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

Birke Bausch, MD; Francesca Schiavi, PhD; Ying Ni, PhD; Jenny Welander, PhD; Attila Patocs, MD, PhD; Joanne Ngeow, MBBS, MPH; Ulrich Wellner, MD; Angelica Malinoc, PhD; Elisa Taschin, BS; Giovanni Barbon, PhD; Virginia Lanza, BS; Peter Söderkvist, PhD; Adam Stenman, MD, PhD; Catharina Larsson, MD, PhD; Fredrika Svahn, MD; Jin-Lian Chen, MS; Jessica Marquard, MS; Merav Fraenkel, MD; Martin A. Walter, MD; Mariola Peczkowska, MD; Aleksander Prejbisz, MD; Barbara Jarzab, MD; Kornelia Hasse-Lazar, MD; Stephan Petersenn, MD; Lars C. Moeller, MD; Almuth Meyer, MD; Nicole Reisch, MD; Arnold Trupka, MD; Matthias Galiano, MD; Simon F. Preuss, MD; Pingling Kwok, MD; Nikoletta Lendvai, MD; Gani Berisha, BS; Özer Makay, MD; Carsten C. Boedeker, MD; Georges Weryha, MD; Karoly Racz, MD; Andrzej Januszewicz, MD; Martin K. Walz, MD; Oliver Gim, MD; Giuseppe Opocher, MD; Charis Eng, MD, PhD; Hartmut P. H. Neumann, MD; for the European-American-Asian Pheochromocytoma-Paraganglioma Registry Study Group

**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 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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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_000975.4); 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 millennium. 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. By 2010 and 2011, additional susceptibility genes for pheochromocytomas and paragangliomas were reported, including SDHA (succinate dehydrogenase subunit A [RefSeq NM_004168.3]; VHL, SDHB, SDHC, and SDHD (analyzing all exons)); 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]). 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. 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.

### 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. For all registants, 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. 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.

#### 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, SDHD, 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. 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 for Cancer Research in Padua, Venice, Italy; and Washington University School of Medicine, St Louis, Missouri.

### 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.
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. 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. The DNA variants classified as of unknown significance (class 3), likely benign (class 2), or likely pathogenic (class 4), or certainly pathogenic (class 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).

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).

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 \( (P = .33) \).

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

<table>
<thead>
<tr>
<th>Germline Mutation, No. of Probands With Same Mutation/Nationality</th>
<th>Age at Diagnosis, y</th>
<th>Sex</th>
<th>Family History</th>
<th>Paraganglionic Phenotype</th>
<th>No. of Clinical Variables Suggesting Heritability</th>
<th>Nucleotide Change</th>
<th>ACMG Variant Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDHA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1/Germany</td>
<td>66</td>
<td>M</td>
<td>Negative</td>
<td>Extra-adrenal, thoracic</td>
<td>1</td>
<td>c.1A&gt;C</td>
<td>5</td>
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<tr>
<td>1/Germany</td>
<td>30</td>
<td>M</td>
<td>Negative</td>
<td>Adrenal, unilateral</td>
<td>1</td>
<td>c.1A&gt;T</td>
<td>5</td>
</tr>
<tr>
<td>1/Germany</td>
<td>27</td>
<td>F</td>
<td>Negative</td>
<td>Carotid</td>
<td>2</td>
<td>c.2T&gt;G</td>
<td>5</td>
</tr>
<tr>
<td>1/Germany</td>
<td>34</td>
<td>M</td>
<td>Negative</td>
<td>Adrenal, unilateral</td>
<td>1</td>
<td>c.3G&gt;C</td>
<td>5</td>
</tr>
<tr>
<td>5/Germany</td>
<td>15-37</td>
<td>M and F</td>
<td>Negative and positive</td>
<td>Adrenal, unilateral; extra-adrenal, retroperitoneal; carotid; and jugular</td>
<td>1-3</td>
<td>c.91C&gt;T</td>
<td>5</td>
</tr>
<tr>
<td>2/Sweden</td>
<td>20 and 47</td>
<td>M and F</td>
<td>Negative</td>
<td>Extra-adrenal, retroperitoneal; adrenal, unilateral</td>
<td>0-2</td>
<td>c.223C&gt;T</td>
<td>5</td>
</tr>
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<td>Jugular</td>
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<td>c.296A&gt;G</td>
<td>5</td>
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<td>Negative</td>
<td>Jugular</td>
<td>2</td>
<td>c.457-1G&gt;A</td>
<td>5</td>
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<tr>
<td>1/Turkey</td>
<td>17</td>
<td>M</td>
<td>Negative</td>
<td>Extra-adrenal, retroperitoneal</td>
<td>2</td>
<td>c.566G&gt;A</td>
<td>4</td>
</tr>
<tr>
<td>1/Germany</td>
<td>43</td>
<td>M</td>
<td>Negative</td>
<td>Carotid</td>
<td>1</td>
<td>c.622T&gt;C</td>
<td>4</td>
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<tr>
<td>1/Sweden</td>
<td>64</td>
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<td>c.629G&gt;A</td>
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<td>1/Germany</td>
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<td>F</td>
<td>Negative</td>
<td>Jugular</td>
<td>1</td>
<td>c.778G&gt;A</td>
<td>4</td>
</tr>
<tr>
<td>1/Poland</td>
<td>33</td>
<td>M</td>
<td>Negative</td>
<td>Extra-adrenal, pelvic</td>
<td>2</td>
<td>c.820G&gt;A</td>
<td>5</td>
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<tr>
<td>1/Germany</td>
<td>27</td>
<td>F</td>
<td>Negative</td>
<td>Adrenal, unilateral</td>
<td>1</td>
<td>c.830C&gt;T</td>
<td>3</td>
</tr>
<tr>
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<td>Negative</td>
<td>Jugular</td>
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<td>c.940G&gt;A</td>
<td>4</td>
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<tr>
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<td>c.1115C&gt;G</td>
<td>3</td>
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<td>Negative</td>
<td>Carotid</td>
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<td>c.1177G&gt;A</td>
<td>3</td>
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<td>M</td>
<td>Negative</td>
<td>Adrenal, bilateral</td>
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<td>c.1283_1298del</td>
<td>5</td>
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<td>30</td>
<td>F</td>
<td>Negative</td>
<td>Adrenal, pelvic</td>
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<td>c.1316G&gt;A</td>
<td>4</td>
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<td>F</td>
<td>Negative</td>
<td>Jugular</td>
<td>2</td>
<td>c.1334C&gt;T</td>
<td>5</td>
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<tr>
<td>1/Germany</td>
<td>48</td>
<td>M</td>
<td>Negative</td>
<td>Carotid, bilateral</td>
<td>1</td>
<td>c.1340A&gt;G</td>
<td>5</td>
</tr>
<tr>
<td>2/Germany and United States</td>
<td>28 and 49</td>
<td>M</td>
<td>Negative</td>
<td>Jugular; adrenal, unilateral, malignant</td>
<td>1</td>
<td>c.1361C&gt;A</td>
<td>4</td>
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<tr>
<td>1/Germany</td>
<td>44</td>
<td>M</td>
<td>Negative</td>
<td>Extra-adrenal, retroperitoneal</td>
<td>1</td>
<td>c.1432_1432 + 1del</td>
<td>5</td>
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<tr>
<td>1/Germany</td>
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<td>F</td>
<td>Negative</td>
<td>Jugular</td>
<td>1</td>
<td>c.1766G&gt;A</td>
<td>5</td>
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<tr>
<td>3/Germany, United States, and Turkey</td>
<td>42-65</td>
<td>M and F</td>
<td>Negative</td>
<td>Extra-adrenal, retroperitoneal, multiple; carotid; and vagal</td>
<td>1-2</td>
<td>c.1799G&gt;A</td>
<td>4</td>
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<tr>
<td>1/Poland</td>
<td>39</td>
<td>M</td>
<td>Negative</td>
<td>Adrenal, unilateral</td>
<td>1</td>
<td>c.1979C&gt;G</td>
<td>3</td>
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<tr>
<td>TMEM127</td>
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<td>4/United States and Germany</td>
<td>35-58</td>
<td>M and F</td>
<td>Negative and positive</td>
<td>Adrenal, unilateral</td>
<td>0-1</td>
<td>c.3G&gt;A</td>
<td>5</td>
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<tr>
<td>1/Germany</td>
<td>68</td>
<td>M</td>
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<td>Adrenal, unilateral</td>
<td>0</td>
<td>c.73A&gt;T</td>
<td>5</td>
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<tr>
<td>1/Poland</td>
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<td>F</td>
<td>Negative</td>
<td>Adrenal, bilateral</td>
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<td>c.131T&gt;G</td>
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<td>1/Germany</td>
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<td>c.215T&gt;A</td>
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<td>Carotid</td>
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<td>c.325T&gt;C</td>
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<tr>
<td>1/Germany</td>
<td>45</td>
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<td>c.410-1G&gt;C</td>
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<td>Extra-adrenal, retroperitoneal</td>
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<td>c.413T&gt;G</td>
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<tr>
<td>1/Hungary</td>
<td>22</td>
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<td>c.419G&gt;A</td>
<td>5</td>
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<tr>
<td>1/Germany</td>
<td>76</td>
<td>M</td>
<td>Negative</td>
<td>Adrenal, unilateral</td>
<td>0</td>
<td>c.462C&gt;G</td>
<td>4</td>
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<tr>
<td>1/Hungary</td>
<td>51</td>
<td>F</td>
<td>Negative</td>
<td>Adrenal, bilateral, carotid, malignant</td>
<td>3</td>
<td>c.464T&gt;A</td>
<td>5</td>
</tr>
<tr>
<td>1/Germany</td>
<td>26</td>
<td>F</td>
<td>Negative</td>
<td>Adrenal, unilateral</td>
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<td>c.518T&gt;C</td>
<td>4</td>
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<tr>
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<td>Adrenal, unilateral</td>
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<td>c.532dup</td>
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<td>Positive</td>
<td>Adrenal, bilateral</td>
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<td>c.543,555dup</td>
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</tr>
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<td>2/Turkey and Hungary</td>
<td>26 and 47</td>
<td>F</td>
<td>Negative</td>
<td>Adrenal, unilateral; adrenal, bilateral</td>
<td>1</td>
<td>c.572del</td>
<td>5</td>
</tr>
<tr>
<td>1/Sweden</td>
<td>55</td>
<td>F</td>
<td>Negative</td>
<td>Adrenal, unilateral</td>
<td>0</td>
<td>c.665C&gt;T</td>
<td>3</td>
</tr>
</tbody>
</table>

(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* | Nucleotide Change | ACMG Variant Class^a | SDHA | TMEM127 | MAX | SDHAF2
--- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---
MAX
1/Germany | 36 | F | Negative | Adrenal, unilateral | 1 | c.73C>T | 5 | SDHA | TMEM127 | MAX | SDHAF2
1/Germany | 23 | F | Negative | Adrenal, unilateral | 1 | c.146C>G | 5 | SDHA | TMEM127 | MAX | SDHAF2
3/Germany and Poland | 32-38 | M and F | Negative and positive | Adrenal, unilateral; adrenal bilateral | 1-3 | c.223C>T | 5 | SDHA | TMEM127 | MAX | SDHAF2
1/Germany | 50 | F | Positive | Adrenal, bilateral | 2 | c.242,243del | 5 | SDHA | TMEM127 | MAX | SDHAF2
1/Germany | 21 | M | Negative | Adrenal, unilateral | 1 | c.292dup | 5 | SDHA | TMEM127 | MAX | SDHAF2
1/Germany | 26 | M | Negative | Adrenal, bilateral | 2 | c.307G>T | 5 | SDHA | TMEM127 | MAX | SDHAF2
SDHAF2
1/United States | 25/ | F | Positive | Carotid, vagal | 3 | c.232G>A | 5 | SDHA | TMEM127 | MAX | SDHAF2

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

p Suggesting Heritability: 1, likely pathogenic; 2, probably pathogenic; 3, variant of unknown significance; 4, likely benign; 5, benign.

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 | SDHA (n = 38) | TMEM127 (n = 29) | MAX (n = 11) | SDHAF2 (n = 1)
--- | --- | --- | --- | ---
Family history^b | 1/29 (3) [0-20] | 2/20 (10) [2-33] | 2/8 (25) [5-64] | 1/1 (100)
>1 | 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^c | 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 | 4/14 (31) [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 Indicates index patients carrying an SDHA (n = 29), TMEM127 (n = 20), MAX (n = 8), or SDHAF2 (n = 1) germline mutation.

^b 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 familial 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 (1 in 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.
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.5, 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 adenreal tumors, for which MAX and TMEM127 mutation carriers are at highest risk, is bilateral adrenalec-toomy 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 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 hydratase (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 (KIF1Bβ) 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.29,40 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 mutation 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).

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 (SDHAF2) 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.
ARTICLE INFORMATION

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Author Affiliations: Department of Medicine II, Freiburg University Medical Center, Albert-Ludwigs University, Freiburg, Germany (Baust); Veneto Institute of Oncology, Istituto di Ricovero e Cura a Carattere Scientifico, Padova, Italy (Schiavi, Taschini, Barbon, Lanza, Opocher); Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio (Ni, Chen, Marquard); Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden (Landelvä); Department of Surgery, Region Östergötland, Linköping, Sweden (Gimm); Genomic Medicine Institute, Lerner Research Institute and Tausigg Cancer Institute, Cleveland Clinic, Cleveland, Ohio (Eng); Section for Preventive Medicine, University Medical Center, Albert-Ludwigs University, Freiburg, Germany (Neumann).

Author Contributions: Drs Bausch and Schiavi share first authorship. Drs Gimm, Opocher, Eng, and Neumann share senior authorship. Drs Bausch and Neumann had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Bausch, Patoc, Wellner, Reisch, Preuss, Boedeker, Eng.


Drafting of the manuscript: Bausch, Ngeow, Wellner, Meyer, Makay, Boedeker, Gimm, Eng.


Obtained funding: Malinoc, Berisha, Opocher.


Study supervision: Larsson, Boedeker, Rac, Januszewicz, Neumann.

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REFERENCES


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**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 (*EGLIN* [*PHD2*], *EPAS1* [*HIF2A*], *KIF1B*, *MET*, and *SDHAF2*). 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.

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