CLINICAL STUDY

Frequency of somatic MEN1 gene mutations in monoclonal parathyroid tumours of patients with primary hyperparathyroidism

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Abstract

Objectives: Investigation of small numbers of parathyroid tumours by X-chromosome inactivation analysis suggests that the majority of them are monoclonal lesions most likely caused by a somatic mutation. Somatic mutations in the MEN1 gene located on chromosome 11q13 have recently been identified in 12–17% of solitary parathyroid tumours in patients with sporadic primary hyperparathyroidism, and they may be the precipitating genetic defect leading to monoclonal cell proliferation in these tumours.

Design: To determine the prevalence of MEN1 gene mutations in monoclonal parathyroid neoplasias we investigated 33 parathyroid tumours of patients with primary hyperparathyroidism for clonality and mutations in the MEN1 gene.

Methods: X-chromosome inactivation analysis was used to assess the clonal status of the tumours, direct sequencing of the complete coding region was applied to identify mutations in the MEN1 gene. Somatic mutations in the MEN1 gene were identified in nine cases. Six of them were found in the relatively large second exon of the MEN1 gene (A49D, 193del36, 402delC, 482del22, 547delT, W126X). One was found in exon 5 (904del9), one in exon 7 (Y327X) and one in exon 9 (R415X). Of the monoclonal tumours, 5 out of 19 (26%) harboured a somatic MEN1 gene mutation.

Conclusions: In summary, 73% of the solitary parathyroid adenomas were monoclonal. In 26% of the monoclonal tumours a somatic MEN1 gene mutation has been identified. However, for 74% of monoclonal tumours of the parathyroids the underlying genetic defects are still not known.

CLINICAL STUDY

Introduction

The molecular pathophysiology of parathyroid neoplasia is still incompletely understood. A solitary adenoma is found in 80–85% of the patients with primary hyperparathyroidism whereas multiglandular enlargement has been observed in 15–20% (1). It is widely accepted in tumour biology that neoplasms originate from a single cell with a somatic mutation conferring a growth advantage upon it and its progeny (2). To date, two genetic abnormalities have been identified in benign parathyroid tumours. In a small subset of parathyroid tumours, activation of the PRADI (parathyroid adenoma 1)/cyclin D1 oncogene has been found. It is caused by the rearrangement of the parathyroid hormone (PTH) promotor in proximity to the PRADI gene, a cell cycle regulator (3, 4). Moreover, somatic mutations of the MEN1 (multiple endocrine neoplasia type 1) gene located on chromosome 11q13 have recently been identified in 12–17% of solitary parathyroid tumours of patients with sporadic primary hyperparathyroidism (5–7). The finding that the tumours with somatic MEN1 gene mutations show allelic loss on 11q13 very likely indicates that they are monoclonal neoplasias. However, loss of heterozygosity (LOH) on 11q13 in microdissected parathyroid tumours is not necessarily associated with a monoclonal pattern of X-chromosome inactivation analysis (8, 9), which suggests that LOH may not be a singular event.

X-chromosome inactivation analysis is a different method to study clonality of neoplasias. It is based on the functional inactivation of one of the two X-chromosomes in all female somatic cells in early embryo genesis by DNA methylation at CpG sites (10). The established pattern of X-chromosome inactivation in one cell is passed on to its progeny. In a polyclonal tissue, random

inactivation of paternal and maternal X-chromosomes can be found whereas monoclonal cell expansions show an identical pattern of inactivation of either the paternal or the maternal X-chromosome.

Two previous studies which investigated clonality of parathyroid tumours by X-chromosome inactivation analysis in 8 patients each indicate that the majority of the solitary adenomas are monoclonal lesions (11–13). However, the few tumours tested in these studies were only defined by histopathological rather than molecular criteria and somatic mutations were not determined. Therefore, the percentage of somatic mutations in the MEN1 gene in monoclonal parathyroid neoplasms is not known to date. Moreover, whereas clonality was previously found in 75% to 90% of solitary parathyroid tumours, LOH on 11q13 was only found in 27% to 39%. These discrepant results raise the question whether the examined tumours with neither MEN1 gene mutations nor LOH on 11q13 are monoclonal neoplasias too. If so, this would imply that the majority of clonal cell expansions in the parathyroids are caused by other somatic mutations rather than the MEN1 gene mutations.

The intention of this study was, therefore, to test the hypothesis whether nearly all parathyroid tumours are monoclonal neoplasms and to answer the question as to what percentage MEN1 gene mutations can be found in monoclonal cell expansions. Evidence for clonality in a parathyroid tumour with a somatic mutation in the MEN1 gene would identify this somatic mutation as a likely precipitating genetic defect initiating the monoclonal cell expansion.

**Subjects and methods**

Thirty-three patients referred for parathyroidectomy for primary hyperparathyroidism in a consecutive series between April 1998 and February 1999 were included in the study. The morning before the operation, blood was collected for determination of total and ionized calcium, phosphate, creatinin and intact parathyroid hormone (IMMLITE Intact PTH Enzyme Immuno- metric Assay, DPC, Biomedical Analytics, GmbH, Gottingen, Germany) as well as for extraction of leucocyte DNA. The intraoperative procedure included exploration of at least two parathyroid glands in the case of a solitary adenoma and all four glands in the case of more than one enlarged gland. Half of the enlarged gland was used for histopathological analysis and the other half was snap-frozen in liquid nitrogen and stored at −80 °C. The diagnosis of parathyroid adenoma or hyperplasia respectively was made by histological analysis. Criteria for a parathyroid adenoma are evidence of a capsule, almost complete absence of fat cells, and normal non-hyperplastic parathyroid tissue at the rim or in other removed parathyroid glands (14). According to these criteria, 31 patients had a solitary adenoma whereas in two cases parathyroid hyperplasia was diagnosed. All female patients with primary hyperparathyroidism were postmenopausal, three receiving hormone replacement therapy. None of them had clinical evidence of other endocrinopathies or a family history of other MEN1 manifestations. One patient was on haemodialysis for three years before kidney transplantation. However, she did not develop secondary but primary hyperparathyroidism which manifested shortly after kidney transplantation. Informed consent to participate in the study was obtained from all patients before surgery.

**X-chromosome inactivation analysis**

Frozen parathyroid tissue was mechanically disrupted and digested with proteinase K. Leucocyte DNA was extracted using the QIAamp blood kit (Qiagen, Chatsworth, CA, USA). X-chromosome inactivation analysis was repeated with microdissected paraffin-embedded parathyroid tissue for all tumours initially identified as polyclonal. For this purpose, microscopically identified hypercellular areas were circled on the glass slide. Tissue from the marked areas of three consecutive slides was microdissected and digested with proteinase K for extraction of DNA.

To determine random versus nonrandom X-chromosome inactivation we used a PCR approach described by Allen et al. (15). Conditions were the same as described by Krohn et al. (16). Briefly, amplification of a variable number of tandem repeats in the first exon of the androgen receptor (HUMARA) was studied after digestion with the methylation-sensitive restriction enzyme, HpaII. Two distinct PCR fragments are found in the case of heterozygosity for the tandem repeats. Nonrandom X-chromosome inactivation results in one diminished allele after amplification of the digested DNA, and is suggestive of monoclonality. A polyclonal origin with random X-chromosome inactivation would lead to reduction of both alleles. For detection of the PCR products, a 6-carboxy-fluorescein-labelled forward primer was used (Perkin Elmer Applied Biosystems, Foster City, CA, USA). PCR products were quantified on an ABI 373 Genetic Analyzer (Perkin Elmer Applied Biosystems) with the GeneScan software. The ratio of the numbers for the two alleles was calculated, and the ratio of the undigested DNA was divided by the ratio of HpaII-digested DNA, according to Delabesse et al. (17). If necessary, the resulting number was inverted to give a ratio greater than 1. The resulting index is close to 1 if the tissue is polyclonal. An index of 2 is more than two times the s.d. above the average index for a group of samples of polyclonal thyroid tissue (16) and was chosen as the lowest index indicating monoclonality.

**Sequencing**

One hundred nanograms extracted tumour DNA were amplified in a 50 µl PCR. The reaction was carried out with the Prime Zyme PCR kit (Biometra, Diagnostic Prod.
Results

Analysis of the clinical data

Mean age of the 33 patients with primary hyperparathyroidism was 62 ± 9 years, mean values (±standard deviation) of serum total calcium were 2.98 ± 0.22 mmol/l (normal range: 2.02–2.60 mmol/l), of ionized calcium 1.59 ± 0.16 mmol/l (normal range: 1.12–1.23 mmol/l), of phosphate 0.77 ± 0.14 mmol/l (normal range: 0.84–1.45 mmol/l), of intact parathyroid hormone 150 ± 63 pg/ml (normal range: 10–65 pg/ml) and of creatinin 93 ± 19 μmol/l (normal range: female 44–80 μmol/l, male 53–97 μmol/l).

Comparison of the clinical and biochemical parameters (age, serum total and ionized calcium, phosphate, creatinin and parathyroid hormone levels) revealed no significant differences between patients with or without MEN1 gene mutations and polyclonal or monoclonal tumours (P values between 0.06 and 0.9, see Table 2).

X-chromosome inactivation analysis

X-chromosome inactivation was assessed for tumour tissue of 31 women (29 with a solitary adenoma, 2 with hyperplasia). Twenty-eight subjects (90%) were heterozygous for the (CAG)n-polymorphism on exon 1 of the

Table 1 List of the primer pairs used together with the annealing temperatures and the PCR product lengths.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Annealing temp. (°C)</th>
<th>Product (base pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-1</td>
<td>AACCTTAGCGGACCCTGGG</td>
<td>ACGGGGAAATGAGTGGAGGC</td>
<td>58</td>
<td>336</td>
</tr>
<tr>
<td>2-2</td>
<td>AACCTTAGCGGACCCTGGG</td>
<td>TAAATGTTCCACCTGAGG</td>
<td>58</td>
<td>304</td>
</tr>
<tr>
<td>3</td>
<td>ATCTGAAGGTTGGGTCACAGG</td>
<td>AAATGGGAAGTCCCTTGGTG</td>
<td>59</td>
<td>401</td>
</tr>
<tr>
<td>4</td>
<td>ACAATGATATCGACTTCAGCC</td>
<td>CAGGGGAGCTGAGTGGAGGC</td>
<td>60</td>
<td>289</td>
</tr>
<tr>
<td>5/6</td>
<td>GTTAGTAATTTCCTGAGCTGGG</td>
<td>TGTCCTAGGCAGCTGTTAGG</td>
<td>60</td>
<td>479</td>
</tr>
<tr>
<td>7</td>
<td>CTTAGGATCCTTGGCTCTACAC</td>
<td>GATGGAGGGGAAGAAAGGAC</td>
<td>60</td>
<td>218</td>
</tr>
<tr>
<td>8</td>
<td>TTTCTACACCTACAGGCTCC</td>
<td>GCCAGGACACTGATTAGCAG</td>
<td>59</td>
<td>330</td>
</tr>
<tr>
<td>9</td>
<td>CCTACCTTTTTCAGGGGGCCAGG</td>
<td>ACCACCTGTAATGGCCGCGAG</td>
<td>60</td>
<td>317</td>
</tr>
<tr>
<td>10-1</td>
<td>AACCTTGGCTTACCTTGGTCTCT</td>
<td>AGGACAGTCACCGAGGCTT</td>
<td>58</td>
<td>255</td>
</tr>
<tr>
<td>10-2</td>
<td>CAGGCTTGTACGTGTGAGGA</td>
<td>CCCACAAGGCGGTCCGAAGTC</td>
<td>58</td>
<td>341</td>
</tr>
</tbody>
</table>

* No t-test due to low number.

Table 2 Clinical data of the patients (age, serum total calcium (Ca total, normal range: 2.02–2.60 mmol/l), ionized calcium (Ca ion, normal range: 1.12–1.23 mmol/l), phosphate (PO₄, normal range: 0.84–1.45 mmol/l), PTH (normal range: 10–65 pg/ml), and creatinin (normal range: female 44–80 μmol/l, male 53–97 μmol/l) levels) expressed as means ± s.d. Comparison of patients with parathyroid adenomas or hyperplasia, with or without MEN1 gene mutations, and with polyclonal or monoclonal parathyroid tumours. Differences were not statistically significant.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Ca total (mmol/l)</th>
<th>Ca ion (mmol/l)</th>
<th>PO₄ (mmol/l)</th>
<th>PTH (pg/ml)</th>
<th>Creatinin (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma (n = 31)</td>
<td>61 ± 9</td>
<td>2.98 ± 0.22</td>
<td>1.59 ± 0.16</td>
<td>0.77 ± 0.14</td>
<td>174 ± 146</td>
</tr>
<tr>
<td>Hyperplasia (n = 2)</td>
<td>68 ± 13 *</td>
<td>2.95 ± 0.2 *</td>
<td>1.58 ± 0.25 *</td>
<td>0.75 ± 0.12 *</td>
<td>211 ± 50 *</td>
</tr>
<tr>
<td>Mutation (n = 9)</td>
<td>61 ± 9</td>
<td>2.91 ± 0.19</td>
<td>1.52 ± 0.13</td>
<td>0.78 ± 0.18</td>
<td>119 ± 47</td>
</tr>
<tr>
<td>No mutation (n = 24)</td>
<td>61 ± 9</td>
<td>2.99 ± 0.22</td>
<td>1.59 ± 0.11</td>
<td>0.76 ± 0.12</td>
<td>167 ± 65</td>
</tr>
<tr>
<td>Monoclonal (n = 19)</td>
<td>61 ± 10</td>
<td>2.96 ± 0.24</td>
<td>1.60 ± 0.18</td>
<td>0.82 ± 0.12</td>
<td>181 ± 163</td>
</tr>
<tr>
<td>Polyclonal (n = 9)</td>
<td>63 ± 11</td>
<td>3.02 ± 0.1</td>
<td>1.65 ± 0.11</td>
<td>0.66 ± 0.1</td>
<td>258 ± 65</td>
</tr>
</tbody>
</table>

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androgen receptor. Three patients with a solitary parathyroid tumour were not informative for the polymorphism. Nineteen of twenty-six solitary adenomas (73%) showed nonrandom X-chromosome inactivation. In one of the 19 tumours nonrandom X-chromosome inactivation could only be identified after repetition of the analysis with microdissected paraffin-embedded tissue following histological examination. The two cases with parathyroid hyperplasia showed random inactivation (Table 3). Examples of one random and non-random X-chromosome inactivation pattern, respectively, are shown in Fig. 1.

Analysis of the MEN1 gene

Mutational analysis revealed alterations in the MEN1 gene in 14 of 33 examined parathyroid tumours. Two variations were found within intronic sequences (2249C/G, 5146G/A). They were both present in tumour and leucocyte DNA. 2249C/G is located 39 base pairs upstream of the start codon. It was identified in three patients and is most likely a polymorphism. 5146G/A is located 31 base pairs upstream of exon 5. As the patient (58 years old) with this germline genetic variation had neither a history nor clinical symptoms of other endocrinopathies suggestive of MEN1 it seems unlikely that this genetic variation represents an intronic mutation of putative splice sites leading to a MEN1 phenotype. Furthermore, 80% of the patients with MEN1 show symptoms before the age of 50 (18). However, it cannot be excluded completely that this patient suffers from a sporadic form of MEN1 with a parathyroid adenoma as the only manifestation. Three polymorphisms were found in exons 3, 9 and 10 (R171Q, D418D, K588K). R171Q and D418D are polymorphisms which have previously been reported in healthy subjects with frequencies of 1.4% and 42% respectively (19). The silent polymorphism K588K (AAG588AAA) was found in one of the examined cases and has not been reported before. As described for

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Index</th>
<th>Clonality</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solitary adenomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>n.st.</td>
<td>547delT</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>n.st.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>n.i.</td>
<td>A49D</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Female</td>
<td>n.i.</td>
<td>193del36</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Female</td>
<td>n.i.</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>2.42</td>
<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>2.70</td>
<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
<td>2.58</td>
<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>5.99</td>
<td>Monoclonal</td>
<td>W126X</td>
</tr>
<tr>
<td>11</td>
<td>Female</td>
<td>6.03</td>
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<td>n.d.</td>
</tr>
<tr>
<td>12</td>
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<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>14</td>
<td>Female</td>
<td>3.65</td>
<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>16</td>
<td>Female</td>
<td>13.87</td>
<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
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<td>Female</td>
<td>4.09</td>
<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>21</td>
<td>Female</td>
<td>2.43</td>
<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>24</td>
<td>Female</td>
<td>2.82</td>
<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>25</td>
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<td>9.27</td>
<td>Monoclonal</td>
<td>402del1C</td>
</tr>
<tr>
<td>26</td>
<td>Female</td>
<td>4.93</td>
<td>Monoclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>27</td>
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<td>n.d.</td>
</tr>
<tr>
<td>28</td>
<td>Female</td>
<td>4.17</td>
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<td>n.d.</td>
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<td>30</td>
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<td>Monoclonal</td>
<td>904del9</td>
</tr>
<tr>
<td>32</td>
<td>Female</td>
<td>2.56</td>
<td>Monoclonal</td>
<td>482del22</td>
</tr>
<tr>
<td>35</td>
<td>Female</td>
<td>3.23</td>
<td>Monoclonal</td>
<td>R415X</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>1.49</td>
<td>Polyclonal</td>
<td>Y327X</td>
</tr>
<tr>
<td>15</td>
<td>Female</td>
<td>1.53</td>
<td>Polyclonal</td>
<td>n.d.</td>
</tr>
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<td>31</td>
<td>Female</td>
<td>1.33</td>
<td>Polyclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>33</td>
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<td>Polyclonal</td>
<td>n.d.</td>
</tr>
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<td>1.50</td>
<td>Polyclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>36</td>
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<td>1.43</td>
<td>Polyclonal</td>
<td>n.d.</td>
</tr>
<tr>
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<td>n.d.</td>
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<tr>
<td>Hyperplasia</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
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<td>Polyclonal</td>
<td>n.d.</td>
</tr>
<tr>
<td>18</td>
<td>Female</td>
<td>1.21</td>
<td>Polyclonal</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

n.d., mutation not detected; n.st., not studied due to male sex; n.i., not informative due to homozygosity.
5146G/A, it may be possible that this germline genetic variation results in an aberrant splicing of the tumour RNA. However, this patient, aged 57 years, showed no other MEN1 manifestation making a sporadic MEN1 variant relatively unlikely.

Mutations in the MEN1 gene were detected in nine cases. Six of them were found in the relatively large second exon of the MEN1 gene (A49D, 193del36, 402delC, 482del22, 547delT, W126X). One was found in exon 5 (904del9), one in exon 7 (Y327X, Fig. 2) and one in exon 9 (R415X). Mutations 193del36 (Fig. 2) and 904del9 are in-frame deletion mutations leading to deletion of 12 and 3 amino acids respectively. Mutations 402delC, 482del22 and 547delT are frame-shift deletions which result in altered amino acid sequences and creation of stop codons after 20, 52 and 38 triplets respectively. The deletions and the nonsense mutations (W126X, Y327X, R415X) lead to early truncation of the protein product. One mutation results in an alteration of the amino acid sequence (A49D). In seven of nine sequences with MEN1 gene mutations, the peaks of the mutated sequence are higher than the wildtype sequence peaks. None of the mutations were found in the corresponding leucocyte DNA and are therefore somatic mutations. Five tumours with a somatic MEN1 gene mutation could be identified as monoclonal lesions. One tumour with a somatic MEN1 gene mutation was polyclonal. Two were not informative for the polymorphism of the androgen receptor, one was a male patient. All patients with somatic mutations in the MEN1 gene suffered from primary hyperparathyroidism.

**Discussion**

It is widely accepted that somatic mutations of proto-oncogenes or tumour suppressor genes initiate clonal cell expansion. The intention of this study was to test whether nearly all parathyroid neoplasms are monoclonal lesions and to define the role of the recently described mutations in the MEN1 gene for monoclonal parathyroid neoplasias in the setting of primary hyperparathyroidism.

In this study, 73% of the patients with primary hyperparathyroidism due to a solitary adenoma harboured a monoclonal tumour. Twenty-six percent of the monoclonal parathyroid tumours and 30% of all tested tumours had somatic mutations in the MEN1 gene. Recent studies reported MEN1 gene mutations in 12–17% of solitary parathyroid adenomas (5–7). The methods used for mutational screening were dideoxy-fingerprinting, single strand conformation analysis and direct sequencing. The higher percentage reported in our study could be due to different sensitivities of the methods used. For dideoxyfingerprinting variable sensitivities have been reported (20). Single strand conformation analysis is known to be less sensitive than direct sequencing (21). The study using direct sequencing found somatic MEN1 gene mutations in 13% of solitary parathyroid tumours (7). However, the authors only sequenced the exons 2–10 of the MEN1 gene if LOH on 11q13 was detected. LOH analysis was performed using three microsatellite markers at chromosome 11q13 (PYGM, INT-2 and D11S906) spanning a relatively large area of at least 1.2 Mb (22). Therefore,
this study could have missed tumours with somatic MEN1 gene mutations and very small allelic deletions on 11q13 not detected with the above mentioned technique. Our results suggest that somatic mutations in the MEN1 gene occur more frequently in solitary parathyroid adenomas than previously reported.

Nevertheless, 74% of the identified monoclonal tumours did not have mutations in the MEN1 gene. Therefore, other somatic mutations are likely to play a role in the clonal cell expansions in the parathyroids of patients with primary hyperparathyroidism. Rearrangement of the PRAD1 oncogene may be responsible for a small subset of tumours (11, 23, 24). However, for the majority of parathyroid adenomas the underlying genetic defects are still waiting to be identified. Allelotyping studies of parathyroid neoplasias describe allelic losses on chromosomes 1p, 1q, 6q, 9p, 9q, 11p, 11q, 13q and 15q and less frequently allelic gains on chromosomes 1q, 5q, 16p and 19p (25). These findings strongly suggest the activation or inactivation of other, at present unknown, oncogenes or tumour suppressor genes respectively. Moreover, the variety of chromosomal abnormalities suggests heterogeneous genetic defects leading to parathyroid neoplasms.

In this study, 27% of the tumours tested for clonality showed a polyclonal pattern. X-chromosome inactivation can give false polyclonal results because of contamination with normal tissue. To minimize contamination by polyclonal tissue, we repeated X-chromosome inactivation analysis for solitary adenomas showing random X-chromosome inactivation in snap-frozen tissue using microdissected paraffin-embedded material clearly identified as adenomatous parathyroid tissue. Using this technique one of the previously polyclonal samples

Figure 2 Sequencing data of exon 7 and exon 2 of the MEN1 gene, amplified from parathyroid tumours of two patients with primary hyperparathyroidism. Mutations, marked by an arrow (Y327X – case no. 5, upper panel; 193del36 – case no. 20, lower panel) were detected in tumour tissue but not in the corresponding DNA extracted from peripheral leucocytes, and are therefore somatic mutations.
showed a monoclonal inactivation pattern. Seven solitary tumours repeatedly gave ratios suggesting a polyclonal origin. Besides admixture with normal tissue, a pattern suggestive of a polyclonal origin could also result from an aberrant pattern of DNA methylation or the presence of two clonal expansions with opposing X-chromosome inactivation patterns (12). Furthermore, a comparison of two independent methods for the study of X-chromosome inactivation reported conflicting results in 10% of all cases evaluated (15). In summary, the results of X-chromosome inactivation analysis allow the conclusion that the majority of solitary parathyroid tumours evolve from single cells.

Regarding the MEN1 gene mutations, 85% of the mutations reported in previous studies are inactivating (26). Furthermore, other authors report loss of heterozygosity on chromosome 11q13 (the location of the MEN1 gene) in parathyroid tumours with MEN1 gene mutations (5–7). Therefore, it has been assumed that menin acts as a tumour suppressor. According to Knudson’s hypothesis (27), inactivation of a tumour suppressor only occurs after mutation in one allele and loss of the wildtype allele. Looking at our sequencing data, it must be stated that the peaks of the mutated sequences are nearly always higher than the wildtype sequence peaks. In contrast, sequencing data of DNA from thyroid tumours with somatic heterozygous mutations of the thyrotrhopin receptor nearly always give peaks for the mutated allele which are less than 50% of the wildtype sequence peaks (28). This is due to the fact that direct sequencing requires a distribution of mutated and wildtype alleles close to 1:1. As tumour tissue can be contaminated with fibroblasts or blood cells this will further reduce the frequency of the mutated alleles. Thus, it can be difficult to distinguish a small mutated peak from an unspecific background. In the case of a hemizygous mutation, which is assumed for MEN1 gene mutations, dominance of the mutated allele can be expected, as the wildtype allele has been lost. Therefore, the mutations with the mutated peak higher than the wildtype peak are strongly suggestive of hemizygosity. Moreover, 90% of the mutations we found are nonsense mutations (W126X, Y327X, R415X) and deletions (193del36, 402delC, 482del122, 547delT, 904del9) leading to truncation and subsequent inactivation of the protein. In summary, our results are in line with the assumed function of the MEN1 gene product as a tumour suppressor. New mutations in this large gene that can be inactivated throughout its reading frame are being found continuously. In this study, seven of nine mutations we describe (A49D, 193del36, 402delC, 482del122, 547delT, 904del9 and Y327X) have not been reported before.

The clinical characteristics of the patients with or without MEN1 gene mutations in the parathyroid tumours were not different. Therefore, it cannot be concluded that mutations in the MEN1 gene lead to a specific phenotype of very mild or very severe primary hyperparathyroidism. This result confirms other reports which compared the clinical characteristics of patients with or without LOH on 11q13 and with or without MEN1 gene mutations (6, 7). Furthermore, the presence of MEN1 gene mutations in parathyroid tumours of patients with very mild forms of primary hyperparathyroidism found by Carling et al. (7) indicates that loss of function of this putative tumour suppressor is not necessarily associated with an excessive tumour growth.

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