Structural analysis of gene marker loci on chromosomes 10 and 11 in primary and secondary uraemic hyperparathyroidism

Chiho Inagaki¹, Mireille Dousseau¹, Nathalie Pacher¹, Emile Sarfati², Tilman B. Drüke¹ and Jean Gogusev¹

¹INSERM Unité 90 and Département de Néphrologie, Hôpital Necker, and ²Service de Chirurgie Générale, Hôpital Saint Louis, Paris, France

Abstract

**Background.** The genetic molecular anomalies in patients with primary (I°) and secondary (II°) hyperparathyroidism (HPTH) are still largely unknown. In particular, the changes underlying monoclonal growth in the parathyroids of patients with II° HPTH are not well understood.

**Methods.** We screened genomic DNA from a total of 30 patients with I° HPTH and 29 patients with II° uraemic HPTH for possible rearrangements or allelic losses of several gene markers located on chromosome 11p near the PTH gene, namely Ha-ras, IGF-2, WT1, and the PTH gene itself. In addition, two other gene markers, PRAD1 (localized on 11q13) and RET (localized on 10q11) were examined for possible structural alterations. Moreover, we used fluorescence in situ hybridization (FISH) which is another technique to detect numerical alterations of chromosome 11.

**Results.** The results show that one of 13 patients with I° HPTH (8%) exhibited a rearrangement for the PRAD-I gene. Loss of heterozygosity of Ha-ras locus was observed in one of 11 uraemic patients with II° HPTH (9%). Three of 10 patients with I° HPTH (30%) and one of 7 patients with II° HPTH (14%) showed an allelic loss of the WT1 gene. No evidence of rearrangement or allelic loss was detected for the IGF-2, PTH or RET genes respectively. Using FISH method, three normal parathyroid glands, six I° HPTH adenomas and eight II° HPTH hyperplastic glands from uraemic patients were studied with centromeric probe for chromosome 11. Monosomy 11 was observed in one case of I° HPTH and in one other case of II° HPTH.

**Conclusion.** Evidence of loss of heterozygosity for several genes located on human chromosome 11p has been found in a series of parathyroid glands from several patients with I° and II° uraemic HPTH, corresponding to monosomy of chromosome 11 in some cases.

Key words: hyperparathyroidism; primary; secondary; chronic renal failure; haemodialysis; PRADI; Ha-ras; WT1; RET; allelic loss; rearrangement; Southern blot; fluorescence in situ hybridization (FISH)

Introduction

Primary (I°) parathyroid adenomas are benign monoclonal tumours, as demonstrated by Arnold et al. based on X-chromosome-inactivation analysis [1]. Recently it has been shown that secondary (II°) parathyroid hyperplasia due to chronic renal failure can lead to monoclonal, benign tumoural growth as well, at least in case of severe II° hyperparathyroidism (HPTH) [2]. Parathyroid tumorigenesis is still incompletely characterized and understood. A variety of different molecular genetic abnormalities have been implicated in the pathogenesis of parathyroid adenomas, including clonal rearrangement and overexpression of the cyclin D1/PRAD1 oncogene, allelic losses on chromosome arms 1p [3] and 11q [4], and several recently discovered deletions in chromosome regions 6q and 15q [5]. Loss of heterozygosity of the 11q13 region is characteristic for patients with the multiple endocrine neoplasia type I (MEN-I) syndrome [6,7], and germline point mutations of the RET proto-oncogene are involved in the tumourigenesis of MEN-2A [8,9]. Finally, an inactivation of the RB tumour suppressor gene on 13q14 is common in parathyroid carcinoma, but not in I° adenoma [10]. The molecular genetic anomalies involved in the pathogenesis of benign monoclonal tumours in uraemic patients with severe II° hyperparathyroidism (HPTH) are still unknown.

The purpose of the present study was to examine possible rearrangements or allelic losses of genes located on the short arm of chromosome 11 that are near the PTH gene, including the PTH gene itself, and the PRADI and RET genes located on 11q13 and 10q11 respectively in parathyroid gland tissues from patients with I° and II° HPTH. Given the molecular heterogeneity found in I° parathyroid adenomas, we studied...
several genes and gene markers possibly reflecting tumour suppressor genes in these chromosome regions. Since allelic deletions have been frequently found on chromosome 11, we also performed a fluoresence in situ hybridization (FISH) analysis using a centromeric probe.

Subjects and methods

Patients and tumour samples

Parathyroid tissues and venous blood samples were obtained from 30 patients with I° HPTH and 29 patients with uraemic II° HPTH, all of whom had surgery at Saint Louis hospital, Paris from December 1994 to January 1996. Patients with I° HPTH were aged 34–81 years (mean ± SD, 58 ± 14.0 years), and uraemic patients on intermittent haemodialysis treatment with II° HPTH were aged 22–80 years (mean 48 ± 14.8 (SD) years). All patients had serum intact parathyroid hormone (PTH) levels >182 ± 178 pg/ml (mean ± SD) in patients with I° HPTH, and 963 ± 569 pg/ml in patients with II° HPTH (normal range, 10–65 pg/ml).

After surgical removal, tumour tissues were dissected and stored at −80°C until the extraction of DNA. All parathyroid tumours from patients with I° HPTH were classified as adenoma by pathological criteria; none had a feature suggestive of malignancy. Hyperplastic parathyroid glands removed from uraemic patients with II° HPTH were categorized either as diffuse hyperplasia or as nodular hyperplasia. High-molecular-weight genomic DNA was extracted from all parathyroid glands and peripheral venous blood samples by phenol–chloroform method.

DNA probes

We used five probes that have been mapped to chromosome 11, namely the PRAD1, Ha-ras, IGF-2, PTH, WT1 genes and one probe that has been mapped to chromosome 10, namely the RET gene [11]. The following cDNA fragments were used: the 1.4-kilobases (kb) EcoRI fragment of pGEM 7Z f(+) plasmid containing human PRAD1 cDNA [12], the 3.0-kb Ha-ras insert obtained by SacI digestion of pBR322 [13], the 1.0-kb IGF-2 fragment from EcoRI digested pUC12 [14], the 3.7-kb PTH cDNA obtained by EcoRI digestion of pBR322 vector [15], the 1.0-kb WT1 insert from EcoRI digested WT33 plasmid [16], and the RET specific cDNA (clone pBSA3 obtained by Kpn-Sacl digestion of pBSA3 vector) which has been kindly provided by Dr B. Ponder, CRC Human Cancer Genetics Research group, Cambridge, UK [17]. The locus name, the DNA probe, and genomic location were respectively: Ha-Ras (pEJ ras), chromosome 11p15; IGF-2 (pIGF2/8-1), 11p15; WT-1 (WT33), 11p15; PRAD1 (pPL-8), 11q 53; PTH (p20.36, ATCC), 11p15; and RET (pBSA3), 10q11. For chromosome 11p, altered parathyroids were classified as having loss of heterozygosity if loss was seen at any informative locus, and as noninformative only if the patient was constitutionally homozygous for all the loci examined.

The probes were labelled with 32P by the random priming method [18]. The specific activity of the labelled probe was 8 × 106 c.p.m./μg DNA.

Southern blots analysis

Southern blot analysis was performed using 20 μg of parathyroid tissue or peripheral blood DNA from the same patient, digested at completion with several restriction enzymes, including EcoRI, PvuII, HindIII, and BamHI. The DNA fragments were separated by electrophoresis on 0.8% agarose gel, and transferred to nylon filters (Nyborn N, Amersham, UK). Prehybridization was performed at 42°C for 4 h in 6 × SSC (0.15 mol/l NaCl; 0.015 mol/l sodium citrate, pH 7), 0.1% Denhardt’s reagent, 5% (wt/vol) dextran sulphate, sonicated denatured salmon sperm DNA (200 μg/ml), and 50% formamide. Hybridization was performed at 42°C for 16 h in 6 × SSC, 0.5% SDS (sodium dodecyl sulphate), 0.1% Denhardt’s reagent, 5% dextran sulphate, salmon sperm DNA, and 50% formamide. After hybridization the filters were washed for 15 min in 2 × SSC and 0.5% SDS at room temperature, and 15 min in 0.1 × SSC and 0.5% SDS at same temperature. The blots were autoradiographed for 4–7 days at −80°C.

Possible rearrangements or allelic losses of the marker genes studied could not be analysed in all 59 cases, either because the amount of DNA available was too limited or because the analysed loci were uninformative.

Fluorescence in situ hybridization (FISH)

The FISH method used was based on that described by Hopman et al. [19]. Briefly, frozen sections of normal and altered parathyroid glands were cut at thickness of 4–5 μm, fixed in a solution of 4% paraformaldehyde (30 min), rinsed in PBS and dehydrated in series of graded ethanol for 2 min. Biotin-labelled, α-satellite DNA probe for chromosome 11 and appropriate hybridization solutions were provided by Appligene Oncor (Ilkirch, France). The slides were subsequently incubated in protein-digesting solution, rinsed in 2 × SSC, and dehydrated in the series of graded ethanol.

The specific probe in a hybridization mixture was added to each tissue section, covered with plastic coverslip and incubated at 37°C for 2 h in a prewarmed humidified chamber. Post-hybridization washings were carried out at 72°C in 1 × SSC for 5 min. Detection of biotinylated DNA probe that hybridized to the tissue section was visualized by indirect immunofluorescence reaction using fluorescein-labelled avidin, by incubating the slides for 30 min at 37°C. The slides were then washed three times for 2 min each in 40 ml of phosphate-buffered detergent solution (Oncor) at room temperature, counterstained in a solution containing 10 μg/propidium iodide, and mounted in antifade solution.

Sections were examined with an epiphluorescence microscope (Leica DMRB, CRC Human Cancer Genetics Research group, Cambridge, UK). Prehybridization was performed at 42°C for 4 h in 6 × SSC, 0.1% Denhardt’s reagent, 5% dextran sulphate, salmon sperm DNA, and 50% formamide. After hybridization the filters were washed for 15 min in 2 × SSC and 0.5% SDS at room temperature, and 15 min in 0.1 × SSC and 0.5% SDS at same temperature. The blots were autoradiographed for 4–7 days at −80°C.

Possible rearrangements or allelic losses of the marker genes studied could not be analysed in all 59 cases, either because the amount of DNA available was too limited or because the analysed loci were uninformative.
cases screened for rearrangements or allelic losses due to lack of tissue material.

Statistical analysis for FISH

Mean values ± SE were compared by means of \( \chi^2 \) analysis, with \( P < 0.05 \) being regarded as statistically significant. We considered a true monosomy to be present when the percentage of nuclei with 1 signal in the normal parathyroid glands exceeded the mean ± 4 SD (21). Accordingly, a monosomy was diagnosed when more than 50% of the nuclei counted showed only 1 or no signal for chromosome 11.

Results

Southern blot hybridization

The findings of gene rearrangements and deletions made with Southern blot analysis of our series of samples from patients with I° and II° HPTH are summarized in Table 1. After EcoRI digestion of autologous control peripheral blood leukocyte DNA (PBL), the PRAD-1 probe hybridized to 4.7-kb, 2.8-kb, and 2.4-kb bands. In comparison, the hybridization pattern of DNA from the adenomatous tissue showed the presence of a supplementary band of 3.3 kb (Figure 1A). Thus one of 13 patients with I° HPTH (8%) showed a structural aberration compatible with a rearrangement of the PRAD-1 gene. In contrast, no such abnormality was detectable in DNAs extracted from the uraemic parathyroid tissues from patients with II° HPTH (Table 1).

Comparatively, Southern blot analysis of Ha-ras gene on DNA extracted from tissues of uraemic patients with II° HPTH showed loss of heterozygosity of this gene in one of 11 samples (9%). As shown in Figure 1B, after the digestion of DNA of this hyperplastic tissue with PvuII restriction enzyme, a loss of the 3.4 kb band was observed, compared with the restriction pattern of DNA from the peripheral blood. In addition, the Ha-ras specific genomic probe revealed a decreased band intensity in tumour DNA, compared to the peripheral blood leukocytes DNA, which is consistent with the presence of a single gene copy. Conversely, when control DNA and DNA from patients with adenomatous I° HPTH was digested with PvuII restriction enzyme and hybridized with Ha-ras gene cDNA, no structural abnormality was detectable in any of the samples studied.

The findings of Southern blot analysis using WT1 genomic probe are shown in Table 1 and Figure 1C. When the blots were hybridized with a WT1-specific probe, a loss of the 5.0 kb WT1 band was detected in DNA restricted with the EcoRI enzyme in three of 10 patients with I° HPTH (30%) and in one of seven patients with II° HPTH (14%) (Figure 1C). For comparison, autologous peripheral blood DNA digested with the EcoRI enzyme generated a hybridization pattern containing 14.3-kb, 5.5-kb, 5.0-kb, 3.6-kb, 3.3-kb, and 1.8-kb bands. A representative pattern of hybridization with loss of the 5.0-kb band in three patients with I° HPTH is shown in Figure 1C. Loss of heterozygosity of the WT1 gene was also detected in the DNA of one hyperplastic gland from a patient with II° HPTH, compared with control PBL DNA (not shown). Moreover, the intensity of hybridizing bands in DNA from adenomatous and hyperplastic tissues was half that in control DNA, also suggesting a single gene copy.

Finally, Southern blot analysis of the same panel of DNA samples, i.e. stemming from the parathyroid tissues of patients with I° and II° HPTH, did not allow to detect any rearrangement or allelic loss of the IGF-2, PTH or RET genes respectively.

FISH analysis

Fluorescence in situ hybridization (FISH) with centromere-specific probe for chromosome 11 was performed on specimens randomly selected from one normal parathyroid gland and from both I° and uraemic II° HPTH tissues. Table 2 shows the distribution of FISH findings in these tissues.

Using the above-defined criteria, in normal parathyroid gland tissue, 51.9 ± 2.8% of nuclei contained two hybridization signals, 37.8 ± 1.0% one signal, and 10.3 ± 2.1% no signal. None contained three signals (Table 2).

For nuclei containing two signals, the distribution of hybridization signals was between 15.6 and 60.1% in parathyroid tissues from the six patients with I° HPTH, and between 8.6 and 68.6% in tissues from the

<table>
<thead>
<tr>
<th>Gene marker</th>
<th>I° HPTH</th>
<th>Allelic loss</th>
<th>II° HPTH</th>
<th>Allelic loss</th>
<th>Total</th>
<th>Allelic loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ha-ras</td>
<td>8</td>
<td>0</td>
<td>11</td>
<td>1</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>IGF-2</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>PTH</td>
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<td>0</td>
<td>9</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
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<td>3</td>
<td>7</td>
<td>1</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>RET</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>PRAD1</td>
<td>13</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>22</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1. Frequency of loss of heterozygosity of gene marker loci (Ha-ras, IGF-2, PTH, WT1, RET and PRAD1) located on chromosomes 10 and 11 in parathyroid tissue of patients with I° and II° HPTH respectively.
Loss of heterozygosity in hyperparathyroidism

Fig. 1. Southern blot analysis of three different patients’ blood leukocyte samples (control, lane PBL [peripheral blood leukocytes]) and of parathyroid tissues (lane T [tissue]). DNA hybridized with PRADI, Ha-ras and WT1 probes. DNA (20 μg) was digested with EcoRI and probed with the PRADI cDNA (A), the Ha-ras cDNA (B), and WT1 cDNA. In (A), DNA samples from patient with I° HPTH, in (B) DNA samples from uraemic patient with II° HPTH, and in (C) DNA samples from patient with I° HPTH.

Table 2. Percentage of chromosome 11 copy numbers determined by fluorescence in situ hybridization (FISH) in tissues from patients with I° HPTH or II° HPTH and in normal parathyroid gland tissue

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Percentage of centromere signals per nucleus (mean ± SE)</th>
<th>Nuclei examined (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>I° HPTH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>case 1</td>
<td>9.9 ± 1.0</td>
<td>41.9 ± 0.3</td>
</tr>
<tr>
<td>case 2</td>
<td>19.7 ± 4.9</td>
<td>64.8 ± 3.0</td>
</tr>
<tr>
<td>case 3</td>
<td>8.8 ± 1.7</td>
<td>31.1 ± 0.3</td>
</tr>
<tr>
<td>case 4</td>
<td>10.8 ± 0.9</td>
<td>31.2 ± 0.6</td>
</tr>
<tr>
<td>case 5</td>
<td>10.0 ± 1.1</td>
<td>36.2 ± 4.9</td>
</tr>
<tr>
<td>case 6</td>
<td>5.3 ± 0.7</td>
<td>44.3 ± 2.0</td>
</tr>
<tr>
<td>II° HPTH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>case 1</td>
<td>6.7 ± 0.1</td>
<td>24.8 ± 6.1</td>
</tr>
<tr>
<td>case 2</td>
<td>8.7 ± 2.2</td>
<td>35.7 ± 4.8</td>
</tr>
<tr>
<td>case 3</td>
<td>32.8 ± 1.4</td>
<td>58.5 ± 2.5</td>
</tr>
<tr>
<td>case 4</td>
<td>9.0 ± 1.1</td>
<td>37.3 ± 0.4</td>
</tr>
<tr>
<td>case 5</td>
<td>7.4 ± 1.7</td>
<td>32.3 ± 2.4</td>
</tr>
<tr>
<td>case 6</td>
<td>8.7 ± 3.1</td>
<td>37.1 ± 0.3</td>
</tr>
<tr>
<td>case 7</td>
<td>5.6 ± 1.5</td>
<td>30.3 ± 1.1</td>
</tr>
<tr>
<td>case 8</td>
<td>10.2 ± 2.3</td>
<td>32.9 ± 0.1</td>
</tr>
<tr>
<td>Normal parathyroid glands</td>
<td>10.3 ± 2.1</td>
<td>37.8 ± 1.0</td>
</tr>
<tr>
<td>Mean ± 4 SD</td>
<td>25.1</td>
<td>44.6</td>
</tr>
</tbody>
</table>

eight patients with II° HPTH (Table 2). In one case of adenomatous I° HPTH tissue, parathyroid cells within the section exhibited a signal distribution of as much as 64.8 ± 3.0% to nuclei containing only one signal. Similarly, in one case of II° HPTH, there was an elevated signal distribution of 58.5 ± 2.5% in nuclei containing only one signal, revealing a monosomy of chromosome 11 in this pathological parathyroid gland sample.

Figure 2 shows parathyroid cell nuclei in normal tissue (Figure 2A) exhibiting two hybridization signals per nucleus using FISH technique, and in hyperplastic tissue from patient 3 with II° HPTH (Figure 2B) exhibiting only one hybridization signal per nucleus. This pattern indicates the presence of chromosome 11 monosomy in patient 3. The same monosomy pattern was observed in patient 2 having I° HPTH.

Discussion

In the present study, four of the 27 parathyroid tissues sampled from patients with I° HPTH (11%), and two of the parathyroid tissues obtained from patients with...
Fig. 2. Fluorescence in situ hybridization (FISH) on a slide containing paraformaldehyde-fixed section of (a) normal parathyroid gland tissue and (b) hyperplastic parathyroid gland tissue from a uraemic patient with II° hyperparathyroidism. After hybridization with chromosome-11-specific probes, parathyroid cell nuclei (in gray) exhibit two specific signals (in white) in normal tissue (a), but only one specific signal (in white) in hyperplastic tissue (b).

II° HPTH had evidence of various allelic losses on chromosome 11, as detected by Southern blot analysis. These results are in agreement with previous observations demonstrating partial deletions on chromosome 11q or entire allelic loss of this chromosome in 25–29% of sporadic parathyroid adenomas [22–24]. The finding of allelic losses in our samples with a slightly lower frequency than in previous studies may be due to the fact that we examined mainly markers on chromosome 11p, near the PTH gene, and only one marker of the chromosome 11q region, namely PRADI. The findings from the Southern blot analysis were further confirmed using FISH with chromosome 11-specific centromeric probe. According to our criteria for the classification of numerical aberrations, we considered monosomy 11 to be the correct cytogenetic diagnosis in the parathyroid tissue sections of one patient with I° HPTH and of one other patient.
with II° HPTH in our series. The FISH results are in accordance with a recent report by Tahara et al. [5], who detected allelic loss of chromosome 11 in 32% of I° parathyroid adenomas using a panel of 10 polymorphic markers distributed along chromosome 11.

These observations are also in line with findings made in parathyroid tumours of patients with the MEN-1 syndrome in which a deletion of the putative MEN-1 tumour suppressor gene on chromosome 11q13 was described [22]. Another study reported loss of heterozygosity of HaRus and Apo-AI genes in two insulinomas removed from patients with the MEN-1 syndrome [6], similar to those present in parathyroid lesions [6,23]. Therefore it had been hypothesized initially that identical allelic deletions on chromosome 11 are involved in the development of monoclonal tumours in various endocrine tissues of a given MEN-1 patient [25] whereas among different MEN-1 families the chromosomal regions of allelic loss could be different. However, recent studies have shown that even in a given MEN-1 patient, variable regions of chromosome 11 loss may occur in different pathological tissues [26], and parathyroid neoplasms can consist of more than one clone [27].

In sporadic parathyroid tumours and hyperplasia, allelic loss at 11q13 is a less frequent finding than in those from MEN-1 patients [22], reflecting on one side the heterogeneity within sporadic tumours and on the other the role that inactivation of a tumour suppressor gene in this chromosomal region has in parathyroid tumorigenesis [28].

In uraemic patients with severe II° HPTH, we [2] and others [29] have shown recently that benign monoclonal tumours develop in a high proportion of parathyroid glands, probably favoured by the existence of polyclonal parathyroid hyperplasia for many years [30]. According to Friedman et al. [22], a similar scenario might occur in MEN-1 patients in whom monoclonal neoplasms might arise on a background of polyclonal proliferation since tumours with allelic loss were larger than those without such loss.

To our knowledge, only two studies have been devoted to the exploration of the possible molecular genetic anomalies underlying monoclonal parathyroid tissue growth in uraemic patients with severe II° HPTH [2,31]. In the first report [31], evidence for an allelic loss on chromosome 11q13 was found in two of 12 parathyroid glands from six patients. Interestingly, the two glands with allelic loss had a significantly greater tissue weight than those without loss of heterozygosity. In the second report [2], allelic 11q13 loss could not be detected in any of the 18 informative hyperplastic parathyroid glands available from 10 uraemic patients. In contrast, monoclonal allelic loss of heterozygosity on the X-chromosome was found in the hyperplastic parathyroid tissue of one uraemic patient.

In the present study we provided evidence for allelic loss in chromosome regions 11p13 and 11p15 in 2 of 25 parathyroid glands removed from uraemic dialysis patients with severe II° HPTH. We found no indication for allelic loss on 11q13, in accord with our previous report [2].

In I° parathyroid adenoma, allelic losses have been detected in loci other than those located on chromosome 11. For example, one or more allelic losses of informative loci on chromosome 1 have been observed in 10 of 25 adenomas (40%) by Cryns et al. [3]. This is the most common molecular genetic abnormality reported to date in such tumours, strongly implicating a presumable tumour suppressor gene on distal 1p (1p32-pter) in parathyroid tumorigenesis. The same group of authors recently reported evidence of the frequent occurrence of loss of heterozygosity in I° parathyroid adenomas on chromosome arms 6q and 15q [5] and on chromosome arm 9p [32].

Since sporadic mutations in the p53 tumour suppressor gene, located on 17p13, represent the single most common genetic alteration in various forms of human cancer [33] such mutations have also been looked for in parathyroid tumours. No p53 point mutations were found in parathyroid adenoma or carcinoma, except in one of nine parathyroid carcinomas with both p53 allelic loss and abnormal nuclear p53 protein staining [34,35]. These findings could implicate the p53 gene in the pathogenesis of a subset of parathyroid carcinomas. However, the major genetic molecular anomaly in the latter clearly is the recently reported loss of the RB gene [10].

Other potentially involved mutations in parathyroid tumours theoretically could implicate somatic genes involved in the regulation of PTH synthesis and secretion. One possible candidate was the recently cloned extracellular Ca\(^{2+}\)-sensing receptor (CaR) [36], with the human CaR gene mapping to chromosome 3q [37]. This hypothesis was derived from the recent observation that familial hypocalciuric hypercalcaemia (FHH), an autosomal dominantly inherited disorder, is associated in most families with a point mutation of the CaR gene [37]. It was conceivable that a mutation of this gene played a role in the pathogenesis of I° parathyroid adenoma since in one study loss of chromosomal DNA encompassing the parathyroid CaR gene occurred in about 10% of sporadic parathyroid adenomas [38]. However, in another study of 44 human parathyroid tumours (23 adenomas, 4 carcinomas, 5 primary hyperplasia, and 12 secondary hyperplasia) no mutations of the CaR gene were detected [39]. These findings indicate that loss of CaR function due to acquired genetic molecular anomalies of its gene probably is a rare event, if it occurs at all, in sporadic parathyroid tumours.

Approximately 20% of Wilms’ tumours manifest allelic losses in the region encompassing the WT1 gene [40]. In our study, an allelic loss of the WT1 gene was detected in parathyroid tissues from three patients with I° HPTH and 1 uraemic patient with II° HPTH. The latter observation is a novel finding, particularly for the case of II° HPTH. This is also true for our detection of an allelic loss of the HaRus oncogene, located on chromosome 11p15, in a patient with II° HPTH.
Obviously the latter finding would imply that a nearby tumour suppressor locus must have been lost.

The MEN-2A and MEN-2B syndromes and familial medullary thyroid carcinoma, which are dominantly inherited cancer syndromes, are all associated with germline mutations of the RET proto-oncogene [41]. Interestingly, point mutations of the RET proto-oncogene were also detected in sporadic medullary thyroid carcinomas [42]. The latter finding is compatible with the hypothesis that point mutations of the RET proto-oncogene might also play a role in the development of sporadic parathyroid tumours. However, such point mutations have not been seen in sporadic parathyroid tumours [43]. In the present study we also failed to provide evidence for allelic loss of the RET gene in parathyroid tumours of patients with II HPTH and extended this to uraemic patients with II HPTH.

Finally, as to possible rearrangements of the PRAD1 gene, which have been found in approximately 5% of II parathyroid adenomas at the molecular level [4,12,44] and in 18% at the protein level [45], we observed a similar frequency at the molecular level in activation of a human oncogene. [4,12,44] and in 18% at the protein level [45], we observed a similar frequency at the molecular level in activation of a human oncogene.

In conclusion, the various types of allelic loss on chromosome 11 observed by us in parathyroid tissues from non-uraemic patients with II HPTH as well as from uraemic patients with severe II HPTH, including monosomy of this chromosome, are in line with similar recent findings made by others. They are compatible with a possible role for the inactivation of tumour suppressor genes located on chromosome 11 in the pathogenesis of benign parathyroid tumours. Since deletions of tumour suppressor genes located on other chromosomes may be involved as well, as has been recently shown for II parathyroid adenomas, it is possible that more than one gene rearrangement or deletion might be implicated in abnormal parathyroid growth. Finally, precise knowledge of individual deletions must be obtained for each parathyroid tumour to achieve the ultimate goal of designing specific therapeutic approaches.

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