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


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. 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. A typical feature of HPT-JT is adenomas and carcinomas of the parathyroid glands, which often have cystic features. 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 and recently the causal HRPT2 gene was isolated through a positional cloning approach. 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 adenomas are in agreement with the observations of somatic loss of the wild type alleles, suggesting that parafibromin has a tumour suppressor function.

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 and its encoded protein menin has been shown to interact with several proteins involved in transcriptional regulation. 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. 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.

Familial isolated hyperparathyroidism (FIHP; OMIM #145000) is defined as hereditary PHPT without other associated neoplasias or endocrinopathies. 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. Based on linkage studies in a few large pedigrees, FIHP has also been suggested to represent genetic variants of MEN1 or HPT-JT syndrome. Following the 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. 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.

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.

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’Étude des Tumeurs Endocrines-GTE/GENEM). 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 osteoarthritis typical for PHPT and recurrent hypercalcuria. 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, 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](http://jmg.bmj.com/)

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 (+) or the presence of a splice mutation IVS2+1G→C (mut). The HRPT2 allele 1 and 2 refers to the polymorphism (IVS2+2B C→T) detected in intron 2 by sequencing. The haplotype marked by a dotted line carries the mutation and is associated with the disease.
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

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

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

**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

<table>
<thead>
<tr>
<th>Family</th>
<th>Affected cases</th>
<th>Histopathology</th>
<th>HRPT2</th>
<th>MEN1</th>
<th>CASR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 female/3 male</td>
<td>Adenoma/hyperplasia</td>
<td>IVS2+1G→C</td>
<td>Wild type</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>2 female/3 male</td>
<td>Adenoma</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
<tr>
<td>3</td>
<td>4 female</td>
<td>Adenoma</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
<tr>
<td>4</td>
<td>2 female</td>
<td>Adenoma/hyperplasia</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
<tr>
<td>5</td>
<td>3 female</td>
<td>Adenoma</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
<tr>
<td>6</td>
<td>1 female/2 male</td>
<td>Hyperplasia</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
<tr>
<td>7</td>
<td>3 female/1 male</td>
<td>Hyperplasia</td>
<td>Wild type</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
</tbody>
</table>

NA, not analysed

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.
families could not exclude the possible involvement of \textit{MEN1} and \textit{HRPT2} loci.\textsuperscript{24}

Mutation analyses of the \textit{HRPT2} and \textit{CASR} genes

Mutation screening of the \textit{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.

![Table 3](http://www.jmedgenet.com)

<table>
<thead>
<tr>
<th>Exon</th>
<th>PCR-primers: 5'→3'</th>
<th>Reverse</th>
<th>Product size (bp)</th>
<th>Annealing temp (°C)</th>
<th>Sequencing primers: 5'→3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1F GAGGACGCT</td>
<td>1R CCCCTTCTT</td>
<td>451 58</td>
<td>1F/1R/1S</td>
<td>CGAAGGAGGA</td>
</tr>
<tr>
<td>2</td>
<td>2F GCTTGTCT</td>
<td>2R AGGCCAGACC</td>
<td>251 62</td>
<td>2F/2R</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3F AAGTGTGG</td>
<td>3R TAGGACAAAAAT</td>
<td>599 62</td>
<td>3S</td>
<td>GTGGTATATCAT</td>
</tr>
<tr>
<td>4–5</td>
<td>4F AAATGTITTT</td>
<td>5R CCTCTACGGTTA</td>
<td>451 62</td>
<td>4F/5R/4-5S</td>
<td>ACCTGAGAAA</td>
</tr>
<tr>
<td>6</td>
<td>6F GCCTTAAAGAC</td>
<td>6R AACCTTGAAGGA</td>
<td>351 58</td>
<td>6F/6R</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7F CAACTATACC</td>
<td>7R TGGAAAGGA</td>
<td>502 58</td>
<td>7F/7R</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8F GTTGGATAA</td>
<td>8R GTTCAACGT</td>
<td>348 58</td>
<td>8F/8R</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>9F ATGTGTGG</td>
<td>9R TACCACTGC</td>
<td>251 58</td>
<td>9F/9R</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10F TACCACTGC</td>
<td>10R TTTCAAACAG</td>
<td>384 58</td>
<td>10F/10R</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>11F TGGAGGTCAAG</td>
<td>11R TTTACCTACGT</td>
<td>251 60</td>
<td>11F/11R</td>
<td></td>
</tr>
<tr>
<td>12–13</td>
<td>12F TGGAGGTCAAG</td>
<td>13R GTATCTCAATAT</td>
<td>713 60</td>
<td>12F/12S</td>
<td>CAGTGTTGTA</td>
</tr>
<tr>
<td>14</td>
<td>14F ATCTTCCCA</td>
<td>14R CCCCATCTCT</td>
<td>321 58</td>
<td>13R/13S</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>15F TTTACCTACGT</td>
<td>15R ATCAATAT</td>
<td>281 60</td>
<td>CAGTGTTGTA</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>16F ACCTTTACAT</td>
<td>16R GAAAGAAGG</td>
<td>401 60</td>
<td>GAAAGTAATTG</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>17F GAGGAGGTCAAG</td>
<td>17R GATCTTACCG</td>
<td>301 60</td>
<td>CCAAGGCA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

The 17 coding exons of \textit{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 MgCl\textsubscript{2}, 1.25 mmol/l of each dNTP and 10 mmol/l oligo-nucleotide primer pair, 1.5 U DNA polymerase (AmpliTaq Gold\textsuperscript{TM}) in a final volume of 20 μl. The thermostabilizing conditions consisted of an initial denaturation of 95°C, followed by 35 cycles of denaturation at 95°C for 40 seconds,
Howell performed as described for the mutation screening of PE Applied Biosystems). Polymerase chain reaction was phoresis on a laser fluorescent sequencing machine (ABI 377, primer was labelled for fluorescent detection after electro-

Database (www.gdb.org). For all markers, the forward polymorphisms (IVS2 by sequencing of 215 normal reference individuals. Two families constitute of a missense mutation and a splice mutation (fig 3). Neither of these mutations was identified or mosaicism in FIHP

During follow up, these patients have not presented with any other endocrinopathies or tumours found in the families. (table 2). At the initial diagnosis there was no evidence of (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 (5–7), no HRPT2 mutation were detected. Previously, these families had been screened for MEN1 mutations with negative results. In an attempt to investigate the third locus involved in FIHP we sequenced 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, in agreement with the likely tumour suppressor function of HRPT2.

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. Therefore, some families with PHPT can initially present as isolated PHPT, but subsequently develop the full blown syndrome of HPT-JT or MEN1, while in others the diagnosis of FIHP remains during follow up. 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. The only missense mutation detected was located at the first ATG, leading to loss of the initiation codon. All of these families were clinically characterised with PHPT, and in addition jaw tumours, renal lesions, or parathyroid carcinoma. 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, whereas in MEN1 families truncating or nonsense mutations are more frequent.

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 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 leucocyte 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.

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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