

ONLINE MUTATION REPORT

SDHB mutation analysis in familial and sporadic pheochromocytoma identifies a novel mutation

A Cascón, A Cebrián, S Ruiz-Llorente, D Tellería, J Benítez, M Robledo*J Med Genet* 2002;**39**:e64(<http://www.jmedgenet.com/cgi/content/full/39/10/e64>)

Pheochromocytoma is a rare, neuroendocrine, chromaffin staining tumour that usually arises within the adrenal medulla, although extra-adrenal pheochromocytomas also appear in ganglia of the sympathetic nervous system. Approximately 10% of pheochromocytomas are hereditary¹ and may be found in association with von Hippel-Lindau disease, multiple endocrine neoplasia type 2, or neurofibromatosis type 1.¹⁻³ Familial paraganglioma (PGL) is a 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 pheochromocytoma and/or paraganglioma susceptibility. While the *SDHD* locus is maternally imprinted, *SDHB* has classical autosomal dominant inheritance. Since Baysal *et al*⁴ first described *SDHD* mutations as being associated with PGL, the screening of such alterations has become routine in the diagnosis of paraganglioma and pheochromocytoma.⁵ Astuti *et al*⁶ reported the first known mutations in the *SDHB* gene that caused susceptibility to familial pheochromocytoma alone and to familial pheochromocytoma 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 PGL.⁸

To investigate further the involvement of *SDHB* and *SDHC* in paraganglioma and pheochromocytoma susceptibility, we searched for germline mutations in 22 patients, 17 with pheochromocytoma, 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 pheochromocytoma and several tumours derived from the sympathetic nervous system. He was diagnosed with adrenal pheochromocytoma 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 pheochromocytoma.

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

- Pheochromocytoma 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 pheochromocytoma 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 pheochromocytoma (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 pheochromocytoma and paraganglioma.

Table 1 Clinical and molecular data of patients analysed. *SDHB* and *SDHC* mutation analysis was performed in 22 consecutive, previously unselected patients with pheochromocytoma and/or paraganglioma, aged 11 to 68 years, with or without a family history and from unrelated families who had tested negative for germline mutations in *VHL*, *RET*, and *SDHD* genes. Genomic DNA was extracted from the patients' blood samples following standard methods. Informed consent was obtained from all patients

Patient ID	Age at onset/sex	Paraganglioma	Pheochromocytoma	Nucleotide change	Amino acid change	Frequency (%)
1	62/M	–	Unilateral	–	–	–
2	68/M	–	Unilateral	IVS2-35A/G	–	7.2
3	36/F	–	Unilateral	–	–	–
4	34/F	–	Unilateral	IVS2-35A/G	–	7.2
5	62/F	–	Unilateral	–	–	–
6	22/M	–	Bilateral	IVS2-33G/A	–	3.3
7	36/F	–	Bilateral	–	–	–
8	51/F	–	Unilateral	IVS2-33G/A	–	3.3
9	38/F	–	Bilateral	GCC→GCA†	A6A	–
10	42/F	–	Unilateral	–	–	–
11	11/M	–	Unilateral	–	–	–
12	42/F	–	Unilateral	–	–	–
13	49/M	–	Bilateral	–	–	–
14	40/M	–	Unilateral	IVS2-33G/A	–	3.3
15	37/M	–	Bilateral	–	–	–
16	14/F*	–	Unilateral	–	–	–
17	48/M	–	Bilateral	IVS4+35ins†	–	–
18	22/F	Para-aortic	–	–	–	–
19	30/M	Abdominal	–	–	–	–
20	36/F	Head	–	IVS2-35A/G IVS2-33G/A CTC→CTT†	7.2 3.3 L7L	–
21	22/F	Para-aortic	Unilateral	IVS2-33G/A	–	3.3
22	32/M*	Para-aortic and carotid body	Unilateral	CGA→TGA	R27X	–

*Patients with a family history.

†Not found in control population.

other studies, which found no *SDHC* mutations in eight families or in 24 unrelated patients⁶ or in 19 unrelated PGL families.⁹ 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 PGL3.¹⁰ Our study confirmed 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 pheochromocytoma. A study of gross deletions remains to be carried out.

Thus, in addition to the *SDHB* mutation previously described by Astuti *et al*⁶ in eight patients with familial pheochromocytoma and/or familial paraganglioma susceptibility, here we report a novel *SDHB* germline mutation found in a Spanish patient with familial pheochromocytoma 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.⁷ 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 other reports had such alterations.^{6,7}

The *SDHB* gene maps to chromosome band 1p35-36.1, a region of frequent allele loss in several endocrine tumours, such as pheochromocytomas, parathyroid adenomas, medullary thyroid carcinomas, and anterior pituitary adenomas.¹¹⁻¹³ and encodes the iron sulphur protein (Ip) of succinate dehydrogenase, a mitochondrial enzyme. Germline mutations in the *SDHB* gene and in the other succinate dehydrogenase subunits, *SDHD* and *SDHC*, have recently been postulated as being associated with hereditary and non-familial paraganglioma or pheochromocytoma.⁴⁻⁸ Its involvement in other tumour types, such as primary nasopharyngeal carcinoma, is currently under study.¹⁴ We can conclude from this study that

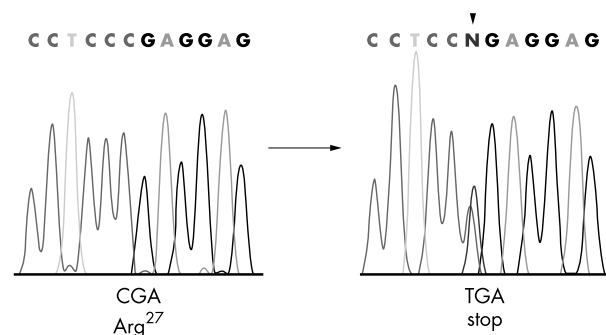


Figure 1 Sequencing chromatogram showing the alteration (denoted by arrowhead). Affected codon and amino acid are indicated below. The analysis was carried out by genomic DNA amplification of peripheral blood leucocytes. The primer pairs for exon amplification of exons 1 to 6 of the *SDHC* gene were 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 CCA GAA TCC TTC CT-3') (251 bp), 4F (5'-TTC CTT TTT AAA ATT GTC TTT GTG TG-3'), 4R (5'-TTC AAA GGA GGC GGA GAC TA-3') (185 bp), 5F (5'-CAG GGG TCC CAG TTT TAT GT-3'), 5R (5'-AGA AAA TGT GCA AAT CCC GA-3') (271 bp), 6F (5'-CGC TTT TCT CTA GAA TCA TGC TG-3'), 6R (5'-AAG ATG ATG CTG GGA GCC T-3') (257 bp). The primer pairs for exon amplification of exons 1 to 8 of the *SDHB* gene (accession number U17248) were as follows: 1F (5'-CTC CCA CTT GGT TGC TCG -3'), 1R (5'-GGC TTT CCT GAC TTT TCC CT-3') (244 bp), 2F (5'-GCG TTA CAT CTG TTG TGC CA-3'), 2R (5'-TTA AGC CTC CCA AGG ATG TG-3') (300 bp), 3F (5'-ACA TCC AGG TGT CTC CGA TT-3'), 3R (5'-AGC CCA ACA GGA ATG AAA TG-3') (197 bp), 4F (5'-CAG CAA GGA GGA TCC AGA AG-3'), 4R (5'-CCC CCA TGC AAA TAA AAA CA-3') (270 bp), 5F (5'-AAA GCT GAG GTG ATG ATG GAA-3'), 5R (5'-AGC TTA TGT TCC CTG CCA AG-3') (272 bp), 6F (5'-GTC TCT CCC GTC ACA AGC TC-3'), 6R (5'-CCT CTT GGA CTT CTG GAT GC-3') (209 bp), 7F (5'-GCA GCT CAG CTA ATC ATC CC-3'), 7R (5'-ACT TCT GGC GTG TCA GCT CT-3') (208 bp), 8F (5'-TGA ACC AGC TGA GGA AGG AG-3'), 8R (5'-TGC TGT ATT CAT GGA AAA CCA 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*.

ACKNOWLEDGEMENTS

We thank the patients participating in this study and the clinicians who provided blood samples and clinical information. We are also grateful for the technical assistance of Rocío Letón. This work was partially supported by the Fondo de Investigaciones Sanitarias project, FIS 00/0118. Alberto Cascón is a postdoctoral fellow of the Excmo Ayuntamiento de Madrid, Spain.

.....

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 Oncológicas, Melchor Fernandez Almagro 3, 28029 Madrid, Spain; acascon@cnio.es

REFERENCES

- 1 **Anderson RJ**, Lynch HT. Familial risk for neuroendocrine tumours. *Curr Opin Oncol* 1993;**5**:75-84.
- 2 **Schimke RN**. Multiple endocrine neoplasia: how many syndromes? *Am J Med Genet* 1990;**37**:375-83.
- 3 **Maher ER**, Webster AR, Richards FM, Green JS, Crossey PA, Payne SJ, Moore AT. Phenotypic expression in von Hippel-Lindau disease: correlations with germline *VHL* gene mutations. *J Med Genet* 1996;**33**:328-32.
- 4 **Baysal BE**, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN, Richard CW 3rd, Cornelisse CJ, Devilee P, Devlin B. Mutations in *SDHD*, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 2000;**287**:848-51.
- 5 **Gimm O**, Armanios M, Dziema H, Neumann HP, Eng C. Somatic and occult germ-line mutations in *SDHD*, a mitochondrial complex II gene, in nonfamilial pheochromocytoma. *Cancer Res* 2000;**60**:6822-5.
- 6 **Astuti D**, Latif F, Dallol A, Dahia PL, Douglas F, George E, Skoldberg F, Husebye ES, Eng C, Maher ER. Gene mutations in the succinate dehydrogenase subunit *SDHB* cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am J Hum Genet* 2001;**69**:49-54.
- 7 **Baysal BE**, Willett-Brozick JE, Lawrence EC, Drovdic CM, Savul SA, McLeod DR, Yee HA, Brackmann DE, Slattery WH III, Myers EN, Ferrell RE, Rubinstein WS. Prevalence of *SDHB*, *SDHC*, and *SDHD* germline mutations in clinic patients with head and neck paragangliomas. *J Med Genet* 2002;**39**:178-83.
- 8 **Niemann S**, Muller U. Mutations in *SDHC* cause autosomal dominant paraganglioma, type 3. *Nat Genet* 2000;**26**:268-70.
- 9 **Baysal BE**, Willett-Brozick JE, Lawrence EC, Drovdic CM, Myssiorek D, Myers EN, Ferrell RE, Rubinstein WS. Genetic heterogeneity in hereditary paraganglioma (PGL): *SDHD* is the primary locus in imprinted PGL pedigrees. Paper presented at 50th Annual Meeting of The American Society of Human Genetics, Philadelphia, 3-7 October 2000. *Am J Hum Genet* 2000;**67**:A385.
- 10 **Willett-Brozick JE**, Astrom K, Lawrence EC, Ferrell RE, Myers EN, Baysal BE. Evidence for recurrent deletions spanning the *SDHC* gene at chromosome 1q21 in families with hereditary paraganglioma type 3 (PGL3). Paper presented at 51st Annual Meeting of The American Society of Human Genetics, San Diego, 12-16 October 2001. *Am J Hum Genet* 2001;**69**:A477.
- 11 **Williamson C**, Pannett AA, Pang JT, Wooding C, McCarthy M, Sheppard MN, Monson J, Clayton RN, Thakker RV. Localisation of a gene causing endocrine neoplasia to a 4 cM region on chromosome 1p35-p36. *J Med Genet* 1997;**34**:617-19.
- 12 **Bender BU**, Gutsche M, Glasker S, Muller B, Kirste G, Eng C, Neumann HP. Differential genetic alterations in von Hippel-Lindau syndrome-associated and sporadic pheochromocytomas. *J Clin Endocrinol Metab* 2000;**85**:4568-74.
- 13 **Correa P**, Juhlin C, Rastad J, Akerstrom G, Westin G, Carling T. Allelic loss in clinically and screening-detected primary hyperparathyroidism. *Clin Endocrinol* 2002;**56**:113-17.
- 14 **Bik-Yu Hui A**, Lo KW, Yat-Yee Chan S, Kwong J, Siu-Chung Chan A, Huang DP. Absence of *SDHD* mutations in primary nasopharyngeal carcinomas. *Int J Cancer* 2002;**97**:875-7.