

ONLINE MUTATION REPORT

Germline and de novo mutations in the *HRPT2* tumour suppressor gene in familial isolated hyperparathyroidism (FIHP)

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Familial forms of primary hyperparathyroidism (PHPT) constitute a broad group of disorders in which PHPT is either a main or an associated feature. With the advances in disease gene identification, some of the genetic abnormalities underlying familial PHPT have been clarified.^{1,2} In hyperparathyroidism–jaw tumour syndrome (HPT-JT; OMIM #145001) the affected family members frequently develop PHPT, ossifying jaw fibromas, and cystic and neoplastic renal lesions.^{3–6} A typical feature of HPT-JT is adenomas and carcinomas of the parathyroid glands, which often have cystic features.¹ This is in contrast to the other forms of familial PHPT in which the parathyroid tumours are generally benign.

The disease locus was first mapped to chromosomal region 1q25–q32 by linkage in affected families^{3,5–7} and recently the causal *HRPT2* gene was isolated through a positional cloning approach.³ The *HRPT2* gene consists of 17 exons encoding an evolutionarily well conserved, 531 amino acid protein named parafibromin. The inactivating mutations demonstrated in the germline of HPT-JT kindreds and as somatic events in some sporadic parathyroid adenomas³ are in agreement with the observations of somatic loss of the wild type alleles,⁶ suggesting that parafibromin has a tumour suppressor function.^{3,6}

The importance of the multiple endocrine neoplasia type 1 gene (*MEN1*) in familial PHPT has been well established. *MEN1* is a tumour suppressor gene located in 11q13,^{8–10} and its encoded protein menin has been shown to interact with several proteins involved in transcriptional regulation.^{11,12} The *MEN1* syndrome (OMIM #131100) is clinically characterised by the frequent development of tumours in the parathyroids, the endocrine pancreas and duodenum, and the anterior pituitary gland. *MEN1*, which is the most common form of hereditary PHPT, is caused by germline mutations of *MEN1*, both in the form of inherited mutations in families and de novo mutations in isolated *MEN1* cases.^{13,14} Less frequently, familial PHPT is seen in familial hypercalcaemic hypercalciuria or the homozygous form of familial hypercalcaemic hypocalciuria (FHH), which are both associated with germline mutations in the calcium receptor gene *CASR* in 3q13–q21.^{15,16}

Familial isolated hyperparathyroidism (FIHP; OMIM #145000) is defined as hereditary PHPT without other associated neoplasias or endocrinopathies.¹⁷ The disease is dominantly inherited and characterised by benign uni- or multi-glandular parathyroid tumours. Germline mutations of the *CASR* gene have previously been reported in a subset of FIHP families.¹⁷ Based on linkage studies in a few large pedigrees, FIHP has also been suggested to represent genetic variants of *MEN1*¹⁸ or HPT-JT syndrome.¹⁹ Following the

Key points

- Familial primary hyperparathyroidism (PHPT) is a heterogeneous disease, both with respect to the clinical presentation and the predisposing genetic defects involved. The main familial forms of PHPT are hyperparathyroidism–jaw tumour syndrome (HPT-JT), multiple endocrine neoplasia (MEN1), and familial isolated hyperparathyroidism (FIHP).
- The recently identified *HRPT2* gene responsible for HPT-JT was screened for mutations in seven kindreds diagnosed with FIHP. Two of the families were found to segregate a constitutional *HRPT2* mutation, including a splice mutation IVS2+1G→C in the first family and a missense mutation 191T→C (L64P) in the second family.
- We were able to document that L64P had occurred de novo in an affected woman who carried the mutation in a somatic and germline mosaic state. The other five FIHP kindreds were negative for germline mutations in all three genes identified for FIHP, *HRPT2*, *MEN1*, and *CASR*.
- In conclusion, we demonstrate that *HRPT2* is a disease gene for 1q linked FIHP and underline the possibility of mutations occurring de novo in this gene. The results further support that a subset of FIHP is a variant of HPT-JT, which has implications for clinical diagnostic purposes and for improved understanding of parathyroid tumour development.

identification of the *MEN1* gene, some families with FIHP have subsequently been shown to segregate constitutional *MEN1* mutations, predominantly missense, suggesting that FIHP can be a variant or partial expression of *MEN1*.^{20–24} The identification of *HRPT2* provides a new and highly relevant candidate for the further elucidation of FIHP genetics. For this purpose, we investigated the possible involvement of *HRPT2* in FIHP families. Our findings demonstrate the involvement of *HRPT2* in a subset of such families.

Abbreviations: FIHP, familial isolated hyperparathyroidism; HPT-JT, hyperparathyroidism–jaw tumour syndrome; MEN, multiple endocrine neoplasia; PHPT, primary hyperparathyroidism

MATERIALS AND METHODS

Families

Genetic analysis was performed on seven kindreds diagnosed with FIHP. The diagnosis of PHPT in the participating individuals was based on extensive and careful investigation in order to exclude the presence of MEN1 or FHH. Informed consent was obtained from all patients and the study was approved by the local ethics committees.

Family 1 is residing in France, and the members have been clinically followed since 1993 (fig 1, table 1). In this family all patients have been screened by a standardised established protocol by the French network on MEN disease (Groupe d'Etude des Tumeurs Endocrines-GTE/GENEM).²⁵ In brief, this included biochemical measurements of serum and urinary calcium, PTH, phosphate, gastrin, insulin, peptide C, glucagon, chromogranin A, pancreatic polypeptide, basic prolactin, and growth hormone, as well as abdominal ultrasound and CT (of the kidneys, adrenal glands, liver, and pancreas), thoracic CT (to search for carcinoids), cervical ultrasound, and pituitary MRI. For the last 2 years, orthopentography has been performed for all members of the family.

The grandfather (I:2) was diagnosed at 59 years of age with PHPT following a period of symptoms suggestive of kidney stones. Two benign hyperplastic parathyroid glands were identified and surgically removed, since which time he has been clinically well. His two children from the second marriage (II:3 and II:5) have both been affected and treated surgically for PHPT. The daughter II:3 was diagnosed with kidney stones at the age of 32 years, and a single parathyroid adenoma, 1.8 cm in size with multiple cystic formations and haemorrhagic foci, was identified at surgery and removed. Nine years later, at 41 years of age, she had a recurrence of PHPT and a second parathyroid adenoma was removed. The patient is presently 53 years old, and has persistent hypercalcaemia at the upper limit with elevated PTH (103 ng/L). She has also developed osteous abnormalities typical for PHPT and recurrent hypercalciuria. Her brother (II:5) was diagnosed at 21 years of age with PHPT following an atypical clinical presentation with mental affective disturbance and kidney stones. A single adenoma was identified at surgery and removed. Eleven years later recurrent PHPT was noted and one additional enlarged parathyroid gland was removed,

following which the patient has been asymptomatic. The grandson (III:4) was treated at 25 years of age for PHPT with removal of one large intrathyroid parathyroid adenoma (1.835 g). The younger brother (III:6) is clinically and biochemically unaffected. The dizygotic twins (III:7 and III:8) are presently 9 years old and have serum calcium levels at the upper limit of normal but are otherwise without symptoms of PHPT. The presence of MEN1 syndrome in this family was excluded by clinical screening for related tumours and by negative *MEN1* mutation analyses in all affected members. No tumours were detected or suspected using orthopentography or renal CT scanning. The observation of two renal cysts in II:3, and one cyst in each of individuals II:3 and III:4 were interpreted as normal findings because of their small size and benign appearance.

Family 2 (fig 2), which has previously been reported to exhibit FIHP linked to the HPT-JT locus,¹⁹ was clinically re-examined in this study. The family members included in the study were biochemically screened, with measurements of serum and urinary calcium, phosphate, albumin, and creatinine. The radiological screening included orthopentography and abdominal ultrasound. This revealed five individuals affected with PHPT related to cystic parathyroid adenomas, but without evidence of other endocrinopathies or neoplasias. The proband (IV:8) was diagnosed with PHPT at 22 years of age, and treated with parathyroidectomy to remove one adenoma. His grandmother (II:2) presented clinically with renal calculi in her forties. After recognition of PHPT at 58 years of age, two parathyroid adenomas were surgically removed, one of which was unusually large (4 g, 40 mm). The proband's father (III:8) developed PHPT at 25 years of age, and a single adenoma was removed. Seventeen years later, at 42 years of age, he had recurrence of PHPT and a second parathyroid adenoma of 1.5 g was removed. He died in 2001 at the age of 51 years following a myocardial infarction. The father's three paternal aunts (II:3, II:4, and II:7) have not shown any clinical or biochemical symptoms of PHPT. Their respective children are similarly unaffected. The proband's aunts (III:2, III:3, and III:6) are clinically unaffected and have normal range total serum calcium levels. Two of his cousins (IV:1 and IV:3) were surgically treated for PHPT at 21 and 19 years of age respectively, with removal of one adenoma in each case.

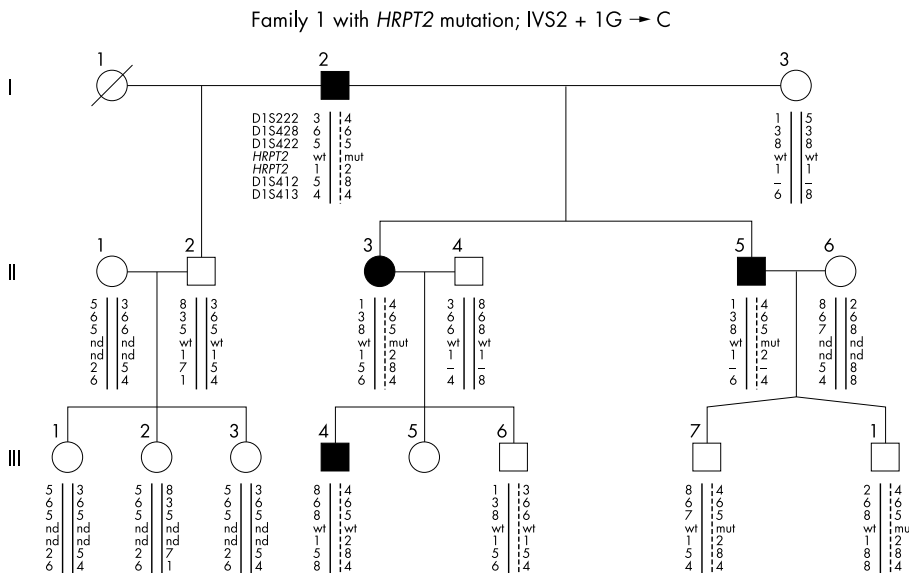


Figure 1 Pedigree of the previously unreported family 1 with autosomal dominant inheritance of isolated PHPT. Affected members operated for PHPT are shown as filled symbols and presently unaffected members as open symbols. The haplotypes defined by microsatellite markers flanking the *HRPT2* gene are shown below each family member genotyped. Sequencing of the *HRPT2* gene showed the wild type sequence (wt) or the presence of a splice mutation IVS2+1G→C (mut). The *HRPT2* allele 1 and 2 refers to the polymorphism (IVS2+28 C→T) detected in intron 2 by sequencing. The haplotype marked by a dotted line carries the mutation and is associated with the disease.

Table 1 Clinical and genetic details of family 1 with FIHP in the study

| Parameter | Family member | | | | | | |
|------------------------------------|---------------|------------|----------------------------|-----------|-------|-----------|-----------|
| | I:2 | II:3 | II:5 | III:4 | III:6 | III:7 | III:8 |
| Sex | M | F | M | M | M | M | M |
| Present age (years) | 79 | 53 | 45 | 27 | 19 | 9 | 9 |
| Age at parathyroid first surgery | 59 | 32 | 21 | 25 | - | - | - |
| Serum calcium (mmol/l) | 2.72 | 2.87 | 3.32 | 3.20 | 2.22 | 2.57 | 2.55 |
| Serum PTH (pg/ml) | 70 | 74 | ND | 138 | 34 | 25.4 | 53.0 |
| No. of pathological glands removed | 2 | 2 | 3 | 1 | - | - | - |
| Histopathological diagnosis | Hyperplasia | Adenoma ×1 | Adenoma ×2/ hyperplasia | Adenoma | - | - | - |
| Disease associated haplotype | Yes | Yes | Yes | Yes | No | Yes | Yes |
| <i>HRPT2</i> sequence | IVS2+1G→C | IVS2+1G→C | IVS2+1G→C | IVS2+1G→C | wt | IVS2+1G→C | IVS2+1G→C |

wt, Wild type sequence

Reference ranges: total serum calcium 2.20–2.60 mmol/l; intact serum PTH 10–60 ng/l.

Table 2 Clinical and genetic details for the seven FIHP kindreds in the study

| Family | Affected cases | Histopathology | <i>HRPT2</i> | <i>MEN1</i> | <i>CASR</i> |
|--------|-----------------|---------------------|---------------|-------------|-------------|
| 1 | 1 female/3 male | Adenoma/hyperplasia | IVS+1G→C | Wild type | NA |
| 2 | 2 female/3 male | Adenoma | 191T→C (L64P) | Wild type | NA |
| 3 | 4 female | Adenoma | Wild type | Wild type | Wild type |
| 4 | 2 female | Adenoma/hyperplasia | Wild type | Wild type | Wild type |
| 5 | 3 female | Adenoma | Wild type | Wild type | Wild type |
| 6 | 1 female/2 male | Hyperplasia | Wild type | Wild type | Wild type |
| 7 | 3 female/1 male | Hyperplasia | Wild type | Wild type | Wild type |

NA, not analysed

The other five cousins (IV:2 and IV:4–7) are presently unaffected, with serum calcium levels within the reference range. However, IV:5, who is presently 16 years old, has had four fractures. Neither of the proband's brothers (IV:9 or IV:10) has clinical or biochemical signs of PHPT. As previously reported, no case of jaw or renal tumour and no evidence of MEN1 has been found in this family¹⁹. Additional screening of III:6 with orthopentography and renal ultrasonography was negative.

FIHP families 3–7 (table 2) have been clinically described and screened for *MEN1* mutations as previously reported.²⁴ They were diagnosed as having PHPT on the basis of the clinical examinations, with findings of hypercalcaemia, increased parathyroid hormone (PTH), and family history of PHPT. The parathyroid lesions consisted of adenomas or hyperplasia. No other neoplastic lesions suggestive of MEN1 or HPT-JT have been found, further supporting the diagnosis of FIHP. Furthermore, previous linkage analyses in these

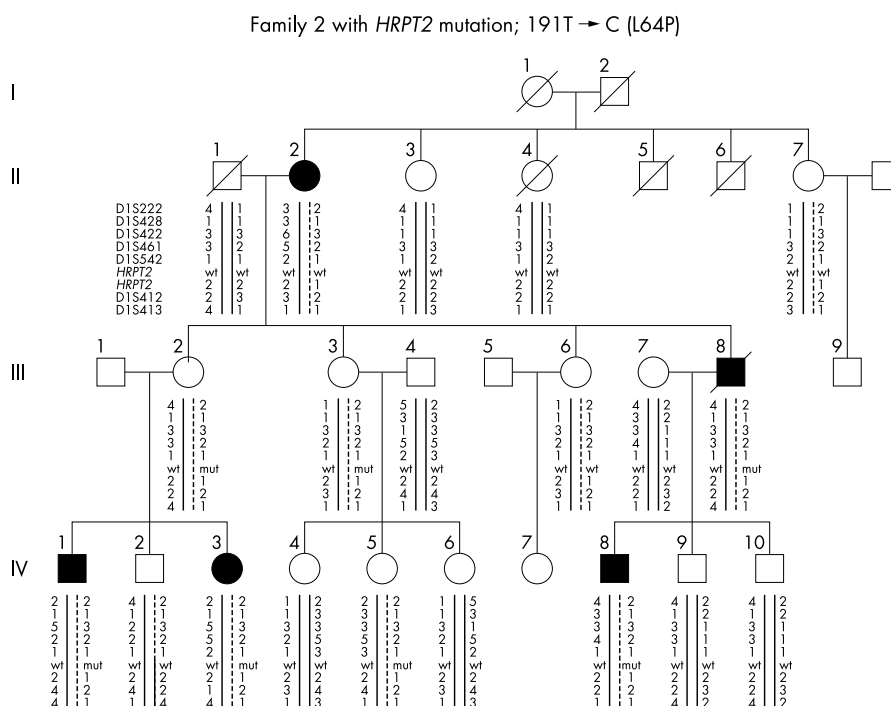


Figure 2 Pedigree and genotypes of the *HRPT2* locus in family 2, diagnosed with FIHP. Affected members operated for PHPT are shown as filled symbols and presently unaffected members as open symbols. The haplotypes defined by microsatellite markers flanking the *HRPT2* gene are shown below each family member genotyped. Sequencing of the *HRPT2* gene showed the wild type sequence (wt) or the presence of a missense mutation L64P (mut) that occurred de novo in II:2. The *HRPT2* allele 1 and 2 refers to the polymorphism (IVS7+33 (GA)₈) detected in intron 7 by sequencing. The haplotype marked by dotted line carries the polymorphism of *HRPT2* and is associated with the disease. As the mutation occurred de novo in II:2, it is present on the affected haplotype in some branches of the family but not in others.

Table 3 Primers and conditions used for mutation detection of *HRPT2*

| Exon | PCR-primers: 5'→3' | | Product size (bp) | Annealing temp (°C) | Sequencing primers: 5'→3' | | | |
|-------|--------------------|-------------------------------|-------------------|------------------------------|---------------------------|----|--------------------|---|
| | Forward | Reverse | | | | | | |
| 1 | 1F | GAGGACGGCT GTTAGTGCT | 1R | CCCCTCTTT CCTTACCCTA | 451 | 58 | 1F/1R/1S | CGAAGGAGGA GGAGGAAGAG |
| 2 | 2F | TAAATGAAT CCAGCCTGAAG | 2R | AGGCCAGACC CTGTCTCT | 251 | 62 | | 2F/2R |
| 3 | 3F | AAAGTGCTGG GATTCTAGG | 3R | TGGACAAAAAT GAAGGTAGG | 599 | 62 | 3S | GTTGTGTATCAT TGTTATTC |
| 4-5 | 4F | AACATGTTTT TGCAGAGCTG | 5R | CTCCTCAGGTTA CTGCAATC | 451 | 62 | 4F/5R/4-5S | ACCTAGAGAA AATCACCATA |
| 6 | 6F | GGCCTAAAGAC ACTGATACC | 6R | CGAACTTAAGA GCAAAGAGG | 351 | 58 | | 6F/6R |
| 7 | 7F | GAATGCCTG CTGTGAAAA | 7R | TGTGAAGGAG CTTGCAATT | 502 | 58 | | 7F/7R |
| 8 | 8F | CTCTGGATCAATA TCTTAGTAGTGG | 8R | GTCTTCAACGT TACTACACTGC | 348 | 58 | | 8F/8R |
| 9 | 9F | ATGGTCATGC TACTGCACTC | 9R | CCAACCCTTAC CCTTAAACA | 251 | 58 | | 9F/9R |
| 10 | 10F | CAGAGATAGTCT TAACCAGCTTC | 10R | CTTCAACATGTG CTACTCACAT | 384 | 58 | | 10F/10R |
| 11 | 11F | AACATGTTTCA TGGAGTAACC | 11R | TGCACTGTTAC GATCTTTTG | 251 | 60 | | 11F/11R |
| 12-13 | 12F | TGGTAACTGA AACTGCAGA | 13R | GTATCTCAATAT CCTACGTACAGG | 713 | 60 | 12F/12S 13R/13S | CAGTGTGGA GAAGATAGTTG CCCAAGCCA CACTGATTAT |
| 14 | 14F | ATCTTCCA TTTTCATCACG | 14R | CCCCATCTCT TAAAAAGCAA | 321 | 58 | | 14F/14R |
| 15 | 15F | TGCCTAAGGGA TTTATAGTAGC | 15R | ACATCATAT GCGCAGAACT | 281 | 60 | | 15F/15R |
| 16 | 16F | GGCGTGATAA ACCCTGAAT | 16R | GAAAGAAGGG AATTAGGGAA | 401 | 60 | | 16F/16R |
| 17 | 17F | GAGGAGTGTAT TTCTAGCTTATC | 17R | GATCAATCTGTGA CCTTCTCA | 301 | 60 | | 17F/17R |

Primers were constructed from alignment of the cDNA sequence (accession no. XM-029845) with the genomic sequence of AL390863 and AL139133. Primers 1F and 17R are located within 5' and 3'UTR regions, while all other primers are from within intronic sequences.

families could not exclude the possible involvement of *MEN1* and *HRPT2* loci.²⁴

Mutation analyses of the *HRPT2* and *CASR* genes

Mutation screening of the *HRPT2* gene was performed by sequencing the entire coding region in two or three affected family members representing each of the seven families. The primers used for amplification and sequencing as well as the annealing temperatures for the amplification are presented in table 3.

The 17 coding exons of *HRPT2* were amplified as 15 different fragments with primers derived from the flanking intronic or 3'/5' UTR regions, to allow detection of mutations in coding regions or affecting the splicing. The DNA (50 ng) was amplified in a mix of 1× *Taq* Gold buffer, 1.5 mmol/l MgCl₂, 1.25 mmol/l of each dNTP and 10 mmol/l oligonucleotide primer pair, 1.5 U DNA polymerase (Amplicon *Taq* Gold™) in a final volume of 20 μl. The thermocycling conditions consisted of an initial denaturation of 95°C, followed by 35 cycles of denaturation at 95°C for 40 seconds,

Germline FIHP mutations reported in this study

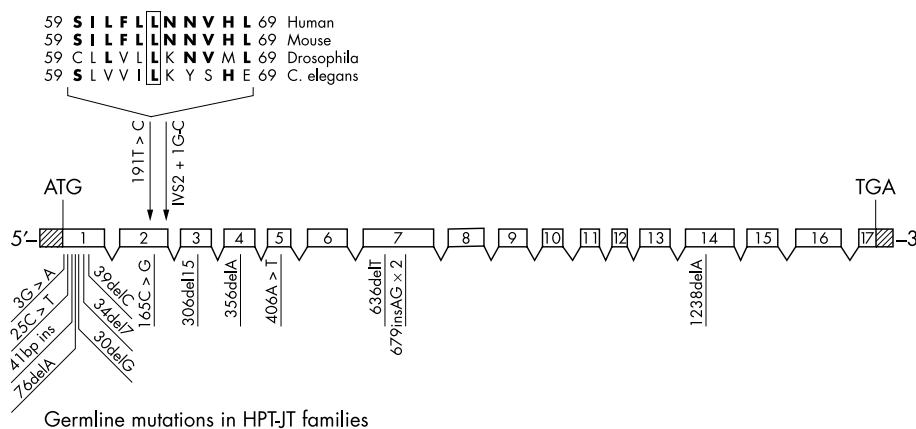


Figure 3 Schematic illustration of the *HRPT2* gene and the germline mutations detected in it. The inactivating mutations reported in HPT-JT families^{3,26} are shown below the corresponding exons. Above is indicated the mutations found in FIHP families. The including missense mutation (L64P) has previously been reported by Carpten *et al*, and the nonsense alteration was identified in this study. The alignment of orthologous sequences of mouse, *Drosophila*, and *Caenorhabditis elegans* are shown above the missense mutation 191T→C (L64P) to demonstrate that the amino acid is conserved among the different species. The positions of nucleotides are numbered from the initiating codon.

annealing at 58–62°C for 30 seconds, and elongation at 72°C for 45 seconds, and a final extension at 72°C for 7 minutes.

Sequencing reactions were performed using the BigDye Terminator cycle sequencing kit (Perkin Elmer) with the following thermocycling conditions: denaturing at 98°C for 5 minutes, followed by 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. The sequencing products were then purified with Sephadex G-50 (Amersham Pharmacia Biotech, Sweden) on MultiScreen-HV Clear Plates (Millipore Corp, Bedford, MA, USA) and thereafter run in either a capillary sequencer ABI Prism 3700 DNA analyser or in an automated sequencer (ABI 377; PE Applied Biosystems, Foster City, CA, USA).

Sequencing of the mutated exon/intron was performed for all available members of families 1 and 2, as well as for the parathyroid adenoma from II:2 in family 2, using forward and reverse sequencing. The mutation in family 1 was confirmed by restriction cleavage assay. Each PCR product of exon 2 was cleaved with *DdeI* (Invitrogen) in a total volume of 100 µl, and separated on 3% agarose gels. The presence of a mutation results in the introduction of a *DdeI* restriction site, leading to the addition of two shorter restriction fragments detected by gel electrophoresis. In addition, the mutation negative status of members II:1, II:7, and III:6 in family 2 was confirmed by sequencing of exon 2 using a different set of flanking primers. Sequencing of *HRPT2* exon 2 and the flanking intronic region was performed on 215 normal unrelated control individuals, comprising 150 controls described by Carpten *et al*³ and the 65 controls reported by Howell *et al*.²⁶

In addition, sequencing of the six coding exons (2–7) of the *CASR* gene was carried out using the same primers and PCR conditions as described elsewhere²⁷ in families 3–7 without identified *HRPT2* mutations.

Genotyping of microsatellite markers

Genomic DNA was extracted from peripheral leukocytes using standard methods and genotyped with polymorphic microsatellite markers. Seven markers from the *HRPT2* region in 1q25–q32 were selected, including cen-D1S222-D1S428-D1S422-D1S461-D1S542-*HRPT2*-D1S412-D1S413-tel. The localisation and order of the markers are based on the information presented in Carpten *et al*³ and in the Genome Database (www.gdb.org). For all markers, the forward primer was labelled for fluorescent detection after electrophoresis on a laser fluorescent sequencing machine (ABI 377, PE Applied Biosystems). Polymerase chain reaction was performed as described for the mutation screening of *HRPT2*. The data were analysed using Genescan software (PE Applied Biosystems).

RESULTS

The seven kindreds studied here all had 2–5 members affected with PHPT related to benign parathyroid tumours (table 2). At the initial diagnosis there was no evidence of other endocrinopathies or tumours found in the families. During follow up, these patients have not presented with any signs suggestive of other familial syndromes such as MEN1 or HPT-JT, thus further supporting the diagnosis of FIHP.

HRPT2 mutations and mosaicism in FIHP

The two *HRPT2* germline mutations identified in the two families constitute of a missense mutation and a splice mutation (fig 3). Neither of these mutations was identified by sequencing of 215 normal reference individuals. Two polymorphisms (IVS2 +28 C→T and IVS7+33 (GA)₈) in intronic sequences were detected in two of the families and in normal reference individuals.

In family 1, the base substitution (G→C) detected at the boundary of exon 2 and intron 2 (IVS2+1G→C; fig 3) is predicted to cause defective splicing. The mutation was found in all four affected family members (I:2, II:3, II:5, and III:4) and in both children (III:7 and III:8) of II:5, who are presently 9 years old and unaffected (fig 1). Furthermore, genotyping of markers from the *HRPT2* region showed that all mutation carriers shared the same disease associated haplotype (fig 1, table 1). Here, the intronic *HRPT2* polymorphism (T/C) revealed at position +28 in intron 2 served as an intragenic marker of the affected haplotype.

In family 2, the missense mutation in exon 2 consisted of a base substitution (CTT→CCT) causing an amino acid change from leucine to proline at position 64 (L64P, fig 3). The mutation was present in four affected family members (III:8, IV:1, IV:3, and IV:8) and in three presently unaffected members (III:2, III:3, and IV:5) (fig 2). One of these mutation carriers is an obligate carrier (III:2) and one has a history of multiple fractures (IV:5). All mutation carriers were also shown to share the same disease associated haplotype of the *HRPT2* region. The polymorphism detected in intron 7 (IVS7+33 (GA)₈) was also present on the affected haplotype, supporting the linkage results. The mutation was not detected either in the oldest affected family member (II:2) or in her three sisters (II:3, II:4, and II:7) and one daughter (III:6). However, two of these individuals (II:7 and III:6) carried the affected haplotype for the entire interval, including the intron 7 polymorphism (fig 2). Interestingly, the mutation (L64P) was also found in the parathyroid adenoma of II:2 but not in her leukocyte DNA. Repeated genotyping and mutation analyses on a new set of DNA samples from this family confirmed the findings in all cases. Taken together, the findings in family 2 suggest that the L64P mutation occurred de novo on the disease associated haplotype in individual II:2. This haplotype was then inherited with the mutation by three of her children (III:2, III:3, and III:8) but without the mutation by her daughter, III:6 (fig 2).

FIHP families negative for *HRPT2*, *CASR* and *MEN1*

In five families (3–7), no *HRPT2* mutation were detected. Previously, these families had been screened for *MEN1* mutations with negative results.²⁴ In an attempt to investigate the third locus involved in FIHP, we addressed the *CASR* gene by screening for mutations in the six coding exons. However, no mutations could be demonstrated.

DISCUSSION

The seven families reported here were all shown to have FIHP, which in two of them was found with constitutional mutations of the *HRPT2* gene. In family 1, the *HRPT2* mutation destroys the splice site at the 5' end of intron 2. In family 2, the missense mutation (L64P) in exon 2 alters an evolutionarily conserved amino acid that is present among orthologous sequences such as mouse, *Drosophila melanogaster*, and *Caenorhabditis elegans* (fig 3). It can be speculated that the effect of the single residue alteration may change the local structure or interfere with the ability for parafibromin to interact with other proteins. In both families the inheritance of microsatellite alleles is in agreement with linkage of PHPT to the *HRPT2* region, as also determined from analyses of intragenic polymorphisms. The mutations are present on the respective affected haplotypes, but were not detected in 215 normal controls. Taken together, these findings suggest the possible pathogenetic importance of the two mutations, thus demonstrating that *HRPT2* is a disease gene for 1q linked FIHP. In further support of the association between FIHP and *HRPT2*, it can be noted that the parathyroid tumours from both families tended to be large and have cystic features,

similar to that seen in HPT-JT. Furthermore, loss of heterozygosity of the wild type 1q alleles in the parathyroid tumours of family members II:2, IV:1, and IV:8 in family 2 has previously been reported,¹⁹ in agreement with the likely tumour suppressor function of *HRPT2*.³

No mutations were identified in the remaining five FIHP families (3–7) in any of the three genes investigated (table 2). However, mutations in regulatory or intronic parts of *HRPT2*, *MEN1*, and *CASR* cannot be excluded by this study. There is also a possibility that other chromosomal regions may contribute to the development of parathyroid tumours in FIHP.

In dissecting the genetic aetiology of FIHP, the diagnostic aspects are of great importance. Familial PHPT is recognised as a genetically heterogeneous disease that can occur either as a separate entity or as part of an inherited cancer syndrome such as MEN1 and HPT-JT.¹ Therefore, some families with PHPT can initially present as isolated PHPT, but subsequently develop the full blown syndrome of HPT-JT or MEN1,^{28–30} while in others the diagnosis of FIHP remains during follow up.^{19–21} In the present study, the FIHP families with *HRPT2* mutations have been followed up for more than 10 years, during which only benign PHPT has been diagnosed, and the possibility of MEN1 and HPT-JT has been carefully evaluated and excluded. Furthermore, affected members from three generations were investigated in both families, including several adult mutation carriers well above the expected age of onset for symptoms detectable at screening.

The missense and splice mutations detected in the study are not representative of the mutation spectra of families with HPT-JT syndrome (fig 3). In the initial publication of the *HRPT2* cloning, 12 of the 13 different germline mutations were predicted to give impaired protein function due to truncation or premature stops.³ The only missense mutation detected was located at the first ATG, leading to loss of the initiation codon.³ All of these families were clinically characterised with PHPT, and in addition jaw tumours, renal lesions, or parathyroid carcinoma.^{3–31} Although based on a limited number of observations, it is striking that the *HRPT2* mutation spectra in FIHP *v* HPT-JT is similar to that observed for the *MEN1* gene. With regard to the *MEN1* gene, FIHP families predominantly have missense mutations,^{20–24} whereas in MEN1 families truncating or nonsense mutations are more frequent.^{13–14}

In one of the FIHP families, the *HRPT2* mutation (L64P) was not found in the initial mutation screening as it had occurred *de novo*. Extensive analysis of additional family members led to the identification of the mutation, which was reported in Carpten *et al*³ and is further described in the present study. As illustrated in fig 2, three of the four children of II:2 inherited the affected haplotype with the disease mutation from their mother (II:2) and in turn passed the same mutated haplotype on to their respective children. However, the same affected haplotype was also inherited to her fourth daughter (III:6) without the mutation. This daughter has recently been thoroughly re-examined clinically and found to be clearly unaffected after examination of the parathyroids, the jaws, and the kidneys. In addition, the same affected haplotype was present without the mutation in one of the mother's sisters (II:7), who is also clinically unaffected. The mother has been surgically treated for PHPT, and genetically she carries the affected haplotype without the mutation in constitutional DNA isolated from her peripheral leukocytes. However, the mutation was present in the tumour DNA, which would suggest that the mother is somatic and germline mosaic for the initiating mutation. In this particular family, the mutation would then have occurred *de novo* during embryogenesis of II:2 on the

chromosome with the affected haplotype. Owing to the mother's mosaicism, this haplotype was then passed three times with the mutation and once without it on to her four children. Similarly, mosaicism in II:2 would explain why the mutation is not present on the affected haplotype in leukocyte DNA although she is clinically affected with PHPT. Mosaicism has previously been observed in other hereditary diseases, for example retinoblastoma and neurofibromatosis, and has then been associated with similar discrepancies between the inheritance of marker alleles and disease mutation.^{32–33}

In summary, we have demonstrated that constitutional mutations in the *HRPT2* gene are likely to be associated with parathyroid tumourigenesis. Specifically, the involvement of *HRPT2* is demonstrated to be a disease gene for 1q linked FIHP. The finding that a subset of FIHP occurs as an allelic variant of HPT-JT has implications for clinical diagnostic purposes and for improved understanding of parathyroid tumour development. It is expected that further functional studies of mutations in the *HRPT2* gene will provide relevant information to better explain the tissue specific involvements of HPT-JT and FIHP.

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