Phaeochromocytoma is a rare, neuroendocrine, chromaffin staining tumour that usually arises within the adrenal medulla, although extra-adrenal phaeochromocytomas also appear in ganglia of the sympathetic nervous system. Approximately 10% of phaeochromocytomas are hereditary and may be found in association with von Hippel-Lindau disease, multiple endocrine neoplasia type 2, or neurofibromatosis type 1. Familial paraganglioma (PGL) is an inherited disorder characterised by the development of highly vascular tumours in the head and neck. Recent studies have related the presence of mutations on structural (SDHC, SDHD) and catalytic (SDHB) succinate dehydrogenase subunits to familial and sporadic phaeochromocytoma and/or paraganglioma susceptibility. While the SDHD locus is maternally imprinted, SDHB has classical autosomal dominant inheritance. Since Baysal et al. first described SDHD mutations as being associated with PGL, the screening of such alterations has become routine in the diagnosis of paraganglioma and phaeochromocytoma. Astuti et al. reported the first known mutations in the SDHB gene that caused susceptibility to familial phaeochromocytoma alone and to familial phaeochromocytoma with head and neck paraganglioma. A recent study reported mutations in the SDHB gene in ~20% cases of PGL and ~3% of sporadic paragangliomas. With regard to SDHC, only one study has identified a germline SDHC mutation in all the affected members of a family as being the underlying cause of PGL3.

To investigate further the involvement of SDHB and SDHC in paraganglioma and phaeochromocytoma susceptibility, we searched for germline mutations in 22 patients, 17 with phaeochromocytoma, three with paraganglioma, and two cases with both (table 1).

**METHODS AND RESULTS**

The patients, with or without a family history and without previous selection, had previously tested negative for VHL, RET, and SDHD mutations (in press). We used amplification analysis followed by sequencing of PCR products. Among the samples examined, we identified six heterozygous variants in the SDHB gene, one nonsense mutation (R27X), two silent mutations (L7L, A6A), and three intronic changes (IVS4+35ins, IVS2-33G/A, IVS2-35A/G). The SDHB mutation was a CGA→TGA (R27X) transition in exon 2 of case 22 (table 1). This R27X change gave rise to a 26 amino acid truncated protein by creating a premature stop codon (fig 1). The patient was a 32 year old male with familial phaeochromocytoma and several tumours derived from the sympathetic nervous system. He was diagnosed with adenalin paraganglioma and para-aortic paraganglioma, both of which were surgically removed, carotid body paraganglioma, and deafness. The patient’s mother was diagnosed with hyperparathyroidism and two affected paternal first cousins were diagnosed with extra-adrenal phaeochromocytoma.

Allele frequencies of the polymorphisms were determined by SSCP or CSGE of 200 chromosomes from a control population (table 1) and IVS4+35ins was not found. Nevertheless, it was not possible to ascribe any deleterious function to this unknown variant because RNA from the patient was not available. IVS2-33G/A and IVS2-35A/G, respectively, had frequencies of 3.3% and 7.2% in controls; no doubly heterozygous subjects were present.

**DISCUSSION**

Analysing the genotypic distribution of both polymorphisms in our small series of patients, we found a higher proportion of IVS2-33G/A heterozygotes among patients. This implies that this polymorphism could be acting as a low penetrance allele in the development of the disease (odds ratio=3.5, 95% CI 1.04 to 11.79). Broader case control studies are necessary in order to examine the role of this change in the pathogenicity of these tumour types.

No mutations in the SDHC gene were found in any of the 22 families tested. These results are consistent with those of

**Key points**

- Phaeochromocytoma is a neuroendocrine tumour that usually arises from chromaffin cells localised within the adrenal medulla, although ~15% of the lesions are found extra-adrenally. These tumours, which produce and secrete catecholamines, are mainly sporadic, but about 10% are hereditary and often associated with germline mutations in the VHL, RET, and NF1 genes and also in the SDHD and SDHB genes.
- Hereditary paraganglioma is a rare inherited disorder characterised by the development of benign extra-adrenal tumours in the head and neck, and which is also associated with SDHB, SDHD, and SDHC mutations.
- These genes encode the catalytic and the two structural subunits of succinate dehydrogenase complex II and although the involvement of SDHD in neuroendocrine tumour susceptibility is well known, there is less evidence of any contribution by SDHB and SDHC.
- We report here the analysis of the SDHB and SDHC genes in the search for germline mutations in patients from unrelated index cases with phaeochromocytoma and/or paraganglioma disorders and with or without a family history. The patients had previously tested negative for germline mutations in VHL, RET, and SDHD and had not been previously selected. We found one previously unreported nonsense SDHB mutation in one case of familial phaeochromocytoma (R27X), two silent mutations (L7L, A6A), and three intronic substitutions (IVS4+35ins, IVS2-33G/A, IVS2-35A/G) in SDHB. We did not find any germline mutations in SDHC.
- This study provides evidence supporting the recommendation of the use of the SDHB gene in genetic screening for phaeochromocytoma and paraganglioma.
other studies, which found no SDHC mutations in eight families or in 24 unrelated patients or in 19 unrelated PGL3 families. However, in another study, the discovery of several intronic polymorphisms led to the identification of large genomic deletions spanning the SDHC gene in two unrelated PGL3 families, and to the conclusion that this kind of recurrent deletion was the likely cause of PGL3. Our study confirms that the contribution of point mutations of the SDHC gene to the susceptibility to developing neuroendocrine tumours remains unclear and suggested that screening for SDHC point mutations may not be a sensitive approach to the determination of predisposition to paraganglioma or phaeochromocytoma. A study of gross deletions remains to be carried out.

Thus, in addition to the SDHB mutation previously described by Astuti et al in eight patients with familial phaeochromocytoma and/or familial paraganglioma susceptibility, here we report a novel SDHB germline mutation found in a Spanish patient with familial phaeochromocytoma that resulted in the loss of this catalytic subunit. The presence of this unrelated mutation is consistent with the idea of there having been no founder mutations in SDHB, since seven different mutations have been described in this gene. We also note the importance of SDHB mutations in familial versus sporadic cases, since no germline mutations were found in this study in sporadic cases and only 3-4% of subjects tested in sporadic cases, since no germline mutations were found in this study in sporadic cases and only 3-4% of subjects tested in familial cases with familial paraganglioma or phaeochromocytoma. Its involvement in other tumour types, such as primary nasopharyngeal carcinoma, is currently under study. We can conclude from this study that

![Schematic diagram showing the alteration](http://jmg.bmj.com/)

**Figure 1** Sequencing chromatogram showing the alteration (denoted by arrowhead). Affected codon and amino acid are indicated below. The analysis was carried out by genomic DNA amplification of peripheral blood leucocytes. The primer pairs for exon amplification of exons 1 to 6 of the SDHC gene were designed on the basis of its genomic sequence (accession number AH006319) and were as follows (numbers in brackets indicate the amplicon sizes): 1F (5′-CAC ATG ACA CCC CCA ACC-3′), 1R (5′-CTC CCA GTC CCA CTG AAG TC-3′) (138 bp), 2F (5′-TCT ATC CCT TCA CCC CTA AAA A-3′), 2R (5′-AGC GAG ACT CCG TCT CAA AA-3′) (205 bp), 3F (5′-GAT TAC AGG CCT GAG CAA CC-3′), 3R (5′-CTG GCT GAA TCC TCC CT-3′) (257 bp), 4F (5′-TCT CTT TTG AAA ATT GTC TTT GTG TG-3′), 4R (5′-TCT AAA GGA GGC CTA GTA-3′) (185 bp), 5F (5′-CAG GGG TGC CAG TTA TCT C-3′), 5R (5′-CTG GCT CCA AGG AGG TGC TC-3′) (270 bp), 6F (5′-AGC CCA ACA TCT CAG GAA AG-3′), 6R (5′-AGC ACA AGC TCC TGC TCT TG-3′) (272 bp), 7F (5′-AGC ACA AGC TCC TGC TCT TG-3′) (209 bp), 8F (5′-AGA GAA AGC TGA GAA AGG AG-3′), 8R (5′-GTC TAT CCT TGC TCT CGA ACA A-3′) (275 bp)
the participation of the Ip subunit in the development of susceptibility to familial pheochromocytoma is reconfirmed by another familial case with a germline mutation. Despite the evident association of this mitochondrial enzyme defect with tumorigenesis, as yet there is no convincing explanation for such a relation between mitochondrial dysfunction and development of neuroendocrine tumours. Otherwise, our results support the recommendation of conducting SDHB germline mutation searches in routine genetic screening of kindreds that test negative for germline mutations in other genes associated with these pathologies, such as RET, VHL, or even SDHD.

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AUTHORS’ AFFILIATIONS
A Cascón, A Cebrián, S Ruiz-Llorente, D Tellería, J Benítez, M Robledo, Department of Human Genetics, Spanish National Cancer Center (CNIO), Madrid, Spain
Correspondence to: Dr A Cascón, Department of Human Genetics, Centro Nacional de Investigaciones Oncologicas, Melchor Fernandez Almagro 3, 28029 Madrid, Spain; acascon@cnio.es

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