Abstract

Objective: Familial isolated primary hyperparathyroidism (FIHP) is defined as hereditary primary hyperparathyroidism without the association of other diseases or tumors. Linkage analyses suggest that different genotypes can lead to the same phenotype of primary hyperparathyroidism. Hereditary syndromes associated with primary hyperparathyroidism are multiple endocrine neoplasia type 1 and type 2 (MEN 1 and MEN 2). In MEN 1, multiple parathyroid adenomas occur in more than 90% of the patients. Therefore, it has been suggested that FIHP could represent a variant or partial expression of MEN 1.

Design: We report on a large FIHP kindred with a MEN1 gene mutation. Nineteen family members (aged 10 to 87 years) were screened. Furthermore, statistical comparison by Fisher’s exact tests of FIHP families with MEN1 gene mutations and MEN 1 families with two or more endocrinopathies was carried out to investigate genotype–phenotype correlations.

Methods: Mutational analysis of leucocyte DNA was carried out by direct sequencing of the complete coding region of the MEN1 gene. Screening of MEN 1 manifestations was carried out by determination of serum calcium, phosphate, parathyroid hormone, prolactin, ACTH, cortisol, IGF-I, gastrin, glucose, insulin, glucagon, serum potassium, aldosterone, plasma renin and urinary hydroxyindoleacetic acid.

Results: We detected an in-frame deletion mutation in exon 8 of the MEN1 gene resulting in the deletion of one glutamine acid residue at position 363. It was found in eight individuals. Two of these family members (aged 42 and 60 years) were operated for primary hyperparathyroidism, and three (aged 13 to 40 years) showed mild hypercalcemia and parathyroid hormone levels within the upper normal range or slightly elevated, without any clinical symptoms. Two individuals (aged 12 and 19 years) were normocalcemic. One could not be tested. None of them had clinical evidence of other MEN 1 manifestations. Statistical comparison of the mutation types in families with FIHP and families with tumors in various endocrine glands these mutation types occur much less frequently (21–34%, \( P < 0.05 \)).

Conclusions: These studies indicate that FIHP can represent a partial MEN 1 variant and is often caused by missense/in-frame mutations.
the MEN 1 patients (10). Therefore, it has been suggested that FIHP may represent a variant or partial expression of MEN 1. Indeed, in a family with FIHP mapping to chromosome 11q13, the location of the MEN1 gene has been described (11). Since the identification of the MEN1 gene as a putative tumor suppressor gene (12), families with FIHP have been screened for mutations within the MEN1 gene. The results are inconclusive. To date, studies of 10 families with FIHP could not find mutations within the MEN1 gene (13–16). On the other hand, MEN1 gene mutations were detected in 7 families presenting with primary hyperparathyroidism (17–23). It should be noted that the patients and family members tested in these studies have not always been investigated for genetic linkage to chromosome 11q13 or for other MEN 1-associated manifestations.

In conclusion, it remains an open question whether FIHP can represent a variant of MEN 1 or rather a distinct entity related to other genetic loci, for instance on chromosome 1q21-32. Furthermore, these studies and findings of other genetic syndromes raise the question whether it is possible to relate a specific phenotype to a specific genotype.

Herein, we report on a large FIHP kindred with a MEN1 gene mutation but no clinical or laboratory evidence of other MEN 1-associated manifestations. This strengthens the hypothesis that FIHP can be a rare variant of the MEN 1 syndrome. Furthermore, reports on FIHP families with MEN1 gene mutations suggest the possibility that this phenotype correlates to a specific MEN1 genotype.

Subjects and methods

The index patient is a 42-year-old female (II/3) presenting with hypercalcemia and nephrolithiasis at the age of 40. Total serum calcium was 2.88 mmol/l (normal range: 2.02–2.60 mmol/l), ionised calcium 1.57 mmol/l (normal range: 1.12–1.23 mmol/l), phosphate 0.71 mmol/l (normal range: 0.84–1.45 mmol/l) and parathyroid hormone 140 pg/ml (normal range: 10–65 pg/ml). Urinary calcium excretion was elevated (14 mmol/day; normal range: <6.25 mmol/day). At the time of diagnosis there was no sign of renal stones or osteoporosis. Ultrasonography of the neck showed a hypodense lesion within the right thyroid lobe, which scintigraphically appeared as a cold lesion. Repeated surgical neck explorations for persisting hyperparathyroidism revealed one parathyroid adenoma of the right upper parathyroid gland and a second parathyroid tumor within the right thyroid lobe which were removed. Control of serum calcium and parathyroid hormone levels one year after the operations were within normal limits. The mother of the patient (I/2) died of an unknown cancer at the age of 71 years, one sister (II/4) has been operated for primary hyperparathyroidism and one brother (II/6) is known to suffer from primary hyperparathyroidism.

Clinical studies of the family members included evaluation of the serum electrolytes, parathyroid hormone, gastrin, glucagon, prolactin, insulin-like growth factor I (IGF-I), adrenocorticotropin (ACTH), cortisol, aldosterone, plasma renin, as well as urinary hydroxyindoleacetic acid concentration. In case of any abnormality, further tests were initiated including secretin stimulation of gastrin, glucose and dexamethasone suppression tests as well as abdominal ultrasonography or magnetic resonance imaging (MRI) scan of the hypophysis.

Informed consent to participate in the study was obtained from all family members.

Mutational analysis

Leucocyte DNA was extracted using the QIAamp blood kit (Qiagen, Chatsworth, CA, USA). One hundred nanograms extracted DNA was amplified in a 50 μl PCR. The reaction was carried out with the Prime Zyme PCR Kit (Biometra, Goettingen, Germany) and 200 nmol/l of forward and reverse primer. Ten primer pairs were used to amplify exon 2 to 10 of the MEN1 gene. PCR conditions were as follows: initial denaturation at 92 °C for 3 min, 35 cycles at 95 °C for 30 s, 58–60 °C for 30 s, 72 °C for 30 s and a final extension of 72 °C for 10 min (24).

Approximately 50 ng of the PCR product was used for direct sequencing with the Applied Biosystems PRISM dye terminator cycle sequencing ready reaction kit (Perkin Elmer, Applied Biosystems, Foster City, CA, USA). The sequence variation detected was confirmed in another blood sample of the subject by repeating the PCR and sequencing reaction.

Statistical analysis

Fisher’s exact tests were applied to compare the frequencies of the different mutation types, i.e. missense/in-frame mutations versus nonsense/frameshift mutations in FIHP families with the mutation types in families presenting with two or more of the typical MEN 1-related endocrinopathies.

Results

Nineteen family members representing four generations (aged between 10 and 87 years) were genetically screened according to the above guidelines. If mutational analysis was positive, the family member was screened by clinical and laboratory investigations as described.

Mutational analysis of leucocyte DNA of the index patient (II/3) revealed an in-frame deletion mutation in exon 8 of the MEN1 gene resulting in the deletion of a glutamine acid residue at position 363. One daughter

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(III/9) and the son (III/10) of the index patient showed the same genetic abnormality. Genetic screening of the sister of the index patient (II/4) who had been operated for primary hyperparathyroidism revealed the same mutation as expected. Furthermore, the mutation E363del was detected in leucocyte DNA from two of four of the children (III/11, III/13) of II/4 as well as in leucocyte DNA of the daughter of III/11 (IV/22). One brother of the index patient (II/6) known to suffer from primary hyperparathyroidism refused all further diagnostic tests. However, blood could be obtained from his son (III/18), which showed the same genetic abnormality. Two brothers (II/5, II/7) and the father (I/1) of the index patient did not harbor the germline mutation E363del (see Fig. 1).

Subsequently, all identified patients and gene carriers were tested for clinical signs and abnormal parameters pointing to the development of primary hyperparathyroidism or other MEN 1-associated endocrinopathies as described in the Methods section. One daughter and one son of II/4 (III/11 and III/13) as well as the son of the index patient (III/10) showed mild hypercalcemia.

Table 1 Clinical data of the MEN 1 patients and mutant gene carriers.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Serum calcium (mmol/l)*</th>
<th>Serum phosphate (mmol/l)*</th>
<th>Parathyroid hormone (pg/ml)*</th>
<th>Parathyroidectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>II/3</td>
<td>42</td>
<td>2.88</td>
<td>0.71</td>
<td>140</td>
<td>Yes</td>
</tr>
<tr>
<td>II/4</td>
<td>60</td>
<td>3.00</td>
<td>0.60</td>
<td>133</td>
<td>Yes</td>
</tr>
<tr>
<td>II/6</td>
<td>48</td>
<td>2.80</td>
<td>0.90</td>
<td>119</td>
<td>No</td>
</tr>
<tr>
<td>III/9</td>
<td>19</td>
<td>2.49</td>
<td>1.13</td>
<td>34</td>
<td>No</td>
</tr>
<tr>
<td>III/10</td>
<td>13</td>
<td>2.61</td>
<td>1.63</td>
<td>91</td>
<td>No</td>
</tr>
<tr>
<td>III/11</td>
<td>34</td>
<td>2.71</td>
<td>1.02</td>
<td>67</td>
<td>No</td>
</tr>
<tr>
<td>III/13</td>
<td>40</td>
<td>2.60</td>
<td>0.99</td>
<td>40</td>
<td>No</td>
</tr>
<tr>
<td>III/18</td>
<td>28</td>
<td>n.a</td>
<td>n.a</td>
<td>n.a</td>
<td>No</td>
</tr>
<tr>
<td>IV/22</td>
<td>12</td>
<td>2.49</td>
<td>1.60</td>
<td>37</td>
<td>No</td>
</tr>
</tbody>
</table>

*Normal range values: calcium 2.02–2.60 mmol/l; phosphate 0.84–1.45 mmol/l; parathyroid hormone 10–65 pg/ml.

n.a., not available.
and parathyroid hormone levels which were within the upper normal range or slightly elevated in the absence of clinical symptoms, indicating the development of primary hyperparathyroidism. Two mutant gene carriers (aged 12 and 19 years) showed no clinical signs or abnormal biochemical parameters. No blood or urine samples for biochemical analyses could be obtained from III/18 (aged 28 years) as he did not attend our clinic (see Table 1). During the three-year follow-up of patients II/3 and II/4, no other MEN 1-associated endocrinopathy was detected. Moreover, no family member showed clinical signs pointing to other genetic syndromes, i.e. hyperparathyroidism-jaw tumor syndrome or MEN 2.

Furthermore, we tested whether this specific phenotype of MEN 1 with primary hyperparathyroidism as the only clinical manifestation is related to a common genotype. To date, there are seven reports about families with FIHP and MEN1 gene mutations; their characteristics are shown in Table 2. They demonstrate a striking predominance of missense or in-frame mutations of the MEN1 gene in families with FIHP. Therefore, we compared the frequencies of the different mutation types, i.e. missense/in-frame mutations versus nonsense/frameshift mutations in FIHP families, with the mutation types in families presenting with two or more of the typical MEN 1-related endocrinopathies, published in five major European, American and Japanese studies (13–15, 17, 25). Indeed, using Fisher’s exact test, missense/in-frame mutations have been found significantly more often in families with parathyroid adenomas only (ratio of missense/in-frame mutations: nonsense/frameshift mutations=7:1) than in five studies of families with two or more endocrinopathies (missense/in-frame mutations: nonsense/frameshift mutations ratio in study 1 (13) = 16 : 31 (P = 0.007); in study 2 (17) = 1 : 3 (P = 0.07); in study 3 (25) = 10 : 36 (P = 0.0007); in study 4 (14) = 13 : 45 (P = 0.0006); in study 5 (15) = 10 : 24 (P = 0.004)).

Discussion

In this study, we report on a family with FIHP in which we detected a mutation of the MEN1 gene in three affected and five unaffected individuals. This result further supports the hypothesis that FIHP can indeed represent a rare variant of the MEN 1 syndrome. Furthermore, we show for the first time that missense and in-frame mutations of the MEN1 gene lead significantly more often to the ‘partial’ MEN 1 phenotype of FIHP than to the classical MEN 1 phenotype of two or more endocrinopathies.

Of the nineteen family members examined in this study, eight were shown to harbor the same mutation leading to the deletion of one glutamic acid at position 363 of the menin protein. Clinical symptoms and/or hypercalcemia pointing to primary hyperparathyroidism were found in five patients, two of them were operated upon and found to have parathyroid hyperplasia or adenomas respectively. In one patient who denied mutational analysis, this genetic variation is highly likely since he suffers from primary hyperparathyroidism and the mutation could be identified in leucocyte DNA of his son (see Table 1).

Screening of all the family members for other MEN 1-associated manifestations was negative. Moreover, follow-up of two affected family members did not show evidence of further endocrinopathies. However, it cannot be completely excluded that other MEN 1-related tumors may still develop, for instance gastrinomas or somatotropinomas, for which the predominant age of manifestation is above 40 years (10). Further follow-up of our patients will answer this question. Nevertheless, to date studies document more than seven families with primary hyperparathyroidism as the only manifestation of MEN 1 (see Table 2). This indicates that FIHP can represent a partial variant of the MEN 1 syndrome.

The MEN1 gene mutations generally described in FIHP families are missense and in-frame deletion mutations leading to an altered amino acid sequence (18–23). Only one nonsense mutation leading to truncation of the gene product, menin, has been described for FIHP families (17). The mutation we found results in the deletion of one amino acid leading to an altered amino acid sequence. These results suggest that missense/in-frame deletion mutations, in particular, may lead to the specific or partial MEN 1 syndrome.

Table 2 Comparison of reported FIHP kindreds with MEN1 gene mutations with respect to mutation type, histological findings, affected members, mutant gene carriers and years of follow-up.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Mutation</th>
<th>Parathyroid histology</th>
<th>No. of affected members (gene carriers)</th>
<th>Follow-up (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shimizu et al.</td>
<td>Y353X</td>
<td>Hyperplasia</td>
<td>3 (no data)</td>
<td>None</td>
</tr>
<tr>
<td>Ohye et al.</td>
<td>L414del</td>
<td>Hyperplasia</td>
<td>4 (no data)</td>
<td>4 to 14</td>
</tr>
<tr>
<td>Fujimori et al.</td>
<td>V194E</td>
<td>Hyperplasia</td>
<td>3 (2)</td>
<td>3.5 to 6.5</td>
</tr>
<tr>
<td>Teh et al.</td>
<td>E255K</td>
<td>Hyperplasia</td>
<td>7 (4)</td>
<td>4</td>
</tr>
<tr>
<td>Poncin et al.</td>
<td>L267P</td>
<td>Not available</td>
<td>4 (no data)</td>
<td>None</td>
</tr>
<tr>
<td>Honda et al.</td>
<td>G305D</td>
<td>Hyperplasia</td>
<td>3 (2)</td>
<td>≤5</td>
</tr>
<tr>
<td>Kassem et al.</td>
<td>Q269P</td>
<td>Hyperplasia/adenoma</td>
<td>14 (3)</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Miedlich et al.</td>
<td>E363del</td>
<td>Hyperplasia/adenoma</td>
<td>6 (3)</td>
<td>3</td>
</tr>
</tbody>
</table>

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phenotype of parathyroid adenomas in the absence of other endocrinopathies. Indeed, statistical comparison of the mutation types in families with FIHP and families with two or more MEN 1-related endocrinopathies reveals a significant difference. In families with FIHP, missense/in-frame mutations have been found in 87.5% of cases whereas in families with more than one endocrinopathy these mutation types occur much less frequently (21–34%).

However, the mutation E363del has been described in other MEN 1 kindreds with more than one endocrinopathy (12, 13). Others (26) describe this genetic abnormality in a lymph node metastasis of a gastrinoma. To date, gastrin levels of all screened members of this MEN 1 kindred are within the normal range. However, continued biochemical screening is mandatory to identify any patients with possible Zollinger-Ellison-Syndrome or other MEN 1-associated manifestations.

The MEN1 gene encodes a 67 kDa protein, menin (12). As the tumors of affected individuals showed loss of the wild-type allele at chromosome 11q13, it has been suggested that menin functions as a tumor suppressor (27). The frequent protein truncation mutations in typical MEN 1 families further support the hypothesis that the gene becomes inactivated consistent with the first hit to a tumor suppressor gene (28). Recently, menin was found to localize to the nucleus, with two independent nuclear localization signals (29). Functional studies by a yeast two-hybrid screen identified JunD as an interacting partner of menin, which points to involvement in transcriptional regulation (30). However, the precise cellular functions of menin are yet to be identified and so are the consequences of the diverse mutations with respect to tumor development.

Other clinical studies of patients with neurofibromatosis type 2 caused by mutations within the so called NF2 gene on chromosome 22 have shown that patients with truncating mutations presented earlier and developed at least two symptomatic CNS tumors in addition to the typical vestibular schwannoma before 30 years, whereas members of other families with mutations that affected a single amino acid had only mild disease (31, 32). These results, in addition to ours, demonstrate that the mutation type may at least partially influence the course and severity of the disease.

In summary, these data suggest that FIHP can indeed represent a partial MEN 1 variant. It is mostly caused by missense/in-frame mutations of the MEN1 gene which may point to an influence of the mutation type on the phenotype of the disease. However, the finding of different phenotypes associated with the same genotype E363del strongly suggests that other genetic and/or epigenetic factors influence the penetrance and clinical manifestation of MEN 1. Moreover, genotype–phenotype correlations could also be masked by several other variables, including different clinical follow-up in different medical centers and lack of information in some families. Last but not least, in vitro analyses of the functional impairment of the protein by different mutations have to be awaited to draw further conclusions.

References


