

# Clinical Predictors for Germline Mutations in Head and Neck Paraganglioma Patients: Cost Reduction Strategy in Genetic Diagnostic Process as Fall-Out

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## Abstract

**Multiple genes and their variants that lend susceptibility to many diseases will play a major role in clinical routine. Genetics-based cost reduction strategies in diagnostic pro-**

**cesses are important in the setting of multiple susceptibility genes for a single disease. Head and neck paraganglioma (HNP) is caused by germline mutations of at least three succinate dehydrogenase subunit genes (*SDHx*). Mutation analysis for all 3 costs ~US\$2,700 per patient. Genetic classification is essential for downstream management of the patient and preemptive management of family members. Utilizing HNP as a model, we wanted to determine predictors to prioritize the most heritable clinical presentations and which gene to begin testing in HNP presentations, to reduce costs of genetic screening. Patients were tested for *SDHB*, *SDHC*, and *SDHD* intragenic mutations and large deletions. Clinical parameters were analyzed as potential predictors for**

**Note:** Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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**finding germline mutations. Cost reduction was calculated between prioritized gene testing compared with that for all genes. Of 598 patients, 30.6% had *SDHx* germline mutations: 34.4% in *SDHB*, 14.2% *SDHC*, and 51.4% *SDHD*. Predictors for an *SDHx* mutation are family history [odds ratio (OR), 37.9], previous pheochromocytoma (OR, 10.9), multiple HNP (OR, 10.6), age  $\leq 40$  years (OR, 4.0), and male gender (OR, 3.5). By screening only preselected cases and a stepwise approach, 60% cost reduction can be achieved, with 91.8% sensitivity and 94.5% negative predictive value. Our data give evidence that clinical parameters can predict for mutation and help prioritize gene testing to reduce costs in HNP. Such strategy is cost-saving in the practice of genetics-based personalized health care.** [Cancer Res 2009;69(8):3650–6]

## Introduction

Multiple genes and their variants that lend susceptibility to many diseases will play a major role in the future. Successful genetics-based medicine must not only serve the patients and families but also be fiscally responsible to society. Cost-reduction in the diagnostic process is an important issue in the setting of multiple susceptibility genes for a disease, even a single tumor type. One model where we can investigate these hypotheses is head and neck paragangliomas (HNP) and their genetic susceptibility.

HNP arise mainly from the carotid, tympanic, jugular, or vagal glomus and represent a challenge for the surgeons. Timely diagnosis is regarded as instrumental for optimal surgical planning, resulting in successful resection while minimizing side effects (1–3).

Three susceptibility genes have been identified as follows: *SDHB*, *SDHC*, and *SDHD*, predisposing to paraganglioma syndrome (PGL) type 4, 3, and 1, respectively. They encode the three subunits of succinate dehydrogenase (4–6). The gene for PGL2 has not yet been identified. Germline mutations of *SDHx* are present in  $\sim 30\%$  of HNP patients (7–14). Rare reports of HNPs occur in multiple endocrine neoplasia type 2 and von Hippel-Lindau disease (VHL; refs. 15–18). These data enable us to identify patients with genetic susceptibility to HNP with management implications for themselves and their families. Genetic susceptibility confers a lifelong risk of multiple paragangliomas and/or pheochromocytomas, and for extraparaganglial neoplasms such as renal cancer, gastrointestinal stromal tumors, medullary thyroid carcinoma, or heman-gioblastomas of the eyes, brain, or spinal cord in carriers of *SDHx*, *RET*, or *VHL* mutations (19–22). Equally important, once a mutation has been identified, relatives can easily be tested for the given mutation and be confirmed or definitively excluded for having a genetic risk. Taken together, the complete palette of potential locations of germline mutations lies in 5 genes totaling 25 “at-risk” exons and also includes large rearrangements/deletions in 4 of these genes (23–25). In the ideal situation, all five genes should be analyzed in the setting of HNP. Currently, in the United States, these cost  $\sim \$4,100$  ( $\$2,700$  for the 3 *SDHx* genes). Because gene testing should only be offered in the setting of genetic counseling, an equally significant issue is the imbalance in supply-demand of genetic counselors and clinicians facile with genetics. Therefore, it would be most helpful to have a logical and cost-saving algorithm for the gene testing approach in HNP. This approach can be generalizable to any disorder with multiple susceptibility genes.

We used the European-American Paraganglioma Registry platform and performed a large consortium study to create a risk assessment model comprising clinical and demographic features to predict the greatest likelihood of having any germline mutation and that would help use the relative risks among the five known susceptibility genes involved. This type of predictive model will help assess heritability (“first-step”) and streamline genetic testing by helping the clinician prioritize which gene to begin testing (“second-step”). Once the gene is identified, it would help the clinician assess risk for subsequent tumors and guide medical management. This strategy would be clinically useful with reduction of costs in the molecular diagnostic process.

## Materials and Methods

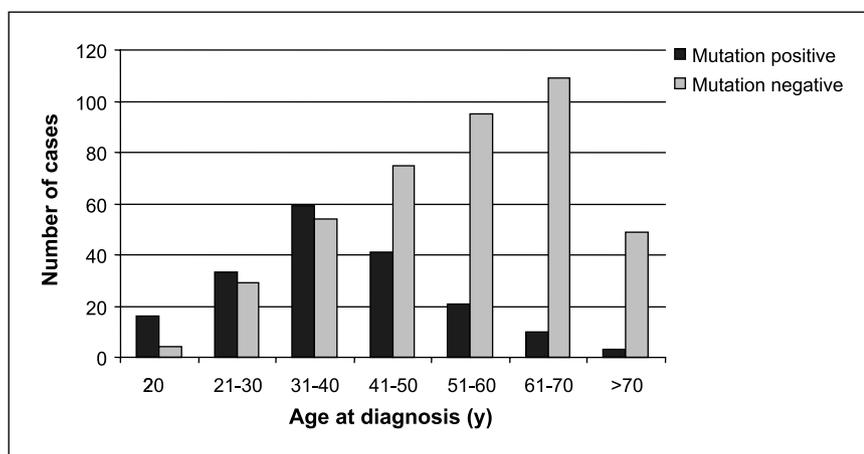
**Patient recruitment.** The European-American Paraganglioma Registry was initiated in 2001 (9, 10, 26), as an integrative collaboration among Freiburg, Warsaw, and Columbus, and for the current study, extended to other centers of excellence for head and neck and skull base surgery in Germany, Poland, Italy, Spain, France, the United Kingdom, Switzerland, Norway, Finland, the Netherlands, the United States, Australia, and New Zealand. In this study, only patients with a presenting diagnosis of HNP (before molecular diagnosis) were included. Individuals with known germline mutation at presentation and families where germline mutation was present in one member were excluded. From each family, we included only the first registered member for this study. Demographic and clinical data including number and biology (malignant/benign) of tumors, previous operations for adrenal and paraganglial tumors (pheochromocytoma), and family history for HNP or pheochromocytoma were recorded.

**Molecular genetic analyses.** Genomic DNA was extracted from 10 mL of EDTA- or ACD-anticoagulated blood using standard methods. The *SDHB*, *SDHC*, *SDHD*, *VHL*, and *RET* genes (for the latter, only exons 10, 11, 13, and 16) were analyzed for intragenic mutations of all exons and splice sites as described (10, 26). Analyses for large deletions were performed by multiplex PCR as described (25). In parallel, we also used commercially available kits for multiplex ligation-dependent probe amplification for *VHL*, *SDHB*, *SDHC*, and *SDHD* (MRC-Holland). As controls, we used blood DNA of  $>1,300$  control cases from the same geographic area as our studied subjects.

**Cost estimates.** United States-based costs of clinical mutation testing of each gene were obtained by averaging costs posted by GeneDx<sup>51</sup> and supplied by the Clinical Diagnostic Laboratories of the University of Pittsburgh and Children’s Hospital of Philadelphia. Costs are similar in Europe and Australia. The components of molecular genetic diagnostics include DNA extraction, PCR and sequencing of each exon, analyses for large deletions/rearrangements, and a report. In the United States, average costs for analyzing intragenic mutations is \$864 for *SDHB*, \$697 for *SDHC*, and \$530 for *SDHD*. Testing for large deletion/rearrangements adds  $\sim \$200$  per gene, for a total cost of \$2,691 ( $\sim \$2,700$ ). In the event *RET* and *VHL* testing are done, then another \$1,400 would be spent.

**Statistics.** To identify a risk assessment model for genetic mutations, we used readily available demographic and clinical features of the patients. We selected age  $\leq 40$  y for this assessment because prevalence of heredity was the greatest compared with apparently sporadic (mutation negative) HNP cases within this decade-interval (Fig. 1). To determine which parameters predicted the presence of a germline mutation in any gene, we compared the group of patients carrying mutations in any of the tested genes to the group who were mutation negative to find “first-step predictors.” We identified six predictors for the presence of a germline mutation: age, gender, number of tumors (solitary or multiple), tumor biology (malignant or benign HNP), previous paraganglial tumors, and family history for HNP or pheochromocytoma. We included these six variables in a logistic regression model and assessed the predictive ability of this model using the

<sup>51</sup> <http://www.genedx.com>



**Figure 1.** Age at diagnosis in apparently sporadic (*mutation negative*) versus hereditary (*mutation positive*) HNP patients shown in decades. Age at diagnosis was inversely proportional to the risk of carrying a germline mutation.

c-statistic. Although malignant HNP was not significant, it was kept in the model because its exclusion would result in a poorer model fit and misclassification of one additional patient (data not shown).

We used the model to develop an algorithm to identify which patients should be genetically tested for mutations. We calculated the model-based probability of any genetic mutation for each patient and used it to determine which patients were unlikely to have genetic mutations and thus should not be tested. When we examined the model-based probability of genetic mutation, we found that the group of patients with the lowest probability of having any mutation was the group who had none of the six risk factors. Therefore, for the first-step prediction, our algorithm is to perform genetic testing only on patients who have at least one of the six risk factors. We calculated the sensitivity, specificity, positive predictive value, and negative predictive value of this algorithm.

When we examined our data using simple frequency tabulations among the subset of patients with at least one of the six risk factors, we found that patients with no family history and a solitary HNP were most likely to have *SDHB*, followed by *SDHD*, followed by *SDHC* mutations. Therefore, among patients with at least one risk factor, we propose the following second-step testing algorithm: Among patients who have no family history and a solitary HNP, test first for *SDHB*, then *SDHD*, then *SDHC*. Among patients who have a family history or multiple HNP, test first for *SDHD*, then *SDHB*, then *SDHC*. Although the tumor biology was associated with involvement of a specific gene (*SDHB* for malignant and *SDHD* for benign tumors), its predictive capacity was much lower than for family history and number of HNP (data not shown).

We compared costs of genetic testing for two different approaches: (a) test all subjects for all three genes; (b) test only subjects with one or more risk factor, in the order described in the previous paragraph. For both approaches, we present costs per patient rounded up to the next whole dollar.

We performed bootstrap analysis to assess the performance of the logistic regression model and the predictive ability of the testing algorithm, and to calculate the cost-reduction of our proposed testing strategy. We selected 1,000 bootstrap samples with  $n = 598$  with replacement from the original data set of 598 registrants. We used these 1,000 samples to repeat all analyses described above. We summarized findings from the bootstrap analyses as the median, minimum, maximum, and 95% confidence interval (CI; represented by the range of results from the 2.5th percentile to the 97.5th percentile).

Analyses were done using SYSTAT v.10 software (SPSS, Inc.) or SAS software (SAS Institute, Inc.).

**Institutional review board for human subjects' protection protocol approvals.** The project was approved by the ethics committees/Institutional Review Boards for Human Subjects' Protection of the respective institutions. Participants provided written informed consent in accordance with accepted standards for each respective country.

## Results

**Registry.** As of October 30, 2007, the European-American Head-and-Neck Paraganglioma Registry comprised 598 unrelated patients. This figure included 328 German, 103 Spanish, 82 Italian,

**Table 1.** Demographic and clinicopathologic features in *SDHB*, *SDHC*, and *SDHD* mutation positive and mutation negative HNP subjects

Clinical parameters	Variables	All	Mutation negative	Mutation positive	<i>SDHB</i>	<i>SDHC</i>	<i>SDHD</i>
Sex	Female	427	334	93 (22%)	31	13	49
	Male	171	81	90 (53%)	32	13	45
Age ≤40 y	No	403	328	75 (18%)	28	11	36
	Yes	195	87	108 (55%)	35	15	58
Tumor biology	Benign	565	404	161 (28%)	50	26	85
	Malignant	33	11	22 (67%)	13	0	9
Multiple	No	516	401	115 (22%)	56	21	38
	Yes	82	14	68 (83%)	7	5	56
Previous pheochromocytoma	No	571	413	158 (28%)	55	26	77
	Yes	27	2	25 (93%)	8	0	17
Family history HNP/pheochromocytoma	No	530	411	119 (22%)	47	23	49
	Yes	68	4	64 (94%)	16	3	45
Total		598	415	183 (31%)	63	26	94

**Table 2.** Association between demographic and clinical features and likelihood of hereditary (*SDHx* associated) HNP

Clinical parameters	Predictors for hereditary HNP	
	Odds ratio (95% CI)	<i>P</i>
Male gender	3.46 (2.10–5.70)	<0.001
Age ≤40 y	3.99 (2.45–6.53)	<0.001
Multiple HNP	10.61 (5.07–22.19)	<0.001
Malignant HNP	2.10 (0.77–5.71)	0.15
Previous Pheochromocytoma	10.86 (2.08–56.62)	0.005
Family history present	37.85 (12.57–114.03)	<0.001

NOTE: Multiple logistic regression analysis was performed as described in the Materials and Methods section. After controlling for all other variables in the logistic regression model, the odds ratio with 95% CIs and *P* value of each independent variable were calculated.

27 French, 23 Polish, 14 Australian/New Zealand, 9 United States, and 12 patients from other countries. Mean age at diagnosis was 49 years (range, 11–87 years); 427 subjects were female and 171 were male. All were of European ancestry, except for three of Maori descent and one of African-American ancestry.

**Germline mutations.** Among all presentations of HNP, 183 (30.6%) had a germline mutation in one of the *SDHx* genes (Supplementary Table S1). No patient had a germline mutation in more than one gene, and none more than one in the affected gene. Mutations of *SDHB* were found in 63 patients (34.4%), including 6 with large deletions and 1 duplication, *SDHC* in 26 (14.2%) including 3 deletions, *SDHD* in 94 (51.4%) including 6 deletions.

Of note, 10 undescribed *SDHC* mutations were detected in addition to 13 novel *SDHB* and 19 novel *SDHD* mutations. The mutations were distributed across all exons of *SDHx*; large deletions were also found for all these genes. Mutations have also been found in one case each in *RET* (Cys620Arg) and *VHL* (Leu135X); these two cases had either a personal or family history of associated tumors characteristic of these diseases and are excluded from further calculations.

**Demographic and clinical predictors for presence of germline mutations.** Demographic and clinical characteristics of all 598 subjects are shown in Fig. 1 and Table 1. Multiple logistic regression analysis indicated that 5 of the 6 variables included in the model were independently associated with presence of a germline mutation in one of the *SDHx* genes (Table 2); the c-statistic of the model was 0.883. Among these 598 subjects, 326 (55%) had at least 1 of the 6 risk factors (first-step predictors), of whom 168 (52%) were mutation positive. No risk factors were present in 272 subjects; however, 15 of these had a pathogenic *SDHx* mutation (5.5% of the 272 with no risk factors, 2.5% of the entire cohort; Supplementary Table S2). Using the approach of testing only patients with at first-step predictors results in 91.8% sensitivity, 61.9% specificity, 51.5% positive predictive value, and 94.5% negative predictive value.

**Costs associated with two testing approaches.** Among these 598 subjects, the cost of genetically testing for all mutations in the three *SDHx* genes is \$2,691 per patient (approach 1). The cost of our two-step procedure (approach 2) is \$1,072 per patient, which represents a 60.2% reduction.

**Bootstrap assessment.** Results from bootstrap analyses were similar to those from analysis of the single sample (Table 3). Our two-step testing approach results in a median cost per patient of \$1,074, or a median decrease of 60.1% (95% CI, 56.7–63.7% reduction) This cost reduction is offset by the number of patients with genetic mutations who would not be tested using this approach. The median

**Table 3.** Internal cross-validation analyses performed by bootstrapping

Variable	Original sample	1,000 bootstrap samples			
		Median	Minimum	Maximum	95% CI
Descriptive information					
No of subjects with one or more risk factors	326	326	287	364	303–350
No of subjects with 0 risk factors	272	272	234	311	248–294
No of positive mutations missed	15	15	3	28	8–23
Percentage of positive mutations missed of all cases	2.5	2.5	0.5	4.7	1.3–3.8
Percentage of positive mutations missed of all positive cases	8.2	8.1	1.5	15.6	4.6–12.2
Logistic regression model performance					
Sensitivity, percent	91.8	91.9	84.4	98.5	87.7–95.4
Specificity, percent	61.9	61.8	53.7	69.2	56.9–66.6
Positive predictive value, percent	51.5	51.5	43.7	58.6	46.5, 56.7
Negative predictive value, percent	94.5	94.5	89.4	98.8	91.6–97.1
C-statistic from multiple logistic regression model	0.883	0.886	0.842	0.936	0.853–0.914
Cost calculations in US\$					
Cost per subject, approach 1	2,691	2,691	2,691	2,691	N/a
Cost per subject, approach 2	1,072	1,074	928	1,239	978–1,167
Cost reduction, expressed as a percentage					
Reduction of approach 2 relative to approach 1	–60.2	–60.1	–65.5	–54.0	–63.7 to –56.7

Abbreviation: N/A, not applicable.

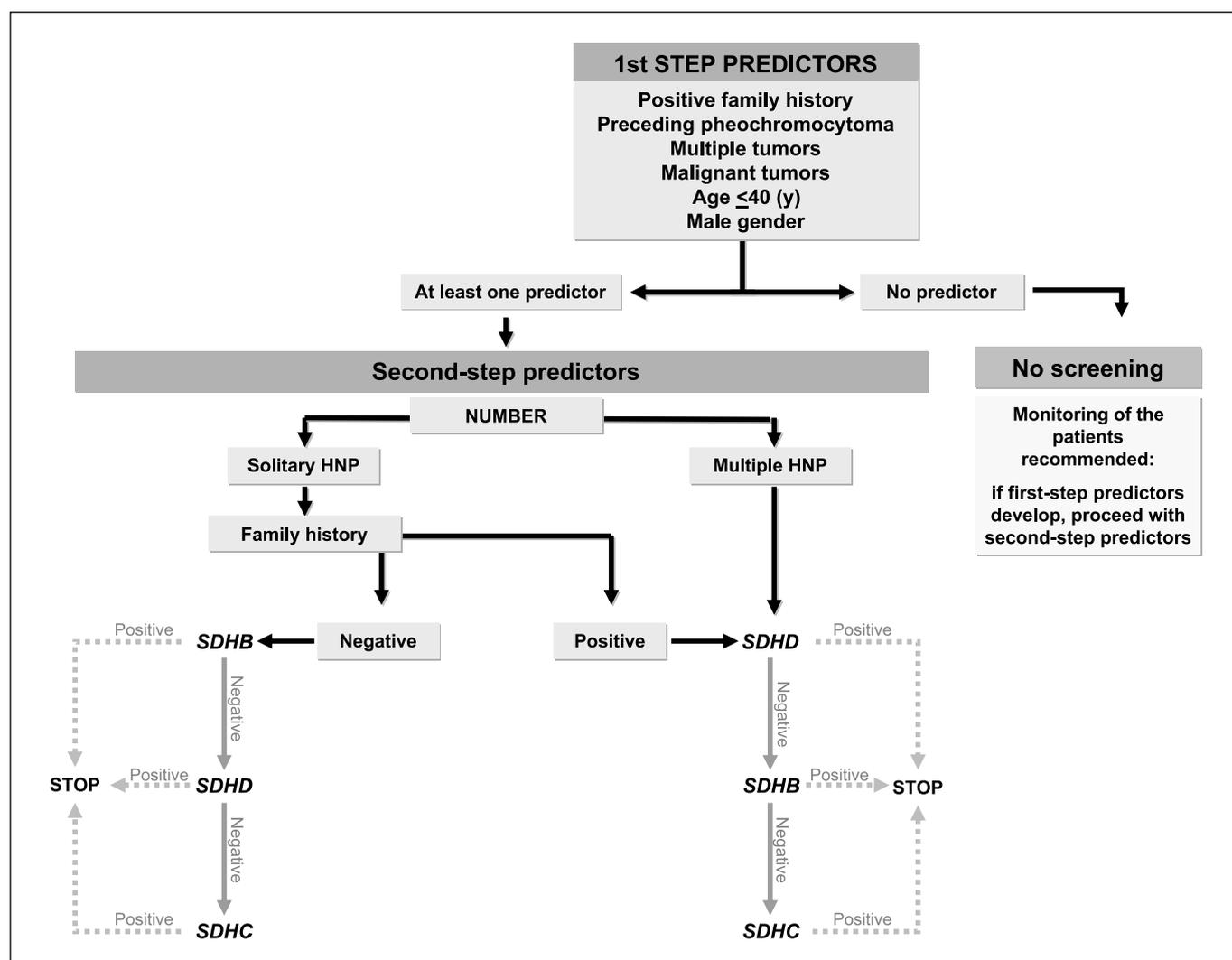
number of “missed” patients using this approach is 15 (95% CI, 8–23), or 2.5% of 598 patients (95% CI, 1.3–3.8%).

## Discussion

This international consortium study comprising 598 patients presenting with previous or current histories of HNP revealed an overall mutation frequency of 30.6%, with virtually all mutations occurring in *SDHD* (15.7%), *SDHB* (10.5%), and *SDHC* (4.3%) gene. Large deletions/rearrangements were found in 16 of 183 (8.7%) *SDHx* gene carriers, specifically in 6 of the 94 (6.4%) mutation carriers of *SDHD*, 7 of 63 (11.1%) of *SDHB*, and 3 of 26 (11.5%) of *SDHC*. Routine testing should therefore include a search for such mutations after a point mutation has been excluded.

The mutation frequency among apparently isolated tumor manifestations is very high. The benefits of knowing of a positive test can be important for the definitive diagnosis, surveillance, and other clinical/surgical management of the patient and family members. Thus, *SDHx* genetic testing in the HNP setting is consistent with the 2003 policy statement for cancer genetic testing from the American Society of Clinical Oncology (27). In the United

States, this strategy is mainly followed in some tertiary care and academic centers, where all HNP presentations are offered bundled (all 3 *SDHx*) gene testing and genetic counseling. However, this would become cumbersome and expensive in the future practice of genetic, and especially genomic, medicine where multiple genes must be assessed for several disorders. Thus, our data might act as a guide for cost-reducing prioritization of gene testing. Priority might be focused on patients presenting under the age of 40 years, male sex, multiple tumors, malignant tumors, preceding pheochromocytoma, and/or known family history for HNP or pheochromocytoma. The sensitivity of such an approach would be 92% (95% CI, 88–95%), with a negative predictive value of 94% (95% CI, 92–97%). Because the narrow 95% CIs around these two parameters were derived from bootstrap validation, we infer that our first-pass model is robust. With our risk assessment model, most hereditary HNP patients could be identified before genetic testing using only clinical parameters but a median of 2.5% could not, based on clinical first-step predictors alone (maximum 4.7% among all bootstrap samples). One strategy to minimize missed mutation carriers may be to reconsider patients for gene testing should they develop any first-step predictors during follow-up.



**Figure 2.** General algorithm for mutation screening of HNP patients. Only those with a first-step predictor being positive enter the main algorithm where second-step predictors are applied.

Further cost reduction was obtained by prioritizing the order in which each *SDHx* gene is tested. In our proposed algorithm (approach 2; Fig. 2), we describe the order in which genes should be tested. Because all patients were found to have one pathogenic mutation in one gene, the testing should stop once a mutation is found.

Of interest are restricted geographic areas where specific mutations due to a founder effect could increase the frequency of hereditary HNP (28–31). All patients from those areas should be screened for the given founder mutation first, before application of our general algorithm. Furthermore, if family pedigree confirms a disease transmission from mother to child, showing lack of parent-of-origin effect seen for *SDHD* mutations, the *SDHB* gene should be tested first, followed by *SDHC*, and if both are negative, *SDHD* gene testing should be performed (32–34). Although the current algorithm apparently seems to contradict a small pilot study (10) for solitary HNP presentations in the absence of family history, it really is not given that the former is a genotype-phenotype association study. For this type of presentation, the current algorithm based on multiple logistic regression modeling does prioritize *SDHB* and *SDHD* over *SDHC*.

Finally, and only in rare situations, if no mutations are detected in any *SDHx* gene, then *VHL* and *RET* testing should be considered.

With the exception of male gender, each of the other significant clinical predictors in our model should not be surprising as each has been previously identified and discussed. Currently, there are no studies identifying male gender as an independent risk factor for germline mutations. Further research in this area is needed to corroborate this first observation.

Identification of other potential genes involved in HNP susceptibility (e.g., the not yet isolated *PGL2* gene) is possible. Our algorithm can be easily adapted with each susceptibility gene by redoing calculations using the same principles outlined here.

Given these data, it would be ideal if all presentations of HNP be referred to cancer genetics professionals for risk assessment and if deemed appropriate, gene testing in the setting of genetic counseling. However, given the market forces against those facile with cancer genetics, our data will serve as a guide to prioritize which HNP patients are referred first to cancer genetics professionals, and for the latter, will also help guide prioritizing which particular gene to begin testing among three potential HNP susceptibility genes. We must seriously consider the trade-off between cost reduction and missing a median of ~8% of mutation

carriers. At a time where American health care costs are skyrocketing, a 60% overall cost reduction in diagnostic processes alone is attractive.

Only the mutation positive carriers need regular clinical follow-up. Currently, there is no international consensus regarding intervals and specific programs. Our consortium sites suggest conventional multisection imaging by magnetic resonance imaging of the skull-base and neck, the thorax, the abdomen, and the pelvis to detect unrecognized paraganglial tumors in time (35–37). This type of surveillance will detect incidental “other” tumors including those of the kidneys, thyroid, and gastrointestinal stromal tumors (19, 22).

In summary, our rational evidence-based approach for stepwise gene testing based on clinical features serves as first-pass strategy, which should be independently validated, and be subject to revision over time by considering other patient factors, such as different nationalities, possible local founder mutations, and changes in costs with new technology availability. In the meantime, our first-pass model could be a useful and cost-saving tool for the future practice of both genetics- and genomics-based personalized health care.

## Disclosure of Potential Conflicts of Interest

No author had any financial and personal relationships with other people or organizations that could inappropriately influence or bias this work.

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