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Bayesian approach to determining penetrance of pathogenic SDH variants

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ABSTRACT

Background Until recently, determining penetrance required large observational cohort studies. Data from the Exome Aggregate Consortium (ExAC) allows a Bayesian approach to calculate penetrance, in that population frequencies of pathogenic germline variants should be inversely proportional to their penetrance for disease. We tested this hypothesis using data from two cohorts for succinate dehydrogenase subunits A, B and C (*SDHA–C*) genetic variants associated with hereditary pheochromocytoma/paraganglioma (PC/PGL).

Methods Two cohorts were 575 unrelated Australian subjects and 1240 unrelated UK subjects, respectively, with PC/PGL in whom genetic testing had been performed. Penetrance of pathogenic *SDHA–C* variants was calculated by comparing allelic frequencies in cases versus controls from ExAC (removing those variants contributed by The Cancer Genome Atlas).

Results Pathogenic *SDHA–C* variants were identified in 106 subjects (18.4%) in cohort 1 and 317 subjects (25.6%) in cohort 2. Of 94 different pathogenic variants from both cohorts (seven in *SDHA*, 75 in *SDHB* and 12 in *SDHC*), 13 are reported in ExAC (two in *SDHA*, nine in *SDHB* and two in *SDHC*) accounting for 21% of subjects with *SDHA–C* variants. Combining data from both cohorts, estimated lifetime disease penetrance was 22.0% (95% CI 15.2% to 30.9%) for *SDHB* variants, 8.3% (95% CI 3.5% to 18.5%) for *SDHC* variants and 1.7% (95% CI 0.8% to 3.8%) for *SDHA* variants.

Conclusion Pathogenic variants in *SDHB* are more penetrant than those in *SDHC* and *SDHA*. Our findings have important implications for counselling and surveillance of subjects carrying these pathogenic variants.

subunits B, C, D (*SDHB*, *SDHC*, *SDHD*) and *SDHAF2*. The high heritability of PC/PGL strongly suggests that germline genetic testing be considered for all affected individuals, enabling predictive genetic testing for at-risk relatives if a pathogenic variant is detected.^{6,7}

Germline mutations in the *SDH* genes are the most common genetic cause of PC/PGLs (MIM:168000,605373,115310 and 614165), occurring in approximately 15% of cases.^{1–3} By comparison, the next most common associated genes are *VHL* (4%–10%), *RET* (1%–5%) and *NF1* (1%–5%). *VHL* and *NF1* are associated with Von Hippel Lindau (MIM:193300) and neurofibromatosis type 1 (MIM:162200), respectively, that include PC/PGL as part of a broader syndrome.

Since PC/PGL are rare tumours,⁸ not only should pathogenic variants be individually extremely rare but their cumulative frequency within disease-associated genes (if fully penetrant) should be <0.0001 in large population cohorts (ie, population prevalence of 1/5000 with allele frequency of 1/10 000 for autosomal dominant disease). Penetrance estimates from cohort studies varies considerably for each gene, ranging from ~3% for *NF1* mutations to 90% for *SDHD* mutations.^{6,7} As a corollary of the observed imperfect penetrance, the frequency of potentially pathogenic variants within the population should be higher than the empiric figure presented above. It is also appreciated that individual pathogenic variants in the same gene may have differing functional impact and hence penetrance, a good example being the *BRCA1* c.5096G>A (p.R1699Q) variant having moderate penetrance as opposed to the highly penetrant c.5095C>T (p.R1699W).⁹ This variable penetrance complicates genetic counselling.

Penetrance for *SDHx* variants has been somewhat controversial. Initial estimates suggested penetrance for *SDHB* variants of between 45% and 77% at ages 40–60 years,^{10–12} likely inflated, however, by inclusion of index cases. Subsequent analyses excluding index cases have suggested a much lower lifetime penetrance for *SDHB* variants of 22%–30%,^{11,13,14} although Rijken *et al*¹⁵ have reported penetrance for *SDHB* variants of 42.1% (34.8%–49.5%) at 70 years and Jochmanova *et al*¹⁶ reported penetrance

INTRODUCTION

Pheochromocytomas (PCs, tumours of the adrenal medulla) and paragangliomas (PGLs, tumours of sympathetic or parasympathetic ganglia) are highly heritable, with 14 PC/PGL susceptibility genes identified.^{1–3} Six of these genes were included in the American College of Medical Genetics recommendations for mandated reporting of incidental findings from clinical exome and genome sequencing^{4,5}: *VHL*, *RET*, succinate dehydrogenase



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of 49.80% (95% CI 29 to 74.9) at 85 years. Family-based penetrance studies in *SDHB* kindreds have suggested penetrance of 26%–35% by age 50 years.^{17–19} A large number of possible confounders might explain these differences, including referral bias, intensity of carrier screening, genotype–phenotype correlation or other genetic and/or environmental modifiers. A recent study of a relatively small number of *SDHA* variants reported penetrance of 39% at 40 years, but significantly less (13%) when index cases were removed.²⁰ Penetrance for *SDHC* variants is as yet unknown.

An elegant approach to estimating penetrance of pathogenic variants was recently proposed by Vassos *et al*²¹ and extended by Minikel *et al*²² and Stessman *et al*,²³ using an algorithm that compares variant allelic frequency in disease cases to its frequency in large population control cohorts such as the Exome Aggregate Consortium (ExAC), and accounting for known disease prevalence and proportion of hereditary cases for that disease.

In this study, we tested the hypothesis that allelic frequencies for pathogenic *SDHA*–*C* variants present in ExAC would be inversely proportional to their penetrance, using two cohorts of PC/PGL subjects in whom genetic testing had been performed. We excluded *SDHD* variants from this analysis since this gene is unsuited to Bayesian methodology due to imprinting and suboptimal coverage in whole exome sequencing.

METHODS

Clinical methods

Australian patients with PC/PGL referred to the Cancer Genetics Diagnostic Laboratory, Royal North Shore Hospital, were tested for *RET*, *VHL*, *SDHA*, *SDHB*, *SDHC* and *SDHD* according to previously published methodology.^{24–26} Genetic testing was triaged initially by an in-house protocol and more recently according to PC/PGL Clinical Practice Guidelines,⁶ with the additional use of tumour *SDHB* immunohistochemistry²⁷ to guide testing of *SDH*

subunit genes. Testing of a sample was performed iteratively and stopped when a variant was identified and considered to be pathogenic/likely pathogenic (P/LP) by one of the following criteria: (1) the variant was described as P/LP in a disease-specific database (ARUP, Leiden Open Variation Database (LOVD) or ClinVar); (2) null variant or missense variant predicted to be damaging or deleterious by at least two in silico tools and a functional study to support damaging effect (eg, in the case of *SDHx* variants, loss of *SDHB* immunostaining in tumour) or (3) the variant was present in multiple affected family members. UK patients were analysed for *SDHB/SDHC/SDHD/VHL* mutations by Sanger sequencing (until 2012) and then mostly by a next generation sequencing assay of *MAX*, *RET*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *TMEM127* or *VHL*.²⁸ *SDHB* and *SDHC* sequence variants were classified as P/LP/benign/variants of uncertain significance by the reporting diagnostic laboratory. The GenBank Accession numbers were as follows: for *SDHA* NG_012339.1, NM_004168.3; for *SDHB* NG_012340.1, NM_003000.2 and for *SDHC* NG_012767.1, NM_003001.3.

Comparison of allele frequencies (table 1) between the Cohort Aus and Cohort UK was performed by G-test of Independence in *DescTools* R package V.0.99.24.

Northern Sydney Local Health District Human Research Ethics Committee (Executive) noted that this project involves the use of existing data for the purpose of publishing figures on the occurrence of pathogenic variants. All subjects had given written informed consent for clinical genetic testing. The data being used are de-identified. Based on this information and in accordance with the National Health and Medical Research Council National Statement 2007—Section 5.1.22, the NSW Supplement to the National Statement—Section 5.1.6 and NSW Health Guideline GL2007_020: Quality Improvement and Ethics Review: A Practice Guide for NSW, this project was assessed as activity not requiring full HREC review.

Table 1 *SDHA*–*C* variants in cases from cohorts 1 (Aus) and 2 (UK) and also present in ExAC

Gene	Variants†	LOVD ID (26)	Number of probands (allele frequencies, %)			Allele frequencies in ExAC (TCGA)	
			Aus (n=575)	UK (n=1240)	Combined, %	Total, %	European (non-Finnish), %
<i>SDHA</i>	c.91C>T, p.Arg31*	SDHA_000013	3 (0.26)	n/a		0.014	0.026
	c.512G>A, p.Arg171His	Novel	1 (0.087)	n/a		0.0009	0
	Non-ExAC variants		5 (0.43)				
	Total		9 (0.78)	n/a	0.78	0.015	0.022
<i>SDHB</i>	c.79C>T, p.Arg27*	SDHB_000150	2 (0.17)	8 (0.32)	0.28	0.001	0
	c.88delC, p.Gln30Argfs*47	SDHB_000017	3 (0.26)	7 (0.28)	0.28	0.0009	0.0018
	c.136C>T, p.Arg46*	SDHB_000021	4 (0.35)	11 (0.44)	0.41	0.0019	0.0018
	c.268C>T, p.Arg90*	SDHB_000001	14 (1.2)	8 (0.32)	0.61	0.001	0.0019
	c.423+1G>A	SDHB_000047	2 (0.17)	3 (0.12)	0.14	0.0009	0.0018
	c.343C>T, p.Arg115*	SDHB_000042	0 (0)	6 (0.24)	0.17	0.0019	0.0037
	c.649C>G, p.Arg217Gly	novel	0 (0)	1 (0.04)	0.028	0.001	0.002
	c.688C>T, p.Arg230Cys	SDHB_000058	0 (0)	1 (0.04)	0.028	0.0009	0.0018
	c.725G>A, p.Arg242His	SDHB_000004	5 (0.43)	3 (0.12)	0.22	0.0028	0.0018
	Non-ExAC variants		60 (5.2)	239 (9.6)	8.20		
	Total		90 (7.8)	287 (11.6)	10.0	0.012	0.014
<i>SDHC</i>	c.77+2dupT	SDHC_000049	0 (0)	1 (0.04)	0.028	0.0009	0.0018
	c.397C>T, p.Arg133*	SDHC_000015	1 (0.087)	5 (0.2)	0.17	0.0028	0.0018
	Non-ExAC variants		6 (0.52)	24 (0.97)	0.83		
Total		7 (0.61)	30 (1.2)	1.0	0.0038	0.0029	

†RefSeq: for *SDHA* NG_012339.1, NM_004168.3; for *SDHB* NG_012340.1, NM_003000.2; for *SDHC* NG_012767.1, NM_003001.3.

ExAC, Exome Aggregate Consortium; SDHx, succinate dehydrogenase subunits A, B and C; TCGA, The Cancer Genome Atlas.

Bold values are totals.

LOVD search method

The LOVD (<http://www.lovd.nl>)²⁹ was manually searched for variants in *SDHA*–*C* subunit genes and retrieved 59 unique variants in *SDHA*, 260 variants in *SDHB* and 66 variants in *SDHC*. Variants common both to LOVD and ExAC (<http://exac.broadinstitute.org>) and absent from The Cancer Genome Consortium (ftp://ftp.broadinstitute.org/pub/ExAC_release/release1/subsets/ExAC_nonTCGA.r1.sites.vcf.gz) were tabulated according to allelic frequency in ExAC.

Penetrance and CI calculation

Bayesian calculation of the conditional probability of disease (penetrance) given the genotype was performed using the following formula²³:

$$P(D|G) = \frac{P(GD) \times P(D)}{P(GD) \times P(D) + P(G\bar{D}) \times (1 - P(D))}$$

where D = disease, G = genotype, and \bar{D} = absence of disease.

The denominator, equivalent to $P(G)$, is the sum of joint probabilities of G with respect to both D and \bar{D} which are mutually exclusive and collectively exhaustive of all possible events.

$P(D|G)$ is the penetrance (the probability of disease given a genotype); $P(GD)$ is the genotype frequency in cases; $P(G\bar{D})$ is the allele frequency in ExAC and $P(D)$ is the general population prevalence for PC/PGL, assumed $\sim 1/3000$.⁸

CI was obtained on the binomial probability as described by Rosenfeld *et al.*³⁰ Upper bound CI for penetrance using upper bound on $P(GD)$ and the lower bound on $P(G\bar{D})$. Lower bound CI for penetrance using lower bound on $P(GD)$ and the upper bound on $P(G\bar{D})$. Data from cases and from ExAC were used to estimate these frequencies.

Figures were plotted by using a modification of Minikel *et al.*,²² and source code is provided online at https://github.com/ericminikel/prnp_penetrance.

RESULTS

Cohort 1 consisted of 575 Australian subjects presenting with PC/PGL for whom genetic testing was performed between 1998 and 2016. Overall, 172 subjects (29.9%) with PC/PGL were diagnosed with a P/LP variant in one of nine genes. P/LP *SDHA*–*C* variants were identified in 106 subjects (nine *SDHA*, 90 *SDHB* and seven *SDHC*). By comparison, P/LP variants in other genes were: 36 *SDHD*, nine *RET*, 15 *VHL*, four *TMEM127*, one *FH* and one *MAX*.

Cohort 2 consisted of 1240 UK subjects presenting with PC/PGL for whom genetic testing was performed between 2001 and 2017.¹⁴ Overall, 446 subjects (36%) with PC/PGL were diagnosed with a P/LP variant in one of nine genes. P/LP *SDHA*–*C* variants were identified in 317 subjects (287 *SDHB* and 30 *SDHC*) and P/LP variants in other genes were 96 *SDHD*, 25 *VHL*, two *RET*, two *FH*, one *TMEM127*, two *MAX* and one *SDHAF2*.

We inferred pathogenicity for each variant from published evidence²⁹ and/or based on segregation or loss of heterozygosity or the absence of protein on immunohistochemistry (table 1, online Supplementary table 1). Criteria for P/LP variants were consistent with standards for the interpretation of sequence variants issued by the American College of Medical Genetics and Genomics (ACMG).³¹

We compared *SDHA*–*C* variants considered P/LP in either cohort against the high confidence variant calls in the ExAC database from which The Cancer Genome Atlas (TCGA) cases

had been removed (obtained from ftp://ftp.broadinstitute.org/pub/ExAC_release/release1/subsets/, 22 June 2017) in order to diminish the risk of confounding by disease inclusion in cases. The allelic frequency of 13 variants that are present in ExAC are shown in table 1 and those that are not reported in ExAC are shown in the online Supplementary table 1. For completeness, we have listed in online Supplementary table 2 all previously reported *SDHA*–*C* variants from ClinVar that are also present in ExAC.

For variants in *SDHB* and *SDHC*, allelic frequencies were not significantly different between Aus and UK cohorts: using G-test of independence, $G=1.2858$, 10 df, $p=0.9995$ and post hoc pairwise G-test found a coefficient value of 0.8897441 between the two cohorts. We therefore combined the two cohorts for subsequent analyses. We note that when all variants (including those not present in ExAC) were considered, *SDHB* variants were collectively more frequent in cohort 2 (23.2%) than in cohort 1 (15.6%) (table 1). This difference was not confined to a particular type of mutation (online Supplementary table 1) and therefore unlikely to be due to any systematic difference in variant detection method. The collective frequency of *SDHC* variants was similar in both cohorts (table 1).

Of these 13 P/LP *SDHx* variants in ExAC, all are individually rare with the exception of *SDHA* variant c.91C>T, p.Arg31* (frequency 1/3036). Although individually rare, when the population frequencies for these variants were combined together (excluding *SDHA* p.Arg31*), the estimated population prevalence of these hereditary PGL syndromes assuming complete penetrance would be 1/6000.

We next applied the principles described in Minikel *et al.*²², using the algorithm described by Stessman *et al.*²³ and with CIs calculated as described by Rosenfeld *et al.*³⁰ to estimate the lifetime penetrance of PC/PGL for *SDHA*–*C* variants taking into account allelic frequencies in our cases versus ExAC controls and estimated population prevalence of these disorders. Our penetrance estimates are shown in figure 1: predicted lifetime penetrance for *SDHB* variants is 22.0% (95% CI 15.2% to 30.9%), for *SDHC* variants 8.3% (95% CI 3.5% to 18.5%) and for *SDHA* variants 1.7% (95% CI 0.8% to 3.8%). Penetrance estimates did not vary significantly by individual allele either in separate cohorts or in the combined analysis (online Supplementary figure 1). Although population stratification is not relevant when considering pathogenic variants causing monogenic disease (in the absence of a founder effect), nevertheless, to account for possible confounding by ethnicity, we also compared allelic frequencies in our cases against the European non-Finnish exome data from ExAC. As shown in the online Supplementary figure 2, exclusion of non-European/Finnish alleles from the control data set did not significantly alter our penetrance estimates although did result in wider CIs due to inclusion of fewer variants (for *SDHA* 1.2%, 95% CI 0.5% to 2.7%; for *SDHB* 20.1%, 95% CI 12.8% to 30.0% and for *SDHC* 10.4%, 95% CI 3.4% to 27.6%).

DISCUSSION

We have systematically addressed the possibility of low penetrance alleles in hereditary PC/PGL syndromes, using a recently described approach for correlating penetrance with allelic frequency.²² Correct assignment of pathogenicity for genetic variants has become an urgent problem facing the clinical genetics community, particularly with increasing use of whole exome/genome sequencing technology.^{31 32} Our study has several notable results: first, P/LP *SDHB*, *SDHC* and *SDHA* variants are

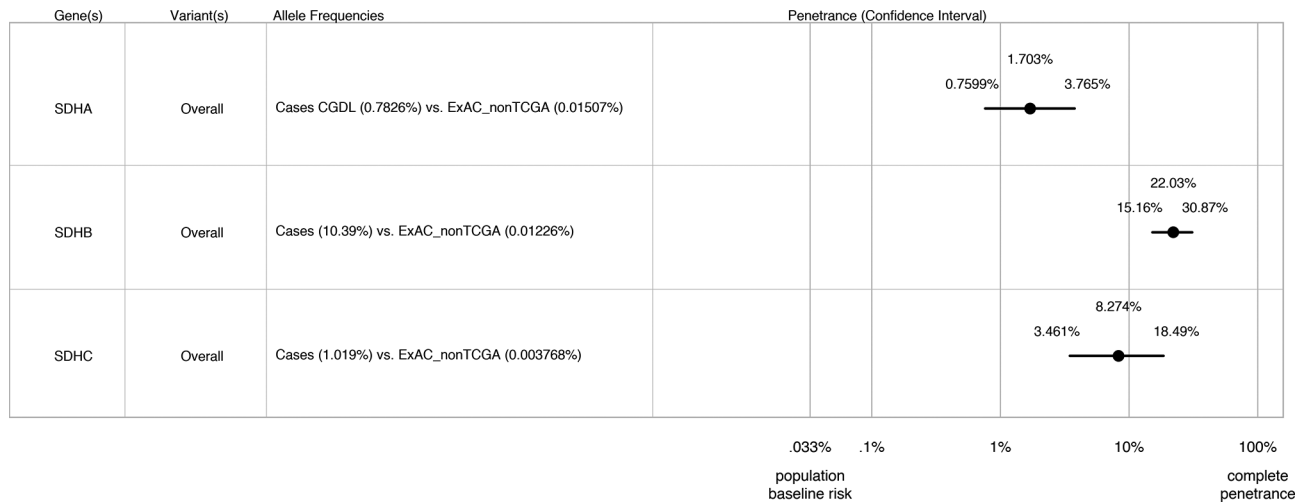


Figure 1 Estimated lifetime penetrance of pheochromocytoma/paraganglioma (PC/PGL) in subjects heterozygous for genetic variants in succinate dehydrogenase subunits A, B and C (*SDHA*, *SDHB* and *SDHC*) from cohorts 1 and 2 combined. The algorithm used to calculate these estimates is based on Minikel *et al*²² and takes into account allelic frequencies in cases versus Exome Aggregate Consortium (ExAC) controls and estimated population prevalence of these disorders.

more common in ExAC than expected; second, our Bayesian estimate of lifetime penetrance for *SDHB* variants is close to empiric data from cohort studies and third, *SDHC* and *SDHA* variants have low penetrance.

Although each are individually rare, the collective frequency of known P/LP *SDHB*, *SDHC* and *SDHA* variants in ExAC was highly surprising and may have several explanations: (1) these hereditary endocrine disorders are more common than previously thought due to the presence either of subpenetrant alleles or incomplete case ascertainment; (2) development of these disorders requires additional genetic modifiers, the absence of which diminishes disease risk in carriers of P/LP alleles; (3) the ExAC database is inadvertently enriched for PC/PGL subjects (unlikely) or (4) that the ExAC database contains sequencing errors (unlikely). On one hand, it is attractive to dismiss these findings as variant calling artefacts present in the ExAC database; however, population frequency estimates for pathogenic *BRCA* mutations inferred in a similar fashion are extremely close to sequencing estimates from a randomly selected Australian patient pool in the Lifehouse study.³³ Again, these estimates and population screening findings are at least twofold higher than previously perceived population estimates. That these variants are more common than expected is non-trivial, if whole exome/genome sequencing is performed at a population level, when apparently healthy subjects carrying so-called pathogenic alleles will outnumber subjects identified on the basis of disease expression³⁴: if indeed present in 0.017% of the population, then ~4000 subjects in Australia and ~11000 subjects in the UK are carrying these P/LP *SDHx* variants.

We deliberately excluded *SDHD* variants from our analysis, since this gene is unsuited to Bayesian methodology due to imprinting and suboptimal coverage in whole exome sequencing. (Only one of 37 different P/LP *SDHD* variants from our cohorts was present in ExAC, data not shown.) Paternally inherited *SDHD* variants are associated with high penetrance of disease³⁵ and would therefore be expected to be rare in the general population.

The finding that *SDHA* c.91C>T, p.Arg31* occurs in ExAC at a population frequency $>10^{-4}$ is at first glance surprising: several reports have shown an association between this variant with either PGLs or gastrointestinal stromal tumours^{36,37}; it is

more frequently reported in PGLs than expected by chance, and bona fide loss of function was inferred from tumoural loss of heterozygosity at this locus and by the absence of *SDHA* assessed by immunohistochemistry.³⁷ However, familial disease appears to be rare in association with this variant²⁰ consistent with low penetrance.

For *SDHB* variants, calculated lifetime penetrance estimates appear close to recent empiric data,¹¹⁻¹⁴ and the lower penetrance estimates for *SDHA* and *SDHC* conform to our anecdotal experience. It is interesting to note that penetrance and risk of multifocal disease seem to be related, that is, ~30% subjects with *SDHB* variants will have more than one PGL or PC, whereas very few subjects with *SDHA* variants develop multifocal disease.²⁰ This deserves further study with larger cohorts of specific genotypes.

Shah *et al*³⁸ recently used whole genome sequence data from 10495 unrelated individuals (with replication in public data from more than 138 000 exomes/genomes in gnomAD) to study population frequency of pathogenic variants in ACMG-recommended 59 gene-condition sets, including *SDHB* and *SDHD*. They found that *SDHB* and *SDHD* P/LP variants were more than 10-fold inflated in the population compared with expected population prevalence of hereditary PC/PGL, and with one possible explanation being that some variants may have been misclassified. The alternate explanation that the inflation is due to incomplete penetrance is supported by our data with some frequent variants being significantly inflated in two clinically ascertained datasets in a consistent manner. Those 13 *SDHx* variants in ExAC that we have observed in our PC/PGL cases all have very strong evidence of pathogenicity in LOVD and/or ClinVar. Indeed, 12 of these variants have a ClinVar star rating of 2 (multiple submitters with assertion criteria). Moreover, nine of these variants are loss-of-function (premature termination or splice site) variants. That these variants are more frequent in the population than expected for the corresponding disease prevalence can only mean either that they are subpenetrant and/or that the disease itself is more common than realised. The fact that our Bayesian estimates for *SDHB* are so close to empiric findings from recent cohort studies¹¹⁻¹⁴ and to family-based studies¹⁷⁻¹⁹ gives us confidence that our estimates for *SDHC* and *SDHA* are also reliable.

Our study has some important limitations. We deliberately chose a validation cohort from a population with close genetic similarity to the discovery cohort,³⁹ and naturally our findings may not apply to populations with different ethnic backgrounds; indeed, it will be interesting to compare allelic frequencies of these variants in populations worldwide. These algorithms may underestimate penetrance for variants not present in ExAC; some studies^{12 14 15} have suggested that certain *SDHB* variants are more penetrant. With respect to using ExAC data as controls, we attempted to minimise confounding by using the data set from which TCGA cases had been removed; it is remotely possible that PC/PGL cases were inadvertently enriched in other cohorts contributing to ExAC (eg, within cardiovascular cohorts). Finally, it is possible that an iterative testing process may have missed combinations of pathogenic variants in two or more genes; although our subsequent experience using massively parallel sequencing approaches suggests that the presence of two germline pathogenic variants is rare (data not shown).

While our manuscript was under review, Maniam *et al*⁴⁰ reported a similar Bayesian approach to calculate penetrance for *SDHA* variants at 0.1%–4.9%, although their study was based on published series of *SDHA* cases rather than as we have done using PPGL case cohorts. Despite these differences in case ascertainment, the similarity of penetrance estimates between the two studies is striking and consistent with our conclusion that pathogenic *SDHA* variants are likely to have low penetrance for disease expression.

We conclude that this approach of using population frequency of suspected P/LP variants in ExAC is extremely useful to validate empiric calculations from cohort studies. Our data suggests that at least for P/LP variants present in ExAC, penetrance is approximately 22% for *SDHB* variants, 8.3% for *SDHC* variants and 1.7% for *SDHA* variants. Our findings will have critical value for genetic counselling and screening of subjects carrying these P/LP variants. By more robust stratification of risk, rational allocation of biochemical and imaging surveillance could reduce both the cost and anxiety associated with carrying a germline mutation.

Electronic database information

- ▶ ExAC Browser, <http://exac.broadinstitute.org>.
- ▶ Mutation Taster, <http://www.mutationtaster.org/>.
- ▶ OMIM, <http://www.omim.org/>.
- ▶ PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>.
- ▶ PROVEAN, <http://provean.jcvi.org>.
- ▶ LOVD, <http://www.lovd.nl/3.0/home>.
- ▶ ClinVar, <https://www.ncbi.nlm.nih.gov/clinvar/>.
- ▶ ARUP, <http://www.arup.utah.edu/database/MEN2/>.

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Contributors MF and RJC-B conceived the study and wrote the manuscript. DEB, TD, CL, ALR, BR and RJC-B were responsible for curating the genetic test results for Cohort 1, and KAA and ERM were responsible for Cohort 2. Additional oversight of the clinical cohorts was provided by JB, AC, AJG, RJH, HM, RS, LT, AT, and KT. DEB, MW and EK confirmed pathogenicity for each variant. DEB, YZ, MF and RJC-B performed the initial analyses, with input from ELD, RWT and AS. All authors had full access to the data, and contributed to review of the manuscript.

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