0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(10):3822–3828
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doi: 10.1210/jc.2007-0709

Succinate Dehydrogenase B Gene Mutations Predict Survival in Patients with Malignant Pheochromocytomas or Paragangliomas

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Context: Pheochromocytomas and paragangliomas may be malignant either at presentation or during recurrence, but the clinical course of malignant tumors is unpredictable.

Objective: The objective was to analyze survival according to clinical characteristics at diagnosis of malignancy and the presence or absence of *SDHB* mutations.

Design: This was a retrospective cohort study.

Setting and Participants: A total of 54 patients with malignant tumors were included. Malignancy was scored according to the presence of metastases or histologically documented lymph node invasion.

Main Outcome Measures: The main outcome was the specific survival after the diagnosis of the first metastasis.

Results: Germline mutations were identified in *SDHB* (n = 23, including 21 patients with apparent sporadic tumors) and *VHL* (n = 1) genes, and two patients had neurofibromatosis 1. Patients were followed up from the diagnosis of primary tumor and from the diagnosis of the first metastasis to the present or to death with medians of 79 [interquartile range (IQR) 24; 190] and 39 [IQR 14; 94] months, respectively. The 5-yr probability of survival after the diagnosis of the first metastasis was 0.55 (95% confidence interval 0.39–0.69). Patients with *SDHB* mutations were younger, more frequently had extra-adrenal tumors, and had a shorter metanephrine excretion doubling time. The presence of *SDHB* mutations was significantly and independently associated with mortality (relative risk 2.7; 95% confidence interval 1.2, 6.4; $P = 0.021$).

Conclusion: *SDHB* mutations, frequent in patients with malignant pheochromocytomas or paragangliomas, are associated with shorter survival. Therefore, *SDHB* genetic testing may be of prognostic value for such patients, even those with an apparent sporadic and/or benign presentation at diagnosis. **(*J Clin Endocrinol Metab* 92: 3822–3828, 2007)**

PHEOCHROMOCYTOMAS (PHs) and paragangliomas (PGLs) are rare neoplasms of chromaffin tissue that appear in the adrenal medulla (PH proper) or in extraadrenal chromaffin tissue (PGL) (1). Patients with PHs and PGLs may also harbor head or neck PGLs of sympathetic or parasympathetic origin (2). Evidence for malignancy may either be present at presentation or appear during recurrence (3–8), malignancy being defined as the presence of metastases, *i.e.* the presence of chromaffin tissue in nonchromaffin organs (9–11). The course of tumor spread is variable, with survival ranging from months to decades (3–8). It is generally accepted that the risk for malignant disease is higher in PGLs than in PHs (4–7, 12, 13), but there is currently no prognostic index of survival for patients with PHs or PGLs and documented metastases.

It was recently reported that patients with chromaffin tumors and mutations in the *SDHB* gene, encoding succinate dehydrogenase subunit B, have a greater risk than patients with no such mutations of extraadrenal and malignant tumors (14–18). Here, we report a comparative study of the presentation and clinical course of 54 patients with malignant PHs or PGLs with or without *SDHB* mutations. Our aim was to analyze survival according to clinical characteristics at diagnosis of malignancy and the presence or absence of *SDHB* mutations.

Patients and Methods

Patients

We reviewed the records of patients diagnosed with a metastatic PH or PGL, either at referral (malignant primary tumor) or during follow-up (subsequent malignant recurrence), in three tertiary referral centers: the

First Published Online July 24, 2007

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Abbreviations: CI, Confidence interval; CT, computed tomography; IQR, interquartile range; MIBG, metaiodobenzylguanidine; MR, magnetic resonance; NF1, neurofibromatosis type 1; PGL, paraganglioma; PH, pheochromocytoma; QMPSF, quantitative multiplex PCR of short fluorescent fragment.

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Hypertension Unit in Hôpital Européen Georges Pompidou and the Department of Endocrinology in Hôpital Cochin, Paris; and the Department of Nuclear Medicine and Endocrine Tumors, Institut Gustave Roussy, Villejuif. Patients with the following criteria were included in the analysis:

1. The presence of a PH or a thoracoabdominal PGL documented by pathology examination after surgery or biopsy, except for one patient who had a urinary metanephrine excretion of 2.5 μmol/d and a [^{123}I]metaiodobenzylguanidine (MIBG) uptake on the primary tumor associated with liver, lung, and bone metastases.
2. The presence of metastases either at presentation or during a recurrence. Distant metastases were documented by histology, or as lesions detected by computed tomography (CT) or magnetic resonance (MR) scans and exhibiting [^{123}I]MIBG or [^{111}In]octreotide uptake. The diagnosis of lymph node metastasis was only accepted if confirmed by histology or in patients with associated extraparaganglionic metastases.
3. The availability of leukocyte DNA and informed written consent for its use for PH/PGL genetic testing.
4. The availability of at least one abdomen or thoracic CT scan and one MIBG scan.

Diagnostic procedures and follow-up

The procedures used for PH/PGL diagnosis and treatment were in accordance with institutional guidelines and have been described previously (5, 7, 15, 17, 19, 20). The urinary excretion of total metanephrines (metanephrine plus normetanephrine) was determined by liquid chromatography, and tumors were classified as hypersecreting if metanephrine excretion was above the upper normal limit (19). In cases with hypersecreting tumors, metanephrine excretion was determined every 6 or 12 months and standardized to urinary creatinine levels to reduce intrapatient variation as a consequence of incomplete urine collection. As previously reported, the metanephrine to creatinine ratio increased exponentially with time. This made it possible to calculate a metanephrine doubling time from the diagnosis of malignancy to latest follow-up [doubling time in months is the ratio of the neperian logarithm of 2 to the slope of the individual regression line] (20). The size of primary tumors was determined from fresh specimens during histological examination when available or, if not, by CT or MR scanning.

In cases with benign primary tumors, malignant recurrence was defined as the appearance of a metastasis (documented at reintervention or by combined biochemical and imaging tests) after complete tumor eradication (documented by negative biochemical and imaging tests) (5). Time to malignant recurrence in months was counted between initial operation and documentation of the first metastasis. Follow-up imaging tests were usually performed every 6 or 12 months after the diagnosis of malignancy. The site and size of metastases were determined by CT or MR scanning and MIBG scintigraphy or [^{111}In] octreotide scintigraphy.

Genetic testing

The PH/PGL genetic testing was performed as previously recommended (17). The diagnosis of neurofibromatosis type 1 (NF1) was based on phenotypical criteria (21). All exons of the *SDHB, VHL*, and *SDHD* genes, and exons 10, 11, 13, 14, 15, and 16 of the *RET* gene were directly sequenced. Cases in which no mutations were detected by direct sequencing of the coding exons were screened for large rearrangements in the *SDHB, VHL*, or *SDHD* genes by quantitative multiplex PCR of short fluorescent fragments (QMPSFs) (22, 23).

Statistical analysis

Quantitative values are reported as means ± SD or median [interquartile range (IQR)], as appropriate. Probabilities and median times of survival are reported with 95% confidence interval (CI). Differences in baseline characteristics between patients with or without *SDHB* mutations were assessed using the unpaired Student's *t* test or Mann-Whitney *U* test for quantitative variables, and the χ^2 test or Fisher's exact test for qualitative variables. Survival was analyzed as the number of months from the diagnosis of the first metastasis to last follow-up (patient's death or last visit before November 2005). The following data were recorded for all patients: age, sex, mutational status, site and grade of the primary tumor, metanephrine levels, presence of distant metastasis at diagnosis of malignancy, and number of metastatic organs. Median survival durations were estimated using the Kaplan-Meier method, and survival curves were compared between groups using the log-rank test. Variables associated with survival with a P value < 0.20 and well-documented prognostic factors, *e.g.* the presence of distant metastases at diagnosis of malignancy and the number of metastatic organs, were entered into a multivariate analysis using the semi-parametric Cox's model. Variables that are collinear or for which data were incomplete were not included in the final multivariate model. Point-wise CIs for survival probabilities fitted by the Cox's model are reported using the log-log survivorship function. All analyses were performed using SAS Statistical Software (version 8.2; SAS Institute Inc., Cary, NC). A P value < 0.05 was considered significant.

Results

Of 72 patients with malignant PHs or PGLs, 18 were not included because the diagnosis of the primary tumor was not documented by histology (n = 5), because evidence of distant metastasis or of biopsy proven lymph node metastases was lacking (n = 12), and/or because no germline DNA was available (n = 3) (one patient met two exclusion criteria), leaving 54 patients for the present analysis.

Clinical characteristics

Metastases were detected when the primary tumor was diagnosed in 24 of the 54 patients, whereas 30 patients had apparent benign primary tumors and subsequent metastatic recurrence (Table 1). For the 30 patients with an apparent benign primary tumor, the median time to the first metastasis was 52 months [IQR 38; 109]; this group included four patients who developed a first metastasis more than 20 yr after the diagnosis of the primary tumor. Patients with metastases at diagnosis of the primary tumor were older (42.1 ± 13.3 *vs.* 34.5 ± 13.7 yr; $P = 0.044$) at first operation, and more of them were men (17 of 24 *vs.* 12 of 30; $P = 0.024$) than patients with subsequent metastatic recurrences. Other bioclinical and tumoral characteristics did not differ between the two groups. Most patients had hypersecreting tumors. None of the patients had any cancer other than PHs or PGLs.

TABLE 1. Baseline characteristics

No. of patients	54
No. of males	29 (54)
No. with apparent sporadic tumors	49 (91)
No. with NF1/or germline mutations in *SDHB*/*SDHD*/*VHL*/*RET* genes	2/23/0/1/0
Age at diagnosis of primary tumor (yr)	37.9 ± 13.9
Age at diagnosis of malignancy (yr)	42.0 ± 13.8
No. with hypertension at presentation	42 (78)
No. with hypersecreting primary tumors	48 (89)
Urinary metanephrine excretion at diagnosis of primary tumor (μmol/d)[a]	46 [22; 88]
No. with extraadrenal primary tumors	25 (46)
No. with malignant primary tumors	24 (44)
Diameter of the largest primary tumor (mm)	67 [60; 110]
Urinary metanephrine excretion at diagnosis of malignancy (μmol/d)[a]	16 [7; 47]
Metanephrine excretion doubling time (months)[a]	13 [8; 46]

Values represent the number of patients (%), means ± SD, or median [IQR].

[a] In patients with hypersecreting tumors.

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TABLE 2. Germline mutations affecting the *SDHB* gene

cDNA nucleotide	Amino acid change	Exon	Unrelated cases	First description (Ref.)
c.17_35del19	p.Ala6fs	1	1	This article
c.127G>C	p.Ala43Pro	2	1[1]	15
c.137G>A	p.Arg46Gln	2	3[2]	14
c.166_170delCCTCA	p.Pro56fs	2	1[1]	16
c.167C>T	p.Pro56Leu	2	1	23
c.268C>T	p.Arg90X	3	1	24
c.423+1G>C	splice defect	Intron 4	1[1]	25
c.587G>A	p.Cys196Tyr	6	1[1]	26
c.620_621delTG	p.L207fs	6	3[3]	15
c.688C>T	p.Arg230Cys	7	1[1]	15
c.689G>A	p.Arg230His	7	3[2]	17
c.725G>A	p.Arg242His	7	1	26
c.758G>A	p.Cys253Tyr	7	2[1]	17
c.1-?_72+?del	DEL EXON 1		2	27
c.73-?_843+?del	DEL EXON 3–8		1	This article

[a] *Superscript numbers* in column 4 indicate the number of patients previously reported in Ref. 17.

Genetic data

For five patients there was phenotypical evidence and/or a family history suggesting a genetic disease. Two patients had diagnostic criteria for NF1, one had a retinal hemangioblastoma, but no mutation or deletion of the *VHL* gene, and two patients had a family history of PGLs (both had *SDHB* mutations). Of the 49 patients with apparent sporadic tumors, 22 had a germline mutation affecting the *SDHB* (n = 21) or *VHL* (n = 1) genes. Overall, 26 patients, including 12 with a malignant primary tumor and 14 with metastatic recurrences, had germline mutations or large deletions in *SDHB* (n = 23) or *VHL* (467A>G, pY156H, n = 1) genes or phenotypical evidence of NF1 (n = 2). No mutations in the *RET* or *SDHD* genes were detected. Patients with a gene mutation, regardless of the nature of the mutation, were younger and more frequently had bilateral tumors than patients without mutation, but the two groups did not differ in metanephrine excretion, primary tumor size, or primary tumor status (benign or malignant).

The germline mutations identified in the *SDHB* gene are presented in Table 2. They include eight different missense mutations in 13 patients (14, 15, 17, 24, 25), one nonsense (26) and one splicing mutation (27) in one patient each, and three different frame-shift mutations in five patients (15, 16). One of these frame-shift mutations was a deletion of 19 bases in the first exon (Fig. 1A) that has never been reported previously. QMPSF detected large deletions in *SDHB* in three patients, two with a deletion, including the first exon (28), and one with a deletion from exons 3–8 (Fig. 1B). Clinical features according to the *SDHB* genetic status are given in Table 3. The *SDHB* mutation carriers were younger, had a shorter metanephrine excretion doubling time, and more frequently had extraadrenal tumors than the *SDHB* mutation-negative patients. The uptake of MIBG and radiolabeled octreotide did not differ between patients with or without *SDHB* mutations. A total of 54 patients underwent at least one [^{123}I]MIBG scintigraphy; MIBG uptake was seen in 19 of 23 patients with *SDHB* mutations and 28 of 31 with no *SDHB* mutation. There were 23 patients who underwent an Octreoscan; radiolabeled octreotide was taken up in two of 16 patients with *SDHB* mutations and two of seven with no *SDHB* mutation. The median time from the diagnosis of primary tumor to the documentation of a first metastasis was 4 months in patients with *SDHB* mutations and 20 months in patients without. There was no difference in presentation or outcome between the 28 patients with sporadic tumors and the three patients with familial tumors, two patients with NF1 and one patient with a *VHL* mutation, but no *SDHB* mutation. We found no relevant genotype-phenotype correlation among patients with *SDHB* mutations.

Metastatic spread

The first diagnosed distant metastasis affected a single organ in 32 patients: the skeleton (n = 17, including seven patients with subsequent lymph node metastases); liver (n = 8, including three patients with subsequent lymph node me-

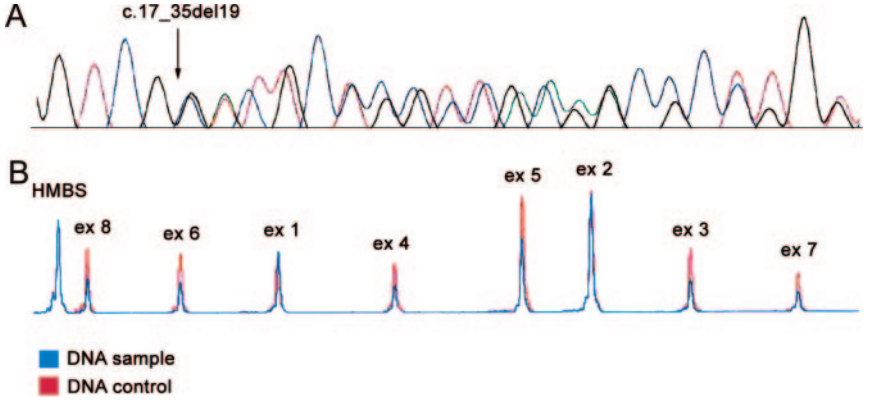

FIG. 1. Two novel *SDHB* germline mutations detected by direct sequencing (A), with an electropheretogram showing a deletion of 19 nucleotides in the first exon of the *SDHB* gene (c.17_35del 19; p.Ala6fs), and by QMPSF (B). Fluorescence profiles of the patient (*blue*) and those of a control subject (*red*) are superimposed. The relative heights of the peaks are determined after adjustment of the two peaks obtained with the control PCR (HMBS amplicon) from patient and control DNA, to the same peak height. A large deletion, including the exons 3–8, is revealed by the halving of the corresponding peaks.

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TABLE 3. Patient features according to *SDHB* mutation status

Characteristics	*SDHB* positive (n = 23)	*SDHB* negative (n = 31)	*P* value [a]
No. of males	14 (61)	15 (48)	0.363
No. with apparent sporadic tumors	21 (91)	28 (90)	0.902
Age at diagnosis of primary tumor (yr)	33.7 ± 13.0	41.0 ± 13.9	0.053
Age at diagnosis of malignancy (yr)	36.2 ± 11.3	46.4 ± 14.0	0.006
No. with hypersecreting tumors	19 (83)	29 (94)	0.206
Urinary metanephrine excretion at diagnosis of primary tumor (μmol/d)[a]	44 [18; 62]	56 [22; 89]	0.510
No. with extraadrenal tumors	16 (70)	9 (29)	0.003
No. with malignant primary tumors	10 (43)	14 (45)	0.902
Diameter of largest primary tumor (mm)	60 [50; 110]	73 [60; 105]	0.633
Time to diagnosis of malignancy (months)	4 [0; 41]	20 [0; 105]	0.140
No. with distant metastasis at diagnosis of malignancy	20 (87)	21 (68)	0.103
Urinary metanephrine excretion at diagnosis of malignancy (μmol/d)[a]	27 [8; 47]	14 [5; 47]	0.584
Metanephrine excretion doubling time (months)[a]	8 [5; 13]	36 [11; 134]	0.034
Follow-up after diagnosis of malignancy (months)[a]	28 [10; 49]	55 [25; 121]	0.044
Died during follow-up	14 (61)	12 (39)	0.107

Values represent number of patients (%), means ± SD, or median [IQR].
[a] In patients with hypersecreting tumors.

tastases); lungs (n = 4, including two patients with subsequent lymph node metastases); pancreas (n = 1, patient with subsequent lymph node metastases); or were intraarterial tumor extensions (n = 2). Nine patients had multiple distant metastases at diagnosis of malignancy, and in 13 patients the malignancy was first diagnosed by observation of invasion of lymph nodes. Including all patients and all lengths of follow-up, the most frequent metastases involved lymph nodes (n = 38), the skeleton (n = 37), liver (n = 25), and lungs (n = 21). Median time from the diagnosis of primary tumor to diagnosis of metastases was 33 months [IQR 0; 64] for lymph nodes, 36 months [IQR 10; 68] for skeleton, 34 months [IQR 1; 100] for liver, and 47 months [IQR 17; 98] for lung metastases. A few patients had metastases in the brain (n = 1), peritoneum (n = 5), pancreas (n = 2), breast (n = 1), stomach (n = 1), or pituitary (n = 1). Among the 38 patients who had lymph node invasion at some time during follow-up, only three had no other metastatic invasion.

Survival

All included patients were followed up to November 2005 or death. Median times from the diagnosis of the primary tumor and from the diagnosis of the first metastasis to last follow-up were 79 [IQR 24; 190] and 39 months [IQR 14; 94], respectively. For the 26 patients who died during follow-up, median time from the diagnosis of the primary tumor to death was 76 months [IQR 10; 124] and from the diagnosis of the first metastasis to death was 38 months [IQR 10; 63]. Causes of death were progression of metastatic disease (n = 14), complications of catecholamine hypersecretion (intestinal obstruction in six, malignant hypertension in one, pulmonary edema in one), iatrogenic complications (one perioperative death, one aplasia after chemotherapy), and unknown (n = 2).

The 5-yr probability of survival after the diagnosis of the first metastasis was 0.55 (95% CI 0.39–0.69) (Table 4). It was 0.36 (0.15–0.57) in *SDHB* mutation carriers and 0.67 (0.47–0.81) in the absence of *SDHB* mutation (relative risk 2.6; $P = 0.019$). Median survival after the diagnosis of the first metastasis was 42 months (95% CI 35–79) for the *SDHB* mutation carriers and 244 months (63–244) for patients with no *SDHB* mutation (Fig. 2). Survival tended to be lower for patients aged 40 yr or more at diagnosis of primary tumor, and patients with hypersecreting tumors, with malignant primary tumors or with distant metastases at diagnosis of malignancy, than for patients without these characteristics. It

TABLE 4. Survival probabilities and relative risk of death according to patient characteristics

		5-yr survival probability (range)	Relative risk (95% CI)	*P* value
All patients		0.55 (0.39–0.69)		
Male sex	Yes	0.54 (0.33–0.71)	1.1 (0.5–2.4)	0.850
	No	0.57 (0.34–0.74)		
Age ≥ 40 yr at diagnosis of primary tumor	Yes	0.45 (0.23–0.64)	1.8 (0.8–4.0)	0.146
	No	0.64 (0.43–0.79)		
SDHB mutation present	Yes	0.36 (0.15–0.57)	2.6 (1.1–5.9)	0.019
	No	0.67 (0.47–0.81)		
Hypersecreting tumor	Yes	0.51 (0.34–0.66)	4.7 (0.6–35.6)	0.100
	No	0.87 (0.36–0.98)		
Extraadrenal primary tumor	Yes	0.57 (0.33–0.75)	0.9 (0.4–2.1)	0.842
	No	0.54 (0.34–0.71)		
Malignant primary tumor	Yes	0.44 (0.22–0.65)	1.7 (0.8–3.8)	0.186
	No	0.62 (0.42–0.77)		
Distant metastasis at diagnosis of malignancy	Yes	0.49 (0.31–0.65)	2.2 (0.8–6.5)	0.137
	No	0.72 (0.42–0.89)		
More than one metastatic site	Yes	0.51 (0.26–0.71)	1.2 (0.5–2.8)	0.632
	No	0.58 (0.38–0.73)		

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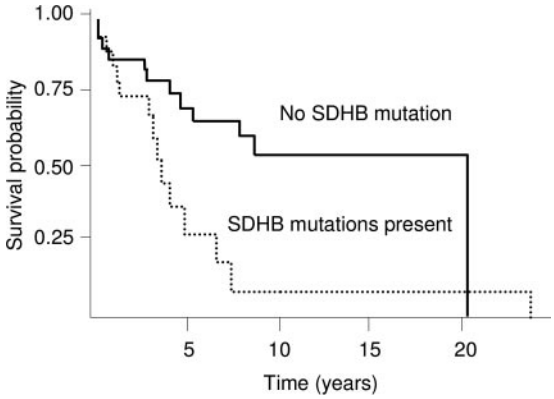

FIG. 2. Probability of survival according to mutation status.

was very similar for men and women, and for patients with adrenal (PH proper) and those with extraadrenal (PGL) primary tumors. We used multivariate analysis to test age at diagnosis, the presence of a *SDHB* germline mutation, malignancy at presentation, the number of metastatic sites, and the presence of distant metastases at diagnosis of malignancy. Only the presence of *SDHB* mutations was significantly and independently associated with survival. The relative risk of death was 2.7 (95% CI 1.2, 6.4; $P = 0.021$) for patients carrying *SDHB* mutations.

Discussion

PH and PGL are on average malignant in 5–15% of cases (9–11). Patients with PH/PGL may suffer malignant recurrences, so measures of overall frequency of malignancy increase with increasing follow-up. In five large series with mean follow-ups of 5–15 yr (577 patients, including 59 with malignant tumors at some time during follow-up), the prevalence of malignant tumors at presentation was 4.2%, and the overall incidence of malignancy, including subsequent metastasis in patients with apparent benign tumors at presentation, was 10.2% (3, 4, 6–8). Extraadrenal tumors, *i.e.* PGLs, are more frequently malignant than PH proper. In five large series reporting the rate of malignancy according to primary tumor site, 43 of 598 PHs (6.7%) and 26 of 83 PGLs (23.9%) were malignant (4, 6, 7, 12, 13). We have previously shown that patients with PHs or PGLs who carry *SDHB* mutations have a high relative risk of developing extraadrenal and/or malignant tumors, (14, 15, 17), a finding that has been confirmed by several teams in Europe, America, and Australia (16, 18, 23, 29). However, where malignancy is documented, there is no known predictor of survival, and all studies of malignant PH/PGL have indicated that the evolution of the disease is unpredictable, with survival ranging from a few months to several decades (3–8).

We describe a series of 54 patients with malignant PHs or PGLs, and this is a number of patients similar to that for all previous series combined. All were followed from the diagnosis of malignancy to November 2005 or death. Four patients had evidence of a first metastasis more than 20 yr after resection of the primary tumor, which underlines the need for a life-long follow-up of patients operated on for PHs or PGLs (3–8). All patients had lymph node or distant metastases documented at histology, and/or, in the case of distant metastases, by imaging studies. In decreasing order of frequency and increasing order of time from diagnosis of the primary tumor, metastases were documented in lymph nodes, the skeleton, liver, and lungs. A total of 34 patients underwent at least one reintervention at the site of the primary tumor and/or at the site of metastases, 21 patients underwent metabolic radiotherapy using one or several doses of [^{131}I]MIBG, 19 underwent at least one tumor embolization, and 12 received chemotherapy using various drug combinations. Because many patients received multiple therapies in various sequences, the therapeutic response to reinterventions, MIBG therapy, embolization, or chemotherapy could not be analyzed according to the presence or absence of *SDHB* mutations This series was appropriate for the analysis of survival of malignant PH/PGL patients according to clinical, tumoral, and genetic characteristics.

Of our 54 patients with malignant PH/PGL, 23 (43%) had an *SDHB* gene mutation, including three cases with large deletions of one or several coding exons (Table 2 and Fig. 1). The same frequency of mutations or deletions was found in patients with apparent sporadic tumors, *i.e.* without syndromic presentation or family history of PH/PGL (21 of 49 patients, 43%); this strongly supports the value of *SDHB* mutation testing in all patients with PHs or PGLs (18, 29, 30). Extraadrenal tumors at presentation were more than twice as frequent in *SDHB* mutation carriers than in patients without *SDHB* mutations (Table 3). Survival was significantly and independently related to the presence of *SDHB* mutations, but not to the site of the primary tumor (Table 4). This suggests that the more aggressive outcome of PGLs than PHs described in the literature reflects undiagnosed *SDHB* mutations. Many studies reporting this finding were conducted before the era of comprehensive genetic testing. This finding does not exclude the primary tumor site having prognostic value, with patients harboring PGLs being more prone to subsequent malignancy and having a shorter survival than those with PHs. However, our study included only patients with documented malignant tumors. A large cohort of patients with PH/PGL at risk for malignancy would be needed to assess the respective value of *SDHB* mutation status and primary tumor site to predict subsequent metastases. Besides, the small numbers of *SDHB*-negative patients with PHs (n = 22) and with PGLs (n = 9) in our study precluded a meaningful comparison of survival by tumor site independent of *SDHB* mutation status. *SDHB* mutation carriers with malignant PH/PGL were younger than patients with no *SDHB* mutations, but only the presence of *SDHB* mutations, not age at diagnosis, was significantly associated with survival (Tables 3 and 4). Median time from presentation to evidence of a first metastasis was 4 months for patients with *SDHB* mutations and 20 months in patients without. *SDHB* mutation carriers also displayed a faster progression of catecholamine hypersecretion with a shorter metanephrine excretion doubling time than *SDHB* mutation-negative patients (Table 3). In multivariate analysis, the presence of *SDHB* germline mutations was consistently and independently associated with survival. Therefore, our study demonstrates that the presence of *SDHB* germline mutations is associated with a poor outcome in patients with malignant PH/PGL. As

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far as we know, this is the first example involving endocrine tumors in which the presence of a mutation has a prognostic value independent of tumor stage or tumor burden.

Our study includes a relatively large number of patients with malignant PHs or PGLs followed up to the present or death, and uses a robust definition of malignancy and sensitive methods for PH/PGL genetic testing. However, it is retrospective, and, therefore, there are risks of referral biases and nonstandardized follow-up and therapeutic management. The constitution of a prospective cohort involving all patients with PH/PGL in a geographically defined area, regardless of tumor status at referral, is feasible. However, given the rarity of validating events, *i.e.* the presence of metastases at presentation or during follow-up, and the possibility of very late metastatic recurrences, it would be a very long-term project.

Our findings have important clinical implications. First, a sporadic presentation does not exclude the presence of *SDHB* mutations, probably because *SDHB*-related PH/PGL has a low penetrance (16, 23, 29), so all patients with PH/PGL should be offered genetic testing for *SDHB* mutations (31, 32). Second, all patients with PH/PGL, including those with apparent benign tumors at presentation, and particularly *SDHB* mutation carriers, should be subject to indefinite follow-up because they are exposed to the risk of malignant recurrence. Third, in cases in which malignancy is documented, either at presentation or during a recurrence, the identification of germline *SDHB* mutations is predictive of a rapid metastatic spread; therefore, such cases probably require aggressive management combining surgery, metabolic radiotherapy, and, possibly, chemotherapy.

Acknowledgments

We thank Dr. P. Saugier-Veber and Professors M. Tosi and T. Frébourg (Institut National de la Santé et de la Recherche Médicale U614, Institut Fédératif de Recherche Multidisciplinaires sur les Peptides, Faculty of Medicine, Rouen, France) for their advice about adapting the quantitative multiplex PCR of short fluorescent fragment technology to the search for large rearrangements of the pheochromocytoma/paraganglioma susceptibility genes. We also thank Professor G. Chatellier for his helpful suggestions and careful reading of the manuscript.

Received March 28, 2007. Accepted July 12, 2007.

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This work was supported by Grant AOM 02068 from the Assistance Publique-Hôpitaux de Paris, Délégation à la Recherche Clinique, for the Cortico and Medullo-surrenale: les Tumeurs Endocrines network.

Disclosure Statement: The authors have nothing to disclose.

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