Genetic Testing in Head and Neck Paraganglioma: Who, What, and Why?

Shankar K. Sridhara[1] Murat Yener[2] Ehab Y. Hanna[2] Thereasa Rich[3] Camilo Jimenez[4] Michael E. Kupferman[2]

[1] Department of Otolaryngology Head & Neck Surgery, Dwight D Eisenhower Army Medical Center, Fort Gordon, Georgia, USA
[2] Department of Head and Neck Surgery, University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[3] Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[4] Department of Endocrinology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Address for correspondence Michael E. Kupferman, MD, Department of Head and Neck Surgery, University of Texas MD Anderson Cancer Center, 1400 Pressler Street, Unit 1445, Houston, Texas 77030, USA (e-mail: mekupfer@mdanderson.org).

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Abstract

Background Genetic testing in head and neck paragangliomas (HNPG) can have profound implications in patient and family counseling.

Methods Retrospective review was performed of patients with HNPG at a cancer care center from 1970 to present. Patient demographics, disease patterns, outcomes, and genetic mutations were analyzed.

Results We identified 26 patients with available genetic testing results. Sixteen had mutations. Succinate dehydrogenase gene, sub unit D (SDHD) accounted for 75% of mutations, of which P81L accounted for 75%. The remainder had SDHB mutations. Patients with mutations were younger (average age 39.5 years versus 48.4 years), 63% (versus 40%) had multiple tumors, 94% (60%) had at least one carotid body tumor, and family history was positive in 38% (20%).

Conclusion Patients suspected of heritable HNPG should undergo testing first at the SDHD and SDHB loci, and those with younger age, multiple tumors, carotid body tumors, and positive family history are more likely to have mutations.

Keywords
- paraganglioma
- carotid body tumor
- genetic testing
- mutation
- succinate dehydrogenase

Introduction

Paragangliomas (PG) are autonomic nervous system tumors arising from neuroectoderm-derived catecholamine-secreting cells known as paraganglia. Head and neck paragangliomas (HNPG) are generally nonfunctional and often present as a painless neck mass. The most substantial shift in the understanding of HNPG over the past decade has been the identification of genetic mutations associated with these tumors.

The most common hereditary syndromes involving PG and/or pheochromocytoma include multiple endocrine neoplasia type 2, von Hippel–Lindau disease, and neurofibromatosis type 1. More recently, germline mutations in the succinate dehydrogenase (SDH) gene family have been found to cause a high risk primarily for PGs. Succinate dehydrogenase is a mitochondrial enzyme complex with an important role in oxidative phosphorylation and intracellular oxygen sensing and signaling.[1] Within the succinate dehydrogenase complex, mutations in three genes, *SDHB*, *SDHC* and *SDHD*, account for the vast majority of PG syndromes.

Because genetic testing in HNPG has relatively recently become an area of fertile investigation, the full spectrum of knowledge in this subject is not yet comprehensively established, and the majority of published literature is from

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European centers. Our objective in this study was to evaluate the results of genetic testing in HNPG at a tertiary cancer care institution in the United States, with the goal of offering a cost-effective recommendation for performing genetic testing in this challenging disease.

Methods

After Institutional Review Board approval was obtained, the charts of patients evaluated for HNPG at MD Anderson Cancer Center (MDACC) from 1970 to present were identified. These patient records were then individually reviewed for patient demographics, tobacco and alcohol use, family history, presentation, tumor characteristics, imaging characteristics, treatment rendered, outcomes, and genetic testing results. Data was recorded in a de-identified manner using random three-digit numerical codes to maintain anonymity.

Data was analyzed to further characterize patients who did have germline mutations and to compare these patients with those who did not have mutations. Chi-square analysis, Fisher's exact test, and Welch *t*-test were performed as indicated to compare categorical variables between the two groups.

Results

The demographics of all patients ($N = 177$) with HNPG who were evaluated at MDACC from 1970 to present are presented in ►**Table 1**. Results of genetic testing were available for 26 patients. Of these, 16 were identified to have germline mutations. The *SDHD* gene locus accounted for 75% of the mutations, of which 75% had the common mutation, P81L. *SDHB* mutations accounted for 25% of the mutations identified. ►**Table 2** details the genetic mutation and pertinent clinical characteristics of each patient identified to have a germline mutation.

Table 1 Demographics of All Patients Evaluated at MDACC with HNPG from 1970 to Present

Demographic	Data
Average age (range) in years	43.5 (6 to 81)
Male (%)	68 (38.4%)
Family history	23 (13.0%)
Location, % (n)	
– Carotid body – Jugulare – Vagal – Tympanic	47% (83) 16% (28) 11% (19) 8% (14)
Presenting symptom	
– Hearing loss – Neck mass	19% (34) 18% (32)
Secreting tumor	7 (4.0%)
Surgery at MDACC	53 (30.0%)
Complications	2 (1.1%)

Abbreviations: HNPG, head and neck paragangliomas; MDACC, MD Anderson Cancer Center.

We next compared the clinical characteristics of patients harboring mutations to those who tested negative for mutations. Patients who had germline mutations were diagnosed at an average age of 39.5 years. This is in contrast to patients without mutations, with a trend toward an older age at diagnosis (average age of 48.4 years, $p = 0.06$). Multiple tumors were present in 88% of patients with mutations, as compared with 40% of those without mutations ($p = 0.01$). Patients with mutations had bilateral tumors significantly more often than patients without mutations, ($p = 0.004$). Concurrent or subsequently identified tumor sites outside the head and neck included pheochromocytoma, mediastinum, abdominal and pelvic PG, and metastatic disease to the spine. Those with mutations had a statistically higher rate of presence of at least one carotid body tumor (100% versus 60%, $p < 0.01$). Although a family history of PG was also suggestive of presence of germline mutation, a statistically significant relationship could not be achieved. ►**Table 3** provides a comparison of various clinical characteristics between patients with and without mutation.

Discussion

HNPG is a disease with known potential for significant morbidity and mortality, and knowledge of its genetics has become a fertile area of investigation. Martin et al describe two main mechanisms to explain the pathogenesis of *SDH* mutations causing PG. The first is that *SDH* mutations cause dysregulation of hypoxia-induced factors (HIFs), thereby yielding a cellular response mimicking that of hypoxia, which is known to cause carotid paraganglial hypertrophy. The second is that *SDH* mutations cause inactivation of some of the factors, such as the prolyl hydroxylase EglN3, that mediate apoptosis in paraganglionic cells.[2] Improved diagnosis and understanding of heritable cases is crucial for proper counseling and follow-up of patients, and for early detection and screening of disease in family members. Our study results suggest that patients diagnosed before the age of 40 years and those with multiple or bilateral tumors, particularly at the carotid body, regardless of presence of family history for PG, should strongly be recommended for genetic testing.

In a review of data from a multinational European registry of PG and pheochromocytoma, Schiavi et al found that there were significantly more carotid body tumors and the age at diagnosis was significantly younger in patients with *SDHC* mutations as compared with sporadic cases.[3] Netterville et al suggest a high rate of familial cases amongst vagal paragangliomas in a series in the United States.[4] Burnichon et al report that in their prospective series of 445 patients with PG, those with germline mutations were significantly younger and more frequently had multiple or malignant PG as compared with those without mutations.[5] Indeed, other studies have yielded similar findings as well.[2,6,7] Neumann et al report on clinical predictors of *SDHx* mutation based on their multinational European registry. They found that predictors for an *SDHx* mutation are family history (odds ratio [OR], 37.9), previous pheochromocytoma (OR, 10.9), multiple

Table 2 Clinical and Genetic Characteristics of Patients with Germline Mutations

Patient #	Mutation	Location	Prior treatment	Mgmt at MDACC
1	SDHD P81L	Left vagale, right carotid body	None	Radiation
2	SDHD 94_95 DELPC	Left carotid body, right vagale	None	None
3	SDHB V140F	Carotid body (family h/o metastatic disease)	Surgery	None
4	SDHD P81L	Carotid body (bilat), h/o left maxillary PG	Radiation and surgery	None
5	SDHD P81L	Carotid body (h/o treated tympanicum)	Surgery	None
6	SDHD P81L	Carotid body (bilat)	None	Surgery with embolization
7	SDHD P81L	Carotid body (bilat), spine	Surgery	Chemotherapy
8	SDHB L111V	Carotid body (bilat)	None	Surgery with embolization
9	SDHD, mutation undocumented	H/o resected bilat carotid body and mediastinal PG, presented with atrial PG	Surgery	Surgery
10	SDHD P81L	Carotid body (bilat), left vagale, right jugulare	None	None
11	SDHB C.166_170delCCTCA	Carotid body (mets to nodes)	Surgery	Surgery with embolization
12	SDHD Trp43X	Carotid body (bilat), bilat pheo, retroperitoneum	Surgery and radiation	Surgery with embolization, adjuvant IMRT to 58Gy
13	SDHD P81L	Carotid body (bilat)	None	None
14	SDHD P81L	Jugulare, left carotid body	Surgery	None
15	SDHB c.286G > A (G96S)	Tympanicum, carotid body	None	Surgery
16	SDHD W66X	Carotid body (bilat), bilat pheo, sympathetic chain	None	Surgery

Abbreviations: h/o, history of; IMRT, intensity-modulated radiation therapy; MDACC, MD Anderson Cancer Center; PG, paraganglioma.

Table 3 Comparison of Clinical Parameters Between Patients with Germline Mutations and Patients without Germline Mutations

	Mutation present	Mutation absent	*p* value
Male % (n)	43.8 (7)	50 (5)	0.76
Average age (yrs, SD)	**39.6 (10.5)**	**48.4 (11.1)**	**0.06**
Carotid body % (n)	**100 (16)**	**60 (6)**	**0.014**
Surgical treatment % (n)	75 (12)	70 (7)	0.78
Family history % (n)	37.5 (6)	20 (2)	0.42
Average size (cm, SD)	3.6 (2.0)	4.3 (1.7)	0.46
Bilateral % (n)	**81.25 (13)**	**20 (2)**	**0.004**
Secretory/pheo % (n)	12.5 (2)	20 (2)	0.63
Distant mets % (n)	31.25 (5)	20 (2)	0.67

Abbreviation: SD, standard deviation.

HNPG (OR, 10.6), age less than 40 years (OR, 4.0), and male gender (OR, 3.5).[8] It is unusual that our dataset demonstrates a high rate (40%) of multiple tumors among patients who were not found to have mutation; it is possible that some of these patients did indeed have mutations for which genetic testing was unavailable at the time of evaluation. Whether some of these patients should undergo retesting at a later time for newly described mutations remains unclear.

From a cost perspective, the findings have substantial implications for the health system. Given that the cost for mutational screening at all 3 main *SDH* loci (*SDHD, SDHB,* and *SDHC*) is approximately $2,700 per patient, Neumann at al suggest screening only selected cases in a stepwise fashion based on these predictors and, in so doing, report 60% cost reduction, with 91.8% sensitivity and 94.5% negative predictive value.[8] ►**Table 4** summarizes findings of several studies with regards to clinical factors associated with presence of germline mutation.

Our findings suggest that for patients with HNPG, genetic testing should first be performed at the *SDHD* locus, and then the *SDHB* locus prior to testing at other loci. Individual genetic testing should, of course, be tailored to personal and family

Table 4 Clinical Factors Associated with Presence of Germline Mutation

Study	Factors associated with mutation
Schiavi et al 2005[3]	Young age, carotid body tumors
Martin et al 2007[2]	Age < 50 years, family history, multiple tumors
Fakhry et al 2008[7]	Young age, family history, multiple tumors
Burnichon et al 2009[5]	Young age, multiple tumors, malignant tumor
Neumann et al 2009[8]	Age < 40 years, family history, multiple tumors, previous pheochromocytoma, male gender
Hermsen et al 2010[6]	Age < 50 years, family history, multiple tumors, male gender, carotid body tumors
Present study	Young age, multiple tumors, bilaterality, carotid body tumor

history. These findings corroborate the majority of published literature, which demonstrates *SDHD* to be the most common mutation, followed by *SDHB* and then *SDHC*. Within a European registry of patients with PG, the prevalence of underlying *SDHx* mutations among 121 patients with HNPG was 28% (7% *SDHB*, 4% *SDHC*, and 17% *SDHD*).[3] In a series reported by Boedeker et al, 5 *SDHC*, 13 *SDHB*, and 45 *SDHD* gene mutations were found amongst 195 patients with HNPG.[9] In a large prospective series by Burnichon et al, 130 *SDHD*, 96 *SDHB*, and 16 *SDHC* mutations were found amongst 445 patients with PG.[5] It should be noted, however, that some studies have found a higher proportion of *SDHB* mutations. Hermsen et al found equal numbers of *SDHB* and *SDHD* mutations in their series,[6] and Ricketts et al report a significantly higher number of *SDHB* mutations than *SDHD* mutations in their series from Birmingham, United Kingdom.[10] By contrast, our data and the published literature suggest that testing at the *VHL* and *RET* foci in cases of HNPG is not warranted on a routine basis and should only be considered if family history suggests such a syndrome. In another series reported by Boedeker et al, only 12 patients were found to have hereditary non-*SDHx* HNPG of a total of 809 patients with HNPG and 2,084 *VHL* registrants, 11 in the setting of germline *VHL* mutations, and 1 of a *RET* mutation.[11] ►**Table 5** demonstrates the distribution of germline mutations within the *SDH* family as seen in various studies.

Multiple tumors were not always clinically apparent at initial presentation in our patients. Rather, some of these diagnoses of multiple and distant tumors were made during subsequent follow-up evaluation. This is perhaps the greatest implication of genetic testing for a patient with HNPG; knowledge of clinical characteristics of certain PG syndromes can help guide follow-up and surveillance of patients. For example, since it is known that *SDHD* mutations are associated with multifocal tumors and *SDHB* mutations are associated with malignant tumors,[12,13] patients who initially present with seemingly sporadic disease can be followed with appropriate surveillance, such as annual physical examinations with imaging as indicated by clinical impression. Havekes et al report on their series of 93 patients with *SDHD*-associated HNPG in whom they performed routine urine screening for pheochromocytoma at initial presentation and at 2-year intervals if initial testing was negative. By using this screening method, they were able to diagnose 19 pheochromocytomas and extra-adrenal PG. However, they note that 37% of these diagnoses were not made on initial screening. The authors conclude that it is paramount to continue routine screening evaluation for patients once they are diagnosed with *SDHD* mutation.[14] Diagnosis of heritable PG syndromes certainly has important implications for family members as well, with regards to screening and early diagnosis and treatment. In this regard, it should be known that *SDH* mutations are generally inherited in an autosomal dominant pattern, but there are parent-of-origin effects with *SDHD* mutations.[15]

Table 5 Distribution of Germline Mutations within the *SDH* Family in HNPG Patients

Study	% SDHD	% SDHB	% SDHC
Schiavi et al 2005[3]	61	25	14
Boedeker et al 2007[9]	71	21	8
Burnichon et al 2009[5]	54	40	6
Hermsen et al 2010[6]	50	**50**	0
Ricketts et al 2010[10]	20	**80**	0
Present study	75	25	0

Abbreviation: HNPG, head and neck paragangliomas.

The primary limitation of our study is its small sample size, which restricted the performance of certain meaningful statistical analysis methods, such as multivariate analysis, and of statistical comparison between the *SDHD* and *SDHB* groups. Given the rarity of this tumor and the fact that gene testing has only been available since 2005, it is difficult to generate a large number of subjects, even at a well-established tertiary cancer facility. As such, continued contribution to the growing body of literature in this subject justifies conducting and reporting studies as best as possible, despite less than ideal sample size. It is also important to pursue genetic testing when clinically appropriate so that larger series can be generated for future study. Since our institution is a tertiary cancer care institution that tends to draws complex patients from domestic and international origins, a second limitation of our study is patient selection bias. Studies of this rare disease entity are frequently performed at such institutions, however, because smaller institutions may not be able to generate adequate sample size to perform meaningful investigation.

Conclusion

Knowledge of genetic mutations is helpful in counseling the kindred of those with this potentially morbid disease. Our data supports the routine elective genetic screening of patients who are younger than age 40, have multiple or bilateral tumors (especially if at least one site is carotid body), or who have positive family history for PG or related condition. Our results suggest that patients suspected of heritable HNPG should undergo genetic testing first at the *SDHD* and *SDHB* loci, unlike familial PGs at other sites, which are also associated with *VHL* and *RET* mutations. Our findings have implications for long-term monitoring for secondary tumors, counseling family members, and cost-efficient mutational screening.

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