Clinically Guided Genetic Screening in a Large Cohort of Italian Patients with Pheochromocytomas and/or Functional or Nonfunctional Paragangliomas

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Purpose: The aim of the study was to define the frequency of hereditary forms and the genotype/phenotype correlations in a large cohort of Italian patients with pheochromocytomas and/or functional or nonfunctional paragangliomas.

Design: We examined 501 consecutive patients with pheochromocytomas and/or paragangliomas (secreting or nonsecreting). Complete medical and family histories, as well as the results of clinical, laboratory, and imaging studies, were recorded in a database. Patients were divided into different groups according to their family history, the presence of lesions outside adrenals/paraganglia considered syndromic for VHL disease, MEN2, and NF1, and the number and types of pheochromocytomas and/or paragangliomas. Germ-line mutations in known susceptibility genes were investigated by gene sequencing (*VHL, RET, SDHB, SDHC, SDHD*) or diagnosed according to phenotype (*NF1*). In 160 patients younger than 50 yr with a wild-type profile, multiplex ligation-dependent probe amplification assays were performed to detect genomic rearrangements.

Results: Germline mutations were detected in 32.1% of cases, but frequencies varied widely depending on the classification criteria and ranged from 100% in patients with associated syndromic lesions to 11.6% in patients with a single tumor and a negative family history. The types and number of pheochromocytomas/paragangliomas as well as age at presentation and malignancy suggest which gene should be screened first. Genomic rearrangements were found in two of 160 patients (1.2%).

Conclusions: The frequency of the hereditary forms of pheochromocytoma/paraganglioma varies depending on the family history and the clinical presentation. A positive family history and an accurate clinical evaluation of patients are strong indicators of which genes should be screened first. (***J Clin Endocrinol Metab* 94: 1541–1547, 2009**)

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Abbreviations: HNPGL, Head and neck PGL; MEN2, multiple endocrine neoplasia type 2; MLPA, multiplex ligation-dependent probe amplification; NF1, neurofibromatosis type 1; PGL, paraganglioma; Pheo, pheochromocytoma; SDH, succinate dehydrogenase; sPGL, secreting PGL; VHL, von Hippel-Lindau (disease).

Pheochromocytomas (Pheos) and paragangliomas (PGLs) are neural crest-derived tumors. The former are found in the adrenals, develop from chromaffin cells, and generally secrete catecholamines. Chromaffin tumors, located outside the adrenals, in the abdomen and the thorax, are named PGLs. In the present manuscript these tumors will be referred to as "secreting PGLs" (sPGLs) to distinguish them from PGLs of the head and neck (HNPGLs), which are parasympathetic in origin and, for the vast majority, nonsecreting.

Susceptibility to Pheos and PGLs is an established component of three genetic syndromes, von Hippel-Lindau (VHL) disease, multiple endocrine neoplasia type 2 (MEN2), and neurofibromatosis type 1 (NF1), which are caused, respectively, by germline mutations of the tumor-suppressor gene *VHL*, the protooncogene *RET*, and the *NF-1* gene, which also functions as a tumor suppressor (1, 2). These syndromes are characterized, in addition to Pheo/PGL, by lesions involving organs other than the adrenals or the paraganglia. These lesions will be referred to as "syndromic." More recent work has highlighted the importance of *SDHB*, *SDHC*, and *SDHD* genes that encode the B, C, and D subunits of succinate dehydrogenase (SDH), also known as mitochondrial complex II (3–5). Mutations of the *SDHB*, *SDHC*, and *SDHD* genes are associated with three clinical syndromes referred to as PGL4, PGL3, and PGL1, respectively. These syndromes are mostly characterized by HNPGLs variably associated with Pheo/sPGL. At present, no other lesions have been identified as typical components of these syndromes.

Hereditary forms were once believed to represent around 10% of all Pheos/PGLs, but in 2002, Neumann *et al.* (6) reported germ-line mutations involving four known susceptibility genes in 66 (24%) of 271German/Polish patients with clinical findings suggestive of sporadic tumors. This report led to the recommendation that all patients with Pheo/sPGL be screened for hereditary disease. However, the cost implications of this proposal are considerable, and the reasoning that supports it must be critically analyzed before such a policy is adopted (7, 8). Systematic mutation analysis of all of the currently known susceptibility genes is considerably expensive, and some type of clinical prescreening is recommended to identify the most likely target of the mutation. Nevertheless, selection of patients with Pheo/PGL for genetic testing is a complex process.

Family history can be of some help in identifying a hereditary form. A positive history may be highly informative (*e.g.* a relative with an already established diagnosis or lesions typical of syndromic disease) or partially informative (a relative with a single Pheo/PGL). The clinical presentation can also provide useful clues, but any decision must rely on a thorough knowledge of the possible genes involved, their characteristics (mode of transmission, penetrance), and especially the correlated clinical pictures. Unfortunately, these latter may be extremely variable; whereas some patients present associated syndromic lesions that are diagnostic for VHL, MEN2, and NF1, in others, when these syndromic lesions are absent, only the number, type, and association of Pheos, sPGLs, or HNPGLs has to be considered. In the presence of only one Pheo/PGL, only a positive family history suggests a hereditary form, whereas the presence of multiple or recurrent Pheos/PGL strongly suggests a genetic form that has to be further defined by the genetic analysis. In fact, different syndromes can sometimes share a common clinical picture as, for example, bilateral/recurrent Pheos in VHL or MEN2 and association of HNPGLs with sPGL in PGL4 or PGL1.

The Pheochromocytoma/Paraganglioma Working Group of the Italian Society of Endocrinology recently performed genetic testing in a large cohort of Italian patients with secreting and/or nonsecreting PGLs. Our objective was to characterize the frequency and distribution of genetically determined Pheos and PGLs in this geographic area and to identify phenotypic clues that can be used to guide decisions on the use of genetic testing in patients with these tumors.

Patients and Methods

The study protocol was preapproved by the institutional review boards of all participating centers, and each participant provided written informed consent to all study procedures (including genetic testing) and to publication of the results. Unless otherwise stated, all commercial products mentioned below were used according to manufacturers' instructions.

Patients

The study population consisted of 501 consecutive patients (adults and children) with Pheo and/or PGLs visited in the 17 endocrinology or hypertension centers of the Italian Pheochromocytoma/Paraganglioma Network between January 1, 2003, and December 30, 2007. The only inclusion criterion was a past or new diagnosis of Pheo or PGL. Upon enrollment, each participant was evaluated according to a well-established protocol that included complete personal and family histories, clinical evaluation, and a panel of laboratory tests and imaging studies aimed at detecting lesions considered diagnostic for VHL, MEN2, and NF1 (syndromic lesions). Diagnoses of Pheo or sPGL were based on plasma or urinary levels of catecholamines or catecholamine metabolites (mainly metanephrines) and confirmed by iodine^{123}-labeled metaiodobenzylguanidine scintigraphy and/or surgical histology. HNPGLs were diagnosed on the basis of imaging findings (presence in the region of a highly vascular mass on computed tomography, magnetic resonance imaging, ultrasonography, or somatostatin receptor scintigraphy with indium^{111}-labeled pentetreotide, as appropriate) and, when possible, surgical histology.

Malignancy was defined as the presence of metastases outside the paraganglia or adrenals (mostly in bones, lungs, and liver).

On the basis of the results of this workup, participants were classified according to their family history (positive/negative), the presence/absence of syndromic lesions, and the number and type of Pheo/PGL.

Mutation analysis

With the exception of NF1 mutations, which were diagnosed on the basis of phenotype alone as generally accepted (9, 10), all germ-line mutations were documented by the results of genetic testing performed according to standardized protocols in the centers of Florence, Brescia, Padua, or Rome. DNA was extracted from the peripheral blood leukocytes of each patient with the NucleoSpin Blood L kit (Macherey-Nagel, Düren, Germany) and analyzed for germ-line mutations of *RET* (exons 10, 11, 13, 14, 15, and 16), *VHL* (all exons), *SDHD* (all exons), *SDHB* (all exons), and *SDHC* (all exons). For each gene, coding regions and exon-intron boundaries were amplified by PCR as previously described (11). PCR products purified with a commercial kit (PCR purification kit; QIAGEN, Milan, Italy) were subjected to 2% agarose gel electrophoresis with ethidium bromide staining and subsequently sequenced with a genetic analyzer (ABI PRISM 310; Applied Biosystems, Milan, Italy).

TABLE 1. Classification of patients according to family history and clinical presentation

Clinical presentation	Positive family history	Mutation frequency	Negative family history	Mutation frequency
No. of females	38		250	
No. of males	19		194	
Mean age (yr)	39.1		45.7	
Age range (yr)	9–89		5–93	
Associated syndromic lesions		16/16 (100.0%)		50/50 (100.0%)
VHL	9		23	
MEN2	6		17	
NF1	1		10	
Multiple/recurrent Pheo/PGL		27/27 (100.0%)		19/49 (38.8%)
Pheos/sPGL	5		35	
HNPGL	14		10	
Pheos/sPGL + HNPGL	8		4	
Single Pheo/PGL		9/14 (64.3%)		40/345 (11.6%)
Pheo	9		233	
sPGL	1		35	
HNPGL	4		77	
Total	57	52/57 (91.2%)	109	109/444 (24.5%)

Multiplex ligation-dependent probe amplification reactions

A total of 160 patients under 50 yr of age whose DNA sequencing results revealed wild-type *RET*, *VHL*, *SDHB*, *SDHC*, and *SDHD* were subsequently analyzed for genomic rearrangements involving the *VHL* or one of the *SDH* genes. For this purpose, we used commercial kits for multiplex ligation-dependent probe amplification (MLPA)-based assays (SALSA MLPA P016B VHL and SALSA MLPA P226 SDHD; MRC-Holland, Amsterdam, The Netherlands) following the manufacturer's instructions.

The PCR was carried out as follows: 35 cycles of 30 sec at 95 C; 30 sec at 60 C; 60 sec at 72 C; and 20 min of final incubation at 72 C. Amplification products were diluted in HiDi formamide containing 500TAMRA internal size standards (Applied Biosystems, Foster City, CA) and then size-separated with an ABI PRISM 310 Genetic Analyser (Applied Biosystems, Milan, Italy). Electropherograms were analyzed with Coffalyser MLPA DAT software (MRC-Holland).

Statistical analysis

Statistical analysis was based on χ^2 test, and P values <0.05 were considered statistically significant.

Results

Overall, 501 patients were enrolled in the study (288 females and 213 males; mean age, 44.7 yr; age range, 5–93 yr).

Table 1 summarizes the characteristics of the patient groups defined on the basis of family histories and clinical presentations.

Fifty-seven patients had a positive family history. Among these, 16 presented, in addition to Pheo/PGL, with at least one syndromic lesion, 27 presented with multiple or recurrent Pheos/PGLs without any other known syndromic lesion, and 14 presented with only a single Pheo, sPGL, or HNPGL. Germline mutations were found in 100.0, 100.0, and 64.3% of cases, respectively.

Among the 444 patients with a negative family history, 50 presented with at least one syndromic lesion, 49 with multiple or recurrent Pheo/PGL, and 345 with a single Pheo, sPGL, or HNPGL. The germ-line mutation rates in these three subgroups were 100.0, 38.8, and 11.6%, respectively.

On the whole, 91.2% of the patients with a positive family history and 24.5% of those with a negative family history were mutation carriers. A total of 161 (32.1%) of 501 patients harbored germ-line mutations involving *VHL* (48 patients, 9.6%), *RET* (27 patients, 5.3%), *NF1* (as deduced from the phenotype, 11 patients, 2.2%), *SDHB* (24 patients, 4.7%), *SDHC* (4 patients, 0.8%), or *SDHD* (47 patients, 9.4%).

Within the group of 345 patients with a single tumor and negative family history (those most likely to have truly sporadic disease), the subgroup with Pheos/sPGLs and the one with HNPGLs had similar germ-line mutation rates (10.8 and 14.3%, respectively). However, the former subgroup was characterized by lower ages at presentation, with a peak frequency in the second decade *vs.* third decade for HNPGLs (Fig. 1).

Eighty-two percent of germ-line mutations (132 of 161) were found in patients younger than 50 yr. In older patients, about 50% of mutations (14 patients) were found in *SDHD*, whereas the others were found in *VHL* (7 patients), *RET* (5 patients), *NF1*, *SDHB*, and *SDHC* (1 patient each).

Table 2 summarizes the characteristics of the Pheos, sPGLs, and HNPGLs in our 501 patients and the results of the genetic

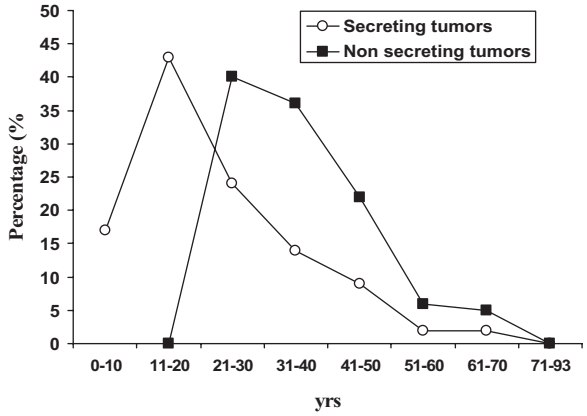

FIG. 1. Percentage incidence of mutations per decades of ages in patients with clinically sporadic secreting tumors (Pheos and sPGLs) (*open circles*) and patients with clinically sporadic nonsecreting tumors (HNPGLs) (*closed squares*).

TABLE 2. Genotype profile of the different clinical presentations as defined by the number and type of Pheos and/or PGLs

	Mutation						
	VHL	**RET**	**NF1**[a]	**SDHB**	**SDHC**	**SDHD**	**Wild-type**
Patient features							
All (n = 501)	48 (9.6%)	27 (5.4%)	11 (2.2%)	24 (4.8%)	4 (0.8%)	47 (9.3%)	340 (67.9%)
Males (n = 213)	23	9	5	11	1	19	145
Females (n = 288)	25	18	6	13	3	28	195
Age at diagnosis (yr)							
Mean	30.0	36.5	42.3	28.7	43.0	39.7	49.6
Range	9–67	18–65	29–62	9–55	16–73	14–69	5–93
Presentation							
Secreting tumors only (n = 380)	44 (12.0%)	27 (7.0%)	11 (2.9%)	19 (5.0%)	1 (0.3%)	7 (1.8%)	271 (71.0%)
Unilateral Pheo (n = 278)	24	16	9	5		4	220
Bilateral Pheo (n = 48)	17	10					21
Single sPGL (n = 36)	2		1	10	1	1	21
Multiple sPGL (n = 4)				2			2
Pheos+ sPGL (n = 14)	1	1	1	2		2	7
Nonsecreting tumors only (n = 106)	2 (1.9%)			4 (3.8%)	3 (2.8%)	28 (26.4%)	69 (65.1%)
Single HNPGL (n = 96)	2			3	3	21	67
Multiple HNPGLs (n = 10)				1		7	2
Secreting and nonsecreting tumors							
Pheo/sPGL + HNPGL (n = 15)	2 (13.3%)	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	12 (80.0%)	0 (0%)

[a] The presence of NF1 mutations was deduced on the basis of phenotype.

analysis, regardless of the family history and the association with other syndromic lesions. These data were analyzed to determine whether the clinical features of the Pheos/PGLs (secreting *vs.* nonsecreting, location, multiplicity, recurrence, tumor combinations) alone can provide indications on the gene that should be examined first.

Pheos and sPGLs were present in 380 patients, HNPGLs in 106 patients, and the association of the two in 15 patients.

Unilateral Pheos (n = 278) were associated with germ-line mutations in 20.7% of cases (8.6% *VHL*, 5.8% *RET*, 3.2% NF1, 1.8% *SDHB*, and 1.4% *SDHD*, respectively). The mutation rate was over twice as high (56.2%) in patients with bilateral Pheos (n = 48), the mutations being found only in *VHL* (35.4%) or *RET* (20.8%) genes.

sPGLs were diagnosed in 54 patients, and among these, 36 were affected by a single sPGL, four by multiple sPGLs, and 14 by the association of Pheo and sPGL. Single sPGLs were associated with a germ-line mutation in 41.7% of cases. These mutations were mostly found in the *SDHB* gene (27.8%) and, much less frequently, also in *VHL*, *NF1*, *SDHC*, and *SDHD* genes. Multiple sPGLs were found associated with germ-line mutations in 50% of cases, and all involved the *SDHB* gene.

Most patients with HNPGLs only had a wild-type genotype [69 (65.1%) of the 106 patients with this type of tumor], but some had mutations involving one of the SDH genes (35 of 106, 33.0%) or, in two cases, *VHL*. There was no evidence of *RET* or *NF1* mutations in this group.

All 15 patients presenting with both a Pheo/sPGL and a HNPGL were affected by a germ-line mutation: 12 (80.0%) of the *SDHD*, one (6.7%) of the *SDHB*, and two (13.3%) of the *VHL* gene.

The vast majority of the 150 mutations diagnosed by genetic analysis (as mentioned above, in 11 patients NF1 was diagnosed clinically, as generally accepted) were missense (68.7%) or nonsense (21.3%) mutations, although we also found frameshifts (4.0%), splicing alterations (4.7%), and two cases of genomic rearrangement (1.3%) both involving total deletion of the *SDHD* gene.

Table 3 shows data on the 25 patients (5.0% of the population) with malignant disease. Over half (16 of 25, 64.0%) had wild-type genotypes. Malignancy rate ranged from 1.9% in HNPGLs to 4.9% in Pheos and 15.0% in sPGLs ($P = 0.008$). Secreting tumors were more likely to be associated with malignant disease than nonsecreting tumors (5.8 *vs.* 1.9%; $P = 0.027$). It is noteworthy that 20.8% (5 of 24) of the patients with *SDHB* mutations had malignant tumors, as compared with less than 5% of those with mutations involving *SDHD* (2 of 47; 4.3%) or *VHL* (2 of 48; 4.2%). No malignant forms were observed in patients presenting germ-line mutations of the *RET*, *NF1*, or *SDHC* genes.

Discussion

The paper published in 2002 by Neumann *et al.* (6) raised interest about the percentage of hereditary PHEO/sPGLs, which they estimated around 24% in a group of patients classified as apparently sporadic.

A more recent study of a large cohort of 314 French patients (8), again all with functional tumors, revealed hereditary disease in 86 (27.4%) cases (including 13 with phenotypically diagnosed NF1). However, the rate of inherited tumors in the subgroup with apparently sporadic presentations was only 11.6%.

These different results might depend not only on the geographic origins of the series but also on the criteria of patient selection and classification. These latter can be particularly dif-

TABLE 3. Clinical characteristics, phenotype, and gene mutations in 25 patients affected by malignant Pheo/PGL

	WT	VHL	SDHB	SDHD	Total	% Malignancy
Gender						
Females	10	1	2	2	15	
Males	6	1	3	0	10	
Mean age at diagnosis (yr)	48.1	28.0	20.8	36.0		
Phenotype						
HNPGL	1	0	0	1	2	1.9 (2/106)
Pheo						
Monolateral	10	1	1	1	13	4.9 (16/326)
Bilateral	3	0	0	0	3	
Extraadrenal PGL						
Single	2	0	2	0	4	15.0 (6/40)
Multiple	0	0	2	0	2	
Intra- + extraadrenal	0	0	0	0	0	0 (0/14)
Secreting + nonsecreting PGL	0	1	0	0	1	6.6 (1/15)
Total	16	2	5	2	25	5.0 (25/501)

WT, Wild type.

ficult for patients affected by Pheo/PGL, especially after the discovery of the PGL syndromes that increase the variability of the clinical presentation (11–17) and the mode of genetic transmission (18, 19).

In the present study, which included patients with both functioning (Pheos/sPGLs) and nonfunctioning (HNPGLs) PGLs, we tried to classify each patient as accurately as possible, according to the two factors that can be assessed before the genetic analysis: family history and overall clinical picture.

Eliciting a family history for these tumors can be difficult. False-negative findings can be caused by gaps and errors in the patient's knowledge of his/her relatives' medical histories, the presence of inherited syndromes with relatively low penetrance (*e.g.* PGL-4 syndrome) (14–17), or the confounding effects of maternal imprinting, which is a feature of the PGL-1 syndrome (19). Nonetheless, a careful family history is of great importance in these patients because it can, when positive, address the genetic analysis to a specific gene or even to a specific mutation. In our series, 57 patients had a positive family history, and among them, 30 belonged to families where a relative had already been diagnosed with a syndromic disease, letting us search for mutations in the correspondent gene. In the others, where a syndromic disease had still to be diagnosed, we had to rely exclusively on clinical presentation. In this group, patients with multiple or recurrent tumors were all mutation carriers.

On a whole, 52 of 57 patients (91.2%) of this subgroup were mutation carriers.

The fact that the remaining five patients were wild-type at genetic screening suggests that other susceptibility genes for Pheo/PGL might indeed exist (20, 21). Of note, each of these five patients was affected by a single adrenal Pheo. Whether this group represents a phenotypic cluster of a mutated, still unknown, susceptibility gene remains to be established.

Even when the family history was negative, the association of Pheo/PGL with a syndromic lesion was the strongest predictor of hereditary disease; in fact, all 57 patients presenting with this clinical picture were found to have a hereditary Pheo/PGL.

In our series, 19 (38.8%) of the 49 patients presenting with multiple or recurrent Pheos/PGLs, a negative family history, and no syndromic lesions were mutation carriers. Therefore, tumor multiplicity and recurrence by itself should be considered a predictor of hereditary disease. Of note, bilateral Pheos were hereditary in more than 50% of patients and exclusively associated with mutations in *VHL* or *RET*. In these patients, the chemical phenotype of the tumors (adrenergic *vs.* noradrenergic) can offer an indication of the gene that should be tested first (22).

Lastly, in the group with a negative family history, no syndromic lesions and single Pheo/PGL (the patients with tumors generally classified as "truly sporadic") germ-line mutations were found in 40 (11.6%) of the 345 patients. When Pheos/sPGLs and HNPGLs were considered separately, the correspondent mutation frequencies were, respectively, 10.8% (29 of 268), a percentage similar to that of the French study (8), and 14.3% (11 of 77), a frequency that is between those observed in similar but smaller cohorts in northern Spain (22.2%) (23) and in France (6.0%) (24). Overall, 24.5% of patients with a negative family history were mutation carriers, a percentage close to that reported by Neumann *et al.* (6).

Our study shows that the frequency of hereditary Pheo/PGL can vary widely, ranging from 11 to 100% depending on the criteria used for patient selection.

In addition to family history, the clinical picture can be of great help in planning the genetic analysis.

In fact, the results of our genetic study demonstrate that type, number, and variable associations of Pheo/PGL can reliably predict which gene or genes are worth being screened, as shown in Fig. 2.

It is interesting to note that, in our study, HNPGLs have been found, either isolated (two patients) or associated to Pheo/sPGL (two patients), in *VHL* mutation carriers. This finding indicates that in patients affected by HNPGLs, genetic analysis should not be limited to the *SDHx* genes but extended to *VHL* when *SDHx* genes are wild-type. Moreover, this clinical finding indirectly supports the hypothesis of a common pathogenesis, namely activation of the hypoxia-linked pathway, in *VHL* and *SDHx*-linked Pheo/PGL (25).

Additional clinical guides to genetic analysis are age at Pheo/PGL presentation and malignancy. In our study, young age and

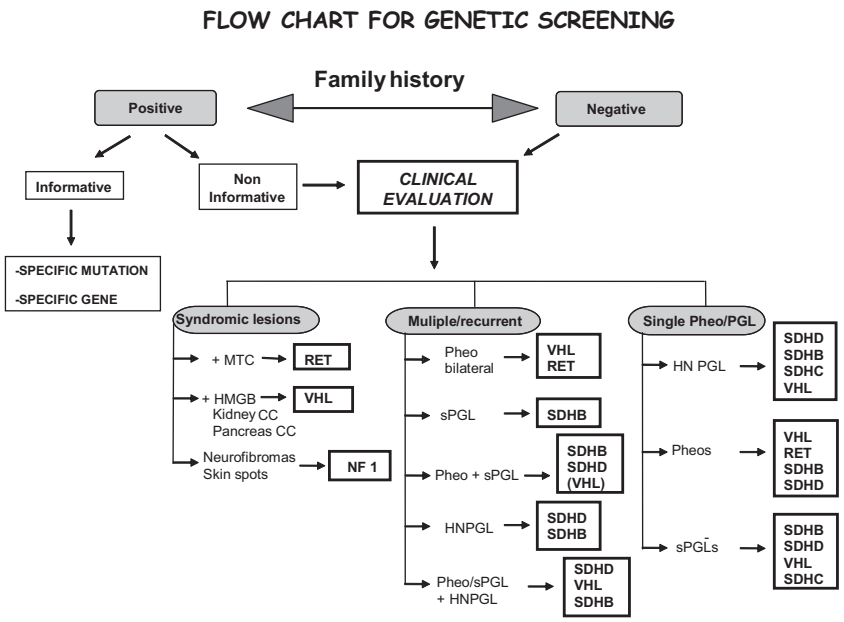

FIG. 2. Flow chart suggested for genetic analysis in patients affected by Pheos or PGLs. The genes reported in the *boxes* are those more likely to be found mutated according to clinical picture. MTC, Medullary thyroid carcinoma; HMGB, hemangioblastomas; CC, cancer/cysts.

metastatic disease are both predictors of *SDHB* mutation, in agreement with other studies (8, 16, 26–28).

Age at presentation also proved to be another predictor of the presence of germ-line mutations in patients with clinically sporadic tumors (Fig. 1). In the subgroup of patients with secreting tumors, genetic forms are most likely when presentation occurs during the second decade of life. Later onset is associated with much lower mutation rates (less than 5% in the fifth decade).

Conversely, in the subgroup of patients with clinically sporadic HNPGLs, the frequency of germ-line mutations is highest in the third decade and still remains high (above 20%) in the fifth decade.

These data suggest that genetic testing should be performed in all young patients with Pheos/sPGLs, even when the tumor appears to be sporadic. Patients with clinically sporadic tumors that occur after the age of 50, regardless of their functionality, might be excluded from genetic testing because the frequency of mutations in these groups is around 5%.

Nonetheless, it is worth mentioning that among the 29 mutation carriers older than 50 yr, 14 (48.3%) had a negative family history and were, therefore, index cases who gave indications for genetic screening of their families.

Our study confirms that malignancy is mostly linked to *SDHB* mutations. Nevertheless, it must be pointed out that other genes may be involved, although to a much lesser extent.

As regards the type of mutations, we found (as reported in Table 3) that the vast majority of those involving *VHL* and *RET* were missense, as expected (1, 29–32), and the same was true for the *SDHB* mutations. In contrast, *SDHD* and *SDHC* mutations were mostly of the nonsense type. Genomic rearrangements were found in only 1.2% (2 of 160) of the patients who were wild-type for all genes in sequencing studies. Although we limited the search for genomic rearrangements to patients under the age of 50 (the group that includes the vast majority of patients with germ-line mutations), such a low frequency suggests to us that this analysis is not worthwhile on a routine basis.

In conclusion, we evaluated a large cohort of Italian patients with Pheos/PGLs. An accurate family history and an extensive clinical evaluation permitted us to separate our patients into different groups and to establish the correspondent incidences of mutations in the known susceptibility genes.

The relative frequency of the different mutations permitted us to construct a comprehensive flow chart (Fig. 2) that, for the first time, takes into consideration not only patients with Pheos /sPGLs but also those with HNPGLs. This flow chart, which is based on the patient's family history and clinical presentation, may represent a guide for the gene analysis strategy.

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