ClinGen Mitochondrial Disease Nuclear and Mitochondrial Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: *SLC19A3, PDHA1, POLG, ETHE1*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50027

Summary of ACMG-AMP Criteria for ntDNA Mitochondrial Disease

Gene	Disease (MONDO ID)	Clinically significant transcript
SLC19A3	Biotin-responsive basal ganglia disease (MONDO:0011841)	NM_025243.4
PDHA1	Pyruvate dehydrogenase deficiency (MONDO:0019169)	NM_000284.4
POLG	Mitochondrial disease (MONDO:0044970)	NM_002693.2
ETHE1	Ethylmalonic encephalopathy (MONDO:0011229)	NM_014297.5

Criterion	ACMG/AMP description	SLC19A3	PDHA1	POLG	ETHE1
PVS1	Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease *Applied per PVS1 flowsheet of Abou Toyoun et al	Applicable	Applicable	Applicable	Applicable

Related publication(s):

Date Approved: April 30, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50027/docs/assertion-criteria for the most recent version.

Clingen_MitoDisease_ACMG_Specifications_v1.pdf

PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	Applicable	Applicable	Applicable	Applicable
PS2_PM6 _Strong	De novo in a patient with the disease and no family history	Applicable	Applicable	Applicable	Applicable
PS3	Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product	Not applicable	Not applicable	Not applicable	Not applicable
PS3 _Supporting	In vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product	Specified- see PS3 table	Not applicable	Not applicable	Specified - see PS3 table
PS4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Not applicable	Not applicable	Applicable	Not applicable
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	Not applicable	Specified - see PM1 table	Not applicable	Not applicable
PM2	Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium	<0.00005 (<0.0050%)	0.0000092 (<0.00092%)	<0.0005 (<0.05%)	<0.00002 (<0.0020%)

PM3	For recessive disorders, detected in trans with a pathogenic variant (Follow SVI guidance for PM3)	Applicable	Not applicable	Applicable	Applicable
PM4	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants	Applicable	Applicable	Applicable	Applicable
PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	Applicable	Applicable	Applicable	Applicable
PM6	Assumed *de novo*, but without confirmation of paternity and maternity	Applicable	Applicable	Applicable	Applicable
PP1	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Specified - see PP1 table	Applicable	Specified - see PP1 table	Applicable
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	Not applicable	Not applicable	Not applicable	Not applicable
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)	Applicable	Applicable	Specified - see PP3 table	Applicable

PP4 _Moderate	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	Not applicable	Not applicable	Specified - see PP4 table	Specified - see PP4 table
PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	Specified - see PP4 table	Specified - see PP4 table	Specified - see PP4 table	Specified - see PP4 table
PP5	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation	Not applicable	Not applicable	Not applicable	Not applicable

ClinGen Mitochondrial Disease Nuclear and Mitochondrial Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: *SLC19A3, PDHA1, POLG, ETHE1*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50027

Rule	ACMG/AMP description	SLC19A3	PDHA1	POLG	ETHE1
BA1	Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium	>0.001 (>0.1%)	>0.00092 (>0.092%)	>0.01 (>1%)	>0.001 (>0.1%)
BS1	Allele frequency is greater than expected for disorder	>0.0005 (>0.050%)	>0.000092 (>0.0092%)	>0.005 (>0.5% - AR)	>0.0002 (>0.020%)
BS2	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	Specified - see BS2 table	Specified - see BS2 table	Specified - see BS2 table	Specified - see BS2 table
BS2 _Supporting	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	Not applicable	Specified - see BS2 table	Specified - see BS2 table	Specified - see BS2 table
BS3	Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing	Not applicable	Not applicable	Not applicable	Not applicable
BS3 _Supporting	Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing	Specified - See BS3 table	Not applicable	Not applicable	Not applicable
BS4	Lack of segregation in affected members of a family	Specified - See BS4 table	Applicable	Applicable	Applicable

---

Related publication(s): **Date Approved:** April 30, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50027/docs/assertion-criteria for the most recent version.

Clingen_MitoDisease_ACMG_Specifications_v1.pdf

BP1	Missense variant in a gene for which primarily truncating variants are known to cause disease	Not applicable	Not applicable	Not applicable	Not applicable
BP2	Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern	Applicable	Not applicable	Applicable	Applicable
BP3	In-frame deletions/insertions in a repetitive region without a known function	Applicable	Applicable	Applicable	Applicable
BP4	Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	Applicable	Applicable	Specified - See BP4 table	Applicable
BP5	Variant found in a case with an alternate molecular basis for disease	Applicable	Applicable	Applicable	Applicable
BP6	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	Not applicable	Not applicable	Not applicable	Not applicable
BP7	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved	Applicable	Applicable	Applicable	Applicable

ClinGen Mitochondrial Disease Nuclear and Mitochondrial Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: *SLC19A3, PDHA1, POLG, ETHE1*

Expert Panel Page: https://www.clinicalgenome.org/affiliation/50027

STRONG EVIDENCE OF PATHOGENICITY

PVS1 Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

*We will utilize SVI's PVS1 recommendation for determining applicable PVS1 strength level (Abou Tayoun et al 2018; PMID:30192042).

STRONG EVIDENCE OF PATHOGENICITY

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

PS2 *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history
Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, *etc.* can contribute to non-maternity
*We will utilize SVI's de novo guidance document.

Related publication(s): **Date Approved:** April 30, 2020

This document is archived and versioned on ClinGen's website. Please check https://www.clinicalgenome.org/affiliation/50027/docs/assertion-criteria for the most recent version.

Clingen_MitoDisease_ACMG_Specifications_v1.pdf

PS3 Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product

Criterion	SLC19A3	PDHA1	POLG	ETHE1
PS3	NOT APPLICABLE	NOT APPLICABLE There is currently no well-established functional assay specifically for PDHA1 or the E1-alpha subunit of PDC.	NOT APPLICABLE There is currently no well-established functional assay specifically for POLG.	NOT APPLICABLE
PS3_ Supp	Transporter assay showing loss of function	-	-	Reduced ETHE1 persulfide dioxygenase
BS3	-	-	-	-
BS3_ Supp	Transporter assay showing no effect on the gene or gene product	-	-	-

PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls

Criterion	SLC19A3	PDHA1	POLG	ETHE1
PS4	REMOVED Autosomal recessive condition	REMOVED X-linked condition	Rarely, pathogenic variants cause disease in an AD manner. For these variants only, presence in: 2 unrelated probands will be considered supporting evidence, 4 unrelated probands will be considered moderate evidence, 16 unrelated probands will be strong evidence. **Note: This will only be utilized when there is segregation evidence supporting autosomal dominant inheritance	REMOVED Autosomal recessive condition

MODERATE EVIDENCE OF PATHOGENICITY

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

Criterion	SLC19A3	PDHA1	POLG	ETHE1
PM1	REMOVED • In Exac, there are no regions that are obviously constrained. • Only 2 variants in general population between 326 and 345, so this may be constrained region but not proven/objective. • There are no regions with a lot of variants and variants are evenly spread throughout.	Located in one of the following functional domains&: •thiamine pyrophosphate (TPP) binding site (aa positions 118Y, 119R, 165G, 167V, 195G, 196D, 197G, 198A, 225N, 227Y, 292H) • α β heterodimer interface (aa positions 160F, 162G, 164N, 169A, 172P, 173L, 176G, 177I, 179L, 180A,183Y, 202G,	REMOVED Well established functional domains exist however no regions are devoid of missense variation.	REMOVED This is a small gene with no known hot-spots or functional domains.

	• About 20 regions without a lot of variants but not proven. • Group agreed to wait until higher resolution constraint scores available to stipulate regions for this criterion.	203Q, 209N, 210M, 213L) • α2 β2 heterotetramer interface (aa positions 88R, 140G, 165G, 166I, 197G, 199A, 200N, 201Q, 202G, 205F, 209N, 213L, 228G, 229M, 230G, 231T, 245R, 296D, 300S) • phosphorylation loop region (aa positions 287Y, 288R, 289Y, 290H, 291G, 292H, 293S, 295S, 296D, 297P, 298G, 299V, 300S, 301Y, 302R, 303T, 304R, 305E, 314S, 315D, 316P)		

&These domains are highly conserved (phastCons scores are mostly 1, phyloP scores are high).

PM2 Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or ExAC

SLC19A3					
ACMG-AMP Criteria	**MAF**	**Prevalence**	**Allelic Heterogeneity**	**Penetrance**	**Notes**
BA1	>0.001 (0.1%)	<1/1,000,000	100%	100%	-
BS1	>0.0005 (0.050%)	<1/1,000,000	50.4% [63/125 (c.1264A>G); PMID: 28696212]	100%	-
PM2	<0.00005 (0.0050%)	-	-	-	Per SVI: Use a threshold an order of magnitude below BS1 threshold

PDHA1					
ACMG-AMP Criteria	MAF	Prevalence	Allelic Heterogeneity	Penetrance	Notes
BA1	>0.00092 (>0.092%)	<1/1,000,000	100%	100%	Utilized biallelic inheritance in WARE calculator (monoallelic cut-off calculated out to even 1 occurrence in gnomAD meeting BA1 so utilized biallelic); for genetic heterogeneity, 84% was used (Patel et al., 2012; page 388)
BS1	>0.000092 (>0.0092%)	<1/1,000,000	10% (Estimated; Patel et al., 2012 - Supp table)	100%	-
PM2	0.0000092	-	-	-	Per SVI: Use a

	(<0.00092%)				threshold an order of magnitude below BS1 threshold
POLG					
ACMG-AMP Criteria	**MAF**	**Prevalence**	**Allelic Heterogeneity**	**Penetrance**	**Notes**
BA1	>0.01 (>1.0%)	1/10,000 (POLG Genereviews)	100%	100%	-
BS1	>0.005 (>0.5%)	1/10,000	50% (estimated)	100%	-
PM2	<0.0005 (<0.05%)	-	-	-	Per SVI: Use a threshold an order of magnitude below BS1 threshold
ETHE1					
ACMG-AMP	**MAF**	**Prevalence**	**Allelic**	**Penetrance**	**Notes**

Criteria			Heterogeneity		
BA1	>0.001 (>0.1%)	<1/1,000,000	100%	100%	-
BS1	>0.0002 (>0.020%)	<1/1,000,000	20% (estimated)	100%	-
PM2	<0.00002 (<0.0020%)	-	-	-	Per SVI: Use a threshold an order of magnitude below BS1 threshold

PM3 For recessive disorders, detected in *trans* with a pathogenic variant
Note: This requires testing of parents (or offspring) to determine phase

Criterion	SLC19A3	PDHA1	POLG	ETHE1
PM3	Use per SVI guidance	REMOVED Not relevant for XL disorders	Use per SVI guidance Note: T251I and P587L are almost always in cis	Use per SVI guidance

*SLC19A3, POLG, ETHE1 will follow ClinGen SVI *in trans* guidance (https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf)

PM4 Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants

PM5 Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before

PM6 Assumed *de novo*, but without confirmation of paternity and maternity
*We will utilize SVI's de novo guidance document.

SUPPORTING EVIDENCE OF PATHOGENICITY

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease

Criterion	SLC19A3	PDHA1	POLG	ETHE1
PP1	For segregation, an affected is defined as an individual who 1) has brainstem or basal ganglia lesions compatible with SLC19A3-related Biotin-responsive basal	-	Further define "affected" as an individual in whom there is objective evidence of manifestations consistent with POLG-related disorders spectrum: Alpers-Huttenlocher syndrome	-

	ganglia disease OR 2) a person with neurodevelopmental regression or MRI lesions compatible with SLC19A3-related Biotin-responsive basal ganglia disease who had significant clinical improvement in either symptoms or MRI lesions from treatment with biotin and thiamine.		(AHS), childhood myocerebrohepatopathy spectrum (MCHS), myoclonic epilepsy myopathy sensory ataxia (MEMSA), ataxia neuropathy spectrum (ANS), and/or progressive external ophthalmoplegia (PEO)	

*All groups will follow ClinGen SVI segregation/LOD scoring guidance

PP2 Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease

Criterion	SLC19A3	PDHA1	POLG	ETHE1
PP2	REMOVED Missense constraint Z-score = -0.37	REMOVED Missense constraint Z-score = 2.57	REMOVED Missense constraint Z-score = -0.74	REMOVED Missense constraint Z-score = 0.96

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)

Criterion	SLC19A3	PDHA1	POLG	ETHE1
PP3/BP4	No gene-specific predictors; agree to utilize REVEL, with thresholds of >0.75 and <0.15 for PP3 and BP4, respectively	No gene-specific predictors; agree to utilize REVEL, with thresholds of >0.75 and <0.15 for PP3 and BP4, respectively	Agree to utilize REVEL, with thresholds of >0.75 and <0.15 for PP3 and BP4, respectively *Will also utilize POLG pathogenicity prediction server if/when live again (PMID: 28480171); both tools (REVEL and server) will have to be in agreement to score	No gene-specific predictors; agree to utilize REVEL, with thresholds of >0.75 and <0.15 f or PP3 and BP4, respectively

PP4 Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.

Strength	SLC19A3	PDHA1	POLG	ETHE1
PP4_Moderate	-	-	1. Mitochondrial DNA depletion showing ≤ 20% of controls, OR 2. Multiple mitochondrial DNA deletions (NOTE:depletion and/or deletion analysis must be performed in muscle and/or liver; other tissues such as blood, fibroblast, and buccal are not acceptable;	Individual has abnormally high urinary ethylmalonic acid AND one of the following: (1) All of the following symptoms present: -Acrocyanosis -Petechiae -Chronic diarrhea -Developmental delay (2) ≥3 or more of the following

			Must be performed in child, as defined as <18 years old) Note: For both scenarios 1 and 2, will only apply if other mtDNA maintenance disorders have been excluded^ (exome sequencing or comprehensive panel-based testing)	biochemical studies: -Abnormally high blood C4-Acylcarnitine esters -Abnormally high blood C5-acylcarnitine -Abnormally high plasma thiosulphate -Abnormally low cytochrome oxidase activity in skeletal muscle (without evidence of other complexes decreased)
PP4	Patient has/had MRI features of Leigh syndrome with clinical response to biotin/thiamine	One of the following criteria are met: (1) Pyruvate radioactive enzyme assay showing decreased (as defined as <3rd percentile of controls) for PDC, activated and decreased ratios (PDC/E3 and/or	1. Mitochondrial DNA depletion showing 20-50% of controls in children (< 18 years of age), AND/OR	Individual has abnoramlly high urinary ethylmalonic acid AND one of the following: (1) 3 of the following features present: -Acrocyanosis -Petechiae

		PDC/CS) in fibroblasts, muscle, and/or lymphocytes; (2) other assays showing decrease in PDC activity (ie: western blot, immunocapture, and activity; commercial kits for research); (3) abnormally high pyruvate and/or pyruvate/lactate ratio	2. COX negative fibers in muscle in children and/or adults Note: Will only apply if other mtDNA maintenance disorders have been excluded^ (exome sequencing or comprehensive panel-based testing)	-Chronic diarrhea -Developmental delay (2) abnormal laboratory studies in 2 of the following biochemical studies: -Abnormally high blood C4-Acylcarnitine esters -Abnormally high blood C5-acylcarnitine -Abnormally high plasma thiosulphate -Abnormally low cytochrome oxidase activity in skeletal muscle, without evidence of other complexes decreased

^The following genes should be excluded: APTX, C10orf2, DGUOK, DNA2, FBXL4, GFER, MGME1, MPV17, OPA1, OPA3, POLG2, RRM2B, SLC25A4, SPG7, SUCLA2, SUCLG1, TK2, TYMP. Genes will be excluded when there are no pathogenic or likely pathogenic variants present in trans that could be causative.

PP5 Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation
Not applicable

STAND ALONE EVIDENCE OF BENIGN IMPACT

BA1 Allele frequency is >0.05 in any general continental population dataset of at least 2,000 observed alleles and found in a gene without a gene- or variant-specific BA1 modification.

See PM2 table for gene-specific BA1 thresholds.

STRONG EVIDENCE OF BENIGN IMPACT

BS1 Allele frequency is greater than expected for disorder
See PM2 table for gene-specific BS1 thresholds.

BS2 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.

Criterion	SLC19A3	PDHA1	POLG	ETHE1
BS2	Observed in a healthy, untreated, adult individual in the homozygous state	Observed in at least two healthy male adults *Note: Individual's phenotype is well-characterized (not just seen	Observed in a healthy adult individual in the homozygous state AND/OR	Observed in a healthy adult individual in the homozygous state

		in database of presumed healthy individuals) AND/OR ≥16 hemizygotes in gnomAD	Normal mtDNA content (1. Must be performed in muscle and/or liver; blood, fibroblast, and buccal not acceptable; 2. Must be performed in children only - defined as <18 years old; 3. A normal level is defined as >50%.)	
BS2_supporting	-	Observed in 4-15 hemizygotes in gnomAD AND/OR Pyruvate radioactive enzyme assay showing normal (defined as >3rd percentile of controls) for PDC, activated and normal ratios (PDC/E3 and/or PDC/CS) in fibroblasts with no evidence of skewed X-inactivation in fibroblasts.	Lack of COX negative fibers in muscle (children and adults)	Normal laboratory values (specific labs outlined in PP4)

BS3 Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing.
See PS3

BS4 Lack of segregation in affected members of a family

Criterion	SLC19A3	PDHA1	POLG	ETHE1
BS4	Lack of segregation in affected and/or treated members of a family.	-	-	-

SUPPORTING EVIDENCE FOR BENIGN IMPACT

BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease

Criterion	SLC19A3	PDHA1	POLG	ETHE1
BP1	REMOVED Some pathogenic variants are missense	REMOVED Some pathogenic variants are missense	REMOVED Some pathogenic variants are missense	REMOVED Some pathogenic variants are missense

BP2 Observed in *trans* with a pathogenic variant for a fully penetrant dominant gene/disorder; or observed in *cis* with a pathogenic variant in any inheritance pattern

Criterion	SLC19A3	PDHA1	POLG	ETHE1
BP2	-	REMOVED X-linked disorder	-	-

.

BP3 In-frame deletions/insertions in a repetitive region without a known function

BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.) See PP3 above.

BP5 Variant found in a case with an alternate molecular basis for disease

BP6 Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation
Not applicable

BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved