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Pathogenicity and Penetrance of Germline *SDHA* Variants in Pheochromocytoma and Paraganglioma (PPGL)

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Germline *SDHA* mutations are reported in a minority of pheochromocytoma/paraganglioma (PPGL) cases but are associated with an increased risk of malignancy, leading some to advocate cascade genetic testing and surveillance screening of "at-risk" first-degree relatives. However, such approaches rely on accurate estimates of variant pathogenicity and disease penetrance, which may have been subject to ascertainment and reporting biases, although the recent provision of large population-based DNA sequence data sets may provide a potentially unbiased resource to aid variant interpretation. Thus, the aim of the current study was to evaluate the pathogenicity and penetrance of *SDHA* variants reported in literature-based PPGL cases by comparing their frequency to those occurring in the Genome Aggregation Database (GnomAD) data set, which provides high-quality DNA sequence data on 138,632 individuals. In total, 39 different missense or loss-of-function (LOF) *SDHA* variants were identified in 95 PPGL index cases. Notably, many of the PPGL-associated *SDHA* alleles were observed at an unexpectedly high frequency in the GnomAD cohort, with ~1% and ~0.1% of the background population harboring a rare missense or LOF variant, respectively. Although the pathogenicity of several *SDHA* alleles was supported by significant enrichment in PPGL cases relative to GnomAD controls, calculations of disease penetrance based on allele frequencies in the respective cohorts resulted in much lower estimates than previously reported, ranging from 0.1% to 4.9%. Thus, although this study provides support for the etiological role of *SDHA* in PPGL formation, it suggests that most variant carriers will not manifest PPGLs and are unlikely to benefit from periodic surveillance screening.

Freeform/Key Words: GnomAD, mutation, paraganglioma, penetrance, pheochromocytoma, *SDHA*

Pheochromocytomas and paragangliomas (PPGLs) are highly heritable tumors but display marked genetic heterogeneity such that ~35% of cases harbor germline mutations in one of ≥15 genes [1–4]. Consequently, genetic testing is recommended in all affected patients with PPGLs irrespective of a relevant family history and increasingly relies on comprehensive disease-targeted gene panels employing next-generation sequencing [1]. Indeed, the identification of a germline mutation in one of the PPGL susceptibility genes may not only have important clinical implications for the patient but may also facilitate cascade testing and periodic surveillance of "at-risk" first-degree relatives, although the appropriate implementation of such screening programs relies on accurate estimates of variant pathogenicity and disease penetrance [1].

Germline mutations in components of the succinate dehydrogenase complex are responsible for a significant proportion of nonsyndromic PPGL cases [5–7]. In particular,

Abbreviations: AF, allele frequency; GIST, gastrointestinal stromal tumor; GnomAD, Genome Aggregation Database; LOF, loss of function; PPGL, pheochromocytoma/paraganglioma.

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mutations in *SDHB* and *SDHD* have been established in large numbers of PPGL cases, providing unequivocal evidence of pathogenicity. Furthermore, the evaluation of cohorts of *SDHB* and *SDHD* variant carriers has facilitated reliable estimates of disease penetrance (*i.e.*, ~20% and ~45% by age 60 years for *SDHB* and *SDHD*, respectively), thereby supporting the likely effectiveness of periodic surveillance of "at-risk" individuals [5, 8, 9].

In comparison with *SDHB* and *SDHD*, mutations in the *SDHA* subunit have only relatively recently been described in patients with PPGLs and are reported to occur at a markedly lower frequency [10–13]. Furthermore, the absence of a relevant family history in most cases indicates a reduced disease penetrance [3, 7, 12, 14]. However, patients with PPGLs harboring *SDHA* mutations are also reported to have an increased risk of malignancy, leading several experts to advocate cascade genetic testing in first-degree relatives to facilitate downstream surveillance screening [15, 16]. Such an approach appears to be supported by the recent reporting of large PPGL series in which 3% to 7% of PPGL cases harbored *SDHA* variants, with estimates of disease penetrance in variant carriers ranging from 10% to 30% [16, 17].

Large-scale population-level DNA sequence data sets provide an invaluable resource to investigate the potential causality of germline variants in hereditary monogenic disease [18, 19]. In particular, quantifying the spectrum and frequency of rare-coding variants in the background population provides a potentially unbiased approach to help establish variant pathogenicity and penetrance, and it avoids many of the ascertainment and reporting biases that may have hampered earlier genetic studies. Indeed, these approaches have enabled the reevaluation of genetic variants associated with several monogenic disease phenotypes, including cardiomyopathy, prion disease, and, more recently, hereditary endocrine disorders [19–22].

Thus, in the current study, we aimed to use the Genome Aggregation Database (GnomAD), which provides high-quality genetic data on 138,632 individuals, to evaluate the pathogenicity and penetrance of germline *SDHA* variants reported in prior studies of PPGLs.

1. Materials and Methods

A. Ascertainment of Cases

PubMed was used to identify PPGL cases reported in the literature in association with heterozygous germline *SDHA* variants (up to February 2018). Only apparently unrelated index cases were included in the analysis. Relevant demographic and clinical information was recorded, as was the presence or absence of a relevant family history [*i.e.*, PPGL/gastrointestinal stromal tumor (GIST) or other relevant cancer]. *SDHA* variants were recorded according to the canonical transcript ENST00000264932. Variants reported as benign or likely benign were recorded but excluded from the overall analysis, whereas variants allocated as "variants of uncertain significance" were included in the analysis but identified as such throughout. The overall frequency of germline *SDHA* variants in PPGL cases was estimated from cohorts reporting germline *SDHA* sequencing results for ≥50 individuals.

B. Database Analysis

The GnomAD database provides high-quality variant calls on 138,632 individuals comprising 123,136 exomes and 15,496 genomes. Details of the data set, including sequencing, filtering, and calibration methods, are available at http://gnomad.broadinstitute.org [18]. All *SDHA* missense and loss-of-function (LOF) variants in the GnomAD cohort were identified (accessed July 2017 to January 2018). LOF variants comprised all single-nucleotide variants predicted to result in nonsense amino acid changes or disruption to canonical donor or acceptor splice sites, as well as small insertions and/or deletions (indels) predicting a frameshift in the encoded protein. Rare *SDHA* variants were defined as having an allele frequency

(AF) <0.05% (*i.e.*, affecting ≤1 in 1000 of the population). Each of the GnomAD missense *SDHA* variants (n = 357) was evaluated using the computational tools SIFT (http://sift.jcvi.org), Provean (http://provean.jcvi.org), and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2). The frequency of the literature-based PPGL *SDHA* variants, together with those reported as pathogenic/likely pathogenic in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), was ascertained in the GnomAD data set. Finally, manual visualization of the variant sequences alongside a multiple-sequence alignment file was used to minimize the possibility that *SDHA* variants represented false-positive artifacts from known pseudogenes (*SDHAP1*, *SDHAP2*, *SDHAP3*). Notably, all rare (AF < 0.05%) missense and LOF *SDHA* variants observed in the GnomAD database occurred only in the heterozygous state. Therefore, the number of times each variant was observed was equal to the number of individuals in GnomAD carrying the variant.

C. Comparison of SDHA Variant Frequency in PPGL and GnomAD Cohorts and Estimates of Disease Penetrance

Odds ratios (together with 95% CIs) comparing the frequency of *SDHA* variants between PPGL and GnomAD cohorts were calculated at http://www.hutchon.net/confidor.htm. Estimates of penetrance were based on the Bayes theorem employing the following validated model [23]:

$$P(D|G) = \frac{P(G|D)P(D)}{P(G|D)P(D) + P\left(G|\bar{D}\right)P(\bar{D})}$$

in which D = disease, G = genotype (*i.e.*, variant under study), and $\bar{D}$ = absence of disease. Thus, in this model, the penetrance or lifetime risk of disease given a specific genotype [$P(D \mid G)$] is equal to the genotype frequency in cases [$P(G \mid D)$] multiplied by the baseline lifetime risk of disease [$P(D)$], divided by overall genotype frequency in the cohort, which is the sum of the joint probabilities of the disease genotype frequency in cases [$P(G \mid D)P(D)$] and controls [$P(G|\bar{D})P(\bar{D})$] [23, 24]. However, we modified the approach such that we used allele frequency in place of genotype frequency [20]. CIs were established based on previously reported methods [24], although we adopted a conservative approach using 95% binomial exact CIs for case and control allele frequencies (calculated from the Clopper-Pearson exact method). Thus, the lower bounds for each penetrance CI were derived by using the lower bound for case and upper bound for control allele frequencies, whereas the upper bounds for each penetrance CI were derived from the upper bound for case and lower bound for control allele frequencies [20, 24]. Use of the 95% CI for both case and control allele frequencies ensures the coverage of the CIs for penetrance will be in excess of 95%. Penetrance estimates were established for individual and combined PPGL cohorts using the complete GnomAD data set as well as subpopulations based on the GnomAD and Exome Aggregation Consortium cohorts.

2. Results

A. PPGL-Associated SDHA Mutations

A total of 95 PPGL index cases with rare heterozygous *SDHA* variants were identified with equal sex distribution and mean age of 40 years (range, 15 to 81 years) (Table 1, Supplemental Table 1). Of these, ~50% presented with head and neck paraganglioma, whereas the remainder manifested either pheochromocytoma or extra-adrenal paraganglioma. Notably, only two index cases had a positive family history of either PPGL or GIST, and in each case, this represented a single affected relative. A small number of additional index cases reported a possible relevant family history, reflecting a single first-degree relative affected with a potentially relevant cancer (*e.g.*, renal cell carcinoma), although in several cases, the *SDHA* carrier status of the affected relative was unknown and in one case was confirmed to be negative.

Table 1. Summary of PPGL Index Cases Associated With *SDHA* Variants Reported in Literature

Characteristic	Value
Index cases, No.	95
Age, mean (range), y	40.0 (15–81)
Female/male, No.	48/47
Primary tumor site, No. (%)[a]	
Head and neck PGL	47 (49)
Other PGL[b]	30 (31)
Pheochromocytoma	19 (20)
Family history, No.	
Positive for PPGL/GIST[c]	2
Positive for other relevant tumor types[c,d]	7
SDHA variant type, No. (%)	
LOF[e]	59 (62)
Missense	36 (38)
Unique *SDHA* variants, No.	39

Abbreviation: PGL, paraganglioma.
[a]One patient had both a mediastinal PGL and carotid body tumor, resulting in n = 96 for primary tumor site.
[b]Refers to all extra-adrenal paragangliomas excluding those affecting the head and neck.
[c]In each index case with an apparent positive family history, a single affected family member was reported.
[d]Other relevant tumors include pituitary adenoma and renal cell carcinoma. However, in some instances, the *SDHA* carrier status of the family member was unknown and in at least one case was known to be negative.
[e]LOF variants include single-nucleotide variants resulting in nonsense amino acid change or canonical splice site disruption, as well as insertions and/or deletions (indels) resulting in a frameshift and premature truncation of the encoded protein.

Among the 95 index cases, 39 different germline heterozygous *SDHA* variants were observed. No patients were observed to harbor homozygous or compound heterozygous *SDHA* mutations. Overall, 62% of individuals harbored LOF *SDHA* variants (*i.e.*, nonsense, splice site, or frameshift), with the remainder expressing missense variants. Notably, >40% of all index cases harbored the p.Arg31Ter nonsense mutation, although >50% of these cases were reported from a single series [17]. Several additional recurrent *SDHA* mutations were observed, including both LOF and missense variants, although most individual *SDHA* variants [28/39 (72%)] were observed in only single PPGL cases (Supplemental Fig. 1).

To establish the frequency of germline *SDHA* variants in patients with PPGL, we identified cohorts of >50 PPGL index cases in which complete *SDHA* sequencing was reported (Supplemental Table 2). Six studies were included, representing 1959 PPGL cases [16, 17, 25–28]. In this combined cohort, 3.6% of all patients with PPGLs harbored a heterozygous *SDHA* variant, although the frequency varied markedly between series (range, 0% to 7.6%). When evaluated by tumor site, the highest frequency of *SDHA* variants was observed in individuals with head and neck paraganglioma (overall, 6.3%; range, 0% to 12.1%), whereas the lowest frequency was observed in patients with adrenal pheochromocytoma (overall, 0.9%; range, 0% to 2.1%) (Supplemental Table 2).

B. SDHA Variants in the GnomAD Cohort

A high cumulative frequency of *SDHA* rare coding-region variation was observed in the GnomAD population, with ~1% of individuals in the cohort harboring a rare heterozygous *SDHA* missense variant (*i.e.*, AF <0.05%), whereas strikingly, ~1 in every 1000 individuals carried a heterozygous LOF *SDHA* allele. Notably, when all GnomAD missense *SDHA* variants were evaluated using SIFT, Polyphen2, and Provean computational tools (n = 357), >75% were predicted to be potentially damaging (*i.e.*, by one or more programs), with only ~22% predicted benign by each of SIFT, Polyphen-2, and Provean.

Next, the number of expected individuals at risk for PPGLs due to *SDHA* mutations in the GnomAD cohort was established. Thus, using the upper and lower bounds of PPGL disease

incidence (*i.e.*, 2 to 5/1,000,000/y) and *SDHA* mutation frequency in PPGL cases (*i.e.*, 1% to 7%), a maximum of ~4 cases (range, 0.4 to 3.8) were predicted (Supplemental Table 3). However, when the GnomAD data set was examined for individuals harboring PPGL-associated *SDHA* variants, the number observed was several orders of magnitude higher than predicted, with ~1 in every 750 of the GnomAD cohort carrying a potentially pathogenic variant. In total, 15 of 39 (40%) of the different PPGL-associated *SDHA* alleles were observed in individuals in the GnomAD cohort, of which the p.Arg31Ter occurred at the highest frequency (Fig. 1A). A similar high number of GnomAD individuals harboring LOF variants reported as pathogenic in the ClinVar database were also observed (Fig. 1B). Taken together, the marked excess of individuals with deleterious *SDHA* alleles in the GnomAD cohort indicated either widespread variant misclassification or low disease penetrance.

C. Pathogenicity and Penetrance of SDHA Variants

Having observed the high background frequency of individuals harboring potentially pathogenic *SDHA* variants in the GnomAD population, we next evaluated whether there was a significant excess of *SDHA* variants in the literature-based PPGL disease cohort relative to the GnomAD controls. We initially focused these studies on p.Arg31Ter as this PPGL-associated variant was observed at the highest frequency in both disease and control cohorts (Fig. 1A). Thus, using the combined cohort of 1959 PPGL cases (Supplemental Table 2), a significant excess of the p.Arg31Ter variant was observed in the disease population relative to the GnomAD controls (Supplemental Table 4), whereas a similar excess was demonstrated in individual PPGL cohorts compared with both "Global" and "European" GnomAD populations, although the extent of enrichment varied between series (Supplemental Table 4). Similarly, a marked excess of LOF *SDHA* variants was observed in the PPGL cohorts when evaluated cumulatively, despite the high number of individuals harboring LOF alleles in the GnomAD population (Supplemental Table 4). Thus, together, these studies provide additional strong support for an etiological role for *SDHA* in PPGL formation.

Finally, we established variant-level estimates of disease penetrance using the allele frequencies of the respective *SDHA* variants observed in disease (*i.e.*, literature-based cohort) and control populations (*i.e.*, GnomAD-based cohorts) (Supplemental Tables 5 and 6). Strikingly, the penetrance estimates were very low, with the majority ranging from 0.1% to 2%, although these varied according to the PPGL and control cohorts used (Table 2). For example, the highest estimates of penetrance were observed for the nonsense p.Arg31Ter variant in the PPGL cohort reported by van der Tuin *et al.* [17] (range, 1.7% to 4.9%) (Table 2), although these estimates remain considerably lower than those reported in the literature.

3. Discussion

The successful implementation of clinical genetic testing requires accurate estimates of variant pathogenicity and disease penetrance to provide appropriate management of the patient and wider family. In this study, we combined genetic data from large disease and population-based control cohorts to provide additional insight into the role of the *SDHA* gene in PPGL formation. Most notably, these studies provide support for the role of *SDHA* in PPGL tumorigenesis while simultaneously demonstrating that variants in *SDHA* are likely associated with a much lower disease penetrance than those reported for other components of the succinate dehydrogenase complex (*i.e.*, *SDHB*/*SDHD*). Thus, *SDHA* appears to act as a low-penetrance risk allele for PPGL formation.

Several important features emerged during this study. First, the observation that most PPGL index cases reported no positive family history of PPGLs/GISTs is supportive of the low disease penetrance, although it is noteworthy that several individuals reported family members with other tumor types, including renal cell carcinoma, in which *SDHA* has been implicated [7]. Thus, future studies should aim to define the full range of tumor phenotypes associated with *SDHA* mutation, which likely extend beyond PPGLs and GISTs. Another

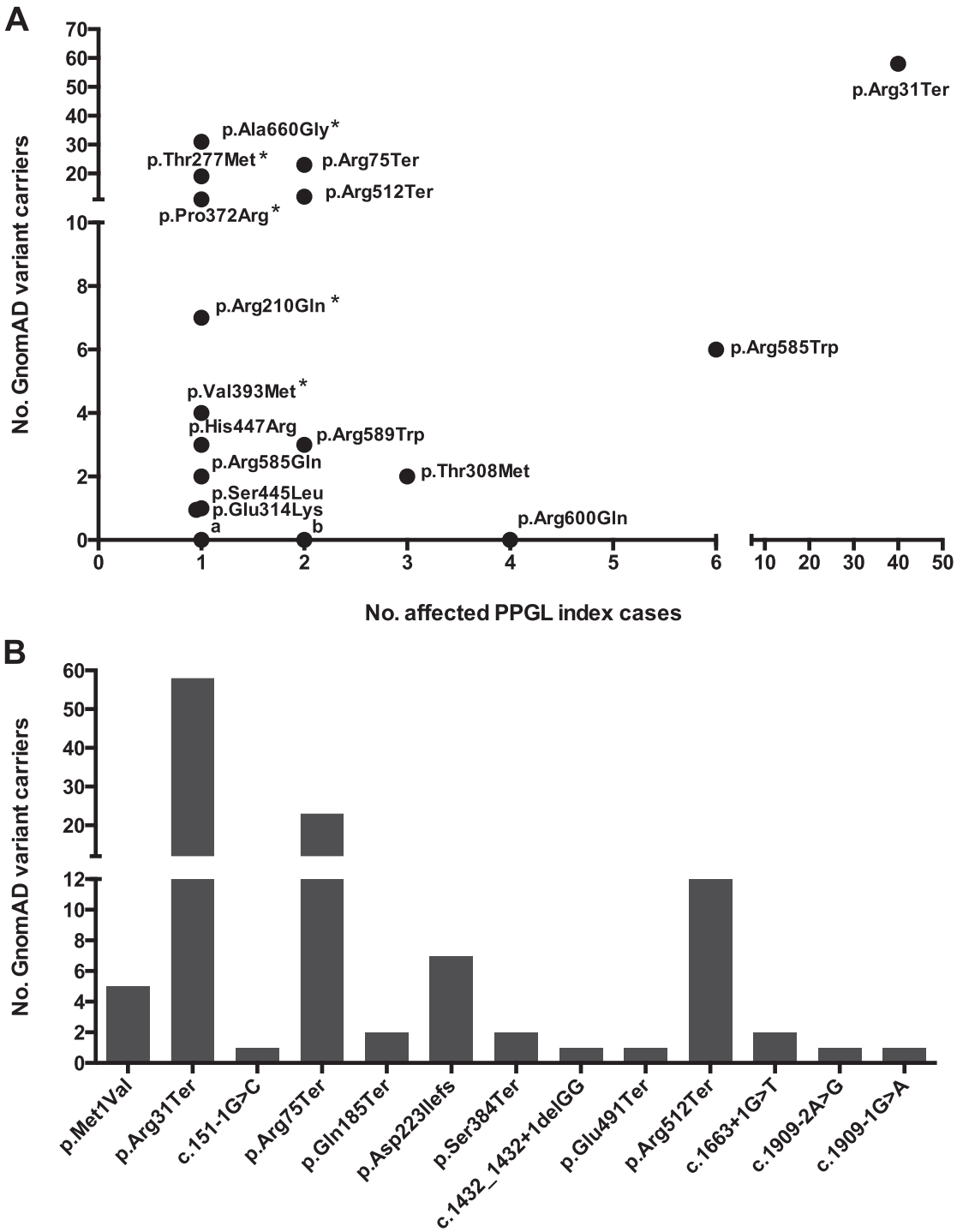

Figure 1. Frequency of PPGL-associated *SDHA* variants in the GnomAD cohort. (A) Of the 39 individual *SDHA* variants reported in the literature in association with PPGL index cases, 15 (~40%) were observed in the GnomAD database. Strikingly, the p.Arg31Ter variant was observed at the highest frequency in both the PPGL and GnomAD cohorts. Several additional *SDHA* variants were observed recurrently in both disease and control cohorts (*e.g.*, Arg585Trp, pThr308Met, p.Arg75Ter, p.Arg512Ter, p.Arg589Trp). In total, 183 individuals in the GnomAD database harbored one of the literature-associated PPGL-associated *SDHA* variants, representing ~1 in 750 of the GnomAD population. Excluding *SDHA* alleles reported as "variants of uncertain significance" in their original report (marked with an "*"), 111 GnomAD individuals harbored likely causative *SDHA* variants (*i.e.*, ~1 in 1250). In contrast, 24 of the PPGL-associated *SDHA* variants were not observed in the GnomAD

database. Of these, 19 variants were observed in single PPGL index cases (denoted "a"), 4 variants were observed twice (denoted "b"), and the p.Arg600Gln variant was observed in 4 unrelated PPGL cases. (B) All pathogenic or likely pathogenic LOF *SDHA* variants reported in the ClinVar database in association with PPGL/Hereditary Cancer Predisposition were identified and their frequency in the GnomAD cohort evaluated. In total, 33 putative LOF alleles were identified, of which 13 were observed in the GnomAD database (shown above). Overall, 116 individuals in the GnomAD cohort harbored one of the LOF *SDHA* alleles, equating to ~1 in 1200 of the cohort. All PPGL-associated variants were observed in the heterozygous state and are described relative to the canonical transcript ENST00000264932.

striking feature of the current study was the high frequency of the p.Arg31Ter variant, which was observed in a disproportionate number of PPGL cases relative to other LOF alleles. Although this may partly reflect the makeup of the specific populations under study (*i.e.*, a high number of p.Arg31Ter cases from the Netherlands), it suggests there may be variant-specific factors that increase tumor risk (*e.g.*, *cis*-acting genetic elements not captured in the current study or influences of the truncating variant on expression of the wild-type allele).

The current analysis only allowed penetrance estimates for *SDHA* variants observed in both disease and control cohorts, with the most reliable estimates (*i.e.*, narrow CIs) obtained for those observed multiple times. Thus, although 15 of 39 unique PPGL-associated SDHA variants occurred in the GnomAD population, the remaining 24 variants were not observed. However, ~80% (19/24) of these variants were observed in single patients with PPGLs, and the appropriate interpretation of such variants remains challenging. For example, ~50% of the different nonsynonymous *SDHA* variants observed in the GnomAD population occurred in single individuals, and consequently, it may not be possible to distinguish disease-causing mutations from those very rare "background" coding variants identified incidentally. In this regard, missense variants present a particular challenge, and it is notable that a high number of such variants were observed in PPGL index cases, frequently affecting single individuals. Thus, the high background frequency of rare missense *SDHA* variants observed in GnomAD indicates that some of the PPGL-associated missense variants may have been susceptible to misclassification. Furthermore, we demonstrate the limited specificity of the computational tools frequently used in support of variant pathogenicity, with most GnomAD missense *SDHA* variants predicted to be deleterious by at least one of the prediction programs. Thus, any high-volume genetic testing for *SDHA* should anticipate the identification of rare missense *SDHA* variants, including those not previously observed in control cohorts, which will remain problematic for interpretation.

There are several potential limitations to the current study. The estimates of penetrance rely on accurate variant allele frequencies in both disease and control cohorts. In the current study, we ascertained the *SDHA* allele frequency in PPGL cases using both individual and combined PPGL cohorts. However, several of the larger cohorts excluded individuals with mutations in more common PPGL-associated genes [16, 17], which in turn will overstate the frequency of *SDHA* variants in unselected PPGL cases (*i.e.*, the true denominator will be underrepresented). Furthermore, it is also possible that these cohorts included PPGL cases in which a genetic diagnosis was considered more likely. Thus, each of these potential limitations will likely overestimate the *SDHA* mutation frequency in cases, and as a consequence, our low estimates of disease penetrance may in fact be overstated. In the future, accurate estimates of *SDHA* mutation frequency in PPGL cases will require the systematic sequencing of large unselected PPGL cohorts. Similarly, for accurate estimates of penetrance, it is necessary that disease and control cohorts are closely matched in terms of population stratification. To address this potential issue, we established allele frequencies not only for the complete GnomAD population but also for additional control cohorts selected to act as suitable comparators (*e.g.*, "GnomAD European" population). Likewise, to ensure our results were not confounded by the unintentional enrichment for relevant disease phenotypes within the control cohort, we evaluated subpopulations in which individuals with known cancers

Table 2. Penetrance Estimates for Recurrent PPGL-Associated *SDHA* Variants

	SDHA Variant Penetrance (%)				Cumulative LOF SNV Penetrance (%)[a]
	Arg31Ter	Arg75Ter	Arg512Ter	Arg585Trp[b]	
Combined PPGL cohort[c] (n = 1959)					
vs GnomAD global	0.90 (0.47–1.69)	0.15 (0.01–0.86)	0.15 (0.00–1.59)	0.58 (0.03–5.47)	0.64 (0.39–1.01)
vs GnomAD European[d]	0.46 (0.24–0.88)	0.09 (0.01–0.58)	0.08 (0.00–0.92)	0.40 (0.02–5.07)	0.39 (0.23–0.63)
vs GnomAD Genomes[e]	0.59 (0.21–1.73)	0.20 (0.01–5.56)	0.05 (0.00–0.10)	0.39 (0.00–36.0)	0.47 (0.22–1.05)
vs ExAC non-TCGA[f]	1.34 (0.55–3.34)	0.07 (0.01–0.43)	0.11 (0.00–1.68)	0.45 (0.02–7.33)	0.60 (0.33–1.06)
Bausch *et al.* (16) PPGL cohort (n = 972)					
vs GnomAD global	0.31 (0.08–0.93)	0.30 (0.02–1.72)	—	—	0.34 (0.15–0.70)
vs GnomAD European	0.16 (0.03–0.48)	0.19 (0.01–1.17)	—	—	0.20 (0.09–0.44)
vs GnomAD Genomes	0.20 (0.03–0.96)	0.40 (0.01–10.6)	—	—	0.25 (0.08–0.72)
vs ExAC non-TCGA	0.45 (0.09–1.86)	0.14 (0.01–0.85)	—	—	0.31 (0.12–0.73)
van der Tuin *et al.* (17) PPGL cohort (n = 393)					
vs GnomAD global	3.38 (1.69–6.41)	—	0.72 (0.01–7.43)	2.85 (0.16–22.3)	1.77 (0.99–3.05)
vs GnomAD European	1.74 (0.86–3.43)	—	0.40 (0.01–4.44)	1.97 (0.09–21.0)	1.08 (0.58–1.92)
vs GnomAD Genomes	2.21 (0.78–6.58)	—	0.25 (0.00–4.78)	1.93 (0.04–73.9)	1.33 (0.56–3.15)
vs ExAC non-TCGA	4.9 (1.96–12.1)	—	0.56 (0.01–7.86)	2.20 (0.09–28.2)	1.66 (0.83–3.18)
vs van der Tuin *et al.* control cohort[g]	1.91 (0.57–7.34)	—	—	—	—

Penetrance estimates are expressed as percent (95% CI). The methods for calculating penetrance estimates together with the respective CIs are described in the Materials and Methods. Baseline lifetime risk of PPGL was estimated to be 0.025% (*i.e.*, 1/4000) based on a midrange incidence estimate of 3 to 3.5/1,000,000 and a ~80-year window of disease susceptibility. Additional details are provided in the footnotes to Supplemental Table 3. The absence of a penetrance estimate (marked —) indicates the absence of the *SDHA* variant in the respective case and/or control cohort or insufficient information to establish control allele frequencies.

[a]The LOF single-nucleotide variant (SNV) penetrance estimate accounts for the cumulative frequencies of all nonsense and canonical splice site *SDHA* variants in the respective disease and control cohorts.

[b]The CIs associated with the penetrance estimates for the Arg585Trp variant are noted to be very wide. Notably, this variant was associated with very low variant allele counts (*i.e.*, one or two) in disease and/or control subpopulations, giving rise to large 95% binomial exact CIs for the respective case and control allele frequencies.

[c]Combined cohort as described in Supplemental Tables 2 and 5.

[d]GnomAD European cohort selected to represent most suitable comparator group as each of the combined and individual PPGL cohorts included individuals of predominantly European origin.

[e]GnomAD Genomes cohort was used to reduce the potential for any confounding from the inclusion of samples for The Cancer Genome Atlas (TCGA). Although GnomAD contains 7208 samples from the TCGA database, none are represented by the 15,496 individuals in whom whole-genome sequencing was undertaken (personal correspondence from GnomAD curators).

[f]Exome Aggregation Consortium (ExAC) non-TCGA cohort provides an alternative comparator group in which all TCGA samples (n = 7601) have been removed from the ExAC population, leaving a remaining cohort of 53,105. This was used to establish population allele frequencies with reduced susceptibility to confounding from the inclusion germline samples from individuals with cancer.

[g]The series reported by van der Tuin *et al.* (17) reported an "in-house" whole-exome control population in which the frequency of the Arg31Ter variant was established. However, no data were provided on other LOF alleles to allow additional allele frequencies to be established.

were excluded, and reassuringly, the penetrance estimates based on these groups did not differ markedly from the larger cohorts.

In summary, these studies support a clear etiological role for *SDHA* in PPGL tumorigenesis while simultaneously indicating that most pathogenic *SDHA* alleles are associated with very low disease penetrance. These studies suggest that undertaking predictive testing in first-degree relatives with subsequent clinical, biochemical, and radiological surveillance in variant carriers is unlikely to provide an effective strategy for PPGL detection. Thus, based on these population-level genetic data, testing of asymptomatic first-degree relatives is not currently recommended. However, it is likely that future large-scale sequencing projects, coupled with detailed phenotype data, will more accurately elucidate the risks in variant

carriers. Furthermore, identifying the potential genetic and/or environmental factors that influence disease expression should be a priority for future studies.

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