

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
Population data	>1% in population databases (gnomAD, ESP) ¹			Absent in population databases (gnomAD, ESP)	Absent in population databases and increased prevalence in affecteds	
Computational and Predictive data (in silico analysis)		Multiple lines of computational evidences suggesting no deleterious impact on gene/gene product ² - 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 ² - 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 ³ : nonsense, frameshift, canonical splice site, initiation codon, single exon or multi-exon deletion/duplication with open-reading frame disruption
Additional data	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 ⁴ of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 2) SDH enzymatic activity abolished without genotyping ⁴ of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 3) Western blot evidence: no SDHB protein detected but without genotyping ⁴ of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 4) Transcriptomic analysis: tumor classification in C1A cluster but without genotyping ⁴ 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 ⁴ of all SDHx genes (SDHA, SDHB, SDHC, SDHD) - negative SDHB and positive SDHA staining with genotyping ⁴ of SDHB, SDHC and SDHD genes 2) SDH enzymatic activity abolished with genotyping ⁴ of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 3) Western blot evidence: no SDHB protein detected with genotyping ⁴ of all SDHx genes (SDHA, SDHB, SDHC, SDHD) 4) Transcriptomic analysis: tumor classification in C1A cluster with genotyping ⁴ of all SDHx genes (SDHA, SDHB, SDHC, SDHD)
Segregation data	Non-segregation with disease		Co-segregation with disease in multiple affected family members	Increased segregation data		
Allelic data		Observed in <i>cis/trans</i> with a pathogenic variant				
Publication data⁵	Increased publication data	Published as benign variant	Published as pathogenic variant	Increased publication data		

¹ Stand-alone evidence of benign impact if >5% in population databases² 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)³ Except variant occurring in the last exon or in the last 50 bps of the penultimate exon. Such variants cannot be interpreted without additional assay⁴ « Genotyping » includes both sequencing using NGS or Sanger and search for large duplication or deletion and implies that no additional SDHx variant was found⁵ Well-documented publications (number of cases published, functional data...)

Variant classification	1 st criterion	2 nd 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