
| II-2: | | | | | |
| 1 | + | + | + | + | |
| II-3: | | | | | |
| 1 | LOH | LOH | LOH | LOH | Wild-type |
| 2 | LOH | − | LOH | − | Wild-type |
| 3 | + | − | + | − | |
| 4 | + | − | LOH | − | Wild-type |
| III-4: | | | | | |
| 1 | LOH | LOH | − | LOH | Wild-type |
| 2 | + | + | + | − | |
| 3 | LOH | LOH | − | LOH | Wild-type |
| III-5: | | | | | |
| 1 | + | + | + | − | |
| 2 | + | + | + | − | |
| 3 | + | + | + | − | |

NOTE.—A plus sign (+) indicates retained heterozygosity, and a minus sign (−) indicates not informative or not done.

**Table 1**

**Clinical and Genetic Details of the Family Members in This Study**

| Patient | Sex | Present Age (years) | Age at Diagnosis (years) | Serum Calcium (mmol/liter)[a] | *MEN1* Mutation | Affected Haplotype | No. of Glands Removed | Complications |
|---|---|---|---|---|---|---|---|---|
| I-1 | Male | Deceased | NA | NA | NA | NA | NA | Renal calculi |
| II-2 | Male | 70 | 66 | 2.86 | Yes | Yes | 4 | Renal calculi |
| II-3 | Male | 52 | 46 | 3.20 | Yes | Yes | 4 | Renal calculi and hypertension |
| II-4 | Female | 71 | 69 | 2.60[b] | Yes | Yes | Declined surgery | … |
| III-4 | Male | 40 | 38 | 2.84 | Yes | Yes | $3\frac{1}{2}$ | … |
| III-5 | Female | 39 | 38 | 3.00 | Yes | Yes | $3\frac{1}{2}$ | … |
| III-8 | Female | 51 | 37 | NA | Yes | Yes | 3 | Renal calculi and hypertension |
| III-9 | Female | 47 | 45 | 2.60[b] | Yes | Yes | Declined surgery | … |
| IV-1 | Male | 16 | … | 2.39 | Yes | Yes | … | … |
| IV-2 | Female | 14 | … | 2.39 | Yes | Yes | … | … |
| IV-3 | Male | 12 | … | 2.45 | No | No | … | … |
| IV-4 | Male | 10 | … | 2.45 | No | No | … | … |
| IV-5 | Male | 28 | … | 2.39 | Yes | Yes | … | … |
| IV-6 | Male | 15 | … | NA | Yes | Yes | … | … |
| IV-7 | Female | 19 | … | 2.39 | No | No | … | … |

NOTE.—NA = not available or not applicable.

[a] Corrected to serum albumin of 40 g/liter (normal range 2.20–2.60 mmol/liter). PTH levels are not given, since these were measured by use of different assays at different centers.

[b] Patient had borderline hypercalcemia and increased PTH levels (113 pg/ml in II-4 and 99 pg/ml in III-9; normal range 10–50 pg/ml).

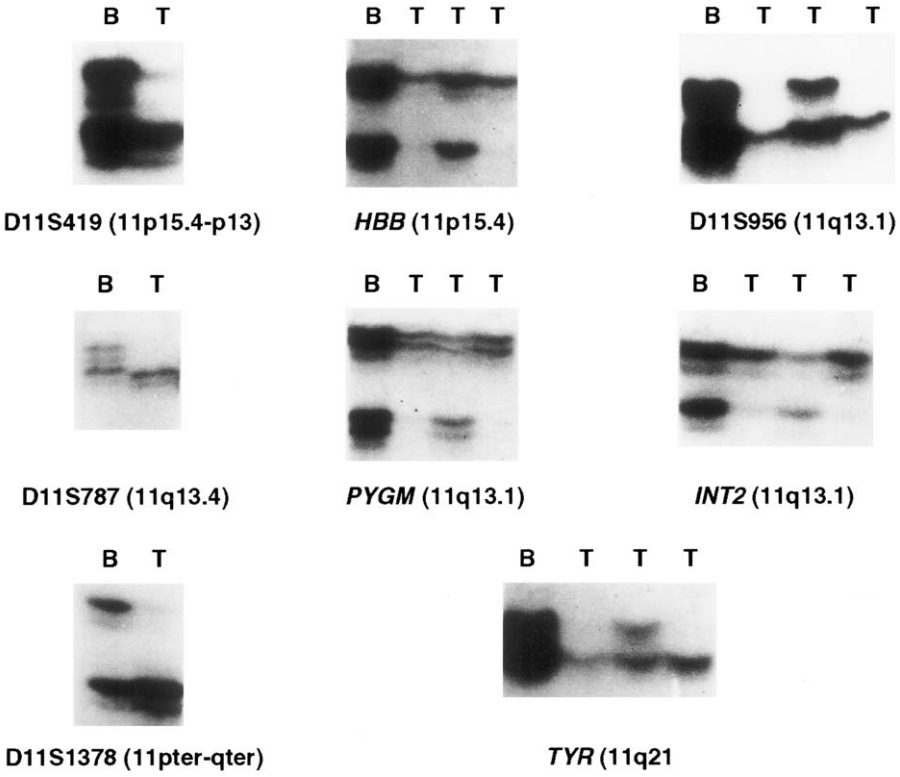


**Figure 2** Autoradiograms showing LOH in the whole of chromosome 11 in parathyroid tumor 1 from patient II-3 (D11S419, D11S787, and D11S1378) and in two of the three tumors from patient III-4 (*HBB,* D11S956, *PYGM, INT2,* and *TYR*). Lane B, Leukocyte DNA. Lane T, Tumor DNA.

sive genetic evidence to confirm that they are a variant of MEN1 (Teh et al. 1998*c*).

To our knowledge, this is the first study to demonstrate that FIHP can occur as a variant of MEN1, and in this family FIHP is associated with a *MEN1* missense mutation. The disease transmission follows an autosomal dominant pattern with high penetrance, as in MEN1. Clinically, the hyperparathyroidism runs a rather mild course, as evidenced by two affected subjects who declined surgery and yet developed no obvious complications. Pathologically, the multiglandular parathyroid disease found is also consistent with that of MEN1 (Teh et al. 1996*b*). Furthermore, LOH results of the parathyroid tumors indicated the involvement of the *MEN1* gene, which has been considered to be a tumor-suppressor gene. The loss of the wild-type alleles in the parathyroid tumors from two individuals is consistent with Knudson's two-hit mutation theory. Thus, in these tumors, one copy of the *MEN1* gene is mutated with E255K, whereas the other copy is lost. We thus propose that FIHP could be divided into at least two forms, on the basis of histopathological and genetic findings. The MEN1 variant is characterized by multiglandular hyperplastic disease resulting from a *MEN1* mutation and, clinically, by a milder course of hyperparathyroidism. The HPT-JT variant characterized by solitary adenomas is linked to the *HRPT2* locus in 1q21-q32 and more frequently presents with profound hypercalcemia or hypercalcemic crisis.

To date, the function of the *MEN1* gene remains unknown. A wide range of *MEN1* mutations, spreading across all nine coding exons, have been reported, although a large proportion of them are frameshift or nonsense, indicating that they are inactivating mutations. The missense mutation found in this family (E255K) has never been reported either in MEN1 families (Basset et al. 1998; Teh et al. 1998*c*; Genome Database) or in sporadic counterparts of MEN1-related tumors, including parathyroid tumors (Heppner et al. 1997; Farnebo et al. 1998). By comparison with the murine *Men1* sequence, this mutation was shown to affect a conserved amino acid (C.L., unpublished data). Although the significance of this mutation, which alters glutamic acid to lysine in codon 255, is not known, our findings suggest that it contributes relatively mildly to parathyroid hyperplasia and not to other MEN1-related neoplasias. However, the family members carrying the mutation should be considered as potential MEN1 pa-

tients and should have close long-term follow-up. Future functional studies of the mutation in the family reported here, compared with others, will provide information relevant to elucidating the biological roles of the *MEN1* gene, in various endocrine tissues.

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## Electronic-Database Information

Accession numbers and URLs for data in the article are as follows:

Genome Database, http://www.gdb.org/ (for *MEN1* mutations [120173])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FIHP [MIM 145000])

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