

Original articles

# Localisation of a gene causing endocrine neoplasia to a 4 cM region on chromosome 1p35-p36

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**Abstract**

The development of some endocrine tumours, such as medullary thyroid carcinomas, pheochromocytomas, anterior pituitary adenomas, and parathyroid adenomas involve a putative tumour suppressor gene located on chromosome 1p32-pter, a region that represents 111 cM. In order to refine the location of this gene, 93 endocrine tumours (39 parathyroid adenomas, 40 anterior pituitary adenomas, seven pancreatic islet cell adenomas, and seven carcinoids) were investigated for loss of tumour heterozygosity (LOH) using the seven polymorphic loci 1pter - D1S228 - D1S507 - D1S234 - D1S476 - D1S220 - D1S207 - D1S206 - 1cen. LOH was detected in 27% of the parathyroid tumours and in 7.5% of the pituitary tumours, but in none of the pancreatic islet cell or carcinoid tumours. In addition, seven of the 10 parathyroid tumours that showed LOH of chromosome 1p facilitated a more precise mapping of this putative tumour suppressor gene; five tumours involved a loss only of the telomeric locus D1S228, whereas two other tumours showed LOH at the centromeric loci D1S507, D1S234, D1S476, and D1S220, but not D1S228. Thus, our results have mapped this tumour suppressor gene implicated in endocrine tumours to a 4 cM region flanked by D1S228 and D1S507 on chromosome 1p35-p36.

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Keywords: endocrine tumours; loss of heterozygosity; chromosome 1p35-p36

The involvement of a tumour suppressor gene located on chromosome 1p in the aetiology of some endocrine tumours, for example, medullary thyroid carcinoma,<sup>1-3</sup> pheochromocytomas,<sup>2,3</sup> anterior pituitary adenomas,<sup>4-6</sup> and parathyroid adenomas,<sup>4,7</sup> has been established by the demonstration of a loss of tumour heterozygosity (LOH). This gene has been mapped to 1p32-1pter,<sup>7</sup> a region that represents 111 cM,<sup>8</sup> and in order to facilitate positional cloning studies of this gene, we

undertook investigations to refine this location. In addition, we investigated the potential role of this gene on chromosome 1p in the aetiology of other endocrine tumours, such as pancreatic islet cell adenomas and carcinoid tumours, which may also occur in association with the multiple endocrine neoplasia type I (MENI) syndrome located on chromosome 11q13.<sup>9-11</sup>

**Materials and methods**

TISSUE SPECIMENS

The 93 endocrine tumours (39 parathyroid tumours (30 from MENI patients and nine from non-MENI patients), seven pancreatic islet cell tumours (all from MENI patients), 40 anterior pituitary adenomas (all from non-MENI patients), and seven carcinoids (one intestinal from a MENI patient and six bronchial from non-MENI patients)) were obtained and stored at -70°C with a matching blood sample. The seven pancreatic islet cell tumours consisted of three insulinomas, three glucagonomas, and one non-functioning adenoma and the subtypes of the 40 anterior pituitary adenomas (14 somatotrophinomas, 15 non-functioning tumours, nine prolactinomas, and two corticotrophinomas) have been previously reported.<sup>6,12-14</sup>

ALLELE LOSS STUDIES

Genomic DNA was extracted from tumours and leucocytes, as previously described.<sup>15</sup> The seven microsatellite polymorphisms from chromosome 1p (D1S228, D1S507, D1S234, D1S476, D1S220, D1S207, and D1S206) were detected by the amplification of genomic DNA from each patient's paired tumour and leucocyte sample with specific oligonucleotide primers flanking the repeat sequence using the polymerase chain reaction (PCR) and LOH was scored as previously described.<sup>5,13,16</sup> All the parathyroid, pancreatic islet cell, and carcinoid tumours were investigated at each of the seven chromosome 1p loci. However, owing to the limited quantities of DNA available from the pituitary tumours, the investigation of these tumours was restricted and only the polymorphisms at D1S228 and D1S507, which were found to define the critical interval from the parathyroid tumour data (fig 1), were investi-

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		Patient							
		1	2	3	4	5	6	7	
1p	Locus								
		D1S228	-	-	-	-	-	+	+
		D1S507	+	+	+	+	+	-	-
		D1S234	+	+	●	●	●	-	-
		D1S476	●	+	+	+	+	●	-
		D1S220	+	●	●	+	●	-	●
		D1S207	+	+	●	+	+	●	●
		D1S206	+	+	+	●	+	●	+
		11q13 loss	N	Y	N	Y*	Y	N*	Y
		13q loss	Y	U	N	N*	N	Y*	Y

Figure 1 Analysis of alleles on chromosome 1p in DNA from leucocytes and parathyroid tumours from seven patients. Patients 4, 5, and 7 suffered from MEN1. The leucocyte and parathyroid tumour alleles were determined as shown in fig 2, and 35 of the 49 leucocyte genotypes determined were heterozygous. The corresponding parathyroid tumour genotypes indicated retention of heterozygosity (+) at 24 loci and allelic deletions (-) at 11 loci. The remaining 14 homozygous loci (black circles) in the leucocytes were not studied in the tumours, as the small amounts of DNA obtained from some tumours limited the loci which could be examined. Allelic deletions of chromosomes 11q13 and 13q are indicated (Y=yes, N=no, U=unknown), and those previously reported<sup>13,14</sup> are indicated by an asterisk. The results indicate that a putative tumour suppressor gene involved in the development of endocrine tumours is located in the interval flanked by D1S228 and D1S507 on chromosome 1p35-p36.

gated in all the pituitary tumours. An assessment of LOH at the chromosome 11q13 loci (D11S480, PYGM, D11S913, D11S1917, and D11S970) and the chromosome 13q12-q14 loci (Rb1.20 and D13S260) in the majority of these tumours has been previously reported,<sup>5,6,13,14</sup> and tumours obtained since these studies were similarly characterised and the results pooled.

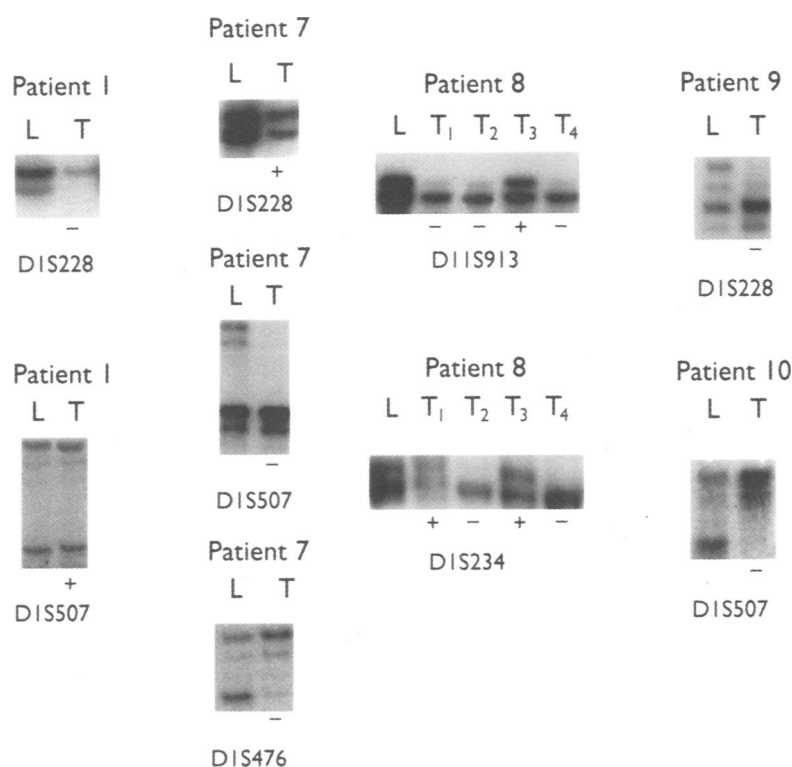
### Results

Thirty-seven of the 39 patients with parathyroid tumours, 33 of the 40 patients with anterior pituitary tumours, all of the seven patients with pancreatic tumours, and all of the seven patients with carcinoid tumours were informative for the 1p loci that were examined. However, LOH was detected only in the parathyroid and anterior pituitary tumours and occurred in 27% and 7.5% of the respective tumours. Interestingly, six of the 28 (21%) MEN1 parathyroid tumours and four of the nine (44%) non-MEN1 parathyroid tumours showed chromosome 1p allele loss and this contrasted to the chromosome 11q13 allele loss in these tumours which were 80% (24 out

of 30) and 33% (three out of nine), respectively. Seven of the 37 (19%) parathyroid tumours (six MEN1 and one non-MEN1) showed allele loss involving both chromosomes 1p and 11q13 (figs 1 and 2). The clinical and histological features, for example, age and sex of the patient and weight of the gland, of these patients were similar to those of patients who showed allele loss of only one chromosome. LOH involving chromosome 1p was observed in only three (two of 13 somatotrophinomas and one of eight prolactinomas) of the 40 anterior pituitary adenomas. Chromosome 1p allele loss was not observed in the pancreatic islet cell tumours or the carcinoid tumours. Seven of the 10 parathyroid tumours that showed LOH of chromosome 1p facilitated a more precise mapping of this putative tumour suppressor gene (fig 1). Thus, in five tumours allele loss involving only the telomeric locus D1S228 was observed whereas in two other tumours LOH at the centromeric loci D1S507, D1S234, D1S476, and D1S220, but not at D1S228, was observed. The combined results from these seven tumours located this putative tumour suppressor gene to an interval flanked by D1S228 and D1S507. The genetic distance between D1S228 and D1S507 has been estimated previously to be 4 cM,<sup>8</sup> and thus our results have helped to define the map location of this gene causing endocrine neoplasia to chromosome 1p35-p36.

### Discussion

Our results which show allele loss of chromosome 1p in 21% of MEN1 and 44% of non-MEN1 parathyroid adenomas are consistent with those of a previous study<sup>7</sup> that located this putative tumour suppressor gene to chromosome 1p32-pter. Our observations of chromosome 1p allele loss in somatotrophinomas and prolactinomas are also in keeping with those previously reported in these subtypes and one corticotrophinoma.<sup>4,6</sup> In addition, our studies have defined a more precise location for this putative endocrine tumour suppressor gene to a 4 cM region, flanked by D1S228 and D1S507, in chromosome 1p35-p36. However, a previous report investigating chromosome 1p LOH in medullary thyroid carcinomas and phaeochromocytomas, which are associated with multiple endocrine neoplasia type 2, indicated that the location of a tumour suppressor gene in these tumours was likely to be in the vicinity of 1cen.<sup>17</sup> Thus, it is possible that there may be two tumour suppressor genes located on chromosome 1p that are involved in the aetiology of some endocrine tumours. The absence of LOH involving chromosome 1p loci in the pancreatic islet cell and carcinoid tumours may either reflect a relatively low level of LOH, which would not have been detected in the small numbers of tumours studied, or a lack of any involvement of chromosome 1p tumour suppressor genes in the development of these tumours. Our definition of a 4 cM region located on chromosome 1p32-pter that is involved in the aetiology of some parathyroid tumours represents an important advance in identifying this putative tumour suppressor



**Figure 2** Allelic deletions on chromosome 1p in six parathyroid tumours (patients 1, 7, and 8), a somatotrophinoma (patient 9), and a prolactinoma (patient 10), none of which has previously been reported.<sup>5,6</sup> Patients 7 and 8 have MEN1. The panels show some of the autoradiographs obtained with the microsatellite polymorphisms at the DIS228, DIS507, DIS234, DIS476, and D11S913 loci. At each represented locus, the leucocytes (L) are heterozygous and the tumour (T) cells have either retained this heterozygosity (+) or developed a loss of heterozygosity (-). The combined LOH studies of chromosome 1p and 11q13 loci from the four parathyroid tumours of patient 8 show that T<sub>1</sub> and T<sub>4</sub> have allelic deletions of chromosome 1p and 11q13, whereas T<sub>2</sub> and T<sub>3</sub> have only an 11q13 loss. These results show that allelic deletions on chromosome 1p are involved in the development of parathyroid and pituitary tumours.

gene. A number of potential candidate genes are located in this region and these include the zinc finger ZFM60,<sup>18</sup> the helix-loop-helix protein Heir-1,<sup>19</sup> the developmental regulator gene Pax7,<sup>20-22</sup> the cell cycle regulator p58,<sup>23</sup> the transcription factor E2F-2,<sup>24</sup> and the transcription factor DAN that has homology to a mouse tumour suppressor gene.<sup>25</sup> Thus our studies, which have identified the region to search for such potential candidate genes, will help in further positional cloning studies aimed at identifying this tumour suppressor gene causing endocrine neoplasia.

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