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Clinical Characterization of the Pheochromocytoma and Paraganglioma Susceptibility Genes *SDHA*, *TMEM127*, *MAX*, and *SDHAF2* for Gene-Informed Prevention

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IMPORTANCE Effective cancer prevention is based on accurate molecular diagnosis and results of genetic family screening, genotype-informed risk assessment, and tailored strategies for early diagnosis. The expanding etiology for hereditary pheochromocytomas and paragangliomas has recently included *SDHA, TMEM127, MAX*, and *SDHAF2* as susceptibility genes. Clinical management guidelines for patients with germline mutations in these 4 newly included genes are lacking.

OBJECTIVE To study the clinical spectra and age-related penetrance of individuals with mutations in the *SDHA, TMEM127, MAX*, and *SDHAF2* genes.

DESIGN, SETTING, AND PATIENTS This study analyzed the prospective, longitudinally followed up European-American-Asian Pheochromocytoma-Paraganglioma Registry for prevalence of *SDHA*, *TMEM127, MAX*, and *SDHAF2* germline mutation carriers from 1993 to 2016. Genetic predictive testing and clinical investigation by imaging from neck to pelvis was offered to mutation-positive registrants and their relatives to clinically characterize the pheochromocytoma/paraganglioma diseases associated with mutations of the 4 new genes.

MAIN OUTCOMES AND MEASURES Prevalence and spectra of germline mutations in the *SDHA, TMEM127, MAX*, and *SDHAF2* genes were assessed. The clinical features of *SDHA, TMEM127, MAX*, and *SDHAF2* disease were characterized.

RESULTS Of 972 unrelated registrants without mutations in the classic pheochromocytoma- and paraganglioma-associated genes (632 female [65.0%] and 340 male [35.0%]; age range, 8-80; mean [SD] age, 41.0 [13.3] years), 58 (6.0%) carried germline mutations of interest, including 29 *SDHA,* 20 *TMEM127,* 8 *MAX*, and 1 *SDHAF2*. Fifty-three of 58 patients (91%) had familial, multiple, extra-adrenal, and/or malignant tumors and/or were younger than 40 years. Newly uncovered are 7 of 63 (11%) malignant pheochromocytomas and paragangliomas in *SDHA* and *TMEM127* disease. *SDHA* disease occurred as early as 8 years of age. Extra-adrenal tumors occurred in 28 mutation carriers (48%) and in 23 of 29 *SDHA* mutation carriers (79%), particularly with head and neck paraganglioma. *MAX* disease occurred almost exclusively in the adrenal glands with frequently bilateral tumors. Penetrance in the largest subset, *SDHA* carriers, was 39% at 40 years of age and is statistically different in index patients (45%) vs mutation-carrying relatives (13%; $P < .001$).

CONCLUSIONS AND RELEVANCE The *SDHA*, *TMEM127, MAX,* and *SDHAF2* genes may contribute to hereditary pheochromocytoma and paraganglioma. Genetic testing is recommended in patients at clinically high risk if the classic genes are mutation negative. Gene-specific prevention and/or early detection requires regular, systematic whole-body investigation.

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Supplemental content

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Preventive medicine has been dramatically improved by cancer genetics. The identification of susceptibility genes for hereditary forms of cancers in the past decades opened avenues for detection of second primary and recurrent tumors. Once a given germline mutation is detected, newly identified carriers are clinically investigated, and thus the diagnosis of tumors in an asymptomatic stage in the relatives of carriers becomes possible. Pheochromocytoma, paraganglioma, and associated inherited diseases served in this direction as important pacesetters. The susceptibility genes—including *RET* as causing multiple endocrine neoplasia type 2 (RefSeq NM_020975.4); *VHL* for von Hippel-Lindau disease (RefSeq NM_000551.3); *SDHD, SDHC*, and *SDHB* (succinate dehydrogenase subunits D [RefSeq NM_003002.3], C [RefSeq NM_003001.3], and B [RefSeq NM_003000.2]) for paraganglioma syndromes types 1, 3, and 4; and *NF1* for neurofibromatosis type 1 (RefSeq NM_000267.3), herein summarized as classic susceptibility genes—have served as effective molecular tools for preventive medicine studies and practice in this field in the first years of the new millenium.[1-8] These studies led to the end of the 10% rule for the frequency of hereditary pheochromocytoma and instead pointed to at least a 24% hereditary fraction.[6,9] By 2010 and 2011, additional susceptibility genes for pheochromocytomas and paragangliomas were reported, including *SDHA* (succinate dehydrogenase subunit A [RefSeq NM_004168.3]), *TMEM127* (transmembrane protein 127 [RefSeq NM_017849.3]), *MAX* (Myc-associated factor X [RefSeq NM_002382.4]), and *SDHAF2* (succinate dehydrogenase complex assembly factor 2 [RefSeq NM_017841.2]).[10-13] In contrast to the classic susceptibility genes, recommendations of the last International Symposium for Pheochromocytoma (2014) do not provide clinical management guidelines for those with mutations in the susceptibility genes analyzed and characterized herein.[14] The main reasons are the limited number of sufficiently large registries, leading to limited clinical data associated with mutations of these genes. The much-needed and important process of returning to the clinical roots is a labor-intensive one. Thus, our comprehensive genetic and clinical characterization study intends to close the gap between finding a mutation in 1 of these 4 new susceptibility genes and the associated clinical data so that gene-informed risk assessment, counseling, and management can be performed.

Key Points

Question What does testing for the *SDHA, TMEM127, MAX*, and *SDHAF2* genes add to effective cancer prevention?

Findings Of 972 participants in the European-American-Asian Pheochromocytoma-Paraganglioma Registry without mutations in the classic pheochromocytoma/paraganglioma susceptibility genes, 58 probands (6.0%) carried certain or likely pathogenic germline mutations that included 29 in *SDHA*, 20 in *TMEM127*, 8 in *MAX*, and 1 in *SDHAF2*. Seven of 63 carriers (relatives and probands) with *SDHA* and *TMEM127* (11%) had malignant pheochromocytomas or paragangliomas.

Meaning Gene-informed prevention and/or early detection requires regular whole-body investigation.

Methods

Study Population

We used the European-American-Asian Pheochromocytoma-Paraganglioma Registry, our population-based registry of unrelated patients presenting with symptomatic, histopathologically confirmed pheochromocytoma and paraganglioma. The Register included patients with head and neck paragangliomas mainly from Germany, Poland, Italy, France, and, for this study, the United States, Sweden, Hungary, Israel, and Singapore.[6-8] For all registrants, DNA was available for genetic testing. Registrants provided demographic and clinical information, including age at diagnosis, sex, location and number of tumors, and family history of pheochromocytomas and paragangliomas. Pheochromocytomas and paragangliomas were differentiated in adrenal pheochromocytomas and extra-adrenal retroperitoneal, pelvic, thoracic, and head and neck paragangliomas. We followed the World Health Organization tumor classification of only lymph node or distant metastasis criteria for malignant pheochromocytoma.[15] Our respective institutions' human subjects protection or ethical committees approved this study. For all patients, written informed consent was documented in accordance with the human subjects protection or ethical committee requirements. The participating centers excluded double registration of any proband in any similar study.

Clinical Screening Program

Patients carrying germline mutations were informed in the context of genetic counseling and were offered early detection surveillance. Imaging included magnetic resonance imaging (MRI) or computed tomography of the skull base and neck, thorax, and abdomen, including the pelvis, or, alternatively, scintigraphy using 131 iodine-labeled metaiodobenzylguanidine, fluorodeoxyglucose F 18-labeled positron emission tomography–computed tomography, or fluorodopa F 18-labeled positron emission tomography–computed tomography. We offered genetic testing to the relatives of the mutation carriers and, in the case of detection of mutations, the same clinical surveillance program. Data from mutation carriers were updated in March to June 2016 for postregistration events.

Mutation Analysis

Genomic DNA was extracted from 10-mL samples of peripheral blood leukocytes. We excluded carriership of mutations of the pheochromocytoma/paraganglioma susceptibility genes *RET* (analyzing exons 10, 11, 13, and 16), *VHL, SDHB, SDHC*, and *SDHD* (analyzing all exons). We performed multiplex ligation-dependent probe amplification analyses for *VHL*, *SDHB, SDHC*, and *SDHD*. We excluded *NF1* by molecular analyses or clinical criteria.[16] The *SDHA, TMEM127, MAX,* and *SDHAF2* genes were analyzed for intragenic mutations by Sanger sequencing. These procedures were performed in the Molecular Genetic Laboratory of the Section for Preventive Medicine, University Medical Center, Freiburg, Germany; Molecular Diagnostic Laboratory for Hereditary Tumors, Veneto Institute

of Oncology, Padova, Italy; Endocrine Genetics Laboratory of the Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary; and Laboratory of the Genomic Medicine Institute, Cleveland Clinic, Cleveland, Ohio.

The samples from Hungary were identified by whole-exome sequencing and were confirmed by Sanger sequencing. The samples from Sweden were analyzed by next-generation sequencing–based multigene panel testing, and mutations were verified by Sanger sequencing as reported. The same procedure was used for the samples in Singapore.[17] For controls, we used the publicly available platforms 1000 Genomes Project or Exome Aggregation Consortium and 140 healthy, anonymous German blood donors for whom exons with DNA variants detected during this study were sequenced.

We classified DNA variants according to the scheme of the American College of Medical Genetics and Genomics.[18] The DNA variants classified as benign (class 1) or likely benign (class 2) were removed from the analysis. The DNA variants classified as of unknown significance (class 3), likely pathogenic (class 4), or certainly pathogenic (class 5) are listed in **Table 1**. For prevalence and other estimates, we used only class 4 and class 5 mutations.

Statistical Analysis

All registrants (index patients) with germline mutations (American College of Medical Genetics and Genomics classes 4 and 5) and all relatives found to carry the family-specific mutations were included for statistical evaluation of clinical characteristics and risk profiles. We performed 2 × 2 comparisons of mutation frequencies or disease features by gene using a Fisher exact 2-tailed test, with $P < .05$ considered to be statistically significant and $P < .10$ considered as a promising finding for future investigation. Age-related penetrance with 95% CIs were calculated using the Kaplan-Meier method. Censored penetrance data were compared using the Peto and Peto modification of the Gehan-Wilcoxon test implemented in R software (http://www.r-project.org).[19]

Results

Molecular Genetic Results

From the European-American-Asian Pheochromocytoma-Paraganglioma Registry, a total of 972 blood DNA samples were available in which germline mutations of the *RET, VHL, SDHB, SDHC*, and *SDHD* genes and clinical manifestations of *NF1* had been excluded. The 972 registrants included 632 female (65.0%) and 340 male (35.0%) patients. Registrants' ages at diagnosis ranged from 8 to 80 years (mean [SD], 41.0 [13.3] years). Seven hundred seventeen had pheochromocytomas or paragangliomas in retroperitoneal, pelvic, or thoracic locations, and 255 had head and neck paragangliomas.

Of the 972 registrants, we found DNA variants of classes 3, 4, and 5 in the *SDHA*, *MAX*, *TMEM127*, and *SDHAF2* genes in a total of 64 patients (Table 1 and eTable 1 in the Supplement). To be conservative, we excluded the 6 patients with class 3 DNA variants from further estimations. Thus, 58 registrants (6.0%) had certain or likely pathogenic mutations, including 29 (3.0%) with *SDHA*, 20 (2.1%) with *TMEM127*, 8 (0.8%) with *MAX*, and 1 (0.1%) with *SDHAF2* mutations (Table 1 and eTable 1 in the Supplement). Nationalities of mutation carriers included 36 German, 8 American, 4 Polish, 4 Turkish, 3 Hungarian, 2 Swedish, and 1 Israeli participants. Novel mutations are represented by 19 of 21 *SDHA* mutations, 11 of 16 *TMEM127* mutations, and 1 of 6 *MAX* mutations. In the subgroup of 255 patients with head and neck paragangliomas, 19 (7.5%; 95% CI, 4.7%-11.6%) were mutation carriers; of the 717 registrants without head and neck paragangliomas, 39 (5.4%; 95% CI, 4.0%-7.4%) were mutation carriers. The distribution of mutations across the different genes differs between these 2 subgroups. Fifteen of 20 patients (75%) in the head and neck paraganglioma subgroup were *SDHA* mutation carriers compared with 14 of 40 (35%) in the subgroup with pheochromocytomas and paragangliomas below this region ($P = .006$). Of note, 3 of 20 patients (15%) had germline *TMEM127* mutations in the head and neck paraganglioma subgroup vs 18 of 40 (45%) in the subgroup with non–head and neck paragangliomas ($P = .03$). No *MAX* mutations were detected in the head and neck paraganglioma subgroup compared with 8 of 40 (20%) in the subgroup with non–head and neck paragangliomas in the ($P = .04$). Similarly, the head and neck paraganglioma subgroup had 1 of 20 *SDHAF2* mutation carriers (5%) in contrast to none in the subgroup with non–head and neck paragangliomas paragangliomas ($P = .33$).

We looked for clinical variables that suggested potential heritable disease, namely, (1) a family history of pheochromocytomas and paragangliomas, (2) younger than 40 years at diagnosis, (3) more than 1 pheochromocytoma or paraganglioma, (4) tumor location outside the adrenal glands, and (5) malignant tumors. As such, 53 of the 58 patients with germline mutations (91%) showed at least 1 such characteristic finding, and 24 (41%) had 2 or more findings (Table 1 and eTable 1 in the Supplement).

Genetic Family Screening and Characterization of Disease Features

Genetic family screening was performed for 13 families consisting of 37 relatives, and 21 relatives were newly recognized as mutation carriers, including 9 for *SDHA*, 9 for *TMEM127*, 3 for *MAX*, and none for *SDHAF2*. Thus, the total number of mutation carriers of the *SDHA*, *TMEM127*, *MAX*, and *SDHAF2* genes in probands and relatives was 79, including 38 with *SDHA*, 29 with *TMEM127*, 11 with *MAX*, and 1 with *SDHAF2*.

Evaluation of clinical data at diagnosis of pheochromocytomas and paragangliomas or at follow-up revealed that imaging was performed for the retroperitoneum in 68 of 79 patients (86%), the pelvis in 65 of 79 (82%), the thorax in 62 of 79 (78%), and the head and neck in 73 of 79 (92%) of the mutation carriers. From these, we were able to characterize the clinical features of *SDHA*, *TMEM127*, *MAX*, and *SDHAF2* disease.

A summary of the information from mutation-positive patients obtained by surveillance imaging and the information obtained for patients who did not undergo surveillance imaging demonstrates clear clinical features associated with mutations in different genes (**Table 2**). Malignant pheochromocytomas and paragangliomas featured across 7 of 63 (11%) *SDHA*

Table 1. Germline Mutations in the *SDHA*, *TMEM127*, *MAX*, and *SDHAF2* Genes and Corresponding Phenotypes in 64 Unrelated Index Patients[a]

Germline Mutation, No. of Probands With Same Mutation/Nationality	Age at Diagnosis, y	Sex	Family History	Paraganglionic Phenotype	No. of Clinical Variables Suggesting Heritability[b]	Nucleotide Change	ACMG Variant Class[c]
SDHA							
1/Germany	66	M	Negative	Extra-adrenal, thoracic	1	c.1A>C	5
1/Germany	30	M	Negative	Adrenal, unilateral	1	c.1A>T	5
1/Germany	27	F	Negative	Carotid	2	c.2T>G	5
1/Germany	34	M	Negative	Adrenal, unilateral	1	c.3G>C	5
5/Germany	15-37	M and F	Negative and positive	Adrenal, unilateral; extra-adrenal, retroperitoneal; carotid; and jugular	1-3	c.91C>T	5
2/Sweden	20 and 47	M and F	Negative	Extra-adrenal, retroperitoneal; adrenal, unilateral	0-2	c.223C>T	5
1/Germany	47	F	Negative	Jugular	1	c.296A>G	5
1/Germany	26	F	Negative	Jugular	2	c.457-1G>A	5
1/Turkey	17	M	Negative	Extra-adrenal, retroperitoneal	2	c.566G>A	4
1/Germany	43	M	Negative	Carotid	1	c.622T>C	4
1/Sweden	64	M	Negative	Adrenal, unilateral	0	c.629G>A	3
1/Germany	53	M	Negative	Jugular	1	c.778G>A	4
1/United States	33	M	Negative	Extra-adrenal, pelvic	2	c.820G>A	5
1/Poland	27	F	Negative	Adrenal, unilateral	1	c.830C>T	3
1/Germany	46	F	Negative	Jugular	1	c.940G>A	4
1/Germany	24	M	Negative	Adrenal, unilateral	1	c.1115C>G	3
1/Germany	63	F	Negative	Carotid	1	c.1177G>A	3
1/Germany	58	M	Negative	Adrenal, bilateral	2	c.1283_1298del	5
1/United States	30	F	Negative	Extra-adrenal, pelvic	2	c.1316G>A	4
1/Germany	20	F	Negative	Jugular	2	c.1334C>T	5
1/Germany	48	M	Negative	Carotid, bilateral	1	C.1340A>G	5
2/Germany and United States	28 and 49	M	Negative	Jugular; adrenal, unilateral, malignant	1	c.1361C>A	4
1/Germany	44	M	Negative	Extra-adrenal, retroperitoneal	1	c.1432_1432 + 1del	5
1/Germany	50	F	Negative	Jugular	1	c.1766G>A	5
3/Germany, United States, and Turkey	42-65	M and F	Negative	Extra-adrenal, retroperitoneal, multiple; carotid; and vagal	1-2	c.1799G>A	4
1/Poland	39	M	Negative	Adrenal, unilateral	1	c.1979C>G	3
TMEM127							
4/United States and Germany	35-58	M and F	Negative and positive	Adrenal, unilateral	0-1	c.3G>A	5
1/Germany	68	M	Negative	Adrenal, unilateral	0	c.73A>T	5
1/Poland	43	F	Negative	Adrenal, bilateral	1	c.131T>G	4
1/Germany	58	F	Negative	Adrenal, unilateral	0	c.215T>A	5
1/Germany	34	F	Negative	Carotid	2	c.325T>C	4
1/Germany	45	M	Negative	Adrenal, bilateral	1	c.410-1G>C	5
1/Germany	66	M	Negative	Extra-adrenal, retroperitoneal	1	c.413T>G	4
1/Hungary	22	F	Negative	Adrenal, bilateral	2	c.419G>A	5
1/Germany	76	M	Negative	Adrenal, unilateral	0	c.462C>G	4
1/Hungary	51	F	Negative	Adrenal, bilateral, carotid, malignant	3	c.464T>A	5
1/Germany	26	F	Negative	Adrenal, unilateral	1	c.518T>C	4
1/Poland	25	F	Negative	Adrenal, unilateral	1	c.532dup	5
1/Israel	33	F	Positive	Adrenal, bilateral	3	c.543_555dup	5
1/Turkey	51	F	Negative	Adrenal, bilateral, extra-adrenal retroperitoneal	2	c.553G>A	4
1/Germany	50	F	Negative	Tympanic	1	c.568G>A	4
2/Turkey and Hungary	26 and 47	F	Negative	Adrenal, unilateral; adrenal, bilateral	1	c.572del	5
1/Sweden	55	F	Negative	Adrenal, unilateral	0	c.665C>T	3

(continued)

Table 1. Germline Mutations in the *SDHA*, *TMEM127*, *MAX*, and *SDHAF2* Genes and Corresponding Phenotypes in 64 Unrelated Index Patients[a] (continued)

Germline Mutation, No. of Probands With Same Mutation/Nationality	Age at Diagnosis, y	Sex	Family History	Paraganglionic Phenotype	No. of Clinical Variables Suggesting Heritability[b]	Nucleotide Change	ACMG Variant Class[c]
MAX							
1/Germany	36	F	Negative	Adrenal, unilateral	1	c.73C>T	5
1/Germany	23	F	Negative	Adrenal, unilateral	1	c.146C>G	5
3/Germany and Poland	32-38	M and F	Negative and positive	Adrenal, unilateral; adrenal bilateral	1-3	c.223C>T	5
1/Germany	50	F	Positive	Adrenal, bilateral	2	c.242_243del	5
1/Germany	21	M	Negative	Adrenal, unilateral	1	c.292dup	5
1/Germany	26	M	Negative	Adrenal, bilateral	2	c.307G>T	5
SDHAF2							
1/United States	25/	F	Positive	Carotid, vagal	3	c.232G>A	5

Abbreviations: ACMG, American College of Medical Genetics and Genomics; *MAX*, Myc-associated factor X; *SDHA*, succinate dehydrogenase subunit A; *SDHAF2*, succinate dehydrogenase complex assembly factor 2; *TMEM127*, transmembrane protein 127.

[a] Indicates status before clinical surveillance imaging.

[b] Includes a family history of pheochromocytomas and paragangliomas; younger than 40 y at diagnosis; more than 1 pheochromocytoma or paraganglioma; tumor location outside the adrenal glands; and malignant tumor.

[c] Classified according to the variant classification system of the ACMG. Class 3 indicates variant of unknown clinical significance; class 4, likely pathogenic; and class 5, certainly pathogenic. Fifty-eight index patients had certain (class 5) or likely pathogenic (class 4) mutations, and 6 index patients had DNA variants of unknown significance (class 3). The latter 6 (5 *SDHA* and 1 *TMEM127*) with DNA variants of unknown clinical significance were not included in additional analyses.

Table 2. Tumor Characteristics of Germline Mutation Carriers of the *SDHA*, *MAX*, *TMEM127*, and *SDHAF2* Genes in 58 Index Patients and 21 Relatives[a]

Variable	Germline Mutation Group, No. of Index Patients + Relatives With Mutation/No. of Mutation Carriers (%) [95% CI][b]			
	SDHA (n = 38)	*TMEM127* (n = 29)	*MAX* (n = 11)	*SDHAF2* (n = 1)
Family history[c]	1/29 (3) [0-20]	2/20 (10) [2-33]	2/8 (25) [5-64]	1/1 (100)
>1 Pheochromocytoma or paraganglioma	3/33 (9) [2-26]	11/28 (39) [22-59]	9/11 (82) [48-97]	1/1 (100)
Adrenal	8/29 (28) [13-48]	20/27 (74) [53-88]	11/11 (100) [68-100]	0/1 (0)
Bilateral adrenal	1/26 (4) [0-22]	10/27 (37) [20-58]	8/11 (73) [39-93]	0/1 (0)
Extra-adrenal retroperitoneal or pelvic[d]	7/26 (27) [12-48]	1/27 (4) [0-21]	1/11 (9) [1-43]	0/1 (0)
Head and neck paraganglioma	15/34 (44) [4-28]	6/27 (22) [3-29]	0/11 (0) [1-43]	1/1 (100)
Malignant pheochromocytoma or paraganglioma	4/34 (12) [4-28]	3/29 (10) [3-29]	1/11 (9) [1-43]	0/1 (0)

Abbreviations: *MAX*, Myc-associated factor X; *SDHA*, succinate dehydrogenase subunit A; *SDHAF2*, succinate dehydrogenase complex assembly factor 2; *TMEM127*, transmembrane protein 127.

[a] Median age (range) at diagnosis in the *SDHA* group was 28 y (8-76 y); *TMEM127* group, 47 y (18-76 y); *MAX* group, 36 y (18-50 y); and *SDHAF2* group, 25 y.

[b] Indicates status after clinical surveillance imaging. Denominators represent the number of mutation carriers for whom imaging information was available.

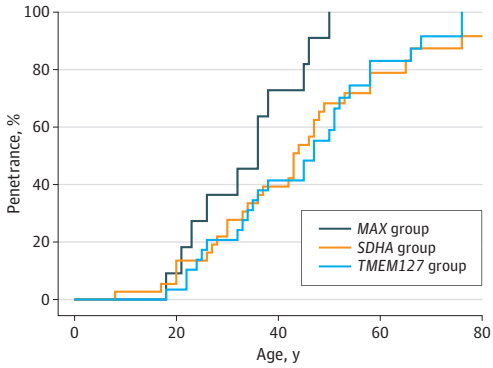
[c] Indicates index patients carrying an *SDHA* (n = 29), *TMEM127* (n = 20), *MAX* (n = 8), or *SDHAF2* (n = 1) germline mutation.

[d] Only 1 of 26 *SDHA* germline mutation carriers (4%) had an extra-adrenal thoracic paraganglioma.

(4 of 34 [12%]) and *TMEM127* (3 of 29 [10%]) mutation carriers, among whom 1 *SDHA* mutation carrier died at 61 years of age, compared with 0 of the 12 *MAX* and *SDHAF2* mutation carriers ($P = .54$) (Table 2). Among the probands and relatives with *SDHA* mutations, 15 of 34 (44%) had head and neck paragangliomas in contrast to 7 of 39 (18%) with mutations in the remaining 3 genes ($P = .02$). All 11 persons with *MAX* mutations had adrenal tumors compared with only 28 of 57 (49%) of those with *SDHA*, *TMEM127*, and *SDHAF2* mutations ($P = .002$). Among those with adrenal disease, bilateral adrenal tumors were found in 18 of 31 (58%) *MAX* and *TMEM127* mutation carriers compared with 1 of 8 (13%) *SDHA* mutation carriers ($P = .04$). Few patients with *SDHA* and *TMEM127* disease had a family history of pheochromocytomas and paragangliomas (3 of 49 vs 2 of 8 for *MAX* mutation carriers; $P = .14$).

Malignant neoplasms in addition to pheochromocytoma or paraganglioma were present in 5 patients with *TMEM127* disease (25%), including 1 with colon cancer; 1, acute myeloid leukemia; 1, pancreatic adenocarcinoma; 1, malignant melanoma; and 2, parathyroid adenoma (in 1 combined with malignant melanoma). In contrast, 1 patient with an *SDHA* mutation had breast cancer, but none of the *MAX* or *SDHAF2* mutation carriers had additional neoplasms.

Figure 1. Age-Related Penetrance of Any Pheochromocytoma and Paraganglioma



No. at risk					
MAX group	11	10	3	0	0
SDHA group	37	35	21	6	1
TMEM127 group	29	28	17	4	0

Kaplan-Meier analysis includes all 57 symptomatic probands (index patients) and 20 relatives (n = 77) with germline mutations of the *SDHA* (succinate dehydrogenase subunit A) (n = 37), *MAX* (Myc-associated factor X) (n = 11), and *TMEM127* (transmembrane protein 127) (n = 29) genes from this study.

Age-related penetrance for *SDHA*, *TMEM127*, and *MAX* mutation–associated tumors is shown in **Figure 1**. This finding is based on 11 *MAX* mutation carriers (8 index patients and 3 relatives), 37 *SDHA* mutation carriers (29 index patients and 8 relatives), and 29 *TMEM127* mutation carriers (20 index patients and 9 relatives). By 40 years of age, the estimated *MAX*-associated penetrance approached 73% (95% CI, 28%-90%) compared with 39% (95% CI, 21%-53%) for *SDHA* carriers and 41% (95% CI, 20%-57%) for *TMEM127* mutation carriers (*MAX* vs *SDHA*, $P = .07$; *MAX* vs *TMEM127*, $P = .03$; *SDHA* vs *TMEM127*, $P = .76$) (Figure 1). Penetrance (any tumor) for *SDHA* mutation carriers was significantly lower in relatives (13% at 40 years; 95% CI, 0%-33%) compared with index patients (45% at 40 years; 95% CI, 23%-60%; $P < .001$). This difference could not be shown for *MAX* (50% [95% CI, 23%-68%] for index patients vs 22% [95% CI, 0%-45%] for relatives at 40 years; $P = .26$) or *TMEM127* mutation carriers (88% [95% CI, 22%-98%] for index patients vs 33% [95% CI, 0%-70%] at 40 years; $P = .69$), but these results have to be interpreted with caution owing to the low case numbers in these subgroups.

Discussion

Although identifying novel genes predisposing to disease is scientifically exciting, rigorously characterizing the clinical context for each gene's content lays the fundamental evidence base for the practice of gene-informed risk assessment, counseling, and medical management and ultimately leads to preventive medicine. The classic genes (*RET*, *VHL*, *NF1*, *SDHB*, *SDHC*, and *SDHD*) predisposing to pheochromocytoma/paraganglioma syndromes exemplify these principles. Although the *SDHA*, *TMEM127*, *MAX*, and *SDHAF2* genes were identified during the past decade, few systematic clinical characteristics are available. In the present study, we rigorously and systematically studied the clinical characteristics of each of these latter genes in our prospectively accruing, longitudinally followed up cohort from the European-American-Asian Pheochromocytoma-Paraganglioma Registry.

Prevalence of germline mutations is an important consideration for offering molecular diagnostics. Together, the prevalence of germline mutations in one of the classic genes is at least 24% and perhaps even greater than 40% when considering all incident cases of symptomatic pheochromocytoma and paraganglioma.[6,20] Our present study shows that all patients presenting with symptomatic pheochromocytoma and/or paraganglioma but without germline mutations in the classic susceptibility genes are candidates for mutations in one of the genes investigated by this study. Herein, we report a 6.0% mutation frequency for the *SDHA*, *TMEM127*, *MAX*, and *SDHAF2* genes combined and, in particular, 3.0% for *SDHA*. Thus, half the germline mutations in this group of genes were in the *SDHA* gene. In contrast, for *TMEM127* (2.1%), *MAX* (0.8%), and *SDHAF2* (0.1%) mutation frequencies, our findings align with the previous reported data of 2.0%, 1.7%, and 0, respectively.[10,21,22]

For management, operation planning, and follow-up of mutation carriers, the risk profiles contribute important information. All identified patients with mutations in 1 of the *SDHA*, *TMEM127*, *MAX*, and *SDHAF2* genes could have tumors in any area where paraganglia are located and therefore need imaging from the skull base to the pelvis. The classic outcome in bilateral adrenal tumors, for which *MAX* and *TMEM127* mutation carriers are at highest risk, is bilateral adrenalectomy with subsequent surgical addisonian disease. Thus, adrenal-sparing surgery, which has shown convincing results for multiple endocrine neoplasia type 2, should be evaluated for *MAX* and *TMEM127* disease.[23] Major permanent adverse effects after surgery must be considered in patients with head and neck paragangliomas. Surgical removal of vagal paragangliomas and large carotid body tumors is associated with a high frequency of permanent loss of function of cranial nerves, leaving patients with hoarseness, difficulties in swallowing and speaking, and aspiration risks that make balanced decisions for the options of surgery and radiotherapy essential.[24]

With regard to screening recommendations, our results suggest that patients with pheochromocytomas or paragangliomas of the retroperitoneum or pelvis should be investigated using MRI of the skull base and neck and that patients who initially have head and neck paragangliomas undergo MRI of the abdomen and pelvis. A complete investigation using MRI should be offered to newly identified mutation carriers. A major question is the interval for routine high-risk surveillance. More than 1 pheochromocytoma or paraganglioma tumor developed in carriers of *TMEM127* and *MAX* mutations, who especially need regular follow-up investigation.

Only current next-generation platforms consisting of the *RET*, *VHL*, *NF1*, *SDHB*, *SDHC*, and *SDHD* genes and the genes analyzed in this study are being offered to individuals with pheochromocytomas and paragangliomas. Typically, the *RET*, *VHL*, *NF1*, *SDHB*, *SDHC*, and *SDHD* genes are represented in most panels offered by various clinical laboratories, both academic and commercial. However, clinicians should be alert that the

panels have great variability in the genes analyzed in this study, ranging from none to all. Thus, panels should include *SDHA, TMEM127*, and *MAX*, with *SDHAF2* being relatively expendable.

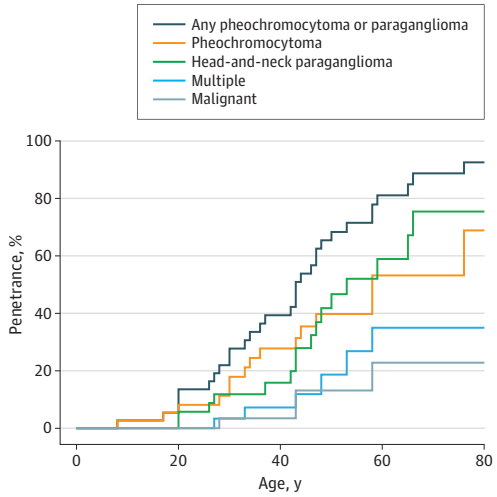
Even after analyzing the 10 listed genes predisposing to pheochromocytomas and paragangliomas, cases with clinical features suggesting heredity but without germline mutations remain. Ongoing efforts using exome and genome sequencing should not only reveal the remaining genes as fumarate hydatase (*FH*), subunits 1 and 2 of the pyruvate dehydrogenase (*PDH1* and *PDH2*), hypoxia inducible factor 1 (*HIF1A*), malate dehydrogenase 2 (*MDH2*), and kinesin family member 1Bβ (*KIF1Bß*) but also lead to an in-depth study of whole-body imaging in a sufficient number of mutation carriers. Genome sequencing will not only reveal intragenic or small indel mutations but also promises to reveal complex, large rearrangements, if any. However, what these sequencing approaches will not reveal are germline nongenetic (eg, epigenetic) alterations.[25]

Strengths and Limitations

Because our study increased the total of known germline mutation carriers of the *SDHA*, *TMEM127*, *MAX,* and *SDHAF2* genes by about 50%, we clearly define new phenotypic features (eTable 2 in the Supplement).[10-13,21,22,26-38] To date, the literature reports only 10 cases of germline *SDHA* mutations across 7 reports[11,29-31,33,35,37]; our series contains 38 cases representing 79% of the known germline *SDHA* mutation carriers. The strength of sample size and meticulous phenotyping facilitated the revelation of new clinical features associated with this gene. We found that the earliest age at onset in *SDHA* mutation carriers was 8 years, previously believed to be 20 years. We uncover a higher proportion of persons with extra-adrenal tumors (22 of 33 [67%]) and especially head and neck paragangliomas (15 of 34 [44%]), whereas the prevalence of adrenal tumors was lower. Although case numbers are still limited, we revealed, for the first time to our knowledge, a 9% (7 of 74 carriers) prevalence of malignant disease among *SDHA, TMEM127*, and *MAX* (without *MAX*, 7 of 63 [11%]) mutation carriers (Table 2). This prevalence is clearly greater than that in the available literature (1% [1 of 109 carriers]; $P = .004$) (eTable 2 in the Supplement). In contrast to previous publications, *SDHA* mutation carriers in the present study were not found to have gastrointestinal tract stromal tumors.[39,40] Two potential reasons explain this finding. First, gastrointestinal tract stromal tumors may have been truly absent in the index patients and relatives of this study. Second, gastrointestinal tract stromal tumors may not have been detected because MRI alone is not the best method to detect them and endoscopy has not been performed systematically.

Age-related penetrance estimations for newly identified susceptibility genes are potentially biased owing to the main inclusion of index cases. In particular, relatives identified with the given mutation may have a considerably lower penetrance compared with index patients, as shown previously for pheochromocytomas and paragangliomas associated with mutations of the *SDHB* gene.[4] For age-related penetrance estimations in our study, we found 3 relatives of *MAX* index patients (total, 11 mutation carriers [27%]), 9 relatives of *SDHA* index patients (total, 38 mutation carriers [24%]), and 9 relatives of *TMEM127* index patients (total, 29 mutations carriers [31%]). Therefore, our present data have to be regarded with caution. Although all-tumor penetrance in *SDHA* probands was higher than in their mutation-carrying relatives, head and neck paraganglioma appears to have a similar penetrance (by site) in *SDHA* disease (median age at penetrance, 53 and 58 years). By 70 years of age, 35% (95% CI, 5%-55%) developed multifocal disease and 23% (95% CI, 0%-42%) had malignant disease (**Figure 2**).

Figure 2. Age-Related Penetrance of Manifestations of *SDHA* Mutations



No. at risk					
Any	37	35	21	6	1
Pheochromocytoma	37	35	21	6	1
Head-and-neck paraganglioma	37	35	21	6	1
Multiple	37	35	21	6	1
Malignant	37	35	21	6	1

Kaplan-Meier analysis includes 37 *SDHA* (succinate dehydrogenase subunit A) mutation carriers stratified by 5 different phenotypic characteristics of pheochromocytoma and paraganglioma.

Of interest, individuals with germline *SDHB, SDHC*, and *SDHD* mutations have been shown to have a high prevalence of head and neck paragangliomas ranging from 45% in *SDHB* mutation carriers to 95% in *SDHC* mutation carriers.[7,8] The only patient in our study with an *SDHAF2* mutation had head and neck paraganglioma. Herein, we show that *SDHA* is not only, in name, a member of this family of genes but is characterized by a high prevalence of head and neck paragangliomas. This observation almost certainly reflects the biology of succinate dehydrogenase constituting the 4 subunits (A-D) and the molecule (*SDHA*F2) that flavinates and activates *SDHA*.

Conclusions

The *SDHA*, *TMEM127, MAX,* and *SDHAF2* genes contribute to hereditary pheochromocytoma and paraganglioma. Genetic testing is recommended in patients at clinically high risk if the patients do not have mutations in the classic susceptibility genes. Gene-specific prevention and/or early detection requires regular systematic whole-body investigation.

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Invited Commentary

Pheochromocytoma and Paraganglioma Susceptibility Genes
Estimating the Associated Risk of Disease

Lauren Fishbein, MD, PhD, MTR; Katherine L. Nathanson, MD

Approximately 40% of the tumors of the autonomic nervous system, pheochromocytomas and paragangliomas (PCC/PGL), are associated with an underlying inherited mutation, more than any other tumor type. Thus, germline mutation testing is recommended for all patients with PCC/PGL. Strong evidence supports an association of susceptibility for PCC/PGL with germline mutations in 10 genes (*FH*, *MAX*, *NF1*, *RET*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *TMEM127*, and *VHL*); mutations in an additional 5 genes also have been associated with disease susceptibility but with lower levels of evidence (*EGLN1* [PHD2], *EPAS1* [HIF2A], *KIF1B*, *MET*, and *SDHAF2*).[1] Even for genes in which an association between mutation and disease has been well established, the frequency of mutations is quite rare; thus, a paucity of data exist on which to base clinical recommendations for patients regarding the risk for developing the first PCC/PGL (eg, if they are identified though familial mutation testing), additional primary PCC/PGLs, metastatic disease, and other tumor types.

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In this issue of *JAMA Oncology*, Bausch and colleagues[2] have sought to describe the clinical characteristics of infrequently mutated susceptibility genes, including *SDHA*,