

ELECTRONIC LETTER

PRKAR1A, one of the Carney complex genes, and its locus (17q22-24) are rarely altered in pituitary tumours outside the Carney complex

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The tumour suppressor gene encoding the cAMP dependent protein kinase A (PKA) type I- α regulatory subunit (RI α), *PRKAR1A*, has been mapped to chromosome 17q22-24 and is often mutated in the Carney complex (CNC),^{1,2} a multiple neoplasia and lentiginosis syndrome inherited in an autosomal dominant manner.^{3,4} The complex was first described as an association of lentiginos, primary pigmented nodular adrenocortical disease (PPNAD), and a variety of endocrine and non-endocrine tumours (cardiac and breast myxomas).⁵⁻⁷ Growth hormone (GH) and prolactin (PRL) secretion abnormalities have been found in over two-thirds of patients with CNC^{8,9}; in some cases, pituitary somatomammotrophic hyperplasia was also seen.⁸ GH producing adenomas (which also secrete small amounts of PRL) have been reported with increased frequency in patients with CNC; it was suggested that tumours in these patients develop in situ from precursor benign hyperplasia, following a sequence of genetic events not unlike the one described in other tissues.^{10,11}

Genes implicated in cyclic nucleotide dependent signalling have long been considered likely candidates for pituitary tumorigenesis.^{12,13} Somatic activating mutations in the *GNAS1* gene, which encodes the α subunit of the stimulatory G protein, lead to increased cAMP production and have been reported in approximately half of sporadic pituitary adenomas associated with acromegaly.¹⁴⁻¹⁶ In addition, methylation abnormalities of the *GNAS1* gene are present in a significant number of pituitary tumours.¹⁷ Patients with McCune-Albright syndrome develop GH and PRL producing pituitary hyperplasia^{17,18} and their pituitary tissue harbours, as other affected tissues in these patients, somatic, activating mutations of the *GNAS1* gene.¹⁹⁻²¹

In the present study, we investigated a large collection of sporadic pituitary adenomas (SPA) from the USA, UK, Japan, and France for loss of heterozygosity (LOH) of the 17q22-24 *PRKAR1A* locus and mutations of the *PRKAR1A* gene. In addition, six index cases that had presented with inherited pituitary tumours (4) or had MEN1-like symptomatology but negative family history (2), and had been screened and found negative for *MEN1* mutations, were also screened for *PRKAR1A* mutations. No significant LOH and no *PRKAR1A* mutations were found in the sporadic tumours or the germline DNA of the patients with the MEN1-like manifestations.

METHODS

Patients and DNA extraction

The institutional review boards of the participating institutions approved the collection of the blood and tumour samples

Key points

- The tumour suppressor gene encoding the cAMP dependent protein kinase A (PKA) type I- α regulatory subunit (RI α), *PRKAR1A*, has been mapped to 17q22-24 and is mutated in Carney complex (CNC), a familial multiple endocrine neoplasia (MEN) syndrome that is associated with inherited pituitary somatomammotrophic adenomas.
- Sporadic pituitary tumours are often associated with *GNAS1* mutations and imprinting defects, suggesting a role for the cAMP pathway in non-inherited tumours, at least those of the GH and/or PRL producing cells.
- In the present study, we collected 73 sporadic pituitary GH and/or PRL producing pituitary adenomas and six cases with a MEN 1-like syndrome, three with familial acromegaly, one with familial non-secretory pituitary tumours, and two with a pituitary tumour and hyperparathyroidism and a negative family history; all had been previously screened and found negative for *MEN1* mutations.
- Twenty paired blood-tumour samples of sporadic cases were studied for loss of heterozygosity (LOH) of the 17q22-24 *PRKAR1A* region using polymorphic markers, including one within the 5' end of the *PRKAR1A* gene. Analysis of each exon of the *PRKAR1A* gene was then performed in 61 tumours from which adequate DNA was available, and in germline DNA from the other six cases.
- LOH was observed infrequently and inconsistently. No coding *PRKAR1A* mutations were found in any of the 61 tumours or any of the genomic DNA from the six cases with MEN 1-like manifestations.
- We conclude that, like menin, the RI α subunit of PKA is not frequently involved in the pathogenesis of pituitary tumours not associated with CNC.

from the patients. None of the patients had CNC according to established criteria.²² DNA was extracted from blood and from frozen or paraffin embedded tumour samples by standard methods, as previously described.²³

In total, we investigated 73 sporadic tumours, 20 of which had matching blood available for LOH analysis. These include 15 GH and 5 PRL secreting tumours (fig 1). All patients had presented with acromegaly or clinically significant hyperprolactinaemia. For an additional collection of 53 tumour specimens, no blood DNA was available. Twenty-two of these tumours contained *GNAS1* mutations, whereas all others were negative for mutations of this gene.

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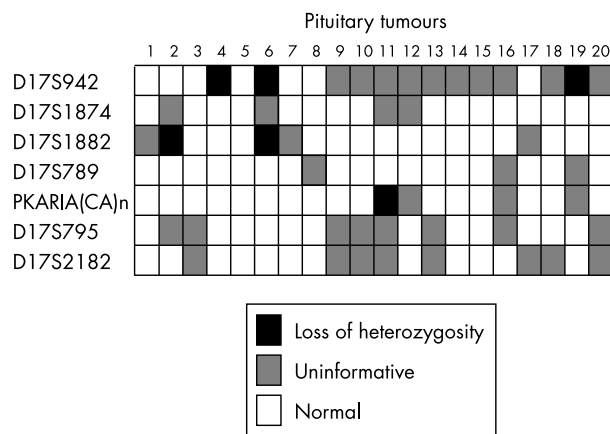


Figure 1 Location of the *PRKARIA* gene on chromosome 17 surrounded by dinucleotide repeat polymorphic markers in a centromere to telomere order. The *PRKARIA* (CA)_n dinucleotide repeat marker is located in the 5' region of the *PRKARIA* gene. Five pituitary tumours showed 17q LOH but in four the LOH zone was not continuous with the *PRKARIA* gene.

Six index cases who had been screened previously for *MEN1* and were not found to have any germline *MEN1* defects were also screened for *PRKARIA* mutations. The first presented at the age of 23 years with amenorrhoea and galactorrhoea owing to a PRL secreting adenoma; her sister had been diagnosed with a corticotrophin producing tumour. The second index case presented at the age of 19 years with acromegaly and a lipoma; his sister and daughter had prolactinoma and acromegaly, respectively. The third index case, the proband of a Brazilian family, presented at the age of 24 years with acromegaly; his brother and dead father also had acromegaly.²⁴ The fourth index case presented at the age of 52 years with a pituitary macroadenoma that was not producing any hormone excess; his dead father had had surgery, too, at the age of 65 for a pituitary tumour. The remaining two patients presented at the ages of 26 and 75 years, respectively, both with somatomammotrophinoma and hyperparathyroidism and no other *MEN1* related abnormalities or no known relative with endocrine tumours.²⁵

Microsatellite and loss of heterozygosity analysis

The polymorphic markers from 17q22-24 surrounding the *PRKARIA* gene are listed in fig 1 in centromere to telomere order. The sequences and genomic order of these primers are available in the genome database online (<http://gdbwww.gdb.org/> and <http://www-genome.wi.mit.edu>). A dinucleotide repeat within the *PRKARIA* gene (*PRKARIA* (CA)_n) was also used, as described elsewhere.^{1,2} For each of the markers, the reverse primer was end labelled with γ -³²P.²³ The microsatellite alterations seen in the tumours were classified as previously described.²³ Briefly, loss of heterozygosity (LOH) was present when only one allele was evident in tumour DNA versus two alleles in the corresponding bands of blood DNA. Microsatellite length instability was present, when multiple bands were seen in the amplified tumour DNA versus one or two in peripheral blood DNA. Specimens that were not successfully amplified were excluded from the analysis; only informative loci in both tumour and blood samples were studied for LOH.

PRKARIA denaturing HPLC (DHPLC) analysis and sequencing

The cDNA structure of human *PRKARIA* was described by Solberg *et al.*²⁶ We recently published the intron-exon structure of *PRKARIA*.² DNA samples were PCR amplified in 30 μ l volumes using *AmpliTag* Gold (Perkin-Elmer); 10 μ l samples were injected into a DHPLC instrument (HELIX, Varian Inc) at

column temperatures recommended by the DHPLC Melt program (<http://insertion.stanford.edu/melt.html>).^{27,28} In samples with LOH or suggestive of heterozygosity on D-HPLC, the remaining 20 μ l was purified using a PCR clean up kit (GFX, Amersham-Pharmacia Biotech) and sequenced using the BigDye Terminator kit (Perkin-Elmer). After electrophoresis on an ABI 377 fluorescent sequencer, the sequence traces were analysed using either Sequencher (Genecodes) or Vector NTI (Informax) (see also references 1 and 2 for conditions).

RESULTS

Loss of heterozygosity analysis for the 17q22-24 *PRKARIA* locus

The location of the tested markers on 17q22-24 and corresponding LOH analysis are summarised in fig 1. There was no large deletion in any one specimen encompassing the whole gene, and there was no consistent deletion among specimens to suggest frequent deletion of part of the gene. One tumour (sample 11) showed LOH for the intragenic *PRKARIA* marker but retained heterozygosity for D17S789 and two markers centromeric to it, whereas the subject was non-informative for the markers telomeric to the *PRKARIA* gene. Interestingly, this tumour was an invasive, grade 3 somatotrophinoma that has been described elsewhere.²⁹ Four additional tumours showed LOH for other markers in the *PRKARIA* region, without, however, losing heterozygosity (sample 4) or producing any information for the *PRKARIA* gene (sample 12).

Other microsatellite abnormalities, such as instability or size discrepancies, were not present.

PRKARIA denaturing HPLC (DHPLC) analysis and sequencing

Heteroduplex (D-HPLC) of the *PRKARIA* gene was performed in all samples with positive (DNA from CNC patients) and negative controls (DNA from previously screened patients) according to the conditions that we have established for *PRKARIA* mutation detection by this technique^{1,2} (data not shown). Because of the lack of abnormalities, we decided to perform sequencing analysis of each *PRKARIA* exon in all samples and in germline DNA from patients with inherited pituitary tumours (n=4) or *MEN1*-like manifestations (n=2). No *PRKARIA* coding sequence mutations were found in any specimens.

DISCUSSION

Pituitary tumours occur with increased frequency among patients with CNC.⁸ *PRKARIA*, the gene mutated in almost half of the patients with CNC,^{1,2} codes for the most abundant regulatory subunit of cAMP dependent PKA, a cellular system highly involved in pituitary cell growth and function.^{13,15,16} In the present study, we investigated the hypothesis that LOH or alterations of *PRKARIA*'s sequence are involved in sporadic pituitary tumours, as well as inherited, non-CNC related pituitary tumours. The results of the experiments described here suggested that the RI- α subunit of PKA is not a significant contributor to tumorigenesis in pituitary cells, as shown by infrequent LOH of the *PRKARIA* 17q22-24 locus and lack of *PRKARIA* mutations in a large international series of pituitary tumours. Although the number of families that was investigated was small, we may also conclude from this study that *PRKARIA* mutations are not responsible for a significant number of non-CNC related inherited pituitary lesions. This observation follows the report of a large family with inherited acromegaly and lack of linkage to the 17q *PRKARIA* locus.^{30,31} Additional studies on families with inherited pituitary tumours, however, need to be performed.

The pattern of a less frequent than expected involvement in sporadic lesions of a tumour suppressor gene that is responsible for a syndrome that predisposes to pituitary tumours is not unusual. *MEN1*, the gene mutated in *MEN1*,³² a syndrome

commonly associated with pituitary tumours and other lesions,³³ is only infrequently mutated in sporadic pituitary tumours.³⁴ Indeed, the tumours caused by germline mutations of tumour suppressor genes are often different from those sporadic lesions that are the result of somatic mutations of these genes.³⁵

It should be mentioned that methylation of the *PRKARIA* gene is a possibility for gene inactivation in the few specimens that showed LOH. Methylation of genes in pituitary adenomas is not infrequent^{36,37} and has been shown to affect tumoral *GNAS1* gene expression.¹⁷ However, *PRKARIA* methylation has not been shown convincingly to date (Sandrini *et al*, unpublished data).

We conclude that *PRKARIA*, a critical component of the PKA signalling pathway, does not appear to be frequently involved through mutation in the pathogenesis of pituitary tumours outside CNC.

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