

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
<b>Population data</b>	>1% in population databases (gnomAD, ESP) <sup>1</sup>			Absent in population databases (gnomAD, ESP)	Absent in population databases and increased prevalence in affecteds	
<b>Computational and Predictive data (in silico analysis)</b>		Multiple lines of computational evidences suggesting no deleterious impact on gene/gene product <sup>2</sup> - missense with non predicted impact on gene/gene product - silent or splice site or intronic variant with non predicted splice impact	Multiple lines of computational evidences supporting a deleterious impact on gene/gene product <sup>2</sup> - missense with predicted impact on gene/gene product - silent or splice site or intronic variant with predicted splice impact	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen (i.e. c.206G>T, p.Gly69Val known as pathogenic and novel missense c.206G>A, p.Gly69Asp)	Same amino acid change as an established pathogenic variant (i.e. c.39G>T, p.Leu13Phe known as pathogenic and novel missense c.39G>C, p.Leu13Phe)	Null variants <sup>3</sup> : nonsense, frameshift, canonical splice site, initiation codon, single exon or multi-exon deletion/duplication with open-reading frame disruption
<b>Additional data</b>	In tumor DNA, loss of the allele carrying the variant	1) Immunohistochemistry evidence: positive SDHB staining 2) No loss of heterozygosity 3) cDNA analysis showing no unexpected exon size nor exon skipping (for likely splicing variants) 4) Transcriptomic analysis: tumor classification not in SDHx-related cluster (C1A)	Loss of heterozygosity in tumor DNA	1) Immunohistochemistry evidence: negative SDHB staining, but without genotyping <sup>4</sup> of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 2) SDH enzymatic activity abolished without genotyping <sup>4</sup> of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 3) Western blot evidence: no SDHB protein detected but without genotyping <sup>4</sup> of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 4) Transcriptomic analysis: tumor classification in C1A cluster but without genotyping <sup>4</sup> of all SDHx genes (SDHA, SDHB, SDHC, SDHD)	1) Immunohistochemistry evidence: SDHB negative and SDHA positive staining with SDHB, SDHC and SDHD sequencing but without search for SDHx large deletions or duplications 2) cDNA analysis : abnormal splicing 3) SDH enzymatic activity abolished with SDHx genes sequencing but without search for SDHx large deletions or duplications 4) Western blot evidence: no SDHB protein detected with SDHx genes sequencing but without search for SDHx large deletions or duplications 5) Transcriptomic analysis: tumor classification in C1A cluster with SDHx genes sequencing but without search for SDHx large deletions or duplications	1) Immunohistochemistry evidence: - negative SDHB staining with genotyping <sup>4</sup> of all SDHx genes (SDHA, SDHB, SDHC, SDHD) - negative SDHB and positive SDHA staining with genotyping <sup>4</sup> of SDHB, SDHC and SDHD genes 2) SDH enzymatic activity abolished with genotyping <sup>4</sup> of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 3) Western blot evidence: no SDHB protein detected with genotyping <sup>4</sup> of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 4) Transcriptomic analysis: tumor classification in C1A cluster with genotyping <sup>4</sup> of all SDHx genes (SDHA, SDHB, SDHC, SDHD)
<b>Segregation data</b>	Non-segregation with disease		Co-segregation with disease in multiple affected family members	Increased segregation data →		
<b>Allelic data</b>		Observed in cis/trans with a pathogenic variant				
<b>Publication data<sup>5</sup></b>	Increased publication data ←	Published as benign variant	Published as pathogenic variant	Increased publication data →		

<sup>1</sup> Stand-alone evidence of benign impact if >5% in population databases  
<sup>2</sup> Combination of different *in silico* tools (missense: PolyPhen2, SIFT, MutationTaster; splice site: MaxEntScan, NNSplice) and Conservation data (nucleotide: phyloP; amino acid: Orthologs conservation, distance between amino acids: Grantham distance)  
<sup>3</sup> Except variant occurring in the last exon or in the last 50 bps of the penultimate exon. Such variants cannot be interpreted without additional assay  
<sup>4</sup> « Genotyping » includes both sequencing using NGS or Sanger and search for large duplication or deletion and implies that no additional SDHx variant was found  
<sup>5</sup> Well-documented publications (number of cases published, functional data...)

Variant classification	1 <sup>st</sup> criterion	2 <sup>nd</sup> criterion
Pathogenic	1 Very Strong	≥1 Strong
		≥2 Moderate
		1 Moderate + 1 Supporting
	≥2 Strong	≥2 Supporting
Likely Pathogenic	1 Strong	≥3 Moderate
		2 Moderate + ≥2 Supporting
		1 Moderate + ≥4 Supporting
	1 Very Strong	1 Moderate
	1 Strong	1-2 Moderate
Likely Benign	1 Strong	≥2 Supporting
		≥3 Moderate
	≥3 Moderate	≥2 Supporting
	1 Moderate	≥4 Supporting
Benign	1 Strong	1 Supporting
	≥2 Supporting	-
	1 Stand-Alone	-
	≥2 Strong	-

Supplemental Figure 1: adjusted ACMG criteria and combination used for SDHB variant classification