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Original article

# Genetic predisposition to pheochromocytoma and paraganglioma: 21 years of experience in the field



Catherine Cardot-Bauters<sup>a</sup>, Marie-Christine Vantyghem<sup>a</sup>, Christine Do Cao<sup>a</sup>, Rachel Desailoud<sup>b</sup>, Michael Joubert<sup>c</sup>, Lucie Coppin<sup>d,e</sup>, Marie-Francoise Odou<sup>d,f</sup>, Pascal Pigny<sup>d,e,\*</sup>

<sup>a</sup> Service d'endocrinologie, diabétologie-métabolisme, hôpital Claude-Huriez, CHU, 59037 Lille cedex, France

<sup>b</sup> Service d'endocrinologie-diabétologie, nutrition, hôpital Sud nord, CHU, 80054 Amiens cedex 1, France

<sup>c</sup> Service d'endocrinologie, CHU Côte de Nacre, 14000 Caen cedex, France

<sup>d</sup> Laboratoire de biochimie « hormonologie-métabolisme-nutrition-oncologie », UF oncogénétique moléculaire, laboratoire de biologie médicale de référence, CHU de Lille, 59037 Lille cedex, France

<sup>e</sup> Inserm UMR 1277, CANTHER, université de Lille, 59045 Lille cedex, France

<sup>f</sup> Inserm, CHU Lille, U1286 – Infinite, université de Lille, 59045 Lille cedex, France

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## ABSTRACT

**Context.** – Pheochromocytoma and paraganglioma (PPGL) are rare neuroendocrine tumors with high heritability, justifying systematic genetic screening for a germline variant in one of the twenty predisposing genes described to date.

**Purpose.** – To describe the experience of one endocrine oncogenetic laboratory over a period of 21 years (2001–2022), from the beginning of PPGL genotyping with Sanger sequencing in 2001 to the implementation of next-generation sequencing (NGS).

**Method.** – The activity database of an academic oncogenetic laboratory was searched to extract patients/relatives identified with a pathogenic variant/likely pathogenic variant (PV/LPV) over a period of 21 years. Clinical and genetic data were compared.

**Results.** – In total, 606 index cases with PPGL and 444 relatives were genotyped. Genotyping of index cases was performed by Sanger sequencing and gene deletion analysis in 327 cases and by NGS in 279. Germline PV/LPV spanning 10 genes was identified in 165 index cases (27.2%). Several recurrent PV/LPVs in *SDHx* were observed in non-related index cases, the most frequent being *SDHD*, c.170-1G>T ( $n = 28$ ). This subgroup showed great phenotypic variability both between and within families in terms of both tumor location and number. Four patients (1.1%) with PV/LPV in *SDHx* had 3PA (Pituitary Adenoma and pheochromocytoma/paraganglioma) syndrome. 258 relatives (58.1%) had inherited a PV/LPV in one driver gene. The rate of PV/LPV carriers who were symptomatic at first imaging evaluation was 32%, but varied between < 20% in *SDHB* and *SDHC* and > 50% in *SDHD*, *VHL* and *MAX*.

**Conclusion.** – Our experience confirmed previously established genotype-phenotype correlations, but also highlights atypical clinical presentations, even for the same genetic variant. These data must be taken into account for optimal patient follow-up and management.

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## 1. Introduction

Paraganglioma (PGL) is a rare neuroendocrine tumor deriving from sympathetic or parasympathetic paraganglia. In the first case, it most frequently develops from thoracic-abdominal-pelvic

paraganglia or adrenals, and overproduces catecholamine. Parasympathetic PGL, on the other hand, develops from head and neck paraganglia and is most often non-secreting. Pheochromocytoma (PCC) should now be classified as adrenal PGL. Taken together, pheochromocytoma and paraganglioma (PPGL) are now considered to be the human tumors with the highest degree of heritability, up to 40% [1], since the number of predisposing genes has steadily increased from 3 (*VHL*, *RET* and *NF1*) in the early 1990s to 6 (additionally, *SDHB*, *SDHC* and *SDHD*) in the early 2000s and around 20 nowadays. Therefore, according to international guidelines, routine genetic testing is recommended in all patients bearing one PPGL,

\* Corresponding author. Laboratoire de biochimie « hormonologie-métabolisme-nutrition-oncologie », UF oncogénétique moléculaire, laboratoire de biologie médicale de référence, CHU de Lille, 59037 Lille cedex, France.

Adresse e-mail : [pascal.pigny@chu-lille.fr](mailto:pascal.pigny@chu-lille.fr) (P. Pigny).

by Sanger sequencing in the 1990s to 2000s and subsequently by gene panel analysis by next-generation sequencing (NGS) [2].

In our lab, genotyping of patients with endocrine tumors started in 1999, due to large local recruitment of patients in the departments of endocrinology and endocrine surgery. Molecular genetic activity then increased, thanks to a campaign for oncogenetic lab funding launched by the Ministry of Health in 2002. The aim of the current study is to describe our activity of PPGL genotyping over a 21-year period, from 2001 to 2022.

## 2. Materials and methods

All subjects (patients and relatives) who were screened for a genetic predisposition for familial PPGL gave their informed consent for genetic testing in accordance with French regulations. Genotyping was performed under supervision of a biologist certified for genetic testing by French Biomedicine Agency (ABM). Results were recorded prospectively in a hospital database to facilitate the data extraction required to draw up the annual activity report required by the ABM and the French National Cancer Institute (INCa). For this study, all patients and relatives identified with a pathogenic variant (PV) or likely pathogenic variant (LPV) in one susceptibility gene from 2001 to 2022 were retrospectively extracted from this database. The following data were collected: demographics, clinical status (symptomatic or asymptomatic, based on imaging and biological screening according to current evaluation guidelines), tumor location, age at tumor onset, genetic testing results, and age at genetic testing.

Initially genomic DNA was extracted with manual kits (QIAGEN and Macherey Nagel). Since 2014, it has been extracted from peripheral blood using the chemagic DNA Blood 4k Kit H24 (PerkinElmer) on the chemagic STAR instrument (PerkinElmer) according to the manufacturer's instructions. From 2001 to 2017, for each proband we used Sanger nucleotide sequencing to sequentially analyze the main PPGL susceptibility genes according to frequency of involvement in the genetic forms of PPGL. Firstly, *VHL*, *SDHB*, *SDHD*, *SDHC* and *RET* were analyzed as previously described [3]. In case of negativity, the genes less frequently involved in familial PPGL (*MAX*, *TMEM127*, *SDHAF2*, *KIF1B*, etc.) were sequentially analyzed by Sanger sequencing, and complementary techniques (MLPA and QMPSF for gene deletion) if appropriate, as they were shown to be PPGL susceptibility genes. The total number of genes analyzed was up to 19 (see below).

Since 2018, a panel of susceptibility genes for PPGL is analyzed in each patient with PPGL by NGS. From 2018 to July 2022, a haloplex design (Agilent Technology) was used for library preparation according to the manufacturer's instructions, to sequence coding exons and flanking intronic sequences of 11 PPGL susceptibility genes: *SDHA* (NM.004168), *SDHAF2* (NM.017841), *SDHB* (NM.003000), *SDHC* (NM.003001), *SDHD* (NM.003002), *TMEM127* (NM.001193304), *MAX* (NM.002382), *KIF1B* (NM.015074), *PHD2 = EGLN1* (NM.022051), *VHL* (NM.000551), and *RET* (NM.020975). In August 2022, the design was updated, with library preparations performed with a SureSelect XT-HS design (Agilent Technology). Eight PPGL susceptibility genes were added: *EGLN2* (NM.080732), *SLC25A11* (NM.003562), *FH* (NM.000143), *MDH2* (NM.0059.18), *EPAS1* (NM.001430), *NF1* (NM.001042492), *DLST* (NM.001933), and *SUCLG2* (NM.003848). After library preparation, targeted sequencing was performed on MiSeq (Illumina). Then, for data analysis, a local bioinformatic pipeline was used for alignment (human reference genome GRCh37/hg19) and BROAD GATK 4 for variant calling. A second analysis was performed with SeqPilot v4.4, SeqNext module (JSI). Variants were annotated using the ACMG-AMP guidelines [4].

When genetic analysis by NGS was negative despite high suspicion of familial PPGL based on immuno-histochemistry and/or clinical presentation, additional sequencing for detection of *VHL* and *SDHx* mosaic mutations was performed, as previously described [5,6]. Genetic testing of relatives was carried out by Sanger sequencing or by MLPA, depending on the nature of the screened variant. Phenotype evaluation for index cases was based on a combination of morphologic examination (CT and/or MRI) and nuclear medicine examination (initially MIBG scintigraphy for PCC, or octreoscan scintigraphy for non-secreting PGL; then F-DOPA PET for PCC and somatostatin analog PET for other PGLs, as PET imaging had become available). Screening for lesions in *SDHx*-mutated relatives was based on clinical examination, measurement of plasma-free metanephrine and initial morphologic work-up (MRI or CT of the skull base and neck, thoracic CT, abdominopelvic CT or MRI), and PET imaging (with the radiotracer according to the causal gene: FDG, DOTATOC or DOPA). For other predisposing genes (*VHL*, *RET*, etc.), screening was performed according to the specific screening recommendations for these pathologies.

Statistical analysis used Prism Software. Categorical variables were analyzed by Fisher's exact test and continuous variables by Student *t* test or Mann-Whitney U test as appropriate. All *P* values were two-tailed, and values less than 0.05 were considered statistically significant.

## 3. Results

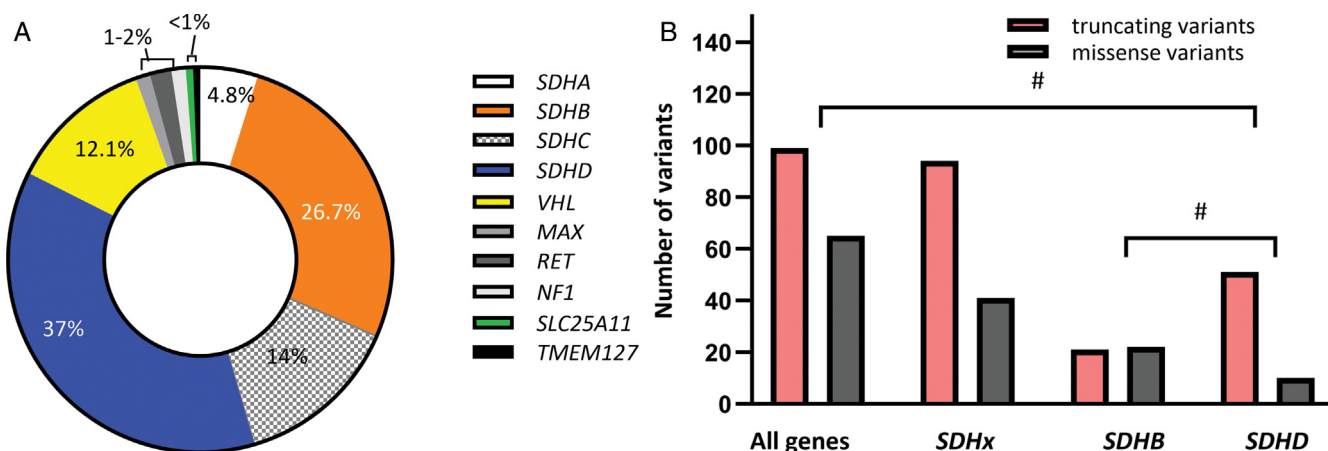
From 2001 to 2022, 606 index cases and 444 relatives were genotyped in our lab. In total, 327 index cases were genotyped by Sanger sequencing and gene deletion analysis and 279 by NGS. Total numbers of subjects tested per susceptibility gene are shown in Table S1. A germline heterozygous PV or LPV in one of the 19 susceptibility genes analyzed was identified in 165 index cases (27.2%) and in 258 relatives (58.1%). For index cases, the proportion of mutated patients was 16.8% (47/279) in those analyzed by NGS versus 36% (118/327) for Sanger plus MLPA.

### 3.1. Propositus with a hereditary PPGL

Demographic data of the mutated probands are detailed in Table 1. Requests for genetic analysis came mostly from the endocrine oncogenetic unit of the university hospital of Lille, France ( $n = 113$ , 68.5%), from other hospitals in the Hauts de France administrative region ( $n = 22$ , 13.3%), or from other areas in France ( $n = 30$ , 18.2%). Median (IQR; range) age at genetic testing was 41 (27; 7–77) years and age at disease onset was 35 (26; 1.5–76) years. The majority of the mutated probands harbored head and neck (HN)-PGL, followed by PCC and then PGL in the thorax, abdomen or pelvis (TAP-PGL). Multiple locations was less frequent. Metastatic disease was present in only 9.1% of the patients at disease onset. The phenotypic description is detailed below.

Patient distribution per mutated gene is shown in Fig. 1a and Table S1. PV/LPVs were distributed across 10 genes. A PV/LPV in one *SDHx* gene was seen in 82.5% of probands, *SDHD* being the most frequently mutated gene. A PV/LPV in *VHL* was seen in 12.1% of probands. Other susceptibility genes were less frequently affected. Details of the various PVs/LPVs are shown in Table 2.

99 index cases (60%) harbored a gene mutation inducing absence or a truncated form of the corresponding protein (i.e., frameshift, exon skipping or splice variants). This suggests that truncating variants are more frequent than missense variants, especially for *SDHD* where they represented 83.6% of the whole (Fig. 1b). Proband with truncating variants more frequently had multiple lesions than those with missense variants (56.5% vs 37.9%,  $P < 0.05$ ).



**Fig. 1.** (a) Distribution of the germline variants identified in index cases according to the susceptibility gene involved. (b) Number of truncating variants (frameshift, exon skipping, nonsense) and missense variants identified in *SDHx* genes. #:  $p < 0.001$ , Fisher's test.

**Table 1**  
Main characteristics of Index cases with familial PPGL.

Variables		Patients (n = 165)
Age at diagnosis (yrs)	Mean ± SD	38.9 ± 16.0
	Median (IQR)	35.0 (26)
Female	Number (%)	91 (55.1)
Request origin	Number (%)	
Lille University Hospital		113 (68.5)
Others		52 (31.5)
Age at genetic testing (yrs)	Mean ± SD	42.2 ± 15.9
	Median (IQR)	41 (27.0)
Molecular genetic methodology	Number (%)	
SANGER		113 (68.5)
MLPA		5 (3.0)
NGS		47 (28.5)
Clinical phenotype	Number (%)	
PCC		28 (17.0)
Single		16 (57.1)
Multiple		12 (42.9)
HN-PGL		88 (53.3)
Single		46 (52.3)
Multiple		42 (47.7)
TAP-PGL		23 (13.9)
Single		19 (82.6)
Multiple		4 (17.4)
HN and TAP-PGL		11 (6.7)
PCC and TAP-PGL		10 (6.1)
PCC and HN-PGL		4 (2.4)
PCC and HN-PGL and TAP-PGL		1 (0.6)
Metastasis at diagnosis		15 (9.1%)

In contrast, there was no difference in metastasis frequency at disease onset.

### 3.2. Relatives with a PV/LPV in one PPGL susceptibility gene

In total, 258 relatives had a PV/LPV: 132 females (51.2%) and 126 males (48.8%), with a median (IQR) age of 36 (34) years at genetic testing. Sixty relatives (23.2%) were under 18 years of age at genetic testing. Relatives' characteristics according to the mutation status are detailed in Table 3. As expected, most had a PV/LPV in *SDHB*, *SDHD*, *SDHC* or *VHL*.

Clinical phenotype was analyzed after removal of the 11 *SDHD* relatives who inherited a PV/LPV from their mother, because of the notion of maternal imprinting [7,8]. Interestingly, the prevalence of clinically symptomatic relatives at first imaging evaluation varied according to the mutated gene: low (<20%) for *SDHB* or *SDHC* PV/LPV, and high (>50%) for *SDHD*, *VHL* or *MAX*. Around 32% of relatives were symptomatic at first evaluation following the genetic

test result, dropping to 16.7% under-18 year-olds, the majority of whom had a TAP-PGL or PCC surgically resected, at 11 years of age for the youngest.

### 3.3. Genotype/Phenotype relationship

To evaluate the genotype/phenotype relationship, we combined probands and clinically symptomatic relatives. Results are shown in Table 4.

Patients carrying an *SDHD* variant presented mostly with HN-PGL, either isolated or associated with other PGL locations. Lesions were frequently multiple at one site with a low rate of metastatic disease. The patients carrying an *SDHB* variant presented mostly with TAP-PGL or HN-PGL, and showed the highest proportion of metastatic disease. The *SDHC* variant carriers were characterized by older age at diagnosis and isolated HN-PGL. *SDHA* variants were rare, and associated with PPGL of any location. The frequency of metastatic disease was high, at 12.5%. As expected, *VHL* patients and also *MAX* patients often presented with multiple PCC. However, in 2 patients with *VHL* PV/LPV, disease was revealed by abdominal PGL at the age of 12 (*VHL*, p.\*214Gly) and 28 (*VHL*, p.G144R), respectively.

### 3.4. Unusual phenotypes

#### 3.4.1. *SDHx* PV/LPV and pituitary adenoma

During the 21-year period, we observed 1 proband and 3 relatives with *SDHx* PV/LPV presenting with combined paraganglioma and pituitary adenoma (3PA syndrome), accounting for 1.1% (4/364) of patients with *SDHx* PV/LPV. Two patients (P1 & P2) belonging to 2 different families had an *SDHD* c.170-1G>T PV, which induces exon 3 skipping. Patient 1 had bilateral carotid body PGL discovered at 68 years of age and a pituitary adenoma (prolactinoma) found at 75 years of age. Patient 2 was a relative who underwent genetic testing at 26 years of age. During follow up, 2 PGLs were discovered at 28 years of age (1 involving the carotid body and 1 the inter-aortico-cavus region) and a pituitary adenoma was found at 39 years of age on cerebral imaging for headache. Blood prolactin was elevated at 312 ng/ml. Patients 1 and 2 received medical treatment and did not undergo surgery. Patient 3 was also a relative, with a p.Q169X truncating variant in *SDHB*. He developed prolactinoma at 36 years of age and carotid body PGL at 38 years of age. Interestingly, loss of the wild type allele of *SDHB* was found in the prolactinoma tissue, but no immunohistochemical study was performed. Patient 4 was a relative with a p.R512\* *SDHA* variant. At 76 years of age, he underwent surgery for a pituitary lesion corres-

**Table 2**  
Pathogenic/Likely Pathogenic variants in the susceptibility genes identified in the probands.

Mutated gene	Observed nucleotide modification	Expected protein modification	Number of index cases	Technique
MAX	c.97C>T	p.R33*	1	Sanger MLPA
MAX	c.145T>C	p.S49P	1	Panel NGS Haloplex
NF1	c.541C>T	p.Q181*	1	Panel NGS XTHS
NF1	c.4546G>T	p.E1516*	1	Panel NGS XTHS
RET	c.1900T>C	p.C634R	2	Sanger
RET	c.1833T>C	p.C611R	1	Sanger
SDHA	c.3G>T	p.M1I	1	Panel NGS Haloplex
SDHA	c.91C>T	p.R31*	1	Sanger
SDHA	c.762.770+17del	p.?	1	Panel NGS Haloplex
SDHA	c.1352G>A	p.R451H	1	Panel NGS XTHS
SDHA	c.1534C>T	p.R512*	1	Panel NGS Haloplex
SDHA	c.1766G>A	p.R589Q	1	Panel NGS Haloplex
SDHA	del ex1-9	p.?	1+1	MLPA/Panel NGS Haloplex
SDHB	c.79C>T	p.R27*	2	Sanger
SDHB	c.137G>A	p.R46Q	1	Sanger
SDHB	c.183T>A	p.Y61*	1	Sanger
SDHB	c.206G>T	p.G69V	1+1	Sanger/Panel NGS Haloplex
SDHB	c.260C>T	p.L88S	1	Sanger
SDHB	c.268C>T	p.R90*	1	Panel NGS Haloplex
SDHB	c.274T>C	p.S92P	1	Panel NGS Haloplex
SDHB	c.277T>C	p.C93R	2	Sanger
SDHB	c.287-1G>C	exon skipping	1	Sanger + MLPA
SDHB	c.287-2A>G	exon skipping	1	Sanger
SDHB	c.293G>A	p.C98Y	1	Sanger
SDHB	c.302G>C	p.C101S	1	Sanger
SDHB	c.343C>T	p.R115*	1	Panel NGS Haloplex
SDHB	c.348delG	p.I117Lfs*18	1+1	Sanger/Panel NGS Haloplex
SDHB	c.423+1G>C	exon skipping	1	Sanger
SDHB	c.487C>T	p.S163P	1	Sanger
SDHB	c.493del	p.E165Kfs*10	1	Panel NGS Haloplex
SDHB	c.505C>T	p.Q169*	2	NGS
SDHB	c.557G>A (mosaicism)	p.C168Y	1	Panel NGS Haloplex/digital PCR
SDHB	c.587G>A	p.C196Y	1	Panel NGS Haloplex
SDHB	c.591delC	p.S198Afs*22	1	Sanger
SDHB	c.596A>G	p.Y199C	1	Sanger
SDHB	c.617A>G	p.Y206C	1	Panel NGS Haloplex
SDHB	c.654G>A	p.W218*	1	Sanger
SDHB	c.689G>A	p.R230H	1	Sanger
SDHB	c.710C>G	p.P237R	1	Sanger
SDHB	c.718.721del	p.L240Tfs*7	1	Sanger
SDHB	c.724C>T	p.R242C	1+1	Sanger/Panel NGS Haloplex
SDHB	c.724C>A	p.R242S	1+1	Sanger/Panel NGS Haloplex
SDHB	c.725G>A	p.R242H	2	Sanger
SDHB	c.757T>C	p.C253R	1	Sanger
SDHB	del ex1-8	p.?	1	MLPA
SDHB	del ex1	p.?	1+1	MLPA/Panel NGS XTHS
SDHB	del ex 3	p.?	1	MLPA
SDHB	c.200+1G>A	exon 2 skipping	1	Sanger
SDHC	c.1A>G	p.M1V	4	Sanger
SDHC	c.43C>T	p.R15*	3+2	Sanger/Panel NGS Haloplex
SDHC	c.122del	p.F41Sfs*6	1	Panel NGS Haloplex
SDHC	c.149.154delGTCCTCinsTATA	p.R50Lfs*46	1	Sanger
SDHC	c.236.239dup	p.G81Cfs*18	1	Sanger
SDHC	c.397C>T	p.R133*	5+2	Sanger/Panel NGS Haloplex
SDHC	c.419G>A	p.G140E	1	Panel NGS Haloplex
SDHC	c.473T>C	p.L158P	1	Sanger
SDHC	del ex 2	p.?	1	MLPA/Panel NGS XTHS
SDHC	c.(-414.?)_(?.21-39)del	p.?	1	MLPA
SDHD	c.3G>C	p.M1V	1	Sanger
SDHD	c.33C>A	p.C11*	1	Sanger
SDHD	c.64C>T	p.R22*	1+1	Sanger/Panel NGS Haloplex
SDHD	c.129G>A	p.W43*	1	Sanger
SDHD	c.170-1G>T	exon skipping	26+2	Sanger/Panel NGS XTHS
SDHD	c.208A>G	p.R70G	2	Sanger
SDHD	c.241.262del	p.P81Afs*47	1	Panel NGS Haloplex
SDHD	c.242C>T	p.P81L	5	Sanger
SDHD	c.305A>G	p.H102R	1	Panel NGS Haloplex
SDHD	c.314G>A	p.W105*	2	Sanger
SDHD	c.338.344delACTATGTinsTCA	p.D113Vfs*21	1	Sanger
SDHD	c.372delC	p.A124Afs*10	1	Sanger
SDHD	c.405delC	p.F136Lfs*32	6+3	Sanger/Panel NGS Haloplex
SDHD	c.432insAT	p.H145Lfs*24	1	Sanger

Table 2 (Continued)

Mutated gene	Observed nucleotide modification	Expected protein modification	Number of index cases	Technique
SDHD	c.445.448dupATCT	p.C150Yfs*42	1	Sanger
SDHD	c.446.449delinsA	p.I149.C150delinsN	1	Sanger
SDHD	del ex 4	p.?	1	MLPA
SDHD	del ex1.4	p.?	1	MLPA
SDHD	c.314+1G>C	exon 3 skipping	1	Sanger
SLC25A11	c.107.108delCA	p.T36Sfs*71	1	Sanger
TMEM127	c.117.120del	p.I41Rfs*39	1	Panel NGS Haloplex
VHL	c.357C>G	p.F119L	1	Sanger
VHL	c.365C>T	p.A122V	1	Sanger
VHL	c.430G>A	p.G144R	1	Sanger
VHL	c.455C>T	p.T152I	1	NGS + Sanger
VHL	c.467A>G	p.Y156C	7+2	Sanger/Panel NGS Haloplex
VHL	c.485G>A	p.C162Y	1	Sanger
VHL	c.492G>C	p.Q164H	1	Sanger
VHL	c.499C>T	p.R167W	4	Sanger
VHL	c.640T>G	p.*214Gext*14	1	Sanger/Panel NGS Haloplex/PCR multiplex

**Table 3**  
Relatives characteristics according to mutation.

Gene	Number of relatives	Age at genetic testing (yrs)	Phenotype <sup>c</sup>		
			Asymptomatic	Symptomatic	Unknown
	n (%)	mean (SD); median (IQR)	n (%)	n (%)	n (%)
SDHB	104 (40.3%)	31.3 (19.4); 31.5 (30.5)	78 (75%)	17 (16.3%)	9 (8.7%)
SDHD	84 (32.5%)	36.4 (20.1); 32 (37)	24 (28.6%) <sup>c</sup>	42 (50.1%) <sup>c</sup>	7 (8.3%) <sup>c</sup>
SDHC	33 (12.8%)	47.0 (18.5); 44.0 (23.5) <sup>b</sup>	28 (84.8%)	2 (6.2%)	3 (9%)
SDHA	7 (2.7%)	53.1 (18.4); 58.0 (29.0) <sup>a</sup>	7 (100%)		
MAX	5 (1.9%)	54.6 (20.0); 51.0 (29.0)		5 (100%)	
VHL	24 (9.3%)	33.6 (20.3); 35.0 (27.5)	12 (50%)	12 (50%) <sup>d</sup>	
NF1	1 (nc)	nc	1 (100%)		
TOTAL	258 (100%)	36.2 (20.5); 36.0 (34.25)			
TOTAL (phenotype)	247	36.7 (20.3); 37.0 (33.0)	150 (60.7%)	78 (31.6%)	19 (7.7%)

nc: not calculated; SD: standard deviation; IQR: interquartile range.

<sup>a</sup> p < 0.05

<sup>b</sup> P < 0.001 vs. SDHB by Dunn's multiple comparison test

<sup>c</sup> for SDHD, analysis of the phenotype was carried out after removal of the 11 probands who received a PV from their mother

<sup>d</sup> 2 relatives with VHL lesions but no PPGL.

**Table 4**  
Phenotypes of all affected subjects\*.

Mutated gene	Number of subjects	Age at clinical evaluation median (IQR)	Topography				Disease extent	
			Adrenal-PGL	HN-PGL	TAP-PGL	Several locations	Multiple lesions at one site	Metastatic disease
SDHA	8	36 (34.5)	1 (12.5)	4 (50)	3 (37.5)	0	1 (12.5)	1 (12.5)
SDHB	61	35.5 (22.5)	5 (8.2)	23 (37.7)	27 (44.3)	6 (9.8)	12 (21.0)	7 (12.3)
SDHC	25	50 (30.5)	2 (8)	21 (84.0)	1 (4)	1 (4)	3 (12.0)	1 (4)
SDHD	103	36 (23.0)	1 (≈1)	79 (76.7)	1 (≈1)	22 (21.4)	76 (73.8)	5 (4.85)
MAX	7	46 (37.0)	6 (85.7)	0	0	1 (14.3)	6 (85.7)	0
VHL	30	29.5 (27.5)	24 (80)	0	2 (6.7)	4 (13.4)	16 (53.3)	2 (6.7)

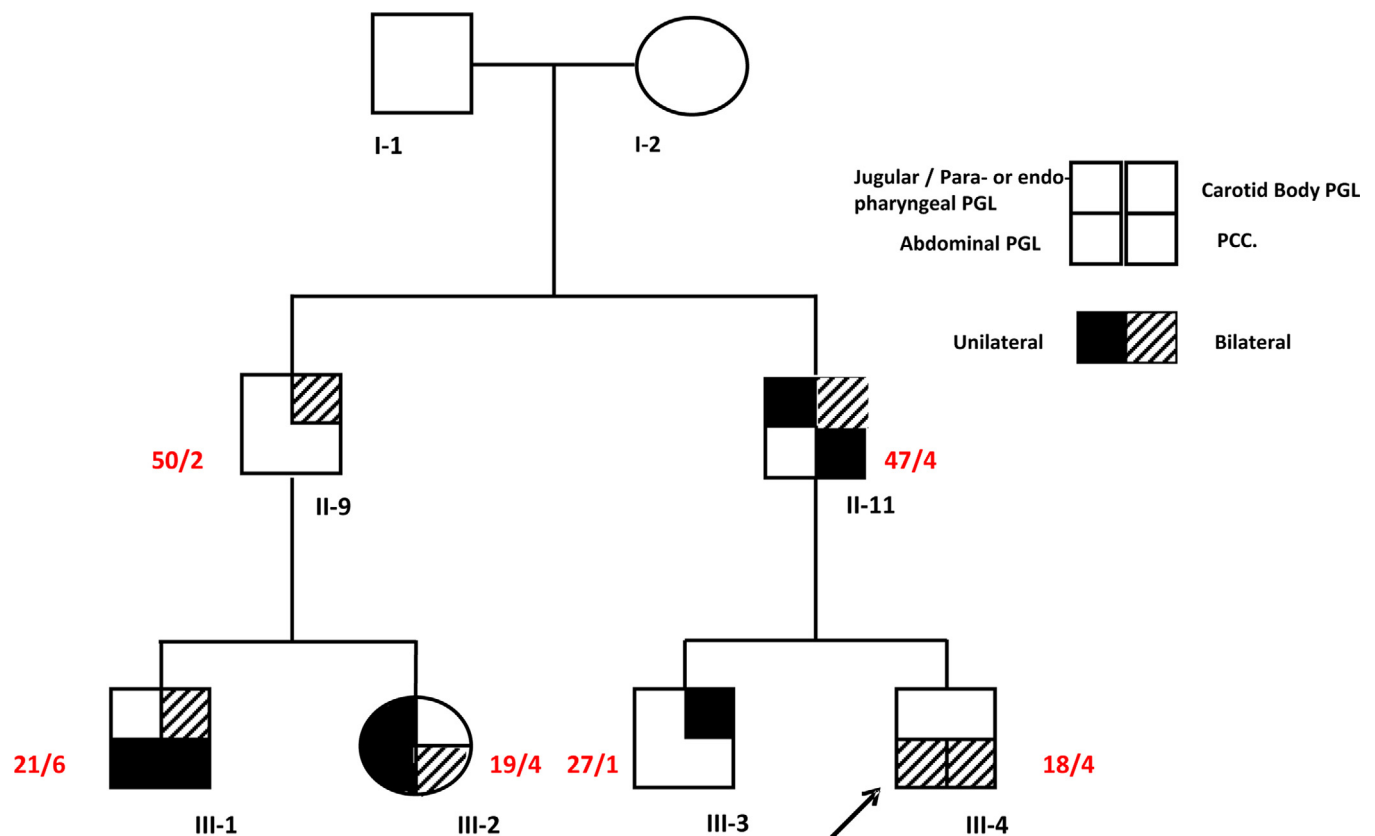
\* Probands and clinically symptomatic relatives. Genes with < 4 affected subjects were excluded from analysis

ponding histologically to a combination of a prolactin adenoma and epithelioid granulomas, which may correspond to a secondary location of the patient's sarcoidosis. The immunohistochemical analysis showed loss of expression of SDHA and SDHB in the endocrine cells.

3.5. Recurrent variants

During the study period, we observed recurrence of certain genetic variants in index cases (Table 2). Twenty-eight of the 61 index cases with an SDHD PV/LPV variant harbored the c.170-

1G>T variant, with a median (IQR) age at diagnosis of 38.5 (26) years (Table S2). Twenty-one presented HN-PGL, often multiple, (61.9%), 3 TAP-PGL and/or PCC and 4 another combination of PPGL. The number of PPGLs varied from 1 to 7, and was not related to age at diagnosis (r=0.05; ns). Metastatic disease was found in 3 index cases (10.7%). Phenotypic heterogeneity was also considerable between families, as illustrated in Sup Fig. 1, with families with only carotid PGL (Family 21), cervical PGL (Family 1), cervical or abdominal PGL (Family 13), or PGLs at 3 anatomical sites (Family 9). Intra-familial heterogeneity was also obvious in Family



**Fig. 2.** Genealogical tree of a family with PPGLs linked to a *SDHD* c.170-1G>T variant, showing great intra-familial phenotype variability. x/y: age at imaging/number of lesions.

28, as shown in Fig. 2. The index case (III-4) presented with bilateral pheochromocytoma (32 and 42 mm diameter) at the age of 16 years, revealed by hypertensive cardiopathy associated with elevated urinary normetanephrine. Two years after surgical resection, a new increase of normetanephrine levels led to the discovery of 2 PGLs in the retro-renal region, at the age of 18. The patient also had a Di George syndrome with a 22q11 microdeletion. Genetic screening was started in the family. His brother (III-3), clinically asymptomatic at 21 years of age, had a negative first imaging evaluation. Six years later, a right carotid body PGL measuring 8 × 6 × 10 mm was found. The father (II-11) had the *SDHD* variant, was probably of paternal origin since the mother (I-2) was wild-type. Clinical and imaging evaluation led to the discovery of a 3 cm diameter left PCC, a 2.5 × 1.5 × 2 cm PGL in the larynx and 2 small carotid PGLs less than 1 cm long, at the age of 47. Only one of his siblings (II-9) had inherited the *SDHD* variant. He was clinically asymptomatic at the age of 50 despite 2 small carotid PGLs measuring respectively 7 and 11 mm in diameter, non-progressive over 10 years' follow-up. His 2 children inherited the *SDHD* variant but presented a more severe phenotype. The son (III-1), who was clinically asymptomatic, had 6 lesions on first imaging evaluation: a left 1.4 cm diameter PCC and 3 inter-aortic-cavus PGLs resected at the age of 21 years, associated with small bilateral carotid PGLs measuring 5.5 × 5.5 × 8 mm and 12 × 14 × 30 mm. His sister (III-2) had 4 lesions at the age of 19 years: a bilateral PCC, a left juxta-adrenal 32 mm long PGL, and a para-pharyngeal PGL measuring 7 × 7 × 8.5 mm.

Seven probands presented the *SDHC* c.397C>T p.R133\* variant. Their mean age at diagnosis was not significantly different from the whole population (41 years); all harbored HN-PGL, the youngest being diagnosed at the age of 14 years, but some degree of phenotypic heterogeneity was also observed (Table S3). Another recurrent variation of *SDHC* was c.43C>T p.R15\*, which was identi-

fied in 5 patients harboring single HN-PGL, one of whom, diagnosed at 64 years of age, also had PCC (Table S4).

### 3.6. *SDHx* mosaicism in patients with PPGL

In collaboration with our colleagues from the Hôpital Européen Georges-Pompidou in Paris, we had the opportunity to report the first *SDHB* mosaic mutation (c.557G>A, p.C186Y) by applying NGS and droplet digital PCR on blood and tumor DNA [9]. Despite an allelic ratio of 15% in blood DNA, the patient was operated on for a functional para-aortic PGL at 24 years of age, with recurrence 4 years later during a second pregnancy [6]. After ten years' follow-up, she presented diffuse liver and bone metastasis. Another female patient diagnosed at 26 years of age with a large HN-PGL, for whom initial genetic analyses were negative, was reassessed at the age of 32. After NGS screening for mosaic mutations, an *SDHD* PV (c.242C>T, p.P81L) was identified with an allele frequency of 2.5% in blood DNA and the result was confirmed by droplet digital PCR.

## 4. Discussion

The present study revealed a germline PV/LPV in one susceptibility gene in 27.2% of probands, which is consistent with some literature data [10] but less than in more recent reports of rates up to 40% by targeted panel sequencing [2]. As described in our sequencing strategy, the number of genes analyzed has increased over the years: from 2001 to 2017, 5 to 9 major genes were analyzed by Sanger sequencing, while since 2018 11 to 19 genes are analyzed by NGS. Increasing the number of genes beyond 11 by using NGS in index cases did not increase the proportion of mutated patients: 16.8% versus 36% for patients analyzed by Sanger plus MLPA. This result, although counterintuitive, may be explained mainly by the

fact that the switch to NGS was accompanied by a generalization of genetic analysis to all PPGL patients, regardless of their clinical presentation. Whereas in the first phase of our study, Sanger analysis was limited to patients with a strong clinical suspicion of familial PPGL [3].

Compared to the distribution of mutated genes in the literature [11], in the present study the proportion of *SDHx* genes was higher. This could be explained by preferential screening of *SDHx* and *VHL* genes on Sanger sequencing in the first time period compared to the more recent gene panel analysis by NGS. In some cases, patients with a severe phenotype (multiple locations, recurrence, metastasis) and negative genetic results on Sanger sequencing were re-analyzed by NGS, which identified germline causative mutations in newly described susceptibility genes. Moreover, the ability to screen for large gene rearrangements, copy number variations and mosaic germline mutations, which were not accessible in the early years, also allowed some hereditary forms to be identified [5,6]. This highlights the interest of renewing investigations in negative situations, in view of the progress in techniques and knowledge [12].

Taking all susceptibility genes together, the proportion of truncating variants was higher than for missense variants in probands. Such a result was previously reported by Bayley et al. [13] in *SDHx* variants. Also, truncating variants were associated with a significantly higher rate of multiple lesions. Different biological scenarios (reactive oxygen species production and/or succinate accumulation) have recently been proposed to explain the differences in phenotype according to the type of *SDHx* variant (missense or truncating) [14].

Regarding genotype-phenotype correlations, we confirmed previously reported trends [15,16], and also added a few nuances. Some variants were recurrent in our cohort, such as *SDHC* c.397C>T or *SDHD* c.170-1G>T. Those variants are referenced many times in pathogenic variant databases such as HGMD® Pro 2023.4 (respectively 18 and 9 references) or LOVD or ClinVar, whereas they are very rare in the general population (respectively ten 280406 and one 280406 alleles in gnomAD (v2.1.1) genome +exome database), confirming their pathogenicity. The present study collected clinical data from several families with the *SDHD* variant (c.170-1G>T). A high degree of phenotypic variability was observed both between and within families. Notably, in Family 28 (Fig. 2), patients with an ultra-severe phenotype (more lesions and more affected sites) coexisted with patients with a mild phenotype. This raises the question of environmental (pesticide) or genetic factors capable of modifying the phenotype.

The 3PA syndrome, associating pituitary adenoma (PA) and pheochromocytoma/paraganglioma (PPGL), is sometimes associated with mutations in PPGL-predisposing genes such as *SDHx* [17]. Four of the 364 subjects (probands and relatives) with a PV/LPV in *SDHx* showed an association of PGL and PA: i.e., a frequency of 1.1%, in line with a previous report [18]. We identified 1 patient with a p.R512\* *SDHA* germline variant whose prolactinoma was immunonegative for both SDHA and SDHB, in line with previous results for other *SDHA* variants [19]. Even if the PA/PPGL association is rare, it is worth keeping in mind. Nevertheless, systematic screening of *SDHx* genes in patients with isolated pituitary adenoma is not recommended. Regarding von Hippel Lindau disease, in 2 out of 30 subjects carrying a PV of *VHL* (6.7%) the disease was revealed at a young age by an abdominal extra-adrenal PGL, a situation that is rare but not non-existent [20]. Identification of the gene involved in PPGL is important for the management of the patient and relatives. However, phenotypic variability seen in *SDHD* variants is a hindrance to personalized imaging screening according to genetic variant. It seems more appropriate to adopt a whole-body imaging strategy in these patients, as recently recommended [21].

In conclusion, our experience confirmed the previously established genotype-phenotype correlations, but also points to the existence of atypical clinical presentations, even for the same genetic variant. These data have to be taken into account for optimal patient follow-up and management. Although NGS enables easy analysis of an ever-increasing number of susceptibility genes, interpretation of the gene variants requires reliable clinical data and complementary tools such as immunohistochemistry on tumor tissue or oncometabolite assays [22].

### Disclosure of interest

The authors declare that they have no competing interest.

### Author contributions

Catherine Cardot Bauters: resources, original draft preparation; Marie Christine Vantuyghem: resources, original draft preparation; Christine Docao: resources, original draft preparation; Rachel Desailoud: resources, original draft preparation; Michael Joubert: resources, original draft preparation; Lucie Coppin: investigation, original draft preparation; Marie Françoise Odou: investigation, resources, formal analysis, original draft preparation; Pascal Pigny: investigation, writing-reviewing and editing.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ando.2024.05.024.

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