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Genetic and Clinical Investigation of Pheochromocytoma

A 22-Year Experience, from Freiburg, Germany to International Effort

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ABSTRACT: Although deceptively simple, the etio-pathogenesis of pheochromocytoma represents a clinical and molecular genetic investigative challenge. Here, we summarize, from a historical point of view, the 22-year-long studies initiated at the University of Freiburg, which developed from a local experience to a national and finally an international effort. All research activities are translational and clinical and hence, registry based and intended to improve the outcome of the patients, whether by improved detection, prevention, or treatment. Major clinical steps are the prospective study on hormone tests and imaging techniques for adrenal and extra-adrenal abdominal tumors as well as the concept of organ sparing and endoscopic tumor resection. Further, we introduced 18-fluoro-dopa positron emission tomography. Population-based registries were used in order to identify germline mutations in the susceptibility genes *VHL*, *RET*, *SDHB,* and *SDHD* in non-syndromic pheochromocytoma. We differentiated distinct clinical features of paraganglioma syndromes associated with *SDHB* and *SDHD* gene mutations. Finally,

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we identified predictors and prevalence of paraganglioma syndromes associated with mutations of the *SDHC* gene.

KEYWORDS: pheochromocytoma; paraganglioma; VHL; MEN; RET; PGL; SDHB; SDHC; SDHD; registry-based research; guidelines for clinical management

INTRODUCTION AND KEY CASE REPORT

Pheochromocytoma and paraganglioma are tumors of similar embryogenesis and histology. However, this group of neoplasias displays important molecular differences and are heterogeneous in their natural history, prognosis, and response to treatment. Terminology in science and practice is divergent. Herein the term *pheochromocytoma* is used for location in the adrenal glands, extra-adrenal, abdominal, and thoracic locations. Nearly all pheochromocytomas are endocrinologically active, releasing catecholamines. The symptoms and signs of pheochromocytoma are mainly on account of hypercatecholaminemia with paroxysmal or persistent hypertension. On account of this, headaches, excessive sweating, and palpitations with or without tachycardia occur as only a few of the cardinal clinical features. In contrast, the term *paraganglioma* is used for non-catecholamine-releasing tumors in the head and neck area. The intercarotic, tympanic, intravagal, and jugular paraganglia are the typical locations. Paraganglioma of the head and neck commonly presents as an asymptomatic tumor mass or with symptoms on account of compression and damage of surrounding structures. Different cranial nerve deficits, bradycardia, hoarsness of voice, and hearing loss are typical symptoms patients report on. More than 25% of pheochromocytomas are hereditary. The classic inherited pheochromocytoma-associated syndromes are multiple endocrine neoplasia type 2 (MEN 2) on account of mutations of the *RET* gene, von Hippel–Lindau disease (VHL) on account of mutations of the *VHL* gene, neurofibromatosis type 1 (NF 1) caused by mutations of the *NF 1* gene and the recently defined paraganglioma syndromes type 1 (PGL 1) and type 4 (PGL 4) caused by mutations of the *SDHD* and *SDHB* genes, respectively.

The Freiburg Pheochromocytoma study was initiated in June 1983 following findings of a 7-cm right adrenal pheochromocytoma in a 46-year-old normotensive male patient seen by one of the authors (HPHN). The patient's sister had undergone surgery for a hemiangioblastoma 4 years earlier and had a son presenting with bilateral metachronous pheochromocytomas of the right adrenal at 13 years of age, and of the left adrenal at 16 years. Two and 5 years before, the disease presentations in the sister and her son were not recognized as hereditary. The findings in our patient, the second male family member with a pheochromocytoma, clearly suggested a hereditary pattern. Abdominal exploration in our patient during surgery revealed a second 1.5-cm left-adrenal pheochromocytoma, not seen in the computerized axial tomography (CT) scan. Both adrenals were resected. Subcutaneous implantation of normal adrenal

cortical tissue in the upper thigh provided ineffective steroid hormone production. However, not believing in lifelong need of steroid medication, the patient induced a nearly life-threatening Addisonian crisis. With appropriate steroid replacement therapy, our patient has remained in good health for the subsequent 22 years. The presence of multiple pheochromocytomas and a hemangioblastoma in this family allowed us to diagnose the autosomal dominantly inherited VHL disease. VHL illustrates many of the facets of pheochromocytoma and associated syndromes, including the circumstances of diagnosis, outcomes of surgical treatment, and requirements for adequate surveillance and follow-up. This case led us to set up our first local registry in Freiburg. New concepts and research activities over the course of the subsequent 22 years were initiated as summarized below.

PHEOCHROMOCYTOMA IN THE CONTEXT OF VHL AND MEN 2

In the 1980s, the two known heritable syndromes containing pheochromocytoma as component tumors were VHL and MEN 2. VHL is an autosomal dominant syndrome occurring in 2.72/100,000 (1/36,000) live births and is characterized by hemangiomas/blastomas, retinal angiomas, pheochromocytomas, and renal cell carcinoma.[1] MEN 2, occurring in 1:200,000 live births, is an autosomal dominant disorder characterized by medullary thyroid carcinoma (MTC), pheochromocytoma, and hyperparathyroidism.[1] Depending on clinical subtype, pheochromocytoma occurs on average in 50% of those affected by MEN 2.

Our first Freiburg study begun with a review of the existing literature in order to formulate the minimal clinical criteria for diagnosis and counseling of patients with VHL, which was important, since the identification of the VHL susceptibility gene was almost 10 years away at that time. In this literature review, we included 239 reported cases and 48 cases from Freiburg resulting in a prevalence of 14% of patients with VHL and associated pheochromocytoma.[2] We performed the first rigorous clinical epidemiological study of VHL in Freiburg and catchment areas and noted that the prevalence was 1:39,000 inhabitants in southwestern Germany (also known as the Black Forest region).[3] Remarkably, unlike the existing literature from elsewhere in the world, we found that the co-occurrence of pheochromocytoma and renal cell carcinoma in the same VHL patient was rare, and that indeed pheochromocytoma predominated in this part of Germany.[3] The latter observation formed the basis of the important concept, described later, of the Black Forest founder mutation in *VHL*.[4] A founder mutation is a particular gene mutation present in a population at increased frequency. The mutation occurred in a small isolated group of "founders," ancestors who gave rise to most of the individuals in the present day population. Further, our meticulous clinical phenotyping led to the later phenotypic classification of VHL type 1 (predominantly with renal clear cell carcinoma) and type 2 (predominantly with pheochromocytoma).

That other non-VHL pheochromocytoma-associated syndromes existed in southwest Germany was soon evident. We were able to clinically screen two families with MEN 2 and detected several asymptomatic tumors including pheochromocytoma.[5]

PROSPECTIVE STUDY FOR THE CLINICAL DIAGNOSIS AND EARLY DETECTION OF PHEOCHROMOCYTOMA

Because the two major pheochromocytoma susceptibility genes, *RET* for MEN 2 and *VHL* for VHL disease were not to be identified until 1993, we decided to collect and update annually clinical phenotypic data for several purposes, which ultimately would lead to improved early detection, diagnosis, and treatment of pheochromocytoma. Such a registry would not only be invaluable for a "gene hunt" but also would help phenotypic characterization after the genes would be identified.

By the time the Freiburg registry reached 19 index cases of familial pheochromocytoma-associated syndromes, MEN 2, and VHL, we were able to perform a unique prospective study for evaluation of diagnostic means for pheochromocytoma. We clinically screened these registrants and their families by comparing various imaging methods, namely, ultrasonography, CT scan, magnetic resonance imaging (MRI), and 131- or 123-iodine-metaiodobenzylgaunidine scintigraphy (MIBG) as well as the hormone analyses for 24-h collection of urine for adrenaline, noradrenaline, and vanillylmandelic acid (VMA) levels, as well as plasma adrenaline, noradrenaline, and chromogranin A levels. The results, published in 1993 in the New England Journal of Medicine,[6] showed a total of 42 new diagnoses of pheochromocytomas found 36 out of 79 participating index cases and relatives. We found that the sensitivity of ultrasonography was 40%, CT scan 76%, MRI 95%, and MIBG scintigraphy 95% and specificities of 95% and higher for all modalities. Sensitivities of hormone analyses were 53% for urine epinephrine, 86% urinary norepinephrine, 64% for urinary VMA, 52% plasma chromogranin A, 33% plasma epinephrine, and 58% plasma norepinephrine. Remarkably, these sensitivity figures remained until recently improved by the group of Graeme Eisenhofer and Karel Pacak, in 1999, by using assays for urinary and plasma metanephrines.[7]

ORGAN SPARING AND ENDOSCOPIC TREATMENT OF PHEOCHROMOCYTOMA

Because our index case (see "Introduction") underwent bilateral adrenalectomy and had a very common side effect of this operation, an Addisonian crisis, self-induced by stopping his replacement steroids, we needed to reconsider alternatives to the then standard-of-care operation. This is particularly

germane for all heritable pheochromocytomas that tend to be bilateral and multifocal. Thus, in 1985, we inaugurated adrenal-sparing surgery as the standard operation. Between 1985 and 1998, 39 patients underwent this procedure in Freiburg. Of these 39, 7 cases had lost the contralateral adrenal gland and 6 additional cases had operation of bilateral pheochromocytoma in one session. Only one of the patients became steroid dependent. Pheochromocytoma relapse was only seen in one case after 6 years.[8]

While adrenal-sparing surgery had good outcomes, it was still an open procedure and considered a major surgery. The door to the next step was opened by the Canadians M. Gagner and co-workers who reported in 1992[9] laparoscopic ("key hole") resection of a pheochromocytoma for the first time. The Austrian urologic surgeon Günter Janetschek was the first who agreed to our suggestion for laparoscopic adrenal-sparing surgery and operated on three patients who were diagnosed in Freiburg with VHL-associated pheochromocytoma, all of whom had bilateral adrenal pheochromocytoma as well as extra-adrenal tumors. The first operation was performed in Innsbruck in August 1997.[10] Reinvestigation with ACTH tests resulted in prompt response of the remnent adrenal tissue in all three cases.[8] In parallel in Essen, the German surgeon Martin Walz independently introduced the technique with retroperitoneal laparoscopic access and included the adrenal-sparing concept. He performed the first adrenal-sparing laparoscopic pheochromocytoma operation in January 1996.[11] The Essen series currently comprises 126 cases (including several diagnosed in Freiburg) with 160 pheochromocytomas and abdominal paragangliomas. Operation time, blood loss, and complication rates are comparable or even superior to open procedures, and cosmetic results are optimal.[12]

18-FLUORO-DOPA AND DOPAMINE PET

Noninvasive assessment of tumors is a mainstay in the clinical management of all pheochromocytomas but are particularly important in heritable cases, especially with the possibility of multiple tumors and the timing of laparoscopic adrenal-sparing surgery. The improvement for imaging of pheochromocytoma was achieved in parallel in Freiburg by Stefan Hoegerle and in Bethesda by Karel Pacak, by using 18-fluoro-dopa (Freiburg) or 18-fluoro-dopamine (Bethesda) positron emission tomography (PET), a method superior to MIBG, for abdominal tumors.[13,14] For head and neck paragangliomas, the same advantage of PET scanning over MIBG was demonstrated soon afterward.[15]

MOLECULAR GENETIC STUDIES OF PHEOCHROMOCYTOMA AND PARAGANGLIOMA PATIENTS

While improvements in clinical surveillance, clinical detection, and surgical treatment are important in improving the well-being of pheochromocytoma

patients, we believed that the earliest detection, or even the predisease detection, of pheochromocytoma would be the most effective. Thus, finding the susceptibility genes for heritable pheochromocytoma would be vital.

Because of our meticulously clinically documented VHL families, we were able to contribute these families to the group at the Massachusetts General Hospital, Boston, to help map the gene to the short arm of chromosome 3 (i.e., 3p).[16] The collaborative groups led by Berton Zbar from Bethesda and Eamonn Maher then at the University of Cambridge, UK, identified the *VHL* gene, a gene which, at that time, had no known function and did not show homology to other genes.[17] Subsequently in another collaboration, a founder effect was detected in the Black Forest VHL families who displayed predominantly pheochromocytomas, all associated with the identical germline mutation *VHL* c. 505 T>C.[4] This mutation was later shown to be associated with a good prognosis similar to that of the general population, the explanation why pheochromocytomas are relatively frequent in the area around Freiburg.[18]

The Freiburg group and others were fortunate to be able to provide clinically well-documented MEN 2 families to the group led by Bruce Ponder at the University of Cambridge, which ultimately identified germline mutations in the *RET* proto-oncogene in MEN 2 (acknowledgment in Mulligan *et al*.[19]). The Freiburg and other families also contributed to the first suggestion that specific *RET* mutations (genotype) were associated with certain clinical features (phenotype).[20] A seminal finding led to the formation of the International *RET* Mutation Consortium led by Charis Eng and Lois M. Mulligan. The Consortium study accrued over 300 MEN 2 families from around the world and were able to definitively observe specific *RET* genotype–clinical phenotype associations.[21] Specifically, *RET* codon 634 mutations were associated with the development of pheochromocytoma. These data also formed the scientific bases for the subsequent practice of clinical cancer genetics.[22,23]

Since 1994, a very fruitful collaboration and friendship was cultured between Hartmut Neumann and Charis Eng, starting when she was a postdoctoral fellow at the University of Cambridge and continuing during her subsequent independent scientific career in Boston, Columbus, Ohio, and recently, Cleveland. In a serendipitous moment when Hartmut Neumann invited Charis Eng to Freiburg to give a lecture as she was completing her postdoctoral fellowship near the end of 1995, both pledged lifelong collegial collaboration on neuroendocrine, including pheochromocytoma, genetics in order to improve the diagnosis, detection, and management of these patients. In these last 11 years, the major focus was to characterize the clinical relevance of mutations in the genes *RET, VHL, SDHB,* and *SDHD*. The autosomal genes encoding the mitochondrial complex II, succinate dehydrogenase, *SDHB, SDHC,* and *SDHD* were shown to be the susceptibility genes for another set of pheochromocytoma syndromes, which we have termed *pheochromocytoma–paraganglioma* (PGL) syndrome.[24–26] Another serendipitous moment occurred in 1995 when Andrzej Januszewicz from Warsaw met Hartmut Neumann in the home of the

prominent nephrologist Friedrich Luft in Berlin. It was then decided that the pheochromocytoma registries of Warsaw and Freiburg become a "joint venture." As of 2005, the registry comprises 570 cases with pheochromocytoma and/or paraganglioma. Of these cases, 60% are German, 35% are Polish, and the remainder from other countries, such as Italy and France. The formation of these joint, mainly population-based, registries have proven extremely helpful in defining specific clinical features associated with mutations in one of several genes associated with pheochromocytoma.

GERMLINE MUTATIONS IN NON-SYNDROMIC PHEOCHROMOCYTOMA

Using clinically well-documented sporadic (not inherited, no family history) pheochromocytoma cases originating from the Freiburg registry, we showed in a pilot study that there was an unexpectedly high frequency of germline mutations in *SDHD* in these sporadic cases.[27] These promising pilot data led us to systematic examination of the entire joint registries for the frequency of unexpected germline *SDH* mutations among non-syndromic pheochromocytomas, a question which is pertinent in clinical cancer genetics practice, including counselling.

In 2002, we reported on 271 unrelated subjects with clinical presentations of adrenal or extra-adrenal abdominal or thoracic pheochromocytoma without evidence of syndromic disease and without a family history.[28] All 8 exons of the *SDHB* gene, all 4 exons of the *SDHD* gene, all 3 exons of the *VHL* gene and exons 10, 11, and 13–16 of the *RET* gene were analyzed for germline mutations in DNA of blood of these patients. Clinical evaluation was performed at presentation and updated. Mutation positive subjects underwent a clinical screening program adjusted to the given disease. Among others, four major findings were obtained. First, we found that 24% of study subjects had an heritable pheochromocytoma disorder, 11% had mutations in *VHL*, 5% *RET,* and 4% each in *SDHB* and *SDHD*. This is a significantly high frequency in apparently sporadic presentations of isolated pheochromocytoma, high enough that one could suggest that all pheochromocytoma patients should be offered genetic testing in the setting of genetic counseling.[29] Second, although mutation frequency decreased steadily by age from 70% in the first decade to 18% in the fifth decade, it is important to note that there were first-time presentations even after the fourth decade, especially associated with *SDHD*. Third, heritable pheochromocytoma, that is, finding a germline mutation, occurred more commonly in patients with multifocal tumors and in those with extra-adrenal pheochromocytoma. However, it should be noted that solitary pheochromocytomas occurred quite commonly at presentation and so, solitary tumors do not necessarily exclude heritability. Finally, during follow-up, only about one-third of the patients developed other syndrome-specific tumors (TABLES 1 and 2).

TABLE 1. Age of the patients and type of tumor at presentation according to genetic status

Variable	MEN 2 ($N = 13$)	*VHL* disease	*SDHD* mutation-associated pheochromocytoma–paraganglioma syndrome ($N = 11$)	*SDHB* mutation-associated pheochromocytoma–paraganglioma syndrome ($N = 12$)	Hereditary disease ($N = 66$)	Non-syndromic disease ($N = 205$)	Total (N = 271)	*P* value*
Age at presentation (year)								
Mean	36.4	18.3	28.7	25.6	24.9	43.9	39.3	<0.001
Range	21–50	5–49	5–59	12–48	5–59	4–81	4–81	
Age at onset ≤ 18 years (no.)	0	20	3	4	27	21	48	<0.001
Type of tumor (no.)								
Multifocal	5	12	4	0	21	5	26	<0.001
Extra-adrenal	0	4	4	6	14	16	30	0.006

*The *P* values are for the comparison of hereditary disease with non-syndromic diseases.
From Neumann *et al*. N. Engl. J. Med. 2002[28]

TABLE 2. Age at presentation of patients with mutations of sporadic disease

		Mutated gene (number of mutations)					
Age (Years)	$N =$	*VHL*	*RET*	*SDHD*	*SDHB*	Number	Hereditary disease (%)*
0–10	10	6	0	1	0	3	70
11–20	47	17	0	2	5	23	51
21–30	31	2	4	3	3	19	39
31–40	44	3	4	3	2	32	27
41–50	56	2	5	1	2	46	18
51–60	51	0	0	1	0	50	2
61–80	32	0	0	0	0	32	0

*Values are the percentages of hereditary cases found by molecular genetic methods.
From Neumann *et al*. N. Engl. J. Med. 2002[28]

DISTINCT CLINICAL FEATURES OF PARAGANGLIOMA SYNDROMES ASSOCIATED WITH *SDHB* AND *SDHD* GENE MUTATIONS

Once we established that germline mutations in one of at least four genes occurred to a significant frequency, we decided that it would be impractical and quite expensive to offer gene testing of all these genes to all presentations of pheochromocytoma. In order to help prioritize gene testing order as well as to facilitate clinical management, we characterized in detail the clinical features (FIG. 1) of those with *SDHB* and *SDHD* mutations. As reported in 2004,[30] using the pheochromocytoma/PGL registry and the newly established head and neck PGL registry, we conducted a study that included 417 patients, 334 with pheochromocytomas and 83 with paragangliomas after exclusion of patients with VHL, MEN 2, and NF 1. In this series, 10% were found to have a germline mutation, 5% each in the *SDHB* gene and *SDHD* gene. Including relatives diagnosed by genetic screening, 100 mutation carriers, 53 of the *SDHB* gene and 47 of the *SDHD* gene, were included for clinical characterization. Age at diagnosis of a first tumor was similar for both genes, 29.8 (SD 15.2) versus 30.6 (14.3) years. Overall, penetrance was similar, 50% by 35 years and 31 years for *SDHB* and *SDHD* mutations, respectively. Head and neck paragangliomas and multifocal tumors were statistically more frequent in *SDHD* mutation carriers. *SDHD* mutation carriers had statistically more intra-adrenal tumors and an earlier onset of HNPs compared to *SDHB* mutation carriers. Malignant pheochromocytoma or paraganglioma occurred in 11 *SDHB* mutation carriers but not in *SDHD* mutation carriers, confirming results of the French study (TABLE 3).[31] Astonishingly, two *SDHB* mutation carriers had renal cell carcinoma, which were added later to a collaborative study with Finnish researchers led by Lauri Aaltonen[32] and one mutation carrier each of both genes had a papillary thyroid cancer.

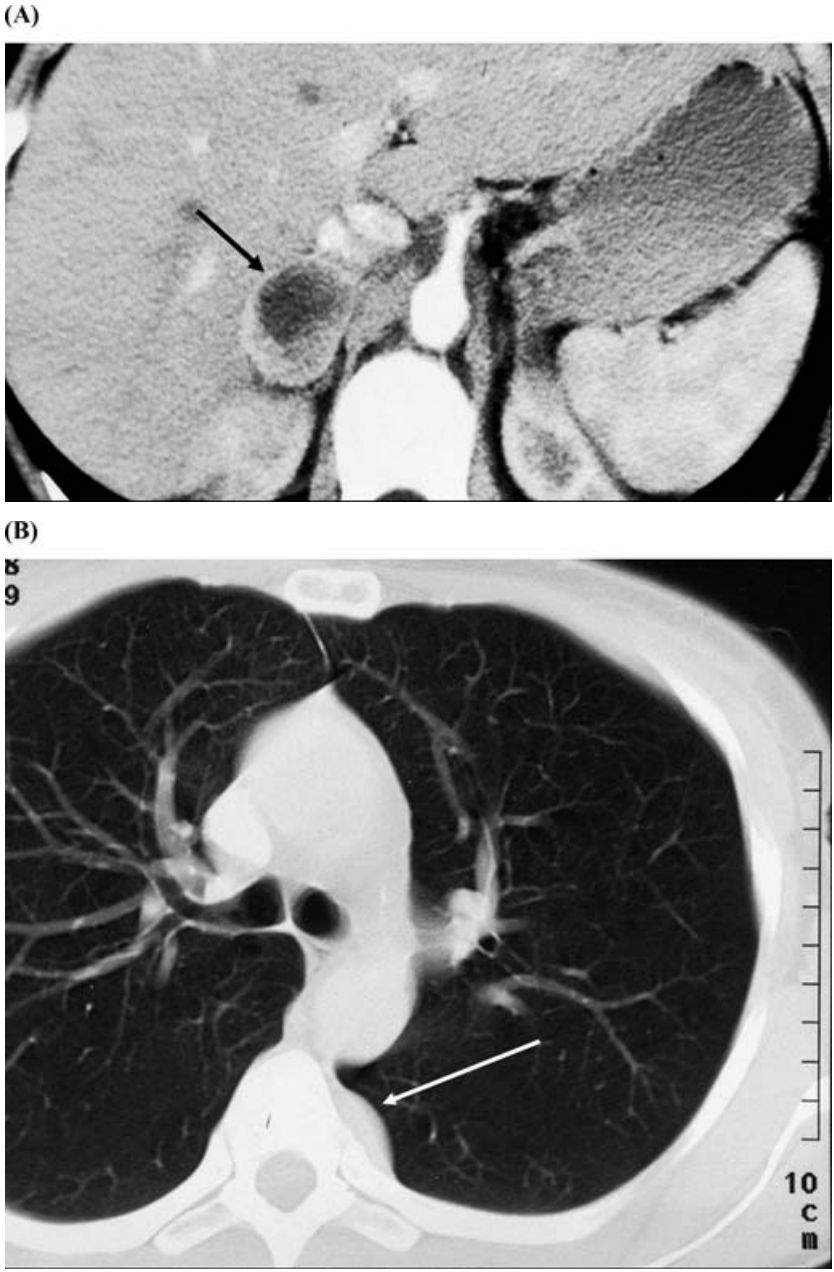
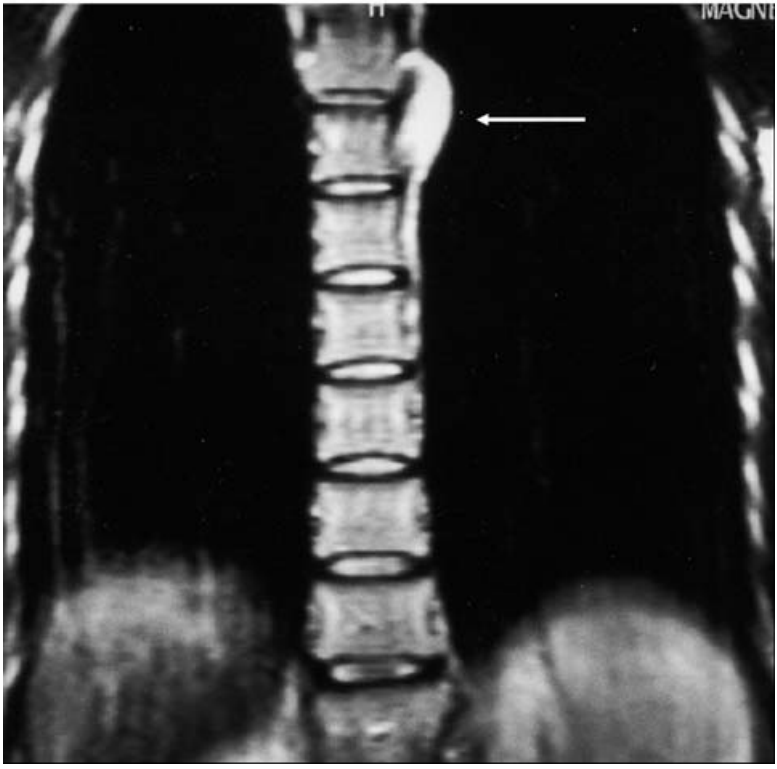


FIGURE 1. Paraganglioma syndrome in a 23-year-old women. This patient showed a mutation in the *SDHD* gene. **(A)** Right adrenal pheochromocytoma in CT (arrow), (**B**) and (**C**) thoracic paraganglioma (arrow) in CT (B) and MRI (C) and, (**D**) paraganglioma of the right glomus jugulare (arrow).

(C)



(D)

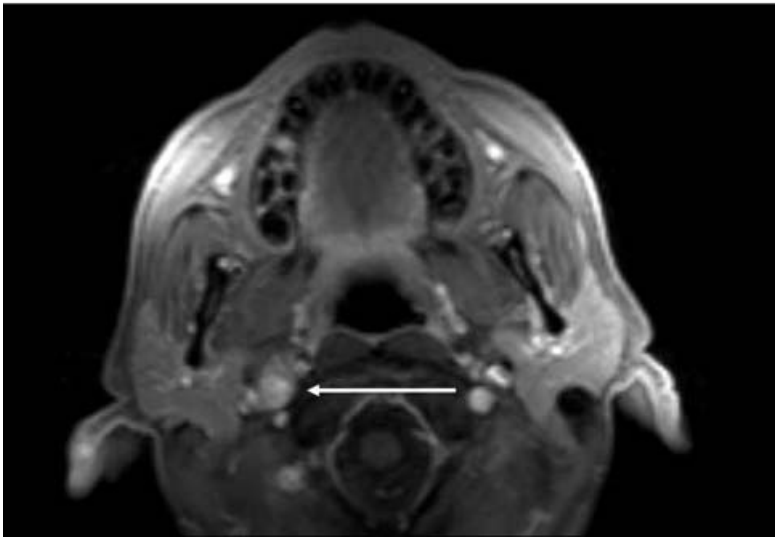


FIGURE 1. continued

TABLE 3. Malignant pheochromocytoma and paraganglioma associated with mutations of the *SDHB* gene (From Neumann *et al*. JAMA 2004[30])

Case	Sex	Location	Status 2004 or at death	Duration of disease (year)	SDHB mutation
1	f	Thoracic	Living 34 years	3	155 del C
2	m	Abdominal extra-adrenal[1]	Living 35 years	20	270 C/G
3	m	Neck	Living 45 years	11	271 G/A
4	m	Adrenal	Living 56 years	6	271 G/A
5	f	Abdominal extra-adrenal	Dead 45 years	32	300–4 del 5bp
6	f	Adrenal[2]	Dead 28 years	11	394 T/C
7	m	Abdominal extra-adrenal	Living 68 years	3	558–3 C/G
8	f	Adrenal[2]	Dead 36 years	2	847 del TCTC
9	m	Neck	Dead 64 years	32	859 G/A
10	m	Neck	Dead 64 years	2	859 G/A
11	m	Abdominal extra-adrenal	Living 66 years	6	899 + 1 GA

[1]Later associated with benign neck paraganglioma and thoracic pheochromocytoma.
[2]Multiple malignant tumors at this location.

PREDICTORS AND PREVALENCE OF A SYNDROME ASSOCIATED WITH MUTATIONS OF THE *SDHC* GENE

Mutations of the *SDHC* gene are rare in the literature at least prior to 2005 and restricted to manifestations with head and neck paragangliomas. We therefore sought to clinically characterize pheochromocytoma and PGL associated with *SDHC* mutations. In our publication in JAMA in 2005, we analyzed the *SDHC* gene in 371 unrelated pheochromocytoma patients who were not carriers of *VHL, RET, SDHB,* and *SDHD* mutations and had clinically no NF 1.[33] None of the pheochromocytoma patients had a germline *SDHC* mutation. This implies that pheochromocytoma presentations, in the absence of PGL, need not be offered *SDHC* testing. In the second part of this study, we analyzed 121 patients with head and neck paragangliomas (HNP) for mutations of the *SDHB, SDHC,* and *SDHD* genes. In this HNP series, mutations frequencies were 4% for *SDHC*, 7% for *SDHB*, and 17% for *SDHD*. Comparing 90 pheochromocytoma subjects without germline mutations to those carrying mutations of the genes *SDHB* ($n = 15$), *SDHC* ($n = 22$), or *SDHD* ($n = 42$) revealed more carotid body tumors in the *SDHC* group than in the sporadic HNP patients. Further, fewer instances of multiple tumors in *SDHC* mutation carriers than in *SDHD* mutation carriers, and no malignant tumors in *SDHC* mutation carriers compared to 6 out of 15 in patients with *SDHB* mutations were noted. There was a younger age at diagnosis in *SDHC* mutation carriers than in sporadic HNP patients (TABLE 4).

CONCLUSIONS

In summary, pheochromocytoma is a disorder in which over the last 22 years much progress has been achieved. Instrumental have been the inherited

TABLE 4. Comparison of major characteristics of head and neck paraganglioma (HNP) subgroup patients

Subgroup	*SDHC*	Sporadic	P (*SDHC* vs. spor.)	*SDHB*	P (*SDHC* vs. *SDHB*)	*SDHD*	P (*SDHC* vs. *SDHD*)	Comparison of all groups*
Patients with tumors (*n*)	22 (100%)	90 (100%)	-	15	-	42 (100%)	-	
Age at diagnosis (median, range)	46 (13–73)	53 (15–83)	0.03	39 (21–66)	0.56	36 (13–67)	0.04	<0.001
Carotid body tumors	13 (59%)	29 (32%)	0.03	10 (67%)	0.74	35 (83%)	0.07	<0.001
Jugular paragangliomas	6 (27%)	33 (37%)	0.46	2 (13%)	0.43	8 (19%)	0.53	0.10
Tympanic paragangliomas	2 (9%)	21 (23%)	0.24	0	0.50	4 (10%)	>0.99	0.04
Vagal paragangliomas	2 (9%)	3 (3%)	0.25	2 (13%)	>0.99	1 (2%)	0.27	0.16
Multiple tumors	2 (9%)	10 (11%)	0.30	4 (27%)	0.20	24 (57%)	<0.001	<0.001
Patients with malignant tumors	0 (0%)	0 (0%)	>0.99	6 (40%)	0.002	0 (0%)	>0.99	<0.001

[n]denotes patients, not tumors.
*For this test as null hypothesis was assumed that there is no difference between the four groups for each parameter.
From Schiavi *et al*. 2005[33]

syndromes, such as MEN 2, VHL, and PGL. Such patients have benefited because of advances on clinical diagnostic means, molecular genetic diagnostic means, and endoscopic surgery.

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