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## **Molecular analysis of the HPJ-JT syndrome and sporadic parathyroid carcinogenesis**

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## *Chapter 7*

Identification of MEN1 and HRPT2 somatic mutations in paraffin-embedded (sporadic) parathyroid carcinomas.

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## ORIGINAL ARTICLE

Identification of *MEN1* and *HRPT2* somatic mutations in paraffin-embedded (sporadic) parathyroid carcinomas

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## Summary

**Objective** Parathyroid carcinoma remains difficult to diagnose. Recently, it has been shown that mutations in the *HRPT2* gene (encoding parafibromin) are associated with the development of parathyroid carcinoma. Although *MEN1* is not typically thought to be involved in carcinoma formation, parathyroid carcinoma may be an extremely rare feature of the multiple endocrine neoplasia type 1 (*MEN1*) syndrome. We recently concluded that loss of heterozygosity (LOH) of the *MEN1* gene is present in a relatively large number of parathyroid carcinomas, often in combination with LOH at the *HRPT2* locus. The aim of this study was to evaluate the role of *MEN1* and *HRPT2* mutations in sporadic parathyroid tumours fulfilling histological criteria for malignancy.

**Patients and design** Formalin-fixed, paraffin-embedded (FFPE) parathyroid carcinoma tissue from 28 cases identified in the period 1985–2000 in the Netherlands was studied. *HRPT2* (27/28 cases) and *MEN1* (23/28 cases) were analysed by direct sequencing.

**Results** Somatic *MEN1* mutations were found in three of 23 (13%) sporadic parathyroid carcinoma cases; these consisted of one missense and two frameshift mutations. One of the latter two cases displayed lymph-node and lung metastases during follow-up. Six *HRPT2* mutations were found in 4/27 cases (15%); five were truncating mutations and one was a missense mutation. Consistent with previously published reports, we found double mutations (2×) and germline mutations (2×) in apparently sporadic parathyroid carcinomas.

**Conclusions** These results suggest that not only *HRPT2* but also *MEN1* mutations may play a role in sporadic parathyroid cancer formation.

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## Introduction

Primary parathyroid hyperparathyroidism (PHPT) has an incidence of one in 1000<sup>1</sup> and may result from a single parathyroid adenoma (80–85%) or from hyperplasia (15–20%), but rarely (less than 1%) from carcinomas.<sup>2</sup> Although parathyroid carcinomas are slow growing, they have a high propensity to recur locally and recurrent disease is difficult to eradicate. Parathyroid carcinoma is also difficult to diagnose because its histopathological features can overlap with those of adenoma.

Whereas PHPT is usually encountered as a nonfamilial disorder, in a minority of cases (5%) it is part of a hereditary syndrome; multiple endocrine neoplasia type 1 (*MEN1*; OMIM 131100) and type 2A (*MEN2A*; OMIM 171400), hyperparathyroidism–jaw tumour syndrome (HPT-JT; OMIM 607393) and familial isolated hyperparathyroidism (FIHP; OMIM 145000) are all hereditary.

*MEN1*, caused by mutations in the *MEN1* gene, is characterized by the occurrence of tumours of the parathyroid (in 95% of patients<sup>3</sup>), pancreatic islet cells and anterior pituitary. *MEN1* consists of 10 exons and encodes a 610-amino-acid protein menin. Menin appears to have a large number of potential functions through interactions with proteins that alter cell proliferation mechanisms.<sup>3</sup> *MEN1* represents a tumour suppressor gene (TSG) and is located on chromosome 11q13. The majority of tumours (95%) show additional loss of heterozygosity (LOH) consistent with Knudson's two-hit theory. The *MEN1* gene is also known to be mutated in a subset (20–30%) of sporadic parathyroid adenomas.<sup>4–6</sup> Two comparative genomic hybridization (CGH) studies investigating physical loss show that somatic loss of chromosome 11q is not a frequent feature in parathyroid carcinomas; however, these studies could not detect possible loss due to homologous recombination.<sup>7,8</sup> Although PHPT represents the most common endocrinopathy in *MEN1*, reaching nearly 100% penetrance by the age of 40,<sup>9</sup> parathyroid carcinoma is an extremely rare feature of the *MEN1* syndrome. So far, only two cases<sup>10,11</sup> of parathyroid carcinomas in *MEN1* mutation carriers have been reported. Therefore, it is assumed that *MEN1* plays no role in the development of parathyroid carcinomas.<sup>12,13</sup> We recently found a considerable percentage of LOH in the chromosome 11q13 region in a cohort of 30 carcinomas by studying polymorphic markers.<sup>14</sup> This led to the idea that the role of *MEN1* mutations in the development of parathyroid carcinomas might be greater than previously believed.

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HPT-JT is an autosomal dominant disorder characterized by parathyroid tumours, ossifying fibromas of the mandible and maxilla, renal hamartomas and cystic kidney disease. The gene causing HPT-JT, localized at chromosome 1q24-q32, is known as the *HRPT2* gene (also known as *Cdc73*) and is thought to function as a TSG. *HRPT2* consists of 17 exons coding for the 531-amino-acid protein parafibromin. Subsequent investigations have revealed that mutations in *HRPT2* are present in 66–100% of sporadic carcinomas.<sup>12,15</sup> This is in contrast to sporadic adenomas, where these mutations are rarely found (1.8%).<sup>12,16–19</sup>

Parathyroid carcinoma is an uncommon cause of PHPT. However, the HPT-JT syndrome is associated with an increased risk of parathyroid carcinoma; 10–15% of the patients develop parathyroid carcinomas.<sup>20</sup>

FIHP is a diagnosis per exclusionem. Germline mutations in the *HRPT2* (5.3%), *MEN1* (17.6%) and *CASR* (11.8%) genes are reported, but the majority of FIHP cases have a still unrecognized cause.<sup>21,22</sup> It is unknown if these remaining cases have a distinct genetic basis.

In this study formalin-fixed paraffin-embedded tissue (FFPE) of parathyroid carcinoma was used for mutation analysis. In such archival tissue, DNA is fragmented, depending on the time of fixation and the length of storage as paraffin blocks. However, in the current study this gave us the opportunity to examine a relatively large number of cases, given the rarity of parathyroid carcinoma. In this cohort of parathyroid carcinomas,<sup>14,23,24</sup> we have identified somatic mutations in *HRPT2* and *MEN1*.

## Materials and methods

### Clinical data and tumour samples

We recently studied 30 parathyroid carcinoma cases from the Netherlands diagnosed in the period 1985–2000.<sup>14</sup> One of these cases (case 30<sup>16</sup>) came from a documented HPT-JT family in which the germline mutation was identified. From the remaining, apparently sporadic, carcinomas we could further study FFPE tumour tissue of 28 cases (24 sporadic primary parathyroid carcinomas, three regional lymph-node metastases, one lung metastasis; 13 females and 15 males in total). Case 22 was not tested for *HRPT2* mutations, and cases 1, 16, 17, 27 and 29 were not tested for *MEN1*, mostly because of limited availability of tissue. Carcinoma features primarily included the presence of vasoinvasion, with or without invasion into the capsule and/or distant metastasis. Three equivocal cases (9, 11 and 25) were diagnosed as carcinomas based on their clinical presentation; vasoinvasion was not found in the histological slides of these cases.<sup>14</sup>

### HRPT2 and MEN1 mutation analysis

DNA extracted from FFPE material was used for polymerase chain reaction (PCR) amplification as described previously.<sup>25</sup> Primers for *HRPT2* and *MEN1* that would specifically amplify and sequence the degraded DNA from FFPE tissue were designed (Table 1). To sequence *HRPT2*, we used 24 primer pairs to cover the 17 exons of *HRPT2* and 15 primer pairs to cover the 10 exons of *MEN1*. Some of the products failed to amplify from FFPE DNA. This is because of the limited fragment size that can be amplified from FFPE tissue,

in which combinations of repetitive sequences, low genomic complexity or either low or high GC content impair possibilities for primer design.

### Immunohistochemical staining of parafibromin

Immunohistochemical staining of parafibromin was described for 26/28 cases.<sup>24</sup>

## Results

### HRPT2 mutation analysis

Twenty-seven of 28 FFPE parathyroid carcinomas were screened for *HRPT2* gene mutations. In 24 of the samples, the sequences of > 75% of the *HRPT2* gene could be analysed in the fragmented DNA, except in case 25 (66% of the sequence analysable) and cases 16 and 26 (69% analysable; in the latter case, two mutations were identified). Exons 15 (101 bp, GC content 29%) and 17 (33 bp, GC content 37%), together comprising 8% of the complete *HRPT2* gene, could not be sequenced in any of the samples. However, exons 1, 2 and 7, known to harbour 85% of all somatic *HRPT2* mutations, were sequenced completely in all samples.

Six mutations were found in four cases (15%, Table 2). Case 1 harboured a germline mutation in exon 2 (c.176C>T), resulting in a change from a serine (an aliphatic amino acid) into a phenylalanine (an aromatic amino acid). Further studies are required to determine a possible pathogenic effect of this mutation.

Case 8, with a frameshift mutation in exon 2 (c.165delC), was described previously in a paper by Howell *et al.*<sup>18</sup> In cases 23 and 26, a somatic mutation in exon 7, c.128G>A, resulting in the formation of a stop codon, was identified. This mutation has also been found in other studies.<sup>18</sup> In both cases, additional frameshift mutations were found in exon 8: c.692\_693insT (germline) and c.693\_694insG for cases 23 and 26, respectively.

In 17 of 27 tumours, a previously described polymorphism (IVS12–86C>T)<sup>18</sup> was identified.

### MEN1 mutation analysis

Twenty-three of the 28 parathyroid carcinomas were screened for mutations in the *MEN1* gene. In most cases, more than 70% of *MEN1* could be analysed. The exceptions were cases 22, 23, 24 and 26, for which 45%, 51%, 61% and 62% were analysed, respectively.

The first part of exon 2 (63 bp) and exon 10 (187 bp), could not be reliably sequenced in any of the samples. Together, these products cover 19% of the coding exons.

Three somatic mutations were found (see Table 2 and Fig. 1), consisting of an (unreported) missense mutation in exon 3 (patient 6: c.646G>T), changing a valine (an aliphatic amino acid) into a phenylalanine (an aromatic amino acid) and two as yet unreported frameshift mutations (patient 18: exon 9, c.1271delG and patient 20: exon 2, c.167\_170del4).

Three polymorphisms in the *MEN1* gene were identified: nucleotide substitutions in exon 4 (c.710G>A), resulting in the change of CGC (arginine) into CAC (histidine), and in exon 9 (c.1269C>T),

**Table 1.** Primer pair choice for FPE genomic sequencing of *HRPT2* and *MEN1* (5'–3'), with product size in base pairs

Exon	Product size (bp)	Primer sequence	Exon	Product size (bp)	Primer sequence
<i>HRPT2</i> mutations			<i>MEN1</i> mutations		
1A	173	CGAGGCGACAAGAGAAGAAG CAGGAGAACTCCCGAAGAT	2A	63	GGGCGGGTGAACCTTAG ACCAAGGAAAGGAGCACCAG
1B	160	ATTGTGGTGAAGGGAGACGA GGGAGGGGTTAAGAAAGAGG	2B	152	CCTGTTTGTCTGGCAGCTGG GGCGCGCATGATAGACAGGTC
2A	166	TGAATCCAGCCTGAAGAGTTG CAGCTCGGACATAAACAGGA	2C	83	CTGGCGGCCTCACCTACTTTC GGAGACCTTCTTACCAGCTCAC
2B	172	GAAGGCCAACCCAGAGAGTA AGGCCAGACCCTGTCTCTTA	2D	163	GCCGTCGACCTGTCCCTCTATC CATGGATAAGATTCCCACCTACTGG
3	162	AGTTGTGTATCATTTTATTCATTCA TGTCTGTTTAAGACTGGGAACAA	3A	77	GCACAGAGGACCCCTCTTCATTAC CTTGCCGTGCCAGGTGAC
4	199	AAAAACCTAAAGCATTTCACCTGT GTTTGGAAATGGGCTTCTGA	3B	132	CTCGCCCTGTCTGAGGATCATG TGGGTGGCTTGGGCTACTACAG
5	199	CAGAAGCCCATTCACAAACT TCCTCAGGTACTGCAATCAAA	4	129	GGGCCATCATGAGACATAATG CTGCCCATTTGGCTCAG
6A	195	TTGGCTCTAAGAGCACTGATACC CCTTCTTTGTGACCTTCCAA	5	41	CCTGTTCCTGGCTCATAAATC CTAGGAAAGGATCATAAATCAGGC
6B	192	TGGCCCTTGATAAAGAGAGA GGCATAAATGAATCCAAGAGG	6	88	GGGTGGCAGCTGGAATATG CTCAGCCACTGTTAGGGTCTCC
7A	195	GGAATGCCTGTCTGTGAAAT CGGGTCACATCTACCTCAGC	7	137	ATCCTCTGCCTCACCTCCAT AGGGTGGTTGGAACCTGATG
7B	176	TGACATAAATGCCCTTAAACAGA TGAAACTTCCACCTAAAAGCAA	8	136	GTGAGACCCCTTCAGAGCCCTAC TGGGAGGCTGGACACAGG
8	236	TGTAGTAGGGAAGAATCGATAGTAAGA AATCTACTGTAAAGCAGTAAAGCAAT	9	165	ATCTGTGCCCTCCCTTCC CACCTGTAGTGGCCAGACCT
9	225	GGTCATGCTACTGCACTCCA GCCACACTGCCTCTCAAGTT	10A	103	CGGCAACCTTGCTCTCACC CCAGGCCCTTGTCCAGTG
10	221	GGCTTTGTATATATTGAACCATCA TCCCTGGAACAAAAGAACAT	10B	184	GGGAGTCCAAGCCAGAGGAG GCCCTTCACTTCTCACTCTGG
11A	171	CAGTGGAGTAACCACTGAGTGAG GGGCTGCAGGAGTCTGAGT	10C	195	GAGGGTCCAGTGCTCAGTTT GGTCCGAAGTCCCCAGTAGT
11B	205	TTTAAAGGAGGGTGCATCTG CGACAGTCTTCAAGAAACATGA			
12	216	GGTTTTTATGACACAGAGTTGTG TGTGGCTTGGGCACTAATAA			
13A	207	GCCCAAGCCACACTGATTAT GAGGTGGTAGCTGCAGGAAT			
13B	219	TGTCTTTATAGGATCTCGAACACC GCCTATAGCAGAGAAACCGAAA			
14A	206	CCATTTTCATCAGCTGGAAT GCATAAGTTTAAGGGGCTGGT			
14B	176	GAAGAAAAGACCAGATGCAACC CGTCATCAACGGCAATAACA			
15	178	CCCCCACCACCTTTTCTACT CACATCATATGCGCAGAACT			
16	198	TGATAACTTGTCTCCACCGTCTC CACAAGCATATTTAGAAATCGGAAT			
17	161	ATTGGCTCCTCCATTCTG GCCAAAAAGTTTGCTTATATGGAT			

A, B and C indicate division of relatively large exons.

**Table 2.** Overview of *HRPT2* and *MEN1* mutations found in apparently sporadic carcinomas. Patient numbers refer to Haven *et al.*<sup>14</sup>

Case	Exon	Nucleotide change	Amino acid change	Somatic/germline
<i>HRPT2</i> mutations				
1	Exon 2	c.176C>T	p.Ser59Phe	Germline
8	Exon 2	c.165delC	p.Tyr55fsX	Somatic
23	Exon 7	c.128G>A	p.Trp42fsX	Somatic
23	Exon 8	c.692_693insT	p.Trp230LeufsX38	Germline
26	Exon 7	c.128G>A	p.Trp42fsX	Somatic
26	Exon 8	c.693_694insG	p.Arg231GlufsX37	ND
<i>MEN1</i> mutations				
6	Exon 3	c.646G>T	p.Val215Phe	Somatic
18	Exon 9	c.1271delG	p.Gly423AlafsX25	Somatic
20	Exon 2	c.167_170del4	p.Thr55ThrfsX62	Somatic

ND, not determined.

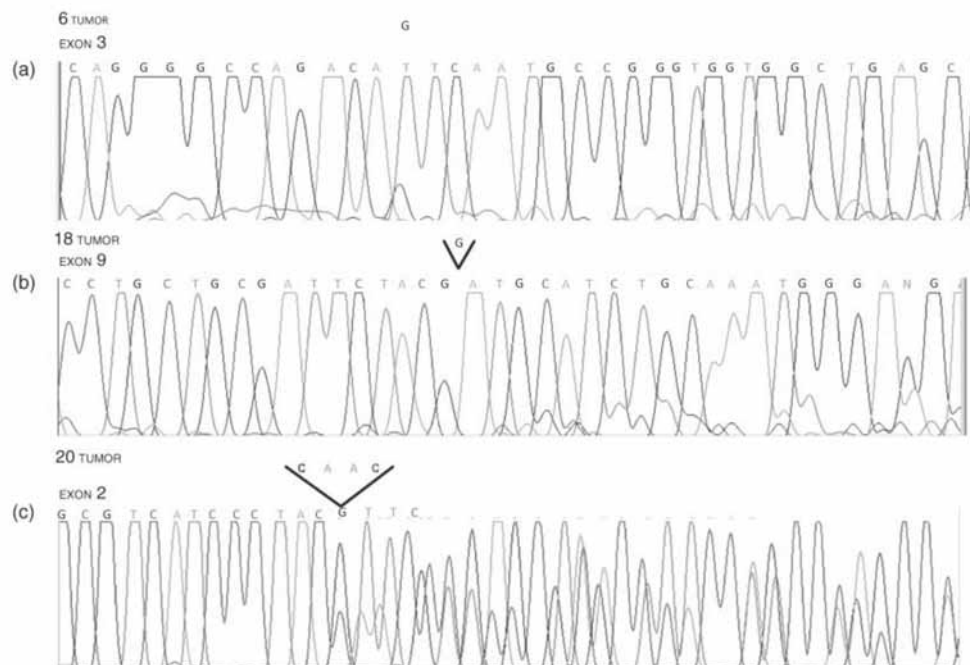


Fig. 1 Sequencing chromatograms of parathyroid carcinomas with somatic *MEN1* mutations: (a) a missense mutation in exon 3 (c.646G>T), resulting in the change of a valine into a phenylalanine (patient 6); (b) a 1-bp deletion in exon 9 (c.1271delG) (patient 18); (c) a 4-bp deletion in exon 2 (c.167\_170del4) (patient 20).

resulting in the change of a GAC (asparagine) into GAT (asparagine), and in exon 9 an (c.1303G>A) ACG into ACA (threonine–threonine) polymorphism was found.

Histology of the three parathyroid carcinomas with *MEN1* mutations is shown in Fig. 2. Notably, the clinical history of patient 18 displayed lymph-node and lung metastases during follow-up. Matched constitutive DNA was analysed to determine the germline *vs.* somatic nature of these mutations; all mutations were found to be somatic.

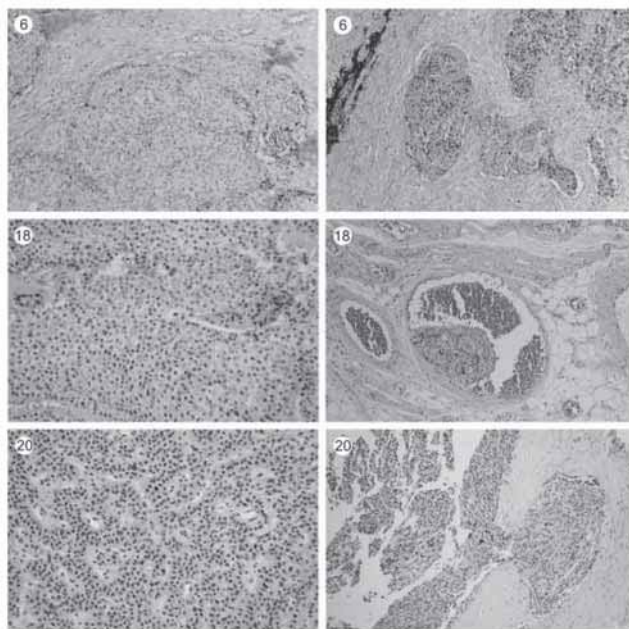
#### Comparison of *HRPT2*, *MEN1* mutated and remaining parathyroid carcinomas

We recently studied the expression profiles of benign and malignant parathyroid lesions with or without *MEN1* or *HRPT2* mutations.<sup>23</sup> A distinct profile was identified for a set of tumours consisting of HPT-JT benign and malignant tumours, including sporadic parathyroid carcinomas. The dominant profile in this subset of tumours appeared to be determined by the abrogation of *HRPT2* function. We identified several differentially expressed genes such as E-cadherin (*CDH1*), histone 1 H1c (*HIST1H1C*) and amyloid beta precursor protein 1 (*APPBP1*). Expression of these genes was confirmed in FFPE tumours using immunohistochemistry. In the current study we related the identified *MEN1* and *HRPT2* mutations with the pre-

vious results for the parathyroid carcinomas (Table 3), including clinical features, immunohistochemical staining results of Ki-67, calcium-sensing receptor (*CASR*), cyclin D1 (*CCND1*), *HIST1H1C*, *APPBP1* and parafibromin<sup>14,23,24</sup> and LOH of *HRPT2* and *MEN1* based on sequence results. Furthermore, we compared the mutation group of tumours with the remaining set of parathyroid carcinomas (Table 4). Combining results in Tables 3 and 4 showed that the *HRPT2* mutated samples, although based on only a small number of samples, showed significantly more *CASR* downregulation and more *CCND1* and *APPBP1* overexpression compared with both *MEN1* mutated carcinomas and the remaining carcinomas.

#### Discussion

As discussed recently by Rubin and Silverberg,<sup>26</sup> the diagnosis of parathyroid carcinoma is difficult, based on clinical and histological grounds; lymph-node and/or distant metastasis denote evident malignancy. Fibrous septa in the tumour, mitotic figures, capsular and vasoinvasive growth remain unreliable features for diagnosis. To identify possible molecular tools to improve the diagnosis of parathyroid carcinomas, we studied a series of 28 cases of parathyroid carcinoma. This relatively large number of tumours could only be obtained by using FFPE tissue of parathyroid carcinomas diagnosed



**Fig. 2** Histology of the three sporadic carcinomas with a *MEN1* mutation. First column: a gross overview of the tumours (magnification  $\times 100$ ). Second column: a detail (magnification  $\times 200$ ) image that shows vasoinvasion (cases 6 and 18) and invasion of the capsule (case 20).

**Table 3.** Parathyroid carcinomas with *HRPT2* or *MEN1* mutations. Patient numbers (as used by Haven *et al.*<sup>14</sup>) and clinical data such as sex, age, diagnosis, metastasis and recurrence are depicted in rows. The presence of vasoinvasion and the mutation status of *HRPT2* and *MEN1* are also included. The results of immunohistochemical staining for Ki-67 (index), CASR (0, downregulation; 1, normal expression), CCND1 (0, normal expression; 1, upregulation), CDH1 (1, normal expression; 2, aberrant expression), APPBP1 (0, normal expression; 1, overexpression), HIST1H1C (0, normal expression; 1, overexpression) and parafibromin are shown.

Patient number	1	8	23	26	6	18	20
Sex	F	M	M	F	M	F	F
Age (years)	30	32	41	80	72	51	52
Diagnosis	Primary	Primary	Reg.L.N.	Primary	Primary	Primary	Primary
Vasoinvasion	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Metastasis/recurrence	NK	Yes	Yes	NK	NK	Yes	NK
<i>HRPT2</i>	Mut	Mut	Mut2	Mut2	No	No	No
<i>MEN1</i>	ND	No	No	No	Mut	Mut	Mut
Ki-67	5	10	9.8	5	9.6	3	3.3
CASR	0	0	0	–	0	1	1
CCND1	1	1	1	1	0	0	1
CDH1	2	2	1	2	2	1	1
HIST1H1C	1	ND	1	0	1	0	1
APPBP1	1	1	1	1	1	0	1
Parafibromin	Global loss	Global loss	Global loss	Focal loss	Global loss	Global loss	Focal loss
seqLOH1q	No	Yes	No/No	No/No	ND	ND	ND
seqLOH11q	ND	ND	ND	ND	Yes	Yes	No

F, female; M, male; Primary, primary tumour; Reg.L.N., regional lymph node; –, no data; NK, not known; Mut, mutation; Mut2, double mutation; ND, not determined; seqLOH, intragenic loss of heterozygosity LOH based on sequencing results.



**Table 4.** Comparison of *HRPT2*/*MEN1* mutated and remaining parathyroid carcinomas. Depicted are the percentages of samples per group that show aberrations. The significant percentages ( $P < 0.05$ ) are shown in bold

		<i>MEN1</i> mutated (%)	<i>HRPT2</i> mutated (%)	Remaining (%)
No. of samples		3	4	21
Ki-67	Average	5.3	7.5	4.8
CASR	Downregulation	33.0	<b>100.0</b>	14.0
CCND1	Overexpression	33.3	<b>100.0</b>	50.0
CDH1	Aberrant	33.3	75.0	28.0
APBBP1	Overexpression	66.6	<b>100.0</b>	50.0
HISTH1C	Overexpression	33.3	66.6	38.0
Parafibromin	Global loss	66.0	75.0	50.0
Parafibromin	Focal loss	33.0	25.0	50.0

in several hospitals in the Netherlands over a period of 15 years. We designed relatively reliable PCRs taking the fragmented DNA in FFPE tissue into account as well as a critical level of DNA input.<sup>27</sup> The parathyroid tumours were revised and studied with regard to morphological and molecular features, including LOH of chromosomes 1q and 11q.<sup>14</sup> We report the presence of inactivating somatic mutations of both *MEN1* and *HRPT2* in sporadic parathyroid carcinomas.

Although *MEN1* mutations are frequently found in familial (95%) and also sporadic adenomas (20–30%), the contribution of germline *MEN1* mutations to the development of parathyroid carcinomas was previously shown in only two *MEN1* syndrome cases. These two parathyroid carcinomas exhibited a concurrent parathyroid adenoma. The number of parathyroid carcinomas reported in patients suffering from the *MEN1* syndrome is thus almost negligible in relation to the prevalence of PHPT in this syndrome. We have found a somatic *MEN1* mutation in 13% of the sporadic parathyroid carcinomas, suggesting that the prevalence of *MEN1* mutated carcinomas as a cause of sporadic PHPT appears to be higher than originally thought. As the prevalence of *MEN1* mutations in sporadic adenomas is higher (30%) than now found in carcinomas (13%), this may indicate that, in time, an adenoma can progress into a carcinoma. The difference in prevalence of parathyroid carcinomas in *MEN1* and sporadic PHPT with somatic *MEN1* mutation may be explained by the regular screening of patients with *MEN1* syndrome. Hyperfunctioning parathyroid glands are thus detected at an early stage and removed before they can progress into carcinoma.

Mutations and subsequent abrogation of *HRPT2* TSG function have recently been associated with sporadic parathyroid carcinomas or with parathyroid carcinomas in the context of HPT-JT syndrome.<sup>15,18</sup> Subsequent immunohistochemical analysis often detected global loss of parafibromin encoded by *HRPT2* in parathyroid carcinomas.<sup>24,26</sup>

We detected six inactivating *HRPT2* gene mutations in only four cases (15%) of our series of parathyroid carcinomas. At least two *HRPT2* mutations are of germline origin, possibly illustrating the incomplete penetrance of the HPT-JT syndrome, which was also reported by others.<sup>12,13</sup> The overall frequency of *HRPT2* mutations detected in this study is substantially lower than that recently described in parathyroid carcinomas (4/4, 6/7 and 10/15),<sup>12,13,18</sup> but

it is still higher than found in parathyroid adenomas (1.8%).<sup>12,16–19</sup> Although exons 1, 2 and 7,<sup>12,15–17</sup> which harbour 85% of all known mutations, were completely screened in all cases, the low mutation frequency could be explained in part by the fact that not all exons could be completely screened because of the nature of the FFPE tissue. Another possible explanation could be the existence of large somatic or germline genomic deletions that were not studied. The latter case, with, for example, a founder mutation in the Dutch population, might explain these results. Finally, we did not address gene silencing of *HRPT2* due to promoter methylation. Part of the apparently sporadic parathyroid carcinoma cases could in fact be familial cases suffering from FIHP. A proportion of FIHP families with parathyroid carcinomas and/or cystic adenomas are found to carry *HRPT2* mutations;<sup>22</sup> however, in the majority of tumours (65%) in an FIHP context, the cause is unknown and may be the result of mutations in a yet unknown (*HRPT1*) gene.<sup>21,22</sup>

Importantly, the selection of parathyroid carcinomas is different compared to the previous studies by Howell,<sup>18</sup> Shattuck,<sup>15</sup> Cetani<sup>12</sup> and co-workers, in which only cases with metastases or recurrence were included. It might be speculated that parathyroid tumours fulfilling only the classic histological features (vasoinvasive growth, fibrous bands, etc.), but without signs of recurrence or metastasis, should be considered as less aggressive carcinomas, in contrast to unequivocal carcinomas with *HRPT2* mutations. Our series of 28 parathyroid carcinomas fulfil the histological criteria for malignancy; however, we do not have complete follow-up to address the above hypothesis. Only one out of four *HRPT2* mutated cases had documented lymph-node metastases. However, in five metastasized parathyroid carcinomas (four with regional lymph nodes and one with a lung metastasis), no *HRPT2* mutations were identified. Notably, the tumours in this study were previously analysed for parafibromin immunoreactivity and all showed global or focal loss of staining,<sup>24</sup> indicative of the diagnosis of parathyroid carcinoma. *HRPT2* mutated parathyroid carcinomas might be different from *MEN1* mutated or remaining tumours. This seems to be supported by the differential immunohistochemical expression of molecules such as CASR, CCND1 and APBBP1. The latter were recently identified as part of a distinct cDNA expression profile in *HRPT2* mutated benign and malignant parathyroid tumours.<sup>23</sup>

In conclusion, we have successfully identified inactivating *HRPT2* somatic mutations in archival sporadic parathyroid carcinomas. Additionally, we report for the first time the presence of *MEN1* somatic mutations in these tumours, suggesting that *MEN1* mutations play a role in the development of parathyroid carcinomas.

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