SDHB/D: CanVIG-UK Gene-Specific Guidance

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For use in conjunction with CanVIG-UK Consensus Specification for Cancer susceptibility Genes of ACGS Best Practice Guidelines for Variant Classification. Evidence lines for which there are no gene-specific recommendations should be reviewed in context of CanVIG-UK Consensus Specification for Cancer Susceptibility Genes.

Evidence towards Pathogenicity

<table>
<tr><th colspan="2">Evidence element and evidence strengths allowed</th><th>Thresholds/data-sources/applications specifically relevant to SDHB, SDHD</th></tr>
<tr><td>PS4: Case-control: The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls</td><td>_VSTR
_STR
_MOD
_SUP</td><td>• For variant data presented in Garrett et al 2021[1] (6,118 cases including data from Birmingham and Leeds NHS laboratories up to 2020; ethnicity of cases not all known)
• if variant frequency in GNOMAD ≤1 in NFE and 0 in all other ethnicities in GNOMAD and 1KGP
→SUP if cases ≥2 (p=0.002), MOD if cases≥4 (p=0.000006)*
• Where there are cases with the variant in addition to those included in Garrett et al 2021[1]
• if variant frequency in GNOMAD ≤1 in NFE and =0 in all other ethnicities in GNOMAD and 1KGP
• and cases are non-overlapping
→SUP if cases ≥3, MOD if cases ≥5*
*These point allocations are very conservative to account for potential heterogeneity in case ethnicity and/or case ethnicities not represented in either gnomAD/1KGP</td></tr>
<tr><td>PP4 (BP5): Phenotypic specificity/case counting (Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology)</td><td>_VSTR
_STR
_MOD
_SUP</td><td>
<table>
<tr><td></td><td></td><td colspan="2">Evidence points [c]</td></tr>
<tr><td></td><td></td><td>SDHB</td><td>SDHD</td></tr>
<tr><td>Phaeochromocytoma/ paraganglioma (must meet PM2 MTAF)</td><td></td><td>+2</td><td>+1</td></tr>
<tr><td rowspan="2">Head & neck paraganglioma</td><td>present</td><td>+0.5</td><td>+3</td></tr>
<tr><td>absent</td><td>-0.5</td><td>-3</td></tr>
<tr><td rowspan="2">Family history [a]</td><td>present</td><td>+3</td><td>+4</td></tr>
<tr><td>absent</td><td>-</td><td>-1</td></tr>
<tr><td rowspan="2">Invasive disease</td><td>present</td><td>+2</td><td>-</td></tr>
<tr><td>absent</td><td>-0.5</td><td>-</td></tr>
<tr><td rowspan="2">Multiple tumours</td><td>present</td><td>+0.5</td><td>+2</td></tr>
<tr><td>absent</td><td>-</td><td>-</td></tr>
<tr><td rowspan="2">SDHB immunohistochemistry (IHC)</td><td>loss</td><td>+3</td><td>+3</td></tr>
<tr><td>normal</td><td>-1</td><td>-2</td></tr>
<tr><td rowspan="2">Succinate:Fumarate ratio (S:F) [b]</td><td>High (>97)</td><td>+3</td><td>+3</td></tr>
<tr><td>Low (<97)</td><td>-</td><td>-</td></tr>
</table>
[a] Presence of paheochromocytoma/paraganglioma in first/second degree relative</td></tr>
</table>

		[b] Validated assay performed in accredited diagnostic laboratory or laboratory publishing on S:F ratio. These evidence points have still been applied conservatively to account for variation in assay performance and lack of replication outside of reference publication. [c] Evidence points have been derived from the likelihood ratios in Garrett et al 2021[1]. For the clinical subphenotypes, the LRs from adjusted multiple regression have been used. The exponent of the lower 95th confidence interval has been used: where this is 0-1, it has been rounded to 0.5 where the point estimate of the exponent is >1. For negative LRs/exponents (i.e. towards benignity), the upper 95th confidence interval is used. <u>Notes</u> • Points contributing to PP4/BP5 should be summed • Where there are multiple cases with the variant reported clinically/in the literature, points for each clinical/molecular subphenotype (family history, head and neck, multiple tumours, invasive tumours, IHC loss, S:F ratio) can only be used once. Points for different subphenotypes can be awarded for different individuals/family members carrying the variant • Where there are clinical data from multiple probands/family members that are discordant for a clinical subphenotype, only the positive score is counted (e.g. for a *SDHD* variant that has been reported 3 times, of which 1 is familial and two are isolated, 4 points are awarded). • If there are conflicting data for two different IHC tests, no points should be awarded for IHC (S:F can still be awarded in this instance, independent of IHC). If IHC and S:F are discordant, the net score can be used. No more than 3 points can be awarded for the combination of IHC and S:F • Points for LOH (as per consensus CanVIG guidance) can be awarded independently to (i.e. in addition to) IHC and S:F, as it is an orthogonal test • If all the data for a variant come from a single case/family OR from a single-centre publication, points for PP4 should not exceed 4 (and the variant should not be classified at higher than likely pathogenic)
PM2: Absent from controls (or at extremely low frequency if recessive) in ESP, 1000GP, or ExAC	_MOD _SUP	For PM2_mod frequency should be below predicted maximum tolerated allele frequency (MTAF) using the threshold below: SDHB: 1.7×10^{-6} ; *SDHD*: 7.3×10^{-7} For PM2_sup follow main consensus specification recommendations (≤0.002% in a cancer-free control series of >50,000 individuals or any equivalent ratio in a larger control series)
PVS1: **Predicted null variant** (in a gene where LOF is a known mechanism of disease)	_VSTR _STR _MOD _SUP	
PS1: Same amino acid change as an established variant	_STR	
PM4: Protein-length-changing variant	_MOD _SUP	
PM5: Novel missense change at an amino acid residue where a different missense change determined to be pathogenic seen before	_MOD _SUP	

<table>
<tr><td>PP3: In silico: Multiple lines of computational evidence support a deleterious effect on the gene or gene product</td><td>_SUP</td><td></td></tr>
<tr><td>PM1, PP2 (/BP1): Enrichment/constraint:
PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease
PM1: Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation</td><td>_STR
_MOD
_SUP</td><td><table><tr><td></td><td>SDHB</td><td>SDHD</td></tr><tr><td></td><td>aa 177-260</td><td>aa 70-114</td></tr><tr><td>Location within enriched region:</td><td>+0.5</td><td>+1</td></tr><tr><td>Location outside enriched region</td><td>-</td><td>-1</td></tr></table>Only apply if PP4 awarded for PHAEO/PGL+MTAF</td></tr>
<tr><td>PS3: Functional: Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product</td><td>_VSTR
_STR
_MOD
_SUP</td><td>No variant-specific ex-vivo assays established as robust.</td></tr>
<tr><td>PP1: Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease</td><td>_VSTR
_STR
_MOD
_SUP</td><td></td></tr>
<tr><td>PS2/PM6: De novo (maternity and paternity confirmed/unconfirmed) in a patient with the disease and no family history</td><td>_STR
_MOD
_SUP</td><td></td></tr>
<tr><td>PM3: in trans with a pathogenic variant (recessive disorders)</td><td>_STR
_MOD
_SUP</td><td></td></tr>
<tr><td>PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation</td><td>_VSTR
_STR
_MOD
_SUP</td><td></td></tr>
</table>

Evidence towards Benignity

<table>
<tr><td>BA1/BS1: Allele frequency is “too high” in ExAC or gnomAD for disorder</td><td>_SA
_STR</td><td><table><tr><td></td><td>SDHB</td><td>SDHD</td></tr><tr><td>BS1</td><td>1.7×10^{-6}</td><td>7.3×10^{-7}</td></tr><tr><td>BA1</td><td>10^{-4}</td><td>10^{-4}</td></tr></table></td></tr>
</table>

BS2: Observation in controls inconsistent with disease penetrance. Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age	_STR _SUP	
BP4: In silico: Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	_SUP	
BP1: Missense variant in a gene for which primarily truncating variants are known to cause disease	_SUP	
BP7: Synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence	_SUP	
BP3: In-frame deletions/insertions in a repetitive region	_SUP	
BS3: Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing	_STR _MOD _SUP	
BS4: Non segregation with disease	_STR _SUP	
BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis	_STR _SUP	
BP6: Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	_STR _SUP	
BP5: Alternate molecular basis for disease	_SUP	

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by
1.1	04/03/22	All	Draft version for consultation with CanVIG	CT	CStAG
1.2	***16/03/22***	PP4	Addition of LOH Clarification of use of S:F in context of contradictory IHC	CT	CStAG
1.3	***24/03/22***	PM2	Application of PM2_sup as per main consensus guidance	AG	CStAG

References

1. Garrett A, Loveday C, King L, et al. Quantifying evidence toward pathogenicity for rare phenotypes: The case of succinate dehydrogenase genes, SDHB and SDHD. *Genetics in Medicine* 2021;24(1):41-50. doi: https://doi.org/10.1016/j.gim.2021.08.004 [published Online First: 30 November 2021]