

## CLINICAL STUDY

# Involvement of the *MEN1* gene locus in familial isolated hyperparathyroidism

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## Abstract

**Background:** Familial isolated hyperparathyroidism (FIHP) is a hereditary disorder characterised by uni- or multiglandular parathyroid disease. A subset of families are likely to be genetic variants of other familial tumour syndromes in which PHPT is the main feature, for example multiple endocrine neoplasia type 1 (MEN 1) and the hyperparathyroidism-jaw tumour syndrome (HPT-JT).

**Objective:** To investigate seven families diagnosed with FIHP, each with two to eight affected family members, to clarify the underlying genetic mechanism.

**Methods:** The entire *MEN1* gene was sequenced for germline mutations and, in addition, tumour specimens were analysed in comparative genomic hybridisation and loss of heterozygosity studies.

**Results:** Two families exhibited *MEN1* mutations, L112V and 1658delG, which were associated with loss of the wild-type 11q13 alleles in all tumours analysed. In the remaining five families, no *MEN1* mutation was identified.

**Conclusion:** These results support the involvement of the *MEN1* tumour suppressor gene in the pathogenesis of some of the FIHP kindreds. However, loss on chromosome 11 was seen in all tumours exhibiting somatic deletions, although in two families the tumour deletions involved 11q distal to *MEN1*. We conclude that the altered *MEN1* gene function is of importance in the development of FIHP.

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## Introduction

Primary hyperparathyroidism (PHPT) is a genetically heterogeneous disease, usually occurring sporadically and arising as a result of a benign adenoma or hyperplasia or, very rarely, from a carcinoma (1, 2). However, several familial forms are also described, the recognition of which is essential for their correct clinical management (3, 4). In the familial forms of the disease, PHPT is either a typical or an associated feature. In multiple endocrine neoplasia type 1 (MEN 1), the hyperparathyroidism-jaw tumour syndrome (HPT-JT) and familial isolated hyperparathyroidism (FIHP), PHPT is seen in most or almost all of the gene carriers. Apart from PHPT, families with HPT-JT present with fibro-osseous jaw tumours, Wilms' tumours, papillary renal cell carcinomas, renal hamartomas, polycystic

kidney disease, kidney cysts and parathyroid carcinoma (5–7), whereas MEN 1 encompasses tumours of the duodenal and pancreatic endocrine tissues and the anterior pituitary gland (8). The *MEN1* tumour suppressor gene was first located to chromosomal region 11q13 (9) and, 9 years later, it was identified by positional cloning (10, 11); the HPT-JT locus (*HRPT2*) has been localized to a limited interval within chromosomal region 1q21–q32 (5–7).

FIHP (OMIM (Online Mendelian Inheritance in Man) 145000) is defined as hereditary PHPT without any other associated endocrinopathies. The disease is autosomally dominantly inherited and characterised by uni- or multiglandular parathyroid disease. The detailed characterisation of the syndromic forms of PHPT has helped to identify some of the genes and chromosomal regions that are involved in the tumourigenesis of FIHP.

**Table 1** Clinical and genetic data of the family members studied.

Family no.	Case no.	Sex	Present age (years)	Present status	S-Ca <sup>a</sup>	S-PTH <sup>b</sup>	Gland weight (mg)	No. of glands removed	Histopathology	MEN1 sequence
1	II-2	F	69	Unaff						wt
1	II-3	M	76	Aff	2.9	Increased	1767 <sup>d</sup>	1	Adenoma	1658delG
1	III-2	F	57	Unaff						wt
1	III-3	F	50	Unaff						wt
1	III-4	F	48	Unaff						wt
1	III-6	F	43	Unaff						1658delG
1	III-11	F	33	Aff	2.8	Increased	370/151 <sup>d</sup>	3.5	Hyperplasia	1658delG
1	III-12	F	42	Aff	2.7	Increased	339/392 <sup>d</sup>	4	Hyperplasia	1658delG
1	III-13	F	49	Aff	3.1	Increased		4	Hyperplasia	1658delG
1	III-14	M	48	Aff	3.0	Increased	1150 <sup>d</sup>	3.5	Hyperplasia	–
1	IV-6	M	21	Aff	2.9	Increased	4417/452 <sup>d</sup>	2	Hyperplasia**	–
2	II-1	F	63	Aff	2.70	150	No surgery	No surgery		L112V
2	II-2	F	61	Aff	2.70	150	No surgery	No surgery		L112V
2	II-3	F	58	Aff	–	–	–	–	–	L112V
2	II-4	F	54	Unaff	2.22	49				–
2	III-1	M	39	Unaff	2.24					wt
2	III-2	M	37	Unaff	2.33					L112V
2	III-5	F	34	Unaff	2.26					wt
2	III-6	M	21	Unaff	2.18					wt
3	II-2	F	76	Aff	2.85	87	–	No surgery		–
3	II-3	F	72	Aff	2.97	197	1800	1	Adenoma	wt
3	III-3	F	49	Aff	2.85	185	2000	1	Adenoma	wt
3	III-4	F	52	Aff	2.93	143	4000	1	Adenoma	wt
4	I-2	F	91 (D)	Aff	3.13	Increased	–	1	Hyperplasia**	–
4	II-1	F	72	Unaff	2.37					wt
4	II-2	F	70	Unaff	2.53					wt
4	II-3	F	67	Aff	2.86	125	696	1	Adenoma	wt
4	III-9	F	49	Uk	2.52	37				–
4	III-10	F	40	Uk	2.57	47				–
5	II-1	F	50	Unaff						–
5	II-2	M	54	Uk						–
5	II-4	F	63	Aff	–	–	–	–		wt
5	II-10	F	55	Aff	2.74	169 <sup>c</sup>	4187 <sup>d</sup>	1	Adenoma	wt
5	III-1	F	26	Uk						–
5	III-2	F	23	Uk						–
5	III-3	F	20	Aff	3.25	84 <sup>c</sup>	382 <sup>d</sup>	1	Adenoma	wt
5	III-4	F	19	Uk						–
6	I-2	M	52	Aff	2.84	58	450	3	Hyperplasia*	wt
6	II-1	F	24	Aff	2.70	78 <sup>c</sup>	700	4	Hyperplasia*	wt
6	II-2	M	28	Aff	3.28	207 <sup>c</sup>	3490	3	Hyperplasia*	wt
7	I-1	M	Unaff							
7	I-2	F	62	Aff	2.9	201	1003/700	2	Hyperplasia	wt
7	II-1	F	34	Aff	2.83	73	100/240/360/160	3.5	Hyperplasia	wt
7	II-2	F	32	Aff	2.85	Increased	300/630/340	3	Hyperplasia	–
7	II-3	F	–	Unaff						–
7	II-4	M	30	Aff	2.97	57	600/240	4	Hyperplasia	–

<sup>a</sup>Total serum calcium (reference range 2.20–2.60 mmol/l) before surgery. <sup>b</sup>Intact serum PTH (reference range 50–65 ng/l) before surgery. <sup>c</sup>S-PTH concentration originally reported as pmol/l. <sup>d</sup>Gland size was reported in mm, not weight.

The weight is calculated with the formula:  $V = 4pr^2/3$  with the tissue density approximated to be 1.

Aff, affected; Unaff, Unaffected; Uk, unknown; wt, wild-type; (D), died aged 91 years; –, not done; \*Nodular hyperplasia; \*\*Hyperplasia as stated in original report.

A few large FIHP families have been associated with mutations in the *MEN1* (12, 13) or the calcium receptor (*CaR*) genes (14), and some additional families have been assigned to the HPT-JT locus on the basis of linkage analysis (15, 16). However, for the vast majority of FIHP families, the genetic background remains unknown and the involvement of known candidate genes has been only partly evaluated (17, 18). In the *MEN1*-related FIHP, the PHPT seems to run a milder course, with the occurrence of multiglandular disease or hyperplasia (12, 13), and in the HPT-JT-related FIHP, solitary adenoma and even parathyroid carcinoma have been demonstrated (15, 16), whereas in a single family with a *CaR* mutation the parathyroid lesions mainly consisted of moderate hyperplasia (14).

In addition to the genetic heterogeneity, the clinical diagnosis of FIHP is challenging. The management of families initially presenting with PHPT has mainly consisted of genetic counselling and the exclusion of other tumours associated with the syndromic forms. In our experience, we have found that some of the families initially presenting with PHPT alone will eventually develop the full-blown MEN 1 or HPT-JT syndrome (19, 20), whereas in others the diagnosis of FIHP will persist (12). Clinically, it is important to identify exactly both the genetic predisposing alteration and the histopathological appearance, because the management of associated patient groups may differ. Here we describe the clinical and genetic findings in seven families apparently with FIHP. On the basis of the analysis of constitutional and tumour specimens, the involvement of the *MEN1* gene locus is demonstrated in a significant proportion of the cases.

## Materials and methods

### Clinical samples

Extensive and careful investigations of all living family members were performed with the purpose of identifying or excluding the presence of other neoplastic lesions suggestive of MEN 1 or HPT-JT. The diagnoses of PHPT in the participating individuals were based on clinical examination, medical records or information from other family members. On the basis of the clinical information, we have divided the families into FIHP and apparent FIHP families. The clinical details of the FIHP patients are shown in Table 1. Tumour samples were available for 16 individuals and retrieved from paraffin blocks or fresh frozen tissues for six of the seven families. To ensure that the analysed samples were suitable for genetic studies – that is, they contained more than 70% tumour cells – representative sections were cut and subjected to histopathological examination. Small pieces were cut from four frozen tumours and embedded in paraffin, and tissue blocks from the remaining 12 cases were routinely stained for histopathological evaluation. Informed consent

was obtained from all patients and the study was approved by the local ethics committees.

### FIHP families

Five of the families in this study, families 1 to 5, were diagnosed as having PHPT on the basis of the clinical examinations, with the findings of hypercalcaemia, increased parathyroid hormone (PTH) and family history of PHPT (Fig. 1). The presence of two or more affected cases older than 50 years and with no other neoplastic lesions suggestive of MEN 1 or HPT-JT further supported the diagnosis of FIHP. For families 3 and 5, the parathyroid lesions consisted of adenomas with glandular weights ranging from 300 to 4000 mg, and in families 1 and 2 the main histopathological finding was hyperplasia; in family 4, both histopathological findings were seen. In family 4, six individuals (I-2, II-1, II-2, II-3; III-9, III-10) were available for genetic studies; among them, two were affected with PHPT (I-2, II-3). In addition, liver cysts and a renal angiomyolipoma were identified in the proband (II-3), and hydropelvis in the proband's mother (I-2), but no other endocrinopathies were recognised. Family 3 presented with four individuals (II-2, II-3, III-3, III-4) affected with PHPT, but only three were available for analysis in this study. Family 5 consisted of eight individuals (II-1, II-2, II-4, II-10, III-1, III-2, III-3, III-4) available for this study, including three cases affected with PHPT (II-4, II-10, III-3). In family 2, three of the sisters were affected by PHPT (II-1, II-2, II-3), but only one had undergone parathyroidectomy (II-3). The affected family members are all above the age of 50 years and have been followed up for more than 10 years without signs of other endocrinopathies. None of the cases in the third generation (III-1, III-2, III-5, III-6) has yet presented with any symptoms suggestive of PHPT. Family 1 has been followed up since 1988, when the father (II-3) and his four children (III-11, III-12, III-13, III-14) underwent surgery for PHPT. Since then, two children of III-13 (IV-5, IV-6) have developed PHPT and in one of them (IV-6) parathyroidectomy has been performed. The sister (II-1) of the proband (II-3) was also affected by PHPT; however, none of her children were clinically affected at the time of the study.

### Families with apparent or suggested FIHP

In families 6 and 7 (Fig. 1) all the affected individuals were diagnosed with PHPT, with no other demonstrated endocrinopathies. However, as only one affected case in each family is above the age of 50 years, the diagnosis of FIHP is not as clear as for the other families mentioned above. There remains a risk that they may develop other features suggestive of a syndromic form of PHPT. In family 6, the father (I-2) and his two children (II-1, II-2) are affected with PHPT. After

parathyroidectomy, the histopathological diagnosis of nodular hyperplasia was confirmed in all the three cases. In family 7, the diagnosis of PHPT was made in four of the six individuals studied. The mother (I-2) was 26 years old at the first operation and has since developed recurrent disease; the most recent operation was performed when she was aged 58 years. Three of her children (II-1, II-2, II-4) have had parathyroidectomy in their twenties. No additional lesions have been noticed, and the parathyroid tumours in this family were mainly diagnosed as hyperplasia.

### Genotyping and linkage analyses

Genomic DNA was extracted from peripheral leucocytes using standard methods and genotyped with polymorphic microsatellite markers. Ten markers covering the HPT-JT region in 1q21–q32 were selected (7, 20), including cen-D1S466, D1S191, D1S408, D1S428, D1S412, D1S413, D1S1660, D1S373, D1S456, and D1S213-tel. Six polymorphic microsatellite markers flanking the *MEN1* locus at 11q13 were analysed: cen-PYGM, D11S4946, D11S4940, D11S4938 and D11S4937, D11S449. The localisation and order of all markers is based on the information presented by Carpten *et al.* (21) and in the Genome Database ([www.gdb.org](http://www.gdb.org)).

All markers were fluorescently labelled for electrophoresis on a laser fluorescent sequencing machine (ABI 377, PE Biosystems, CA, USA). Markers from both sets were eliminated if they failed to amplify reproducibly or gave rise to ambiguous results. For the fluorescence detection, polymerase chain reactions (PCR) were performed in 10 µl reactions containing 20 ng genomic DNA, 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.3), 1.5 mmol/l MgCl<sub>2</sub>, 1.25 mmol/l of each deoxy-NTP, 10 mol/l oligodeoxynucleotide primer labelled with 4, 7, 2', 4', 5', 7'-hexachloro-6-carboxy-fluorescein, 6-carboxy-fluorescein or 4, 7, 2' 7'-tetra-chloro-6-carboxy-fluorescein, and 0.2 units DNA polymerase (Dynazyme/Taq polymerase). In all cases, the thermocycling conditions consisted of an initial denaturation step at 96 °C for 2 min, followed by 30 cycles of denaturation at 96 °C for 30 s, annealing at 55–62 °C for 30–45 s, elongation at 72 °C for 30 s, and a final 5 min extension at 72 °C.

The PCR products were pooled according to the emission spectra of the fluorescent dyes and mixed with formamide and the Genescan 500 size standard (Perkin Elmer) labelled with the dye *N, N, N', N'*-tetramethyl-6-carboxyrhodamine, electrophoresed in 5% polyacrylamide gels, and run on an ABI 377 laser-fluorescent sequencer (Perkin Elmer, CA, USA). The electropherogram results were analysed using the Genescan 3.1 computer software (PE Biosystems).

In the linkage calculations, individuals older than 50 years and without signs or symptoms of PHPT were scored as unaffected, and all clinically unaffected

members younger than 50 years of age were labelled as unknown. Multi-point linkage was calculated as the logarithm of odds (LOD) ratio with the GENEHUNTER program (22). The analyses were performed for individual families and FIHP was modelled as an autosomal dominant trait with 95% penetrance. Conventional cut-off scores for linkage were adopted, where LOD scores exceeding 3.0 signified linkage to a given marker, and those less than –2 excluded linkage.

### Loss of heterozygosity studies

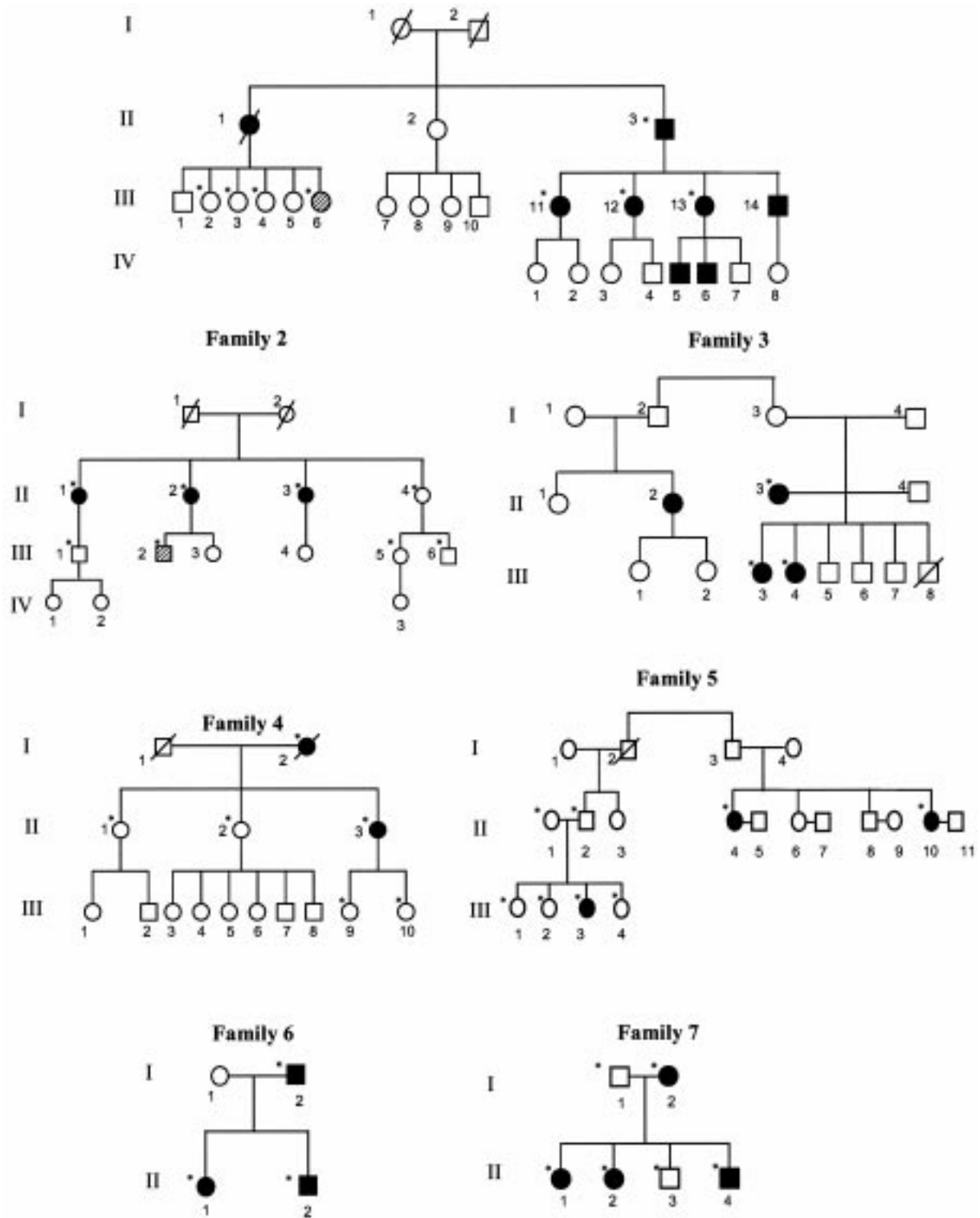
Thirteen matched blood–tumour pairs were genotyped with six polymorphic microsatellite markers in 11q13 and four markers in 1q21–q32. The electropherogram results permitted computerised calculations of relative allele intensities and loss of heterozygosity (LOH) was determined by comparing the intensities of the alleles and confirmed if a reduction of 50% in the signal intensity for the tumour DNA was observed in comparison with the corresponding allele in constitutional DNA.

### Automated sequencing

The nine coding exons of *MEN1* were fully sequenced in all affected members from each of the seven families (Fig. 1). The DNA (50 ng) was amplified in 50 mmol/l KCl, 10 mmol/l (pH 9.0), 2 mmol/l MgCl<sub>2</sub> (Perkin Elmer), 0.1 mmol/l dTTP, DATP, dGTP and dCTP, 0.1 mmol/l of each *MEN1* primer tagged with M13 and 5 U/µl Ampli Tag Gold (Perkin Elmer) in a final volume of 15 µl. The PCR reactions were carried out in a Hybaid Touchdown thermal cycler under the following conditions: an initial denaturation at 95 °C for 15 min was followed by 30 step cycles of denaturation at 96 °C for 30 s, annealing at 62 °C for 20 s, and extension at 72 °C for 30 s, and a final extension for 7 min at 72 °C. The PCR products were subjected to cycle sequencing using the Big Dye Primer cycle sequencing kit (ABI prism, Applied Biosystems, the Perkin Elmer Corporation, Foster City, CA, USA), with the thermocycling conditions as follows: denaturing at 95 °C for 2 min, 15 cycles of 95 °C for 30 s, 55 °C for 30 s and 70 °C for 1 min, 15 cycles of 95 °C for 30 s and 70 °C for 1 min. Finally, the products were run on an ABI 337 automated sequencer.

### Comparative genomic hybridisation and digital image analyses

DNA was extracted from four fresh-frozen tumour tissues and from formalin-fixed paraffin-embedded tumours in the remaining 12 cases, from which 11 were successfully analysed. DNA extraction from 15 to 20 paraffin sections (thickness 5–10 µm) was performed using the QIAamp Tissue Kit (Qiagen). Tumour DNA samples were labelled with fluorescein isothiocyanate-dUTP (FITC) (DuPont, Boston, MA,



**Figure 1** Pedigrees of the seven FIHP families included in this study. Filled symbols indicate affected family members, empty symbols represent currently unaffected individuals, and mutation carriers at risk of developing the disease are marked with hatching. All family members included in the mutation and linkage analyses are marked by asterisks (\*).



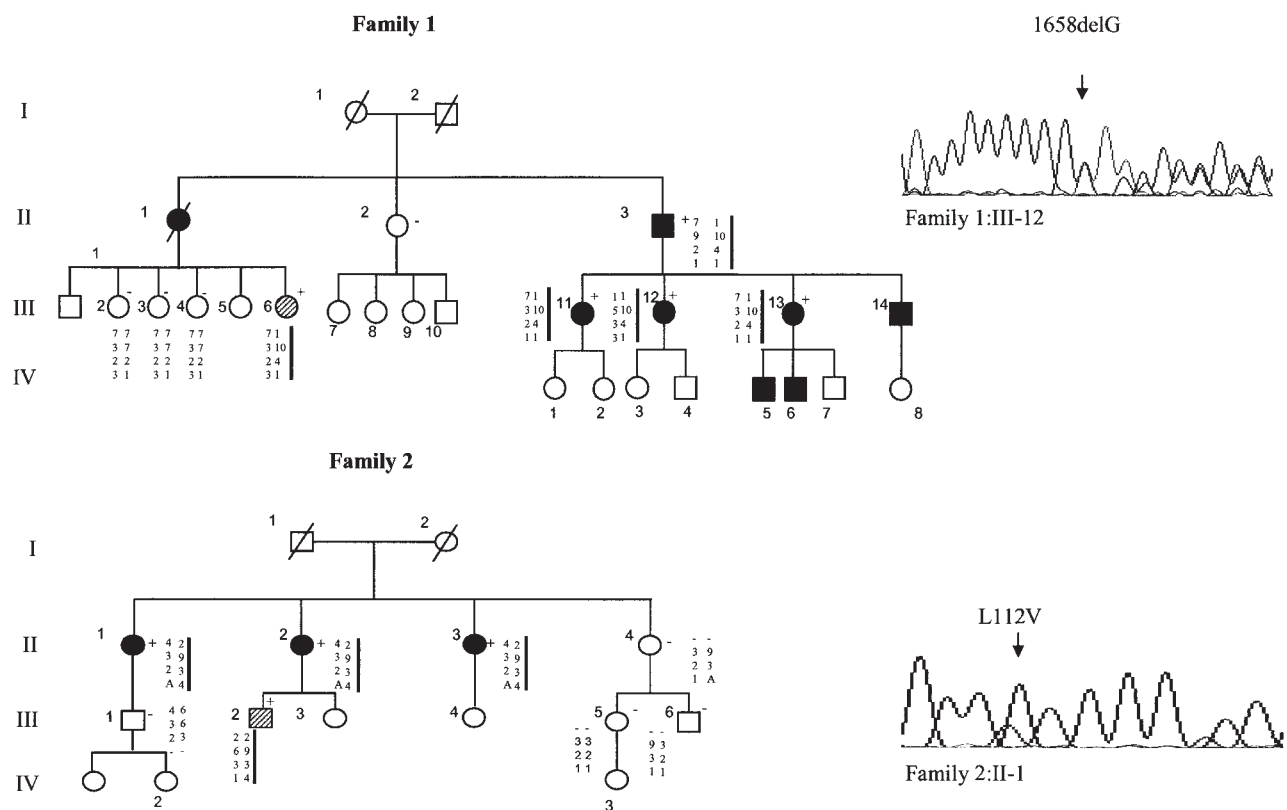
USA) by nick translation, mixed together with Texas Red-labelled normal reference DNA (Vysis Inc., Downers Grove, IL, USA). Tumour and reference DNA were mixed with unlabeled Cot-1 DNA (Gibco-BRL, Bethesda, MD, USA), denatured and applied to slides with denatured metaphases from normal lymphocytes (Vysis Inc.) for 72 h at 37 °C. After hybridisation, the slides were washed in  $0.4 \times \text{SSC}/0.3\% \text{ NP-40}$  at 74 °C for 2 min, and in  $2 \times \text{SSC}/0.1\% \text{ NP-40}$  at room temperature for 1 min. After drying, the slides were counterstained with 4,6-diamino-2-phenylindole (DAPI) (Vysis Inc.), to enable identification. A minimum of 10 (frozen) or 20 (paraffin) three-colour digital images (DAPI, FITC and Texas Red fluorescence) were captured from each hybridisation and microscopic analysis was performed using an Axioplan II images microscope (Carl Zeiss, Jena GmbH, Germany). Images were captured and analysed using the Isis imaging system (MetaSystems GmbH, Altussheim, Germany). DNA sequence copy number changes were detected by analysing the hybridisation intensities of tumour and normal DNAs along the length of all chromosomes in each metaphase spread. The absolute fluorescence

intensities were normalised and the final results were plotted as a series of green-to-red fluorescence ratios; ratios  $< 0.8$  were considered as losses of genetic material. Green-to-red ratios  $> 1.20$  were scored as gains of genetic material. For high-level amplification, the cut-off value of 1.5 was used. The Y chromosome and heterochromatic regions in the centromeric and paracentromeric parts of some chromosomes, and the short arm of acrocentric chromosomes were not included in the evaluation.

## Results

### MEN1 mutation analysis

The nine coding exons of *MEN1* were fully sequenced in all affected members from each of the seven families (Fig. 1). Germline *MEN1* mutations were identified in two of the seven families with PHPT studied (Fig. 2). These mutations consisted of one frameshift mutation in exon 10 (1658delG) and one missense mutation in exon 2, causing an amino acid change from leucine to valine (L112V) (Fig. 2). In family 1, the germline



**Figure 2** Demonstration of two constitutional *MEN1* mutations segregating with the disease phenotype in families 1 and 2. The presence (+) and (-) exclusion of the mutation are indicated next to the pedigree symbols. Filled symbols indicate affected family members, empty symbols indicate currently unaffected family members, and hatched symbols indicate family members at risk. The corresponding chromatogram sequences show the L112V missense and 1658delG frameshift mutations in one affected individual from each family.

1 bp deletion was found in four affected cases and one unaffected case, and the missense mutation L112V was detected in three affected members and one not yet affected member in family 2. The mutations have not previously been described. In addition, a polymorphism in exon 1 (N1755T → A) in families 3 and 6 and a possible intronic polymorphism in one affected member of family 5 (N5248T → C) was detected.

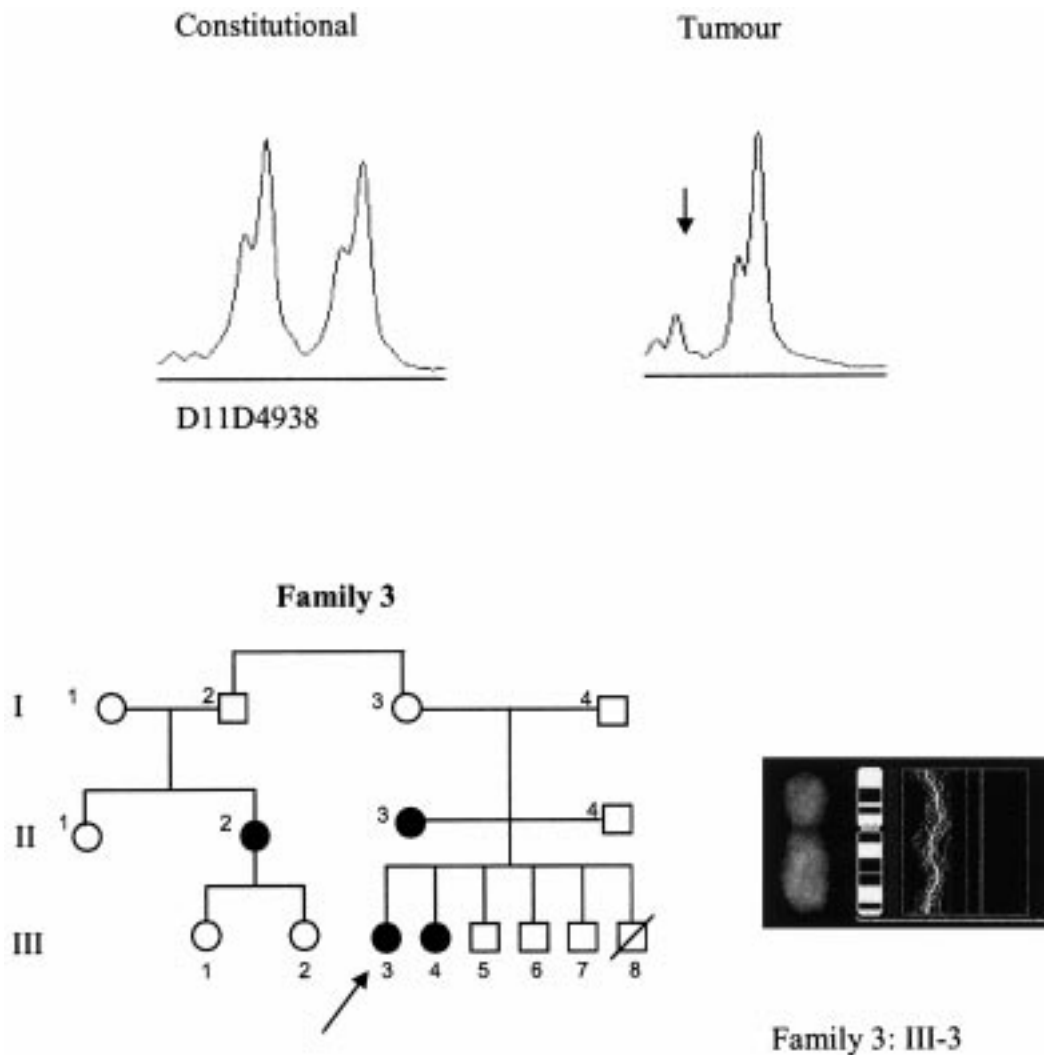
**Comparative genomic hybridisation alterations and LOH studies**

Eleven tumour samples were successfully analysed by comparative genomic hybridisation (CGH). Chromosomal imbalances were identified in eight of the tumours, with gains occurring in chromosomal regions 14q, 16q, 19q and X, and losses preferentially

involving chromosomal regions 11, 15q and 21q. Overall, the copy number changes were infrequent, and the most commonly detected aberrations were loss of chromosome 11 and gain of the X chromosome (Fig. 3 and Table 2). LOH was sought in parathyroid tumours from all families except family 2, from whom no tumour material was available. Polymorphic markers in 11q13 and in 1q21–q32 were used for LOH analysis. In total, 12 of the 13 parathyroid tumours showed LOH at any of the two loci, but no minimal common loss could be concluded (Tables 2 and 3).

**Linkage analyses and haplotyping**

In family 1, the detected 1658delG mutation predicted to cause a truncation co-segregated completely with the inheritance of the affected 11q13 haplotype in all



**Figure 3** Top: Chromatogram showing LOH for D11S4938 telomeric of the *MEN1* gene in the parathyroid tumour from the affected member III-3 in family 3. Bottom: Loss of the entire chromosome 11 demonstrated by CGH in the same tumour.

**Table 2** Summary from CGH and LOH studies of the parathyroid tumours studied.

Family no.	Case no.	Tumour weight of analysed gland (mg)	Genetic data				CGH alteration
			<i>MEN1</i> gene			1q21–q32	
			11q13	D11S4946	germline mutation		
1	II-3	1767	LOH <sup>wt</sup>	–	1658delG	+	
1	III-11	370	LOH <sup>wt</sup>	–	1658delG	+	
1	III-12	339	LOH <sup>wt</sup>	LOH <sup>wt</sup>	1658delG	+	
1	IV-6	4417					– 11
3	II-4	1800	LOH <sup>sa</sup>	–	None	+	– 11, – 15
3	III-3	2000	LOH <sup>sa</sup>	+	None	+	– 11
3	III-4	4000	+	+	None	+	– 11
4	I-2		LOH <sup>sa</sup>	–	None	LOH <sup>n-sa</sup>	
4	III-3	696	LOH <sup>sa</sup>	+	None	+	None
5	II-10	419	LOH <sup>n-sa</sup>	–	None	–	– 11
6	I-1	450	LOH <sup>n-sa</sup>	LOH <sup>n-sa</sup>	None	+	+X
6	II-1	700	LOH <sup>n-sa</sup>	LOH <sup>n-sa</sup>	None	+	None
6	II-2	3490	LOH <sup>n-sa</sup>	LOH <sup>n-sa</sup>	None	+	+X
7	I-2	1003	LOH <sup>n-sa</sup>	–	None	+	– 11, – 21q, + 14, + 16, + 19

wt, wild-type allele; LOH, loss of heterozygosity; sa, shared allele; n-sa, non-shared allele; +, retention of heterozygosity; –, not informative (i.e. constitutional homozygosity).

four affected and one mutation carrier analysed (Fig. 2) Three unaffected members analysed did not carry the disease haplotype and did not have the mutation. The single-point LOD scores were in agreement with a disease locus in 11q13 (0.8 for D11S4946) but not in 1q21–q32 (–1.7 for D1S1660). In addition, loss of the wild-type 11q13 alleles was demonstrated in three of the tumours analysed. In a fourth tumour, loss of chromosome 11 was demonstrated by CGH; LOH could not be analysed because of a lack of constitutional DNA.

The missense mutation in family 2 was demonstrated to co-segregate with the affected 11q13 haplotype in three affected sisters and the one gene carrier, whereas it was not present in four currently unaffected members who did not carry the affected haplotype (Fig. 2). The linkage was further supported by the positive LOD score for the 11q13 region (0.6 for D11S4937) but not for 1q21–q32 (–0.03 for D1S413).

Mutation screening and haplotype analysis in family 3 demonstrated no *MEN1* mutation, but linkage neither to 11q13 nor to 1q21–q32 could be excluded. However, two of the three tumours analysed showed loss of 11q by LOH and CGH, but no alteration in 1q.

Family 4 was negative for *MEN1* mutation, and the haplotype analyses suggested that the disease was not linked to 11q13 in this family. However, the 11q13 LOH identified in one of the tumours analysed involved the marker distal to *MEN1*. Nevertheless, from the haplotyping, a disease locus in the HPT-JT region could not be excluded. Interestingly, clinical examination revealed renal or cystic lesions, or both, in the affected individuals. LOH was detected in 1q of the non-shared allele.

No *MEN1* mutation was seen in family 5, but loss of the non-shared alleles was demonstrated in the one

tumour analysed. From the haplotyping analysis, linkage to either 11q13 or 1q21–q32 could not be excluded.

In each of families 6 and 7, only one affected member is older than 50 years, making the present diagnosis of FIHP uncertain. All four tumours analysed showed loss of the non-shared alleles in 11q involving the *MEN1* locus and retention of 1q. *MEN1* mutation screening was negative, and linkage and haplotyping were in agreement with a disease locus on 11q13 but not in 1q21–q32.

## Discussion

The identification of the *MEN1* tumour suppressor gene has prompted screening for its constitutional mutations in families presenting with FIHP. In this study, we report two novel *MEN1* mutations detected in two of seven families with PHPT only, but without signs or symptoms suggestive of MEN 1 or HPT-JT.

The mutations found in this study segregated with the affected haplotype, supporting their pathogenetic importance and also the hypothesis that FIHP can represent a genetic variant of the MEN 1 syndrome. Some FIHP families may initially present as isolated PHPT, but subsequently develop the full-blown syndrome, as demonstrated by a previously published HPT-JT family (20) and in a study of four apparent FIHP families (19). In this study, the families with detected *MEN1* mutations have been followed up for several years without any evidence of other endocrinopathies. In two families with demonstrated *MEN1* mutation, the diagnosis of FIHP was established at relatively advanced ages. This observation is in agreement with those in two other FIHP families, also associated with



**Table 3** Details of LOH analysis of the parathyroid tumours studied.

Family no.	Case no.	11q13 LOH					1q21–q32 LOH				
		Pygm	D11S4946	D11S4940	D11S4938	D11S4937	D11S449	D1S466	D1S191	D1S408	D1S413
1	II-3	LOH <sup>wt</sup>	–	–	LOH <sup>wt</sup>	–	–	–	–	–	–
1	III-11	–	–	–	–	LOH <sup>wt</sup>	–	–	–	–	–
1	III-12	–	LOH <sup>wt</sup>	–	LOH <sup>wt</sup>	–	–	–	–	–	–
3	II-3	–	–	–	LOH <sup>sa</sup>	–	–	–	+	+	–
3	III-3	–	+	–	LOH <sup>sa</sup>	–	–	+	+	+	+
3	III-4	–	+	–	–	–	–	+	–	+	+
4	I-2	–	–	–	–	LOH <sup>sa</sup>	–	LOH <sup>n-sa</sup>	+	+	+
4	II-3	–	–	–	–	LOH <sup>sa</sup>	–	+	+	+	–
5	II-10	LOH <sup>n-sa</sup>	–	–	–	–	–	–	+	–	+
6	I-2	–	LOH <sup>n-sa</sup>	–	LOH <sup>n-sa</sup>	LOH <sup>n-sa</sup>	–	LOH <sup>n-sa</sup>	+	+	+
6	II-1	–	LOH <sup>n-sa</sup>	–	LOH <sup>n-sa</sup>	–	–	+	+	+	+
6	II-2	LOH <sup>n-sa</sup>	LOH <sup>n-sa</sup>	–	LOH <sup>n-sa</sup>	–	–	LOH <sup>n-sa</sup>	+	+	–
7	I-2	LOH <sup>n-sa</sup>	–	LOH <sup>n-sa</sup>	–	–	LOH <sup>n-sa</sup>	–	+	–	+

wt, wild-type allele; sa, shared allele; n-sa, non-shared allele; LOH, loss of heterozygosity; +, retention of heterozygosity; –, not informative (i.e. constitutional homozygosity or not done).

*MEN1* mutations and PHPT occurring at greater ages than in MEN 1 (12, 13). The phenotypic variation might represent a milder effect of the underlying mutations. Previously, it has also been suggested that the mutations in FIHP families could be functionally milder (12, 13), in the sense that the most commonly occurring mutations are missense and in-frame deletions (23) and may therefore be the cause of partial MEN 1 phenotype expression.

The *MEN1* tumour suppressor gene encodes a 67 kDa protein (10), menin, which is generally localized in the nucleus, with two independent nuclear localization signals (24). The first recognized interacting protein has proved to be the AP1 transcription factor, JunD (25), and the menin–JunD interaction inhibits transcription stimulated by JunD. Some *MEN1* gene mutations have been found to disrupt binding activity, leading to an increase in JunD-activated transcription. Detailed mapping of the specific menin-interacting regions are likely to provide valuable information concerning the functional role of menin. The consequences of FIHP- and MEN 1-associated mutations with respect to tumour development need to be further explored. However, one might speculate that the mutations have less dramatic effects on menin–protein interactions.

The second putative tumour suppressor gene involved in the genesis of FIHP is located on chromosome 1q and is responsible for the HPT-JT syndrome (5). On the basis of the findings of wild-type allele losses in 1q-associated renal tumours (6, 7), we addressed the involvement of the HPT-JT locus in these families by using LOH studies and genotyping. Allelic alterations at 1q were not frequently detected in the tumours investigated, and the information obtained by linkage analysis could not be readily interpreted, because of the size of the families. However, the possibility that the parathyroid tumours in some of the

families harbour small deletions or somatic mutations cannot be excluded, and the identification of the *HRPT2* gene will help to elucidate the role of such a gene in the hereditary forms of parathyroid tumours in FIHP. In one of the families, the clinical picture was suggestive of 1q involvement, because renal features were seen in two patients. However, radiographic examination of the jaws did not reveal the presence of jaw tumours.

It is uncertain at present whether FIHP families with genetic defects other than of the *MEN1* or *HRPT2* genes may exist. In an attempt to investigate further the other regions involved, we extended the tumour studies by applying CGH analysis. Two tumours from one family shared gain of the X chromosome only. Genetic gain of chromosome X has previously been observed in familial parathyroid tumours and, recently, as a recurrent aberration in a study of sporadic parathyroid adenomas (26, 27). The contribution of this genetic alteration to the development of parathyroid tumours in FIHP is at present unknown.

However, six of the eight parathyroid tumours with CGH alterations revealed loss of chromosome 11, either as a single abnormality or together with one or more other alterations. This could suggest that, in the majority of FIHP families, the disorder is caused by one or more different disease genes located on chromosome 11. The lack of demonstrable *MEN1* mutations in five of the seven families analysed underlies the genetic heterogeneity of FIHP. However, we cannot exclude involvement of mutations in other regions of *MEN1*, such as intronic or regulatory parts. Furthermore, the existence of a possible second parathyroid gene in 11q is supported by the LOH outside *MEN1* in families 3 and 4, and by distal 11q deletions in sporadic parathyroid tumours (28). In contrast, the loss of 11 could represent a secondary alteration occurring after the inactivation of the predisposing disease gene.

In summary, FIHP is a genetically heterogeneous disease, caused by the *MEN1* gene in a substantial proportion of cases. The reason why some mutations of the *MEN1* gene are associated with typical MEN 1 expression and give rise to the phenotype is unclear. The identification of the putative HPT-JT gene and future functional studies of *MEN1* and FIHP are likely to shed light on the tissue specific involvement in FIHP.

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