ELECTRONIC LETTER

Hereditary phaeochromocytomas and paragangliomas: a study of five susceptibility genes

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Non-functioning paragangliomas (Pgls), also called chemodectomas, glomus tumours, or non-chromaffin Pgls, are rare, highly vascularised tumours, originating from neural crest derived chief cells of paraganglia in the head and neck region. The main affected sites are the carotid body, a small chemoreceptive organ that senses blood oxygen level, and the jugulotympanic paraganglia. These tumours are typically slow growing and benign but local invasion and distant metastases can occur. Most of the tumours present without causing specific symptoms. Rarely, chemodectomas can secrete catecholamines. Therefore these tumours are diagnosed late on in development when their growth causes symptoms such as dysphagia, bradycardia, or hearing loss. Most chemodectomas occur sporadically whereas 10% to 50% could correspond to familial forms inherited as an autosomal dominant trait with incomplete penetrance. For one susceptibility locus named Pgl1, the tumours are transmitted through the paternal line, a fact suggestive of genomic imprinting.[1] Recently, patients with hereditary chemodectomas were shown to harbour a germline mutation in one of the two genes coding the small (SDHD) and large (SDHC) subunits of cytochrome *b*[2 3] or in the gene encoding the iron sulphur protein subunit of succinate dehydrogenase (SDHB[4]). Gimm *et al*[5] also identified germline mutations of *SDHD* in patients with isolated phaeochromocytomas (Phaeos), thus showing that *SDHD* was a new Pgl and Phaeo predisposing gene. The three subunits encoded by *SDHB, SDHC,* and *SDHD* together with the SDHA flavoprotein subunit are components of the mitochondrial complex II (succinate-coQoxidoreductase) that plays a central part in electron transport and the tricarboxylic acid cycle. Indeed, inherited Phaeos resulting from a germline *SDHD* mutation show a complete selective loss of complex II enzymatic activity and express higher levels of hypoxia induced factors (HIF1α and EPAS1) and VEGF than their sporadic counterparts.[6]

Functioning Pgls, also called extra-adrenal Phaeos or sympathoadrenal Pgls, are rare catecholamine producing tumours arising in chromaffin tissue within sympathetic or other autonomic ganglia. These tumours are mostly intra-abdominal and can develop from the organ of Zuckerkandl or the bladder, for example. Phaeos are therefore a subclass of functioning Pgls affecting the chromaffin cells of the adrenal medulla. Both sympathoadrenal and parasympathetic paraganglia are part of a diffuse neuroendocrine system derived from neural crest cells, a fact which probably explains why functioning and non-functioning Pgls display common histopathological features.[7] Patients with catecholamine secreting tumours often present with severe hypertension that may evolve to hypertensive crisis, arrythmia, and death. Until recently, about 10% of Phaeos were considered hereditary, occurring as an autosomal dominant trait either alone or as part of inherited cancer syndromes such as multiple endocrine neoplasia type 2 (MEN 2), von Hippel Lindau (VHL) disease, or, rarely, neurofibromatosis type 1.[8] The large study recently reported by Neumann *et al*[9] has shown that up to 24% of patients with apparently sporadic isolated Phaeos had a germline mutation of *VHL, RET, SDHD,* or *SDHB*. This finding, if confirmed on independent population samples, will have important clinical consequences for the medical management of patients with Pgls or Phaeos, and their families. Therefore, the aims of our study were (1) to evaluate the percentage of heritable Phaeos and Pgls by looking for germline mutations in the five susceptibility genes already known (*RET, VHL, SDHD, SDHC, SDHB*) in a

Key points

- Non-functioning and functioning paragangliomas (Pgls) are rare tumours of neural crest derived paraganglia. According to their localisation, 10-50% of Pgls are inherited as an autosomal dominant trait with incomplete penetrance. Five susceptibility genes for these tumours are already known: the *RET* proto-oncogene (causing multiple endocrine neoplasia type 2), the *VHL* tumour suppressor gene (causing VHL disease) and the recently described *SDHB, SDHC,* and *SDHD* genes encoding subunits of succinate dehydrogenase.
- We assessed the frequency of *VHL, RET, SDHD, SDHC,* and *SDHB* germline mutations by nucleotide sequencing in 21 patients clinically classified as having apparently sporadic Phaeos or Pgls and in 15 patients clinically classified as having familial tumours.
- Germline nucleotide variations were found in seven of 21 patients (33.3%). Four patients had clearly deleterious mutations in *VHL* (one patient), *RET* (one), *SDHD* (one) and *SDHC* (one with a novel mutation) whereas three patients had polymorphisms of unknown significance. By comparison, 13 of 15 patients with familial tumours had a germline mutation (86.7%) involving *VHL* in 12 whereas one patient had an *SDHB* nucleotide variation.
- Our data confirmed that clinical features suggesting an inherited tumour include a young age at onset (<18), a bilateral phaeochromocytoma, or multiple Pgls.
- The germline mutation rate in patients with apparently sporadic Pgls is at least 19% in western Europe. Therefore, introducing systematic germline mutation testing for the five susceptibility genes is recommended in these patients.

Abbreviations: CT, computed tomography; MEN 2, multiple endocrine neoplasia type 2; NF1, neurofibromatosis type 1; PCR, polymerase chain reaction; Pgl, paraganglioma; Phaeo, phaeochromocytoma; SDHB, iron sulphur protein subunit of succinate dehydrogenase; SDHC, large subunit of cytochrome *b*; SDHD, small subunit of cytochrome *b*; VHL, von Hippel-Lindau disease

Table 1 Distribution of the mutations in the patients and their phenotype

Gene	Mutation	Consequence	Patient's identity	Initial classification and age at diagnosis	Phenotype
VHL	c.499C>G	R167W	1	F - 30	Bilateral Phaeo
			5†	F - 29	Bilateral Phaeo
			6†	F - 15	Unilateral Phaeo
			7	F - 31	Bilateral Phaeo
			13	F - 13	Bilateral Phaeo
			14	F - 15	Bilateral Phaeo
	c.331A>T	S111C	2	F - 39	Unilateral Phaeo
			3	F - 49	Unilateral Phaeo
	c.485G>A	C162Y	4	F - 27	Unilateral Phaeo
	c.500G>A	R167Q	8	F - 43	Left Phaeo and right Pgl
	c.491G>C*	Q164H*	9‡	F - 47	Abdominal Pgl
			10‡	F - 39	Unilateral Phaeo
	c.467A>G	Y156C	18	S - 13	Bilateral Phaeo
RET	c.1900T>C	C634R	17	S - 24	Bilateral Phaeo
SDHD	c.34G>A+c.204C>T	G12S + S68S	15	S - 19	Unilateral Pgl
			16	S - 42	Unilateral Phaeo
	idem; IVS2 – 1G>T	G12S + S68S; aberrant splicing	11	F - 30	Juxtarenal Pgl and cervical Pgl
	IVS2 – 1G>T	aberrant splicing	21	S - 30	Cervical Pgl
SDHC	c.473T>C*	L158P*	19	S - 22	Carotid body Pgl
	c.-12G>A* (promoter)	Decreased expression?	20	S - 53	Interaortico cave Pgl
SDHB	c.263T>C	L88S	12	F - 51	Bladder Pgl

The numbering of the nucleotides begins at the A of the first ATG denoted +1.
F, familial tumour; S, apparently sporadic tumour; Phaeo, phaeochromocytoma; Pgl, paraganglioma.
*The mutation is novel; †, ‡these patients belong to the same family.

sample of patients with non-syndromic Pgls or Phaeos and (2) to define genotype-phenotype correlations in our population sample.

MATERIALS AND METHODS

Patients with Pgl and Phaeo

The patients included in this study were diagnosed with head and neck Pgls or Phaeos or functioning abdominal Pgls at the Department of Endocrinology, Clinique Marc Linquette or at the Department of Endocrine Surgery, Hopital Claude Huriez, CHRU Lille, France. All the patients seen during their postsurgical follow up from 1998 to the middle of 2002 who gave their informed written consent for the genetic analysis were enrolled in the study. The aim of the genetic analysis was to identify heritable cases of Pgls or Phaeos. The patients were clinically classified as having familial tumours if at least two patients of the kindred were diagnosed with Pgls or Phaeos as documented by medical or surgical records, or if the subjects harboured a syndromic Phaeo (associated with a VHL disease or MEN 2). Based on these criteria, tumours of 15 patients corresponding to 13 families were classified as familial tumours. Tumours of 21 patients were classified as apparently sporadic.

Phenotyping

All patients included in the study underwent a careful physical examination including thyroid palpation and a search for cutaneous manifestations of neurofibromatosis type 1 (NF1). The patients' personal and familial histories were recorded by the doctor. The routine screening protocol comprised direct ophthalmoscopy to look for retinal haemangioblastomas (associated with VHL disease) or Lisch nodules (associated with NF1), an abdominal computed tomography (CT) scan, and an MIBG scintigraphy (and when necessary a somatostatin receptor scintigraphy) to localise Phaeos or Pgls and to detect renal or pancreatic lesions associated with VHL disease. Biological studies included measurement of serum and urinary catecholamines and metanephrines, serum chromogranin A, and basal serum calcitonin.

Genotyping

DNA was isolated from peripheral blood using a commercial kit (QIAamp DNA Blood MaxiKit, Qiagen, France) according to the manufacturer's protocol. *SDHD* intronic primers flanking each of the four exons were used for polymerase chain reaction (PCR) amplification of 300 ng DNA, using the experimental conditions described by Baysal *et al*.[2] The PCR amplification of the eight exons of *SDHB* was carried out using the primer pairs and the conditions described by Astuti *et al*.[4] For *SDHC* we designed six intronic primer pairs for exon amplification, except for primer 6R, which was located in the 3′ untranslated region (UTR) of exon 6, according to the genomic sequences.[10] Primer sequences were as follows: 1F: 5′ CGTCACATGACACCCCCAAC 3′ 1R: 5′ CTCCCAGTCCCACTGAA 3′ (amplicon size 140 bp), 2F: 5′ TGATTCTCTTATCTTGCAG 3′ 2R: 5′ ATCTCCAGACTTAGAAACTT 3′ (amplicon size 98 bp), 3F: 5′ TCAAACGGTCATGGTTTTAT 3′ 3R: 5′ CTCTGGCTCCAGAATCCTTC 3′ (amplicon size 154 bp), 4F: 5′ ACTCTCTACTATGGTGTCAT 3′ 4R: 5′ TGTGTAAAAACACATATACAT 3′ (amplicon size 144 bp), 5F: 5′ AGCAGCTGTGACAAGCTACT 3′ 5R: 5′ CTCCCTTCACAGAGAAAATG 3′ (amplicon size 243 bp) 6F: 5′ CTGTTAATGTCCTATTTACT 3′ 6R: 5′ CCAAGGAGATCTGAAAATACA 3′ (amplicon size 293 bp). For *VHL*, the three exons were amplified according to the protocol of Crossey *et al*[11] with some modifications: exon 2 primers: 2f: 5′ GTGGCTCTTTAACAACCTTTGC 3′, 2r: 5′ TGGATACCGTGCCTGACATCA 3′ ; exon 3 primers: 3f: 5′ CATTAGTACAGGTAGTTG 3′, 3r:5′ TACCAT-CAAAAGCTGAGATGAAACAG 3′g. Genomic DNA (300 ng) was amplified by PCR using *Taq* Gold polymerase (Applied Biosystems) and the following experimental conditions: initial denaturation at 95°C for seven minutes; then 35 cycles of 30 seconds denaturation at 95°C, 90 seconds of annealing at 52–60°C, 90 seconds of extension at 72°C; and a final extension step at 72°C for seven minutes. Exons 10, 11, and 16 of *RET* were studied using previously published protocols.[12]

The amplicons were first visualised in an ethidium bromide stained agarose gel before being purified on microcolumns (Microcons YM-50, Millipore) according to the manufacturer's instructions. Both strands of the purified amplicons were

Table 2 Age and phenotype of the patients according to the results of the genetic analysis

	Total	Hereditary proven disease	Sporadic disease	Disease associated with not clearly deleterious mutations of *SDHs*	p value	Phaeo or Pgl associated with *VHL*	Phaeo or Pgl associated with *RET*	Tumours associated with *SDHs*
Number of patients	n=36	n=17	n=15	n=4		n=13	n=1	n=3
Age at presentation (y):								
Mean	33.6	29.1	36.7	41.2	NS	30.0	23.0	27.3
SD	13.7	11.5	14.7	15.6		13.0	–	4.6
Range	13–63	13–49	14–63	19–53		13–47	–	22–30
⩽13	2	2 (11.8%)	0	0		2 (15.4%)		0
⩽18	7	4 (23.5%)	3 (20%)	0	NS	4 (30.8%)		0
⩾50	6	0 (0%)	4 (26.7%)	2 (50%)	NS	0		0
Type of tumour:								
Single	23	7 (30.4%)	12 (52.2%)	4 (17.4%)	<0.05 (χ^2)	5 (38.5%)		2 (66.6%)
Multifocal	13	10 (76.9%)	3 (23.1%)	0	<0.05 (χ^2)	8 (61.5%)	1 (100%)	1 (33.3%)

The p values are for the comparison between hereditary disease and sporadic disease.

sequenced using the ABI PRISM Model 377XL automatic sequencer and the ABI PRISM Ampli*Taq* FS Big Dye Terminator cycle sequencing ready reactions kit (Applied Biosystems, France).

Statistical analysis

For the comparison of means, Student's *t* test was used. For the comparison of frequencies Fisher's unpaired exact test was used for small groups and for larger groups we used the standard χ^2 test. p values <0.05 were considered to be significant.

RESULTS

Prevalence and distribution of germline mutations in patients with apparently sporadic tumours

A total of 21 patients with apparently sporadic Phaeos (13 patients), Pgls (seven patients), or both (one patient) were enrolled in the study. Molecular genetic analysis was performed as follows: direct nucleotide sequencing of *VHL* (three exons) and *RET* (exons 10, 11, and 16) to identify VHL disease or MEN 2, respectively; if negative, *SDHD, SDHB,* and *SDHC* sequencing was performed. A germline sequence variant was found in seven patients: one patient had a *VHL* mutation (14.3% of the mutations), another patient had a *RET* mutation (14.3%), three patients had germline sequence variants in *SDHD* (42.8%), and two patients had germline sequence variants in *SDHC* (28.6%, table 1, patients S).

The sequence variants of *SDHD* seen in three patients comprised the G12S+S68S double variation in two unrelated patients (15 and 16) and a splice acceptor site mutation IVS2–1G>T in one patient (21, table 1). Whereas the G12S variation is considered as a polymorphism,[13] the importance of the G12S+S68S variation is still a matter of debate.[14] Indeed, this variant has already been found in the germline of one patient with a spinal cord Pgl,[15] the germline of two patients with digestive carcinoids considered to be neuroendocrine tumours,[14] and the germline of one patient with a unilateral Phaeo,[16] stressing its potential pathogenic role. However, this G12S+S68S variation also occurred in five controls out of 200 tested,[16] a finding that led us to consider it not to be a clearly deleterious polymorphism. On the contrary, the IVS2–1G>T mutation is pathogenic as it has been found at the germinal level in one patient with a chemodectoma by Dannenberg *et al*[17] and also by us in one woman with a hereditary Pgl (patient 11) in association with the G12S+S68S polymorphism (this study).

Two patients were found to have novel *SDHC* nucleotide variations. One mutation leads to an amino acid substitution of leucine to proline at codon 158 that lies in the third putative transmembrane domain of this protein (fig 1). Interestingly, the L158 is conserved in several distant species such as *Bos taurus* (bullock), the hamster, and *C elegans*[10 18] pointing out the importance of this amino acid for protein structure and function. Moreover, the fact that this variation was not detected in 100 control chromosomes led us to consider the L158P mutation as deleterious. The second variation is a heterozygous G>A change at -12 bp in the proximal promoter of *SDHC* that is GC rich and lacks a classical TATA box[10]; this change was not detected in 100 control chromosomes. The G nucleotide at -12 bp lies near two consensus binding sites for the transcription factor Sp1 (-30,-25; -18,-13) and the G>A mutation creates a *cis* element for the Ikaros *trans* acting factor (-15,-12, GGGA), which was shown to repress the transcriptional activity of several promoters such as *VPAC-1*.[19] Further functional studies are required to determine whether such a mechanism may apply to the *SDHC* promoter. Meanwhile, this G>A variation should be considered as not obviously deleterious. Patient 17 with a *RET* germline mutation was enrolled into the study after the discovery of a bilateral Phaeo operated on at the age of 24. Two months later a thyroidectomy was performed, on the basis of a positive pentagastrin test, showing a bilateral medullary thyroid carcinoma.

In conclusion, the rate of deleterious germline mutations in patients with apparently sporadic tumours reached 19% (four of 21 patients). The subclassification of these patients according to their phenotype showed that two patients out of 13 with Phaeos only (15%) had a germline mutation whereas two patients out of seven with Pgls only (28.6%) had a germline mutation (NS).

Prevalence and distribution of the germline mutations in patients with familial tumours

By comparison, we identified 14 germline mutations in the 15 patients (table 1, patients F) classified as familial Phaeo (11 patients), familial Pgl (three patients), or both (one patient) after clinical investigation. The mutations affected *VHL* in 12 patients (86% of the mutations), *SDHD* in one patient (7%), and *SDHB* in another (7%). The *SDHB* L88S missense mutation was found in a 51 year old woman with a familial malignant Pgl of the urinary bladder (patient 12). A partial cystectomy was performed showing that the tumour infiltrated the vesical detrusor. Two years later an abdominal metastatic lymph node was removed because of a clinical and biological recurrence. Her father was operated on at the age of 56 for a pararenal Pgl; unfortunately, no blood or tumour DNA was available to check the presence of the L88S mutation. The 32 year old daughter of the proband did not inherit the mutation and is at present asymptomatic. This mutation was also found by Astuti *et al*[4] in the germline of one patient with a sporadic Phaeo and in one of 200 control chromosomes. Therefore, the significance of this sequence variant is still uncertain. Overall, we identified 13 deleterious mutations in the 15 patients with familial tumours; the mutation rate was thus 86.7%. This high level of

Table 3 Position of the tumours according to the results of the genetic analysis

	Total	Hereditary proven disease (%)	Sporadic disease (%)	Disease associated with not clearly deleterious mutations of *SDHs*	p value	Phaeo or Pgl associated with *VHL*	Phaeo or Pgl associated with *RET*	Tumours associated with *SDHs*
Number of patients	n=36	n=17	n=15	n=4		n=13	n=1	n=3
Position of the tumours:								
Single; head and neck	2	2(100)	0					2
Single; adrenal	14	4(28.6)	9(64.3)	1(7.1%)	<0.05	4		
Single; extra-adrenal	7	1(14.3)	3(42.85)	3(42.85%)		1		
Multiple: adrenal only	10	8(80)	2(20)		<0.05	7	1	
Multiple: adrenal and extra-adrenal	2	1(50)	1(50)			1		
Multiple: head, neck, and extra-adrenal	1	1(100)	0					1

The p values (Fisher's test) are for the comparison between hereditary disease with sporadic disease.

germline mutations allows us to validate a posteriori our clinically based classification. Based on the results of the genetic analysis, we thus identified 17 patients with inherited Phaeos or Pgls, 15 with sporadic tumours, and four with nucleotide variations of unknown relevance, whose main characteristics are shown in tables 2 and 3. Molecular analysis of the five genes was negative in one 27 year old patient who, however, probably had a familial tumour (right Phaeo operated on at the age of 22, a left adrenal mass discovered in 2002, and a history of bilateral Phaeos in her paternal grandfather).

Phenotype of the patients according to their genotype

The age at presentation of the disease did not differ significantly between patients with hereditary disease and patients without germline mutations. All the patients who presented at the age of 13 years or younger had germline mutations (of *VHL* in 100%) whereas this rate decreased to 57% among patients with an age at diagnosis younger than 18 years. No patients older than 50 years at onset had a clearly deleterious germline mutation. At diagnosis, patients with hereditary disease had multiple tumours more often (58.8%) than those without mutations (20%, $p<0.05$). Interestingly, patients who presented with bilateral Phaeos had an inherited disease in 80% (table 3) in comparison with 28.6% of the patients with unilateral Phaeos ($p<0.05$). In both cases, the inherited tumours were almost always associated with a *VHL* mutation. Indeed, 15.4% of patients with a *VHL* mutation had an extra-adrenal lesion whereas this lesion was present in all the patients with a *SDH* mutation ($p<0.05$). In our hands, the presence of a head and neck Pgl is highly indicative of an inherited disease involving one *SDH* gene. Finally, the mutations that are not clearly deleterious are often seen in patients with an isolated abdominal Pgl.

DISCUSSION

The discovery of new susceptibility genes for familial cancer syndromes leads to an increase of the proportion of inherited tumours among patients presenting with an apparently sporadic tumour. Applied to the field of Phaeos and Pgls, the recent description of germline mutations in three of the four genes encoding subunits of the succinate dehydrogenase, namely *SDHD*, *SDHC,* and *SDHB*, in patients with Pgls or Phaeos has increased the number of susceptibility genes for these tumours to six, which also includes the oncosuppressors *VHL* (involved in VHL disease), *NF1* (neurofibromatosis type 1), and the proto-oncogene *RET* (MEN 2). These results have prompted several groups to look for germline mutations in these susceptibility genes in patients with isolated and a priori sporadic Phaeos or Pgls. Two major studies have been published to date on this subject. The first study, by Baysal *et al*,[20] reported the prevalence of *SDHD*, *SDHC,* and *SDHB* germline mutations in a population of 55 patients from the United States with head and neck Pgls (37 patients with sporadic Pgls and 18 patients with familial Pgls). The authors showed that 8% of the patients with apparently non-familial Pgls had germline mutations in *SDHD* and *SDHB* whereas no germline mutation was found in *SDHC*. In contrast, 70% of the familial cases had mutations in *SDHD* (50% of the families) or *SDHB* (20% of the families). The largest study by Neumann *et al*[9] included 271 patients from central Europe who presented either with non-syndromic Phaeos (241 patients), isolated Pgls (22 patients), or both (eight patients), and in which four susceptibility genes for familial Phaeo (that is, *RET*, *VHL*, *SDHD,* and *SDHB*) were investigated. These authors showed that in patients with apparently sporadic Phaeo or Pgls the rate of germline mutations was as high as 24%. As expected, they also showed that a young age or multiple tumours at the occurrence of the disease are strong indicators of a familial case. Most surprising was the presence of an extra-adrenal tumour among the indicators of heritability. If this rate could be extrapolated to the general population of patients with Phaeos or Pgls, then the old rule stating that 10% of all the Phaeos are inherited will vanish, thus raising the question of introducing systematic germline mutation testing in each patient.[21]

In this context, we wanted to reassess the frequency of germline mutations in a western European sample of patients with apparently sporadic Phaeos or Pgls by studying the five predisposing genes, *SDHD*, *SDHC*, *SDHB*, *RET*, and *VHL*. The results presented here show that seven patients (33%) had a germline nucleotide variation. Based on the nature of this variation, patients could be classified into two groups: (1) four patients (19%) with a deleterious mutation and thus a hereditary tumour; (2) three patients (14%) with sequence variants of unknown significance (including one patient with a familial abdominal Pgl (patient 12)). Accordingly, the mutation rate is at least 19%, a result similar to that reported by Neumann *et al*.[9] Interestingly, we report a new *SDHC* mutation identified in a patient with a Pgl of the carotid body. This mutation is the second germline variation affecting this gene besides the original one described by Niemann *et al*.[3] It thus seems necessary to include this gene in the familial Phaeo or Pgl predisposing survey. Among the 15 patients without mutations, two had multiple tumours and one young patient had multiple tumours and a positive familial history suggesting that they could have familial tumours resulting from non-detectable mutations in the genes studied or involvement of as yet unknown susceptibility genes. Based on the 36 patients included, we also confirmed that multiple tumours are more frequent in patients with familial disease than in the sporadic patients.

Considering that up to 24% of apparently sporadic Phaeos or Pgls are in fact inherited tumours, that patients with germline mutations are prone to develop multiple tumours (adrenal and extra-adrenal), and that the identification of a mutation could lead to a predictive genetic test in the kindred, it seems mandatory to perform a search for germline mutations in the

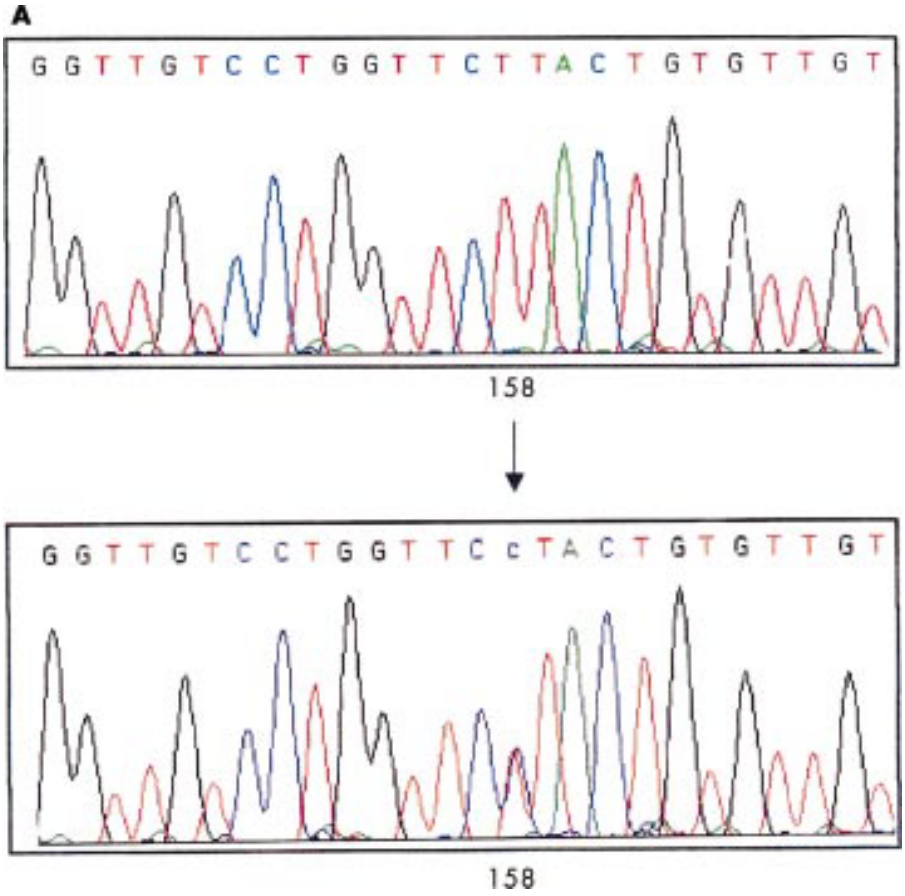

Figure 1 Sequence analysis of the exon 6 of *SDHC* in the germline of (A) a control normal subject; codon 158: Leu, CTT; and (B) patient 19 with a heterozygous mutation L158P, CTT>CCT.

five susceptibility genes so far identified in patients with apparently sporadic Phaeos or Pgls. A reasonable approach would be to test initially young patients with a positive family history of the disease, or with multiple or extra-adrenal tumours. Taking into account (1) the repartition of the germline mutations; (2) the fact that an isolated Phaeo is rarely the first clinical manifestation of MEN 2[22]; and (3) the fact that the diagnosis of NF1 is usually made based on clinical criteria, it seems to us that the genes should be screened in the following order: *VHL*, *SDHD,* and if negative *SDHB, RET,* and *SDHC*.

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