ORIGINAL ARTICLE

Variant type is associated with disease characteristics in SDHB, SDHC and SDHD-linked phaeochromocytoma–paraganglioma

Jean Pierre Bayley,[1] Birke Bausch,[2] Johannes Adriaan Rijken,[3] Leonie Theresia van Hulsteijn,[4] Jeroen C Jansen,[5] David Ascher,[6] Douglas Eduardo Valente Pires,[7] Frederik J Hes,[8] Erik F Hensen,[5] Eleonora P M Corssmit,[9] Peter Devilee,[10] Hartmut P H Neumann[11]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jmedgenet-2019-106214).

For numbered affiliations see end of article.

Correspondence to
Dr Jean Pierre Bayley, Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands; J.P.L.Bayley@lumc.nl

Received 9 April 2019
Revised 9 July 2019
Accepted 24 July 2019

To cite: Bayley JP, Bausch B, Rijken JA, *et al*. *J Med Genet* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jmedgenet-2019-106214

ABSTRACT

Background Pathogenic germline variants in subunits of succinate dehydrogenase (*SDHB*, *SDHC* and *SDHD*) are broadly associated with disease subtypes of phaeochromocytoma–paraganglioma (PPGL) syndrome. Our objective was to investigate the role of variant type (ie, missense vs truncating) in determining tumour phenotype.

Methods Three independent datasets comprising 950 PPGL and head and neck paraganglioma (HNPGL) patients were analysed for associations of variant type with tumour type and age-related tumour risk. All patients were carriers of pathogenic germline variants in the *SDHB*, *SDHC* or *SDHD* genes.

Results Truncating SDH variants were significantly over-represented in clinical cases compared with missense variants, and carriers of *SDHD* truncating variants had a significantly higher risk for PPGL ($p<0.001$), an earlier age of diagnosis ($p<0.0001$) and a greater risk for PPGL/HNPGL comorbidity compared with carriers of missense variants. Carriers of *SDHB* truncating variants displayed a trend towards increased risk of PPGL, and all three SDH genes showed a trend towards over-representation of missense variants in HNPGL cases. Overall, variant types conferred PPGL risk in the (highest-to-lowest) sequence *SDHB* truncating, *SDHB* missense, *SDHD* truncating and *SDHD* missense, with the opposite pattern apparent for HNPGL ($p<0.001$).

Conclusions *SDHD* truncating variants represent a distinct group, with a clinical phenotype reminiscent of but not identical to *SDHB*. We propose that surveillance and counselling of carriers of *SDHD* should be tailored by variant type. The clinical impact of truncating SDHx variants is distinct from missense variants and suggests that residual SDH protein subunit function determines risk and site of disease.

INTRODUCTION

Pheochromocytomas–paragangliomas show the highest level of heritability (approximately 40%) of any human tumour.[1] Germline variants in genes encoding subunits of succinate dehydrogenase (SDH) are the most important risk factors for phaeochromocytoma and extra-adrenal paraganglioma (PPGL) and for head and neck paraganglioma (HNPGL), explaining around two-thirds of hereditary cases.[2]

SDH is a heterotetramer consisting of two catalytic subunits, SDHA and SDHB, and two membrane-spanning subunits, SDHC and SDHD. SDH plays an important role in both the tricarboxylic acid cycle, in which SDHA and SDHB catalyse the oxidation of succinate to fumarate, and in the mitochondrial respiratory chain, where SDHC and SDHD are involved in further electron transfer to reduce ubiquinone to ubiquinol.

SDH subunits show unexplained disease associations, such as the predominance of *SDHD* and *SDHC* variants in HNPGL[3–5] and the association of *SDHB* variants with PPGL,[6] a higher risk of malignancy and renal cell carcinoma.[5 7] In addition, the SDH genes show wide variation in penetrance, with *SDHB*, *SDHC* and *SDHA* exhibiting low penetrance (<25%), whereas *SDHD* displays very high penetrance (>80%).[8–14] Differences in penetrance may be related to modifying genes located on chromosome 11.[15 16] A puzzling aspect of SDH-related disease is that defects in a single protein complex result in marked differences in both tumour location and clinical phenotype.

Although it has long been known that pathogenic variants in the SDH subunit genes (*SDHB*, *SDHC* and *SDHD*) broadly associate with specific phaeochromocytoma–paraganglioma disease subtypes, further understanding of SDHx genotype–phenotype correlations has remained limited. One group has reported a possible link between a low-PPGL phenotype and a single *SDHD* missense variant, p.(P81L),[17 18] but the outcome of those studies was influenced by a prominent founder effect. A better understanding of variant-specific phenotypes would increase the accuracy of clinical prediction, help in the design of optimal surveillance programmes for asymptomatic carriers and improve the targeting of clinical surveillance in symptomatic carriers.

In this study, we wished to explore whether classes of SDH variants or specific SDH variants might explain or partially explain the puzzling disease associations discussed above. We describe the analysis of variant type (missense vs. truncating) and specific variants in 950 PPGL and/or HNPGL patients carrying germline variants in *SDHB*, *SDHC*

or *SDHD*. In order to discern a possible effect of variant type on disease phenotype (occurrence of either pheochromocytoma or paraganglioma) or on age-related tumour risk, only cases with a phenotype (affected cases) were included. Our data reveal several important and novel genotype–phenotype correlations, point to further, still ill-defined correlations and suggest that, mechanistically, residual SDH protein function profoundly influences disease phenotype.

MATERIALS AND METHODS

Patient cohorts

An overall summary of patient cohorts (Germany (DE), Great Britain (GB) and the Netherlands (NL)), assigned to groups with either missense or truncating variants, can be found in online supplementary table 1. Our DE study population consisted of anonymised cases derived from a population-based registry and comprised 384 affected patients with PPGL and/or HNPGL. Patients who came for diagnosis, treatment or re-evaluation and who consented to participate in scientific research studies were systematically included in this registry. Brief details of this patient dataset, differentiated by SDH gene and variant type, can be found in online supplementary tables 2 and 3. This study population showed a high degree of genetic diversity for both *SDHB* and *SDHD*.

The second study population (GB), recruited in the UK, was in the form of a publicly available anonymised dataset describing disease type and age of diagnosis in individuals referred for *SDHB*/*SDHC*/*SDHD* genetic testing at a National Health Service diagnostic laboratory due to a personal or family history of PPGL/HNPGL, as described by Andrews *et al*.[18] The affected individuals in this dataset were diagnosed by routine clinical investigation. This study population showed a high degree of genetic diversity for *SDHB* and a moderate degree of diversity for *SDHD*. Only affected cases (n=366) were included in our analysis. Our analysis was based on the raw patient data found in the supplementary materials, which showed some minor inconsistencies with the published manuscript. The third dataset consisted of Dutch *SDHD* variant carriers (n=200), all of whom had PPGL and/or HNPGL and were mainly recruited via academic hospitals with special expertise in PPGL/HNPGL. In accordance with the Dutch law, approval of an institutional ethics committee was not required because all data were collected for routine patient care. This dataset has been described previously.[19] Details of this patient dataset, differentiated by SDH gene and variant type, can be found in online supplementary table 4. This study population exhibited a very low degree of genetic diversity, with over 90% of all patients explained by only two founder variants.

For patients included in the datasets assembled in Leiden and Freiburg, a diagnosis of paraganglioma or pheochromocytoma was confirmed by imaging with ultrasonography, CT or MRI of the abdomen, MR/CT of the thorax, MR/CT of the head and neck and/or *meta*-iodobenzylguanidine (MIBG), fluorine 18 (18F) dihydroxyphenylalanine (DOPA) whole-body positron emission tomography (PET) or octreotide imaging. Catecholamines or metabolites including noradrenalin, adrenalin and VMA were determined in the plasma or urine. In patients undergoing surgery, the diagnosis was further confirmed using standard pathological analysis of the surgical specimen. Variant carriers from the study by Andrews *et al*[18] were men and women referred to a National Health Service diagnostic laboratory for SDHB/SDHC/SDHD mutation analysis based on a personal or family history of PPGL/HNPGL. Andrews and colleagues collected clinical information using a standard pro forma or from clinical records, research studies or a service evaluation study.

Genetic analysis

Molecular genetic analysis of SDH variants in the DE dataset was carried out using Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) analysis (SALSA MLPA Kit P226; MRC-Holland, Amsterdam, The Netherlands), and in the other datasets as described.[13 18 19] Genotype–phenotype correlations were analysed in individuals with pathogenic or probable pathogenic variants and likely VUS were excluded from the analysis. The variants found in the DE and NL datasets are listed in online supplementary tables 5–7.

Analysis

In order to discern a possible effect of variant type on disease occurrence and age-related tumour risk, only affected cases were analysed. Some individuals were included in both PPGL and HNPGL groups if affected by both tumour types. For the purposes of this study, we defined variants as 'missense' when predicted to result in a single amino acid change and as 'truncating' if they were predicted to have more profound effects on protein structure or function, including truncating variants, splice variants and small or large deletions. The underlying rationale for this dichotomy is that missense variants may still allow SDH and/or complex II assembly and (residual) protein function, whereas truncating variants can reasonably be expected to preclude full complex assembly and protein function. Using descriptive statistics, we first considered the spectrum of missense versus truncating variants found in patients with PPGL and/or HNPGL, comparing variant numbers and the number of patients attributable to each. As this analysis revealed differences in the overall numbers of each variant type and the numbers of patients attributable to each variant, we quantified these differences and compared the frequencies of missense versus truncating variants found in our patient cohort to those reported by the SDH Leiden Open Variation Database (LOVD) databases (https://databases.lovd.nl/shared/genes/SDHB). We further analysed the individual frequencies of PPGL and HNPGL in SDHB, SDHC and SDHD variant carriers, comparing the percentage occurrence of each tumour type by variant type and by gene. Descriptive statistics were also used to explore comorbidity (defined as the co-occurrence of PPGL and HNPGL), patient numbers per gene/variant type and overall trends in gene variant–disease correlations. We also used Kaplan-Meier analysis to explore age-related trends in disease occurrence by gene and variant type.

Statistical analysis

Differences in tumour occurrence based on variant type were analysed using the χ^2 test or the Cochran-Armitage test where ordering was suspected. Age-related tumour risks were estimated using Kaplan-Meier analysis, together with the log-rank test to compare survival distributions between groups. One minus cumulative survival data were used to analyse differences in age of tumour diagnosis in the various patient subgroups. IBM SPSS Statistics V.20.0 for Windows software package or GraphPad (GraphPad Software, La Jolla, California, USA) was used for statistical analysis. A p value of less than 0.05 was considered statistically significant, after Holm-Bonferroni correction for multiple comparisons (38 tests).[20] Significant differences before correction are indicated with an asterisk.

Table 1 Summary of the three datasets, shown as percentage affected cases per gene and cohort (Germany (DE), Great Britain (GB) and the Netherlands (NL)), assigned to groups with either missense or truncating variants

		PPGL			HNPGL		
SDHB	n=448 carriers	DE	GB		DE	GB	
Missense	% affected	55.2	67.6		48.3	38.9	
Truncating	% affected	70.5	80.4		35.2	25.7	
SDHD	n=447 carriers	DE	GB	NL	DE	GB	NL
Missense	% affected	13.7	4.3	15.7	94.1	97.8	97.9
Truncating	% affected	34.6	45.7	11.1	86.5	71.7	100.0
SDHC	n=55 carriers	DE	GB		DE	GB	
Missense	% affected	0.0	0.0		100.0	100.0	
Truncating	% affected	7.4	30.8		92.6	76.9	

A more detailed overview can be found in online supplementary table 1.
HNPGL, head and neck paraganglioma; PPGL, phaeochromocytoma and extra-adrenal paraganglion; SDH, succinate dehydrogenase.

RESULTS

Patient datasets

Exploring correlations of *SDHB*, *SDHC* and *SDHD* variant type with disease, we analysed a total dataset comprising 950 affected cases. A summary of the three datasets is presented in table 1, and detailed overviews of *SDHB*, *SDHC* and *SDHD* gene variants can be found in online supplementary tables 5–7.

Truncating variants are over-represented among SDHx variants

Collections of clinically reported SDHx variants often show a limited diversity that can be partly attributed to founder effects but additional trends are also apparent. Visual assessment of the combined dataset using pie charts suggested that truncating variants are over-represented compared with missense variants (figure 1A–D), a trend particularly evident for *SDHD*. These differences were significant in the case of both *SDHB* and *SDHD* (figure 1E), a result partly reflected in the LOVD SDH databases (figure 2B).[21]

Truncating variants increase PPGL risk

As the above data suggested that particular variant types may play specific roles in disease, we quantified disease occurrence by gene/variant type. In the case of *SDHB* (figure 2A), truncating variants explain a higher proportion of PPGL cases than missense variants ($p<0.05$), whereas they are less frequent in HNPGL cases (figure 2B).

In the case of *SDHD*, truncating variants explain the vast majority of PPGL cases in both the DE ($p<0.01$) and GB ($p<0.001$) datasets (figure 2C). The opposite trend is apparent for HNPGL (figure 2D). Similar trends are not discernible in the NL dataset, probably due to the low diversity of variants.

SDHC has historically made only a modest contribution to HNPGL/PPGL, with a limited number of variants identified since 2000.[4 21] Nevertheless, PPGL in *SDHC* carriers is entirely explained by truncating variants (figure 2E,F), in a pattern reminiscent of *SDHD*, while the distribution of *SDHC* variants in HNPGL is consistent with *SDHB* and *SDHD*.

Variant type influences age-of-disease onset

Using Kaplan-Meier analysis, carriers of *SDHB* truncating variants (figure 3A) showed a slightly earlier age of PPGL diagnosis, which was significant only precorrection. Although no significant effect was apparent for HNPGL (figure 3B), age-related risk showed the reverse pattern to PPGL.

In the case of *SDHD* (figure 3C), carriers of truncating variants showed a significantly earlier age of PPGL diagnosis ($p<0.0001$). The addition of the NL cases (figure 3D) led to the convergence of missense and truncating carriers at older ages ($p<0.0001$), which has a number of possible explanations (see Discussion). No differences were seen in the case of HNPGL (figure 3E). Despite the small number of cases available, *SDHC* appears to follow a pattern comparable to *SDHD* (figure 3F,G), with no reported PPGL cases among missense carriers.

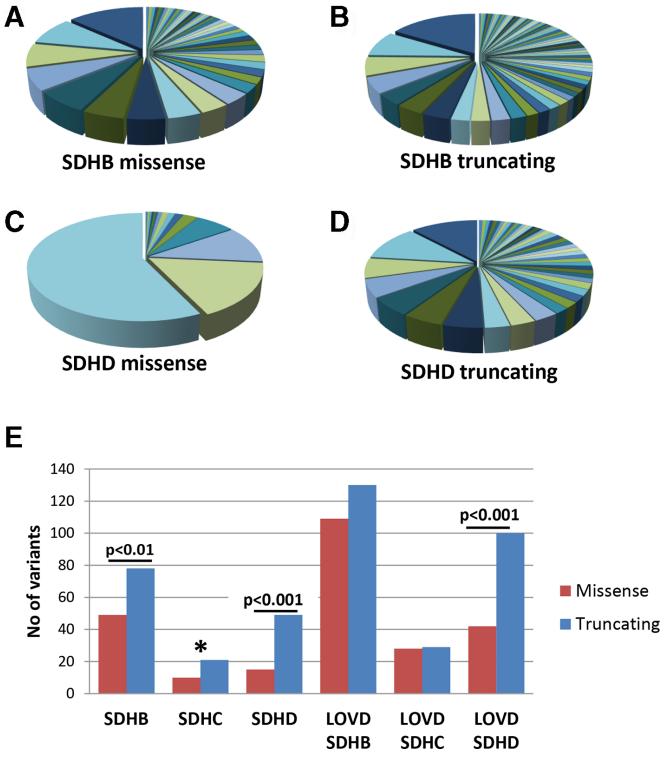

Figure 1 Truncating SDH variants are over-represented in clinical cases. Pie charts illustrating the diversity of *SDHB*/*SDHD* missense and truncating variants in the total cohort, shown as number of carriers per variant: (A) *SDHB* missense (49 variants;195 carriers), (B) *SDHB* truncating (78 variants; 253 carriers), (C) *SDHD* missense (15 variants; 287 carriers), (D) *SDHD* truncating (49 variants;160 carriers); (E) quantification of the total number of clinically reported missense (blue) and truncating (red) variants in the *SDHB*, *SDHC* and *SDHD* genes in the total cohort and in the LOVD SDH databases (https://databases.lovd.nl/shared/genes/SDHB). P values corrected for multiple testing. *Indicates a significant difference before correction. SDH, succinate dehydrogenase.

PPGL/HNPGL comorbidity and number of patients per variant type

We then asked whether variant type influences comorbidity (the co-occurrence of PPGL and HNPGL) in the same patient (figure 4A). Although the overall number of patients with comorbidity was low (*SDHB*, n=25; *SDHD*, n=35), carriers of *SDHD* truncating variants showed markedly elevated comorbidity compared with missense carriers (20% vs 5.2%; $p<0.05$). No difference was seen for *SDHB* variants. Turning to the number of patients per variant type (figure 4B), we found a fivefold difference between *SDHD* missense variants and any other variant type ($p<0.001$). Even after excluding NL founder

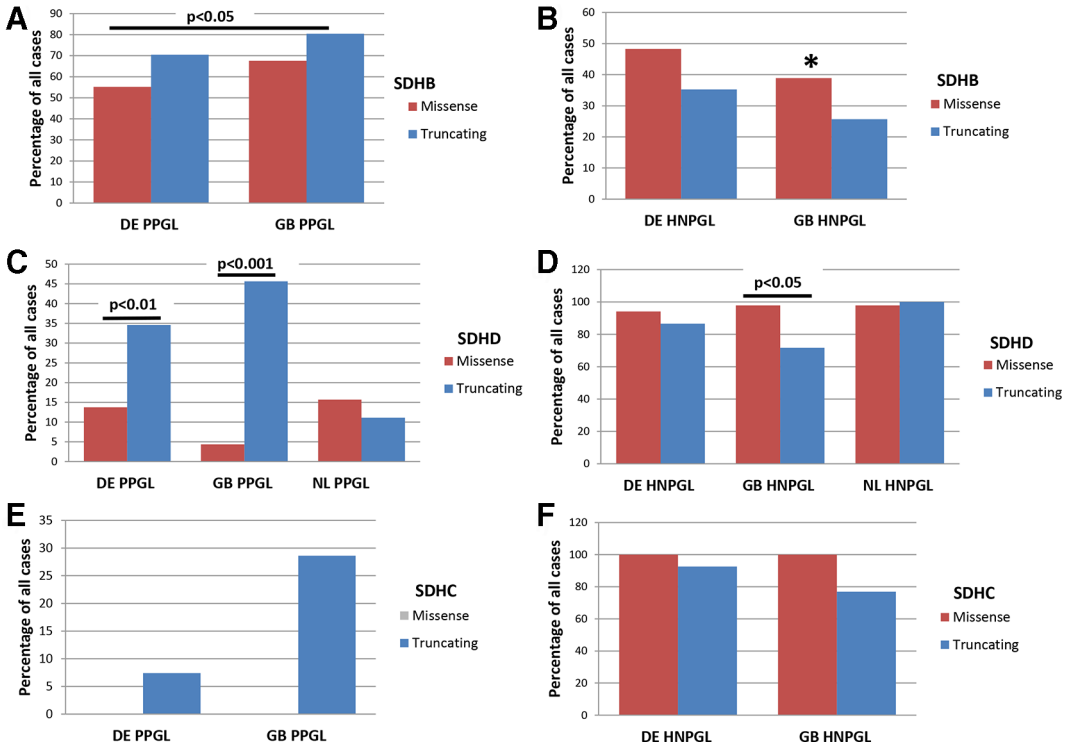

Figure 2 Carriers of missense and truncating SDH variants differ in terms of risk for PPGL and HNPGL: (A) *SDHB* PPGL % affected, (B) *SDHB* HNPGL % affected, (C) *SDHD* PPGL % affected, (D) *SDHB* HNPGL % affected, (E) *SDHC* PPGL % affected, (F) *SDHC* HNPGL % affected. The proportion of affected cases is expressed as a percentage of all affected cases for each gene/variant type category. P values corrected for multiple testing. *Indicates a significant difference before correction. SDH, succinate dehydrogenase.

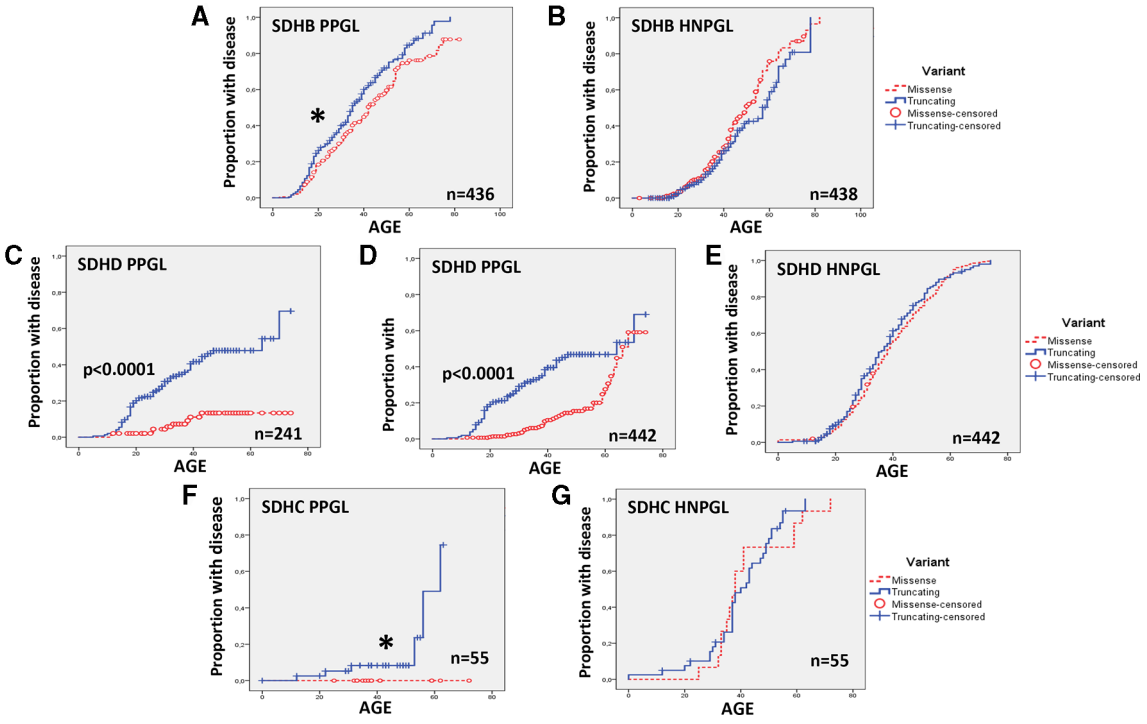

Figure 3 Age of disease onset in carriers of missense and truncating SDH variants: (A) *SDHB* PPGL (DE/GB), (B) *SDHB* HNPGL (DE/GB), (C) *SDHD* PPGL (DE/GB), (D) *SDHD* PPGL – (DE/GB/NL), (E) *SDHD* HNPGL (DE/GB/NL), (F) *SDHC* PPGL (DE/GB) and (G) *SDHC* HNPGL (DE/GB). P values corrected for multiple testing. *Indicates a significant difference before correction. DE, Germany; GB, Great Britain; NL, the Netherlands; SDH, succinate dehydrogenase.

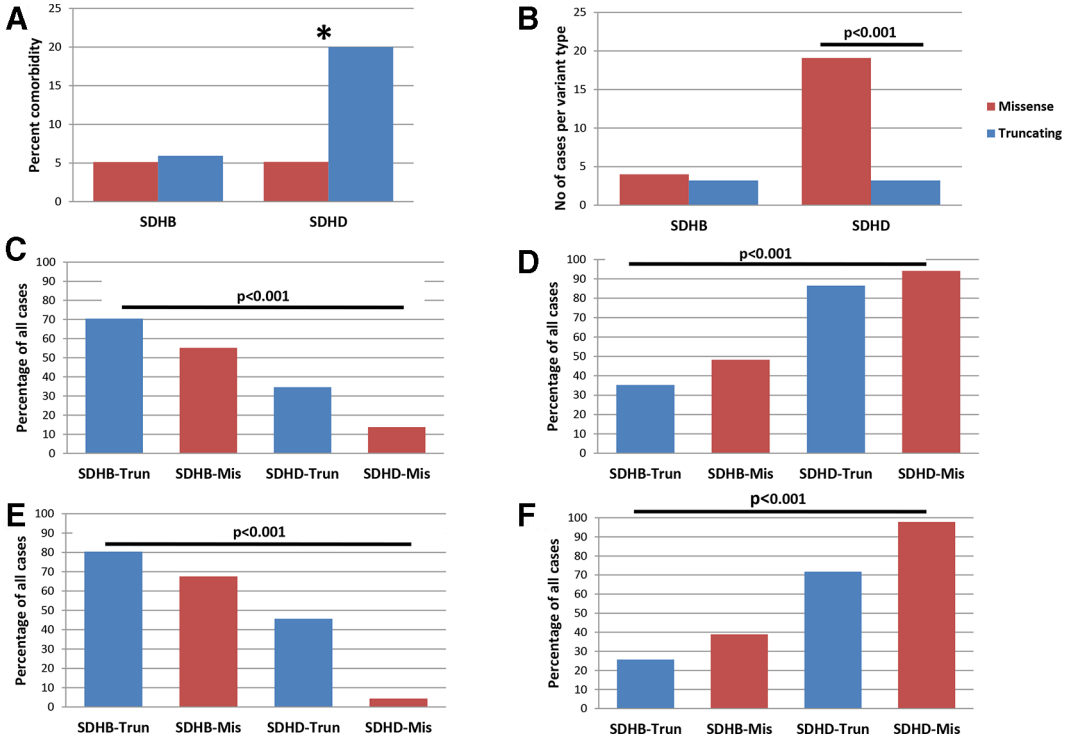

Figure 4 Multifocal disease (patients with both PPGL and HNPGL) in *SDHB* and *SDHD* variant carriers (A). Number of clinically detected cases per variant. (B) Variant type ranked by disease risk: (C) DE PPGL, (D) DE HNPGL, (E) GB PPGL and (F) GB HNPGL. P values corrected for multiple testing. *Indicates a significant difference before correction. DE, Germany; GB, Great Britain; SDH, succinate dehydrogenase.

variants, a threefold difference was still apparent ($p<0.05$; data not shown).

Ranking variant type by disease occurrence

As the above analyses illustrated the impact of variant type on disease, we ranked gene variant types by impact on disease in both the DE and GB datasets and found consistent and opposing patterns for PPGL versus HNPGL. PPGL occurrence took the order (highest to lowest) *SDHB* truncating – *SDHB* missense – *SDHD* truncating – *SDHD* missense (figure 4C,D), whereas the opposite trend was apparent for HNPGL in both datasets (figure 4E,F).

Variant-specific tumour risk

Although it appears that *SDHD* variant type influences tumour risk, due to prominent founder effects (particularly in the missense dataset) it is possible that specific variants act as confounders of apparent differences between missense and truncating variants. In the DE dataset, exclusion of the dominant missense variant p.(Y114C) still led to a clear divergence (figure 5A), although the difference was no longer significant. The divergence between missense and truncating variants was also maintained in the combined DE-GB dataset (figure 5B) after exclusion of all p.(P81L) carriers ($p<0.05$).

Compared with other *SDHD* missense variants (figure 5C), p.(P81L) is associated with a low-PPGL phenotype, as previously reported,[17 18] although due to fewer cases, the effect was only marginally significant ($p=0.045$) before correction. Other variants may also show low PPGL risk. Although insufficient affected carriers were available for most variants, we were able to compare p.(Y114C) ($n=37$) with p.(P81L) ($n=64$). Both of these variants appear to confer a strikingly low-PPGL risk (figure 5D).

DISCUSSION

We found that truncating variants in SDHx genes are clinically over-represented compared with missense variants. As missense changes are the predominant variant type in the human genome (approx. 4:1 compared with all other variants),[22] this imbalance cannot be attributed to general mutational processes. We confirmed the functional significance of this finding by showing that both disease occurrence and age-related tumour risk are influenced by the type of gene variant, profoundly so in the case of *SDHD* and *SDHC* variant carriers. In addition, *SDHB* missense and truncating variants appear to differ in terms of PPGL and perhaps even HNPGL risk, although it is not clear whether differences are mediated by a subtle change in risk across all missense variants or by a specific contribution from a subset of variants.

Patient cohorts

Discerning genotype–phenotype correlations in SDHx cohorts has historically been complicated by the prevalence of founder effects and the scarcity of large, genetically diverse patient populations.[23–30] Although large for a rare disease, our total dataset showed certain disparities. The most prominent was the broad, multicentre nature of patient recruitment in the DE and GB datasets compared with the geographically limited and academic medical centre-based recruitment of patients in the NL dataset.

Do missense *SDHD* variants lead to a later age of onset of PPGL?

An interesting aspect of the NL dataset was the higher frequency and later age of onset of PPGL in *SDHD* missense carriers (figure 3D). Many of these patients, mostly carriers of the Dutch founder variant p.(D92Y), belong to families that were actively

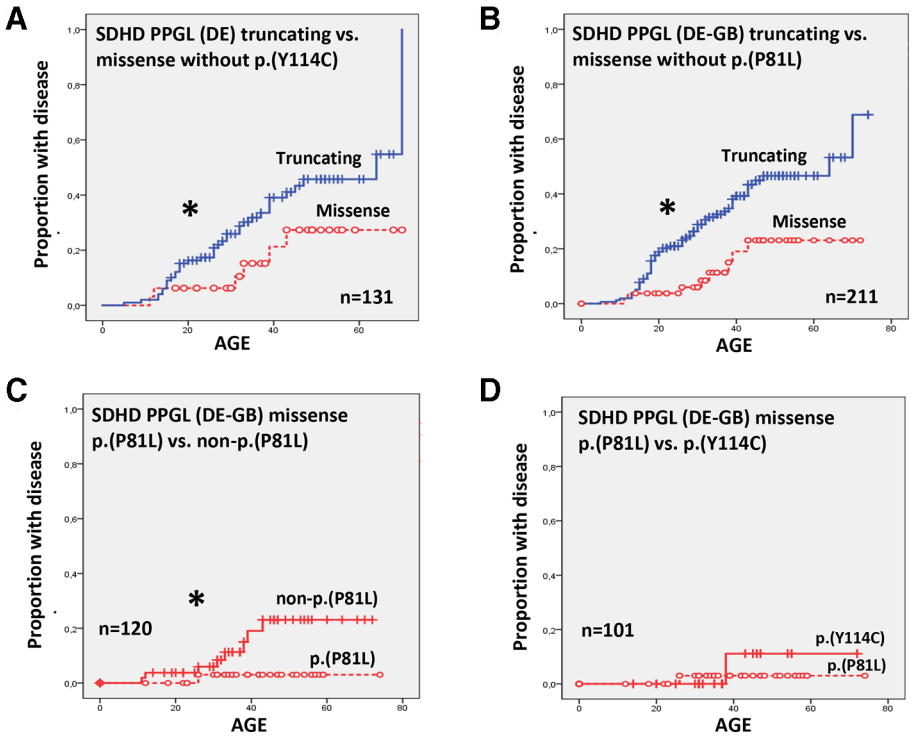

Figure 5 Age of PPGL onset for specific *SDHD* variants: (A) Missense versus truncating variants without p.(Y114C) – DE cohort, (B) Missense versus truncating variants without p.(P81L) – DE–GB cohort, (C) p.(P81L) carriers compared with other non-p.(P81L) missense carriers – DE–GB cohort, (D) p.(P81L) carriers compared with p.(Y114C) carriers – DE-GB cohort. P values corrected for multiple testing. *Indicates a significant difference before correction. DE, Germany; GB, Great Britain; PPGL, phaeochromocytoma and extra-adrenal paraganglioma; SDH, succinate dehydrogenase.

recruited as far back as the 1980s and constitute the cohort in which *SDHD* was originally identified.[3 31] In the intervening decades, these patients have been closely monitored at Leiden University Medical Center. This long-term surveillance may explain apparent differences in PPGL age of onset compared with the DE/GB datasets and, importantly, might thus imply that *SDHD* missense carriers have a propensity to develop PPGL at higher rates and older ages than currently appreciated. However, it is also possible that the p.(D92Y) variant specifically confers an elevated risk for PPGL, but in light of differences in patient recruitment, this question would need to be settled in more comparable patient populations.

PPGL/HNPGL comorbidity

A further confirmation of the profound divergence of *SDHD* missense and truncating variants is seen in the occurrence of PPGL/HNPGL comorbidity. Although cases were relatively rare in our dataset, truncating *SDHD* variants appear to carry an elevated risk for the combined occurrence of PPGL and HNPGL and therefore represent a distinct group within this study.

SDHD missense variants and patient numbers

Although founder variants have been detected in other SDHx genes, *SDHD* missense founder variants are especially prominent and are associated with a primarily HNPGL phenotype and high disease penetrance.[10 18] The *SDHD* founder variants p.(D92Y), p.(P81L), p.(Y114C) and p.(L139P) account for the bulk (>80%) of all patients explained by *SDHD* missense variants in our combined dataset. The large number of patients attributable to these variants may be due to random demographic effects combined with the high penetrance of *SDHD* (87%/70 years).[10]

Individual variants and disease

As previously mentioned, one *SDHD* variant, p.(P81L), has been associated with a low-PPGL phenotype.[17 18] The p.(P81L) phenotype reported by Andrews *et al* was effectively a comparison of missense and truncating *SDHD* variants, due to the overwhelming predominance of the p.(P81L) variant in the GB dataset (38/46 *SDHD* missense carriers). Nevertheless, the reported p.(P81L) low-PPGL phenotype survived comparison with other missense variants in our dataset and suggests that the relatively subtle differences between missense variants in terms of protein function can influence both tumour type and age of diagnosis. The p.(Y114C) variant has been described in detail by Schiavi and colleagues in a study in which only five PPGLs were identified among a total of 262 lesions (1.9%) in 131 affected individuals.[29] Both of these variants therefore show a phenotype that appears to diverge from other *SDHD* missense variants, suggesting that clinically significant differences exist even within a specific gene-variant type.

Clinical implications

The results of our study also have important clinical implications. While our data are not suitable for determining penetrance (i.e., the risk of a variant carrier developing disease) as asymptomatic carriers were not included and index cases excluded, our findings do clearly indicate that carriers of *SDHD* (and likely *SDHC*) truncating variants require closer surveillance than they might currently be receiving. As these carriers have a significantly elevated risk of PPGL compared with carriers of missense variants, they may require more frequent imaging of the thorax and abdomen and regular screening for catecholamines and metabolites compared with current surveillance regimes, depending on

the specific surveillance protocols in use in any given clinical centre.

Underlying biology

An intriguing aspect of our data is that variant type does not broadly elevate general tumour risk but appears to confer specific risks for either PPGL or HNPGL. As variant types can reasonably be expected to impact protein function in different ways depending on their 'severity', we are inclined to explain the differences in phenotype in terms of (loss or relative retention of aspects of) protein function. The influence of variant type and individual variants on phenotype strongly suggests that different variants have a distinct impact on SDH protein function. We therefore speculate that missense variants allow retention of aspects of enzyme activity, with its degree dependant on the individual variant. We further suggest that the cells that give rise to PPGL, the sympathetic paraganglia, thrive in the face of loss of SDH/Complex II protein function, whereas the parasympathetic paraganglia in which HNPGL arises are relatively intolerant of complete loss of SDH protein subunits and are therefore comparatively more tolerant of missense variants. This tissue-specific difference in response to compromise or complete loss of individual SDH subunits may indicate that compromise or loss results in differing rates of enzyme activity, succinate accumulation[32] and/or ROS accumulation[33 34] in the context of tissue-specific sensitivities to these agents. Missense *SDHB* variants appear to confer a slightly higher risk of HNPGL (or specific variants confer this risk), again implying that parasympathetic paraganglia 'prefer' at least residual protein complex assembly and function.

CONCLUSION

In conclusion, variant types show clear correlations with either PPGL or HNPGL. The traditional view in which *SDHD* primarily confers risk for HNPGL while *SDHB* is associated with PPGL should now be revised to include an intermediate category, *SDHD* truncating variants, which confer a significantly elevated PPGL risk compared with *SDHD* missense variants. As patients with *SDHD* truncating variants constitute around 15% of the total dataset, this group represents a significant proportion of all patients currently under clinical surveillance. It is also clear from our data that other variant-specific phenotypes remain to be discovered, but the further dissection and refinement of variant-specific phenotypes will require international multicentre collaboration.

Author affiliations
[1]Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands
[2]Department of Medicine II, University of Freiburg Faculty of Medicine, Freiburg, Germany
[3]Department of Otorhinolaryngology – Head & Neck Surgery, Free University Medical Center, Amsterdam, The Netherlands
[4]Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands
[5]Department of Otorhinolaryngology, Leiden University Medical Center, Leiden, The Netherlands
[6]Department of Biochemistry and Molecular Biology, The University of Melbourne Bio21 Molecular Science and Biotechnology Institute, Parkville, Victoria, Australia
[7]Instituto René Rachou, Fundacao Oswaldo Cruz, Belo Horizonte, Brazil
[8]Department of Clinical Genetics, Leiden University Medical Centre, Leiden, The Netherlands
[9]Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands
[10]Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands
[11]Section for Preventive Medicine, Faculty of Medicine, Albert-Ludwigs-University, Freiburg, Germany

Acknowledgements Statistical advice was provided by Professor Jelle Goeman. We also acknowledge Katrina A Andrews, Eamonn R Maher and colleagues for generously making their dataset publicly available.

Contributors JPB planned the study, analysed the data and wrote the manuscript. PD planned the study and provided supervision. BB, JAR, LTvH, JCJ, FJH, EFH, EPMC and HPHN collected data and edited the manuscript. DA and DEVP analysed data. JPB is responsible for the overall content and guarantees the content of the manuscript.

Funding This work was supported by the Dutch Cancer Society (Grant 2011-5025 to JPB/PD) and by the Jack Brockhoff Foundation (JBF 4186, 2016 to DA); National Health and Medical Research Council of Australia (APP1072476 to DA); Australian Cancer Research Foundation (to DA), Australia Awards – Endeavour Fellowship (to DEVP); Instituto René Rachou (IRR/FIOCRUZ Minas), Brazil and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (to DEVP); and Department of Biochemistry and Molecular Biology, University of Melbourne (to DA).

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

REFERENCES

1 Dahia PLM. Pheochromocytoma and paraganglioma pathogenesis: learning from genetic heterogeneity. *Nat Rev Cancer* 2014;14:108–19.
2 Buffet A, Venisse A, Nau V, Roncellin I, Boccio V, Le Pottier N, Boussion M, Travers C, Simian C, Burnichon N, Abermil N, Favier J, Jeunemaitre X, Gimenez-Roqueplo A-P. A decade (2001-2010) of genetic testing for pheochromocytoma and paraganglioma. *Horm Metab Res* 2012;44:359–66.
3 Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, van der Mey A, Taschner PE, Rubinstein WS, Myers EN, Richard CW, Cornelisse CJ, Devilee P, Devlin B. Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 2000;287:848–51.
4 Niemann S, Müller U. Mutations in SdhC cause autosomal dominant paraganglioma, type 3. *Nat Genet* 2000;26:268–70.
5 Neumann HPH, Pawlu C, Peczkowska M, Bausch B, McWhinney SR, Muresan M, Buchta M, Franke G, Klisch J, Bley TA, Hoegerle S, Boedeker CC, Opocher G, Schipper J, Januszewicz A, Eng C, European-American Paraganglioma Study Group. Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations. *JAMA* 2004;292:943–51.
6 Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, Sköldberg F, Husebye ES, Eng C, Maher ER. Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. *Am J Hum Genet* 2001;69:49–54.
7 Vanharanta S, Buchta M, McWhinney SR, Virta SK, Peçzkowska M, Morrison CD, Lehtonen R, Januszewicz A, Järvinen H, Juhola M, Mecklin J-P, Pukkala E, Herva R, Kiuru M, Nupponen NN, Aaltonen LA, Neumann HPH, Eng C. Early-Onset renal cell carcinoma as a novel extraparaganglial component of SDHB-associated heritable paraganglioma. *Am J Hum Genet* 2004;74:153–9.
8 Solis DC, Burnichon N, Timmers HJLM, Raygada MJ, Kozupa A, Merino MJ, Makey D, Adams KT, Venisse A, Gimenez-Roqueplo A-P, Pacak K. Penetrance and clinical consequences of a gross SDHB deletion in a large family. *Clin Genet* 2009;75:354–63.
9 Hes FJ, Weiss MM, Woortman SA, de Miranda NF, van Bunderen PA, Bonsing BA, Stokkel MPM, Morreau H, Romijn JA, Jansen JC, Vriends AHJT, Bayley J-PL, Corssmit EPM. Low penetrance of a SDHB mutation in a large Dutch paraganglioma family. *BMC Med Genet* 2010;11:92.
10 Hensen EF, Jansen JC, Siemers MD, Oosterwijk JC, Vriends AH, Corssmit EP, Bayley J-P, van der Mey AG, Cornelisse CJ, Devilee P. The Dutch founder mutation SDHD. D92Y shows a reduced penetrance for the development of paragangliomas in a large multigenerational family. *Eur J Hum Genet* 2010;18:62–6.
11 Schiavi F, Milne RL, Anda E, Blay P, Castellano M, Opocher G, Robledo M, Cascón A. Are we overestimating the penetrance of mutations in SDHB? *Hum Mutat* 2010;31:761–2.
12 van der Tuin K, Mensenkamp AR, Tops CMJ, Corssmit EPM, Dinjens WN, van de Horst-Schrivers AN, Jansen JC, de Jong MM, Kunst HPM, Kusters B, Leter EM, Morreau H, van Nesselrooij BMP, Oldenburg RA, Spruijt L, Hes FJ, Timmers HJLM. Clinical aspects of SDHA-Related pheochromocytoma and paraganglioma: a nationwide study. *J Clin Endocrinol Metab* 2018;103:438–45.
13 Rijken JA, Niemeijer ND, Jonker MA, Eijkelenkamp K, Jansen JC, van Berkel A, Timmers HJLM, Kunst HPM, Bisschop PHLT, Kerstens MN, Dreijerink KMA, van Dooren MF, van der Horst-Schrivers ANA, Hes FJ, Leemans CR, Corssmit EPM, Hensen EF. The

penetrance of paraganglioma and pheochromocytoma in *SDHB* germline mutation carriers. *Clin Genet* 2018;93:60–6.
14 Maniam P, Zhou K, Lonergan M, Berg JN, Goudie DR, Newey PJ. Pathogenicity and penetrance of germline *SDHA* variants in pheochromocytoma and paraganglioma (PPGL). *J Endocr Soc* 2018;2:806–16.
15 Hoekstra AS, Addie RD, Ras C, Seifar RM, Ruivenkamp CA, Briaire-de Bruijn IH, Hes FJ, Jansen JC, Corssmit EPM, Corver WE, Morreau H, Bovée JVMG, Bayley J-P, Devilee P. Parent-of-origin tumourigenesis is mediated by an essential imprinted modifier in *SDHD*-linked paragangliomas: *SLC22A18* and *CDKN1C* are candidate tumour modifiers. *Hum Mol Genet* 2016;25:3715–28.
16 Hoekstra AS, Devilee P, Bayley J-P. Models of parent-of-origin tumorigenesis in hereditary paraganglioma. *Semin Cell Dev Biol* 2015;43:117–24.
17 Ricketts CJ, Forman JR, Rattenberry E, Bradshaw N, Lalloo F, Izatt L, Cole TR, Armstrong R, Kumar VKA, Morrison PJ, Atkinson AB, Douglas F, Ball SG, Cook J, Srirangalingam U, Killick P, Kirby G, Aylwin S, Woodward ER, Evans DGR, Hodgson SV, Murday V, Chew SL, Connell JM, Blundell TL, Macdonald F, Maher ER. Tumor risks and genotype-phenotype-proteotype analysis in 358 patients with germline mutations in *SDHB* and *SDHD*. *Hum Mutat* 2010;31:41–51.
18 Andrews KA, Ascher DB, Pires DEV, Barnes DR, Vialard L, Casey RT, Bradshaw N, Adlard J, Aylwin S, Brennan P, Brewer C, Cole T, Cook JA, Davidson R, Donaldson A, Fryer A, Greenhalgh L, Hodgson SV, Irving R, Lalloo F, McConachie M, McConnell VPM, Morrison PJ, Murday V, Park S-M, Simpson HL, Snape K, Stewart S, Tomkins SE, Wallis Y, Izatt L, Goudie D, Lindsay RS, Perry CG, Woodward ER, Antoniou AC, Maher ER. Tumour risks and genotype-phenotype correlations associated with germline variants in succinate dehydrogenase subunit genes *SDHB*, *SDHC* and *SDHD*. *J Med Genet* 2018;55:384–94.
19 Heesterman BL, de Pont LMH, van der Mey AGL, Bayley J-P, Corssmit EPM, Hes FJ, Verbist BM, van Benthem PPG, Jansen JC. Clinical progression and metachronous paragangliomas in a large cohort of SDHD germline variant carriers. *Eur J Hum Genet* 2018;26:1339–47.
20 Gaetano J. Holm-Bonferroni Sequential Correction: An EXCEL Calculator - Ver. 1.2 2013.
21 Bayley J-P, Devilee P, Taschner PEM. The SDH mutation database: an online resource for succinate dehydrogenase sequence variants involved in pheochromocytoma, paraganglioma and mitochondrial complex II deficiency. *BMC Med Genet* 2005;6:39.
22 Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won H-H, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation C, Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285–91.
23 Taschner PE, Jansen JC, Baysal BE, Bosch A, Rosenberg EH, Bröcker-Vriends AH, van Der Mey AG, van Ommen GJ, Cornelisse CJ, Devilee P. Nearly all hereditary paragangliomas in the Netherlands are caused by two founder mutations in the *SDHD* gene. *Genes Chromosomes Cancer* 2001;31:274–81.
24 Simi L, Sestini R, Ferruzzi P, Gaglianò MS, Gensini F, Mascalchi M, Guerrini L, Pratesi C, Pinzani P, Nesi G, Ercolino T, Genuardi M, Mannelli M. Phenotype variability of neural crest derived tumours in six Italian families segregating the same founder SDHD mutation Q109X. *J Med Genet* 2005;42:e52.
25 Peczkowska M, Erlic Z, Hoffmann MM, Furmanek M, Cwikla J, Kubaszek A, Prejbisz A, Szutkowski Z, Kawecki A, Chojnowski K, Lewczuk A, Litwin M, Szyfter W, Walter MA, Sullivan M, Eng C, Januszewicz A, Neumann HPH. Impact of screening kindreds for SDHD p.Cys11X as a common mutation associated with paraganglioma syndrome type 1. *J Clin Endocrinol Metab* 2008;93:4818–25.
26 Cascón A, Landa I, López-Jiménez E, Díez-Hernández A, Buchta M, Montero-Conde C, Leskelä S, Leandro-García LJ, Letón R, Rodríguez-Antona C, Eng C, Neumann HPH, Robledo M. Molecular characterisation of a common SDHB deletion in paraganglioma patients. *J Med Genet* 2008;45:233–8.
27 Bayley J-P, Grimbergen AEM, van Bunderen PA, van der Wielen M, Kunst HP, Lenders JW, Jansen JC, Dullaart RPF, Devilee P, Corssmit EP, Vriends AH, Losekoot M, Weiss MM. The first Dutch SDHB founder deletion in paraganglioma-pheochromocytoma patients. *BMC Med Genet* 2009;10:34.
28 Hensen EF, van Duinen N, Jansen JC, Corssmit EPM, Tops CMJ, Romijn JA, Vriends AHJT, van der Mey AGL, Cornelisse CJ, Devilee P, Bayley JP. High prevalence of founder mutations of the succinate dehydrogenase genes in the Netherlands. *Clin Genet* 2012;81:284–8.
29 Schiavi F, Demattè S, Cecchini ME, Taschin E, Bobisse S, Del Piano A, Donner D, Barbareschi M, Manera V, Zovato S, Erlic Z, Savvoukidis T, Barollo S, Grego F, Trabalzini F, Amistà P, Grandi C, Branz F, Marroni F, Neumann HPH, Opocher G. The endemic paraganglioma syndrome type 1: origin, spread, and clinical expression. *J Clin Endocrinol Metab* 2012;97:E637–41.
30 Bourdeau I, Grunenwald S, Burnichon N, Khalifa E, Dumas N, Binet M-C, Nolet S, Gimenez-Roqueplo A-P. A SdhC founder mutation causes paragangliomas (PGLs) in the French Canadians: new insights on the *SDHC*-Related PGL. *J Clin Endocrinol Metab* 2016;101:4710–8.
31 van Schothorst EM, Jansen JC, Bardoel AF, van der Mey AG, James MJ, Sobol H, Weissenbach J, van Ommen GJ, Cornelisse CJ, Devilee P. Confinement of PGL, an imprinted gene causing hereditary paragangliomas, to a 2-cM interval on 11q22-q23 and exclusion of DRD2 and NCAM as candidate genes. *Eur J Hum Genet* 1996;4:267–73.
32 Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-α prolyl hydroxylase. *Cancer Cell* 2005;7:77–85.
33 Slane BG, Aykin-Burns N, Smith BJ, Kalen AL, Goswami PC, Domann FE, Spitz DR. Mutation of succinate dehydrogenase subunit c results in increased O2.-, oxidative stress, and genomic instability. *Cancer Res* 2006;66:7615–20.
34 Guzy RD, Sharma B, Bell E, Chandel NS, Schumacker PT. Loss of the SdhB, but not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. *Mol Cell Biol* 2008;28:718–31.