# ClinGen PAH Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: **PAH**

Expert Panel Page: [https://www.clinicalgenome.org/affiliation/50015](https://www.clinicalgenome.org/affiliation/50015)

## PATHOGENIC CRITERIA

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Criteria Description</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VERY STRONG CRITERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVS1</td>
<td>Null variant in a gene where loss of function is a known mechanism of disease.</td>
<td>None</td>
</tr>
</tbody>
</table>
| PM3_Very Strong | For recessive disorders, detected in *trans* with a pathogenic variant.  
• 4 compound heterozygotes with 3 P/LP variants OR  
• 2 compound heterozygotes with 2 P/LP variants AND 4 homozygotes OR  
• 3 compound heterozygotes with 2 P/LP variants AND 2 homozygotes | Strength |
| **STRONG CRITERIA** | | |
| PS1 | Same amino acid change as a previously established pathogenic variant regardless of nucleotide change. | None |
| PS2 | *De novo* (paternity confirmed) in a patient with the disease and no family history. | None |
| PS3 | Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect  
• PAH enzyme activity assay demonstrating enzyme activity <50%  
• RT-PCR evidence of missplicing for non-canonical intronic variants | Disease-Specific |
| PS4 | *The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.* | N/A |
| PM3_Strong | For recessive disorders, detected in *trans* with a pathogenic variant.  
• Compound heterozygous with 2 P/LP variants OR  
• Compound heterozygous with 1 P/LP variant AND 2 homozygotes | Strength |
| PP1_Strong | Co-segregation with disease in multiple affected family members: | Strength |

Related publication(s): PMID 30311390  
Date Approved: April 2, 2018

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<thead>
<tr>
<th>MODERATE CRITERIA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PM1</strong> Located in a mutational hot spot and/or critical and well-established</td>
<td>N/A</td>
</tr>
<tr>
<td>functional domain.</td>
<td></td>
</tr>
<tr>
<td><strong>PM2</strong> Absent/rare from controls in an ethnically-matched cohort population sample.</td>
<td>Disease-Specific</td>
</tr>
<tr>
<td>• Threshold: &lt;0.0002 (0.02%).</td>
<td></td>
</tr>
<tr>
<td><strong>PM3</strong> For recessive disorders, detected in <em>trans</em> with a pathogenic variant.</td>
<td>None</td>
</tr>
<tr>
<td><strong>PM4</strong> Protein length changes due to in-frame deletions/insertions in a non-repeat</td>
<td>None</td>
</tr>
<tr>
<td>region or stop-loss variants.</td>
<td></td>
</tr>
<tr>
<td><strong>PM5</strong> Missense change at an amino acid residue where a different missense change</td>
<td>None</td>
</tr>
<tr>
<td>determined to be pathogenic has been seen before.</td>
<td></td>
</tr>
<tr>
<td><strong>PM6</strong> Confirmed de novo without confirmation of paternity and maternity.</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>PP4_Moderate</strong> Plasma Phe &gt;120 μmol/L and exclusion of a defect of BH4 cofactor</td>
<td>Strength;</td>
</tr>
<tr>
<td>metabolism.</td>
<td>Disease-Specific</td>
</tr>
<tr>
<td><strong>PP1_Moderate</strong> Co-segregation with disease in multiple affected family members</td>
<td>Strength</td>
</tr>
<tr>
<td>• 2 affected segregations + 0 unaffected segregations</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SUPPORTING CRITERIA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PP1</strong> Co-segregation with disease in multiple affected family members</td>
<td>Disease-Specific</td>
</tr>
<tr>
<td>• 1 affected family member + 3 unaffected segregations</td>
<td></td>
</tr>
<tr>
<td><strong>PP2</strong> Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>PP3</strong> Multiple lines of computational evidence support a deleterious effect on the gene or gene product</td>
<td>None</td>
</tr>
<tr>
<td><strong>PP4</strong> Phenotype specific for disease with single genetic etiology.</td>
<td>None</td>
</tr>
</tbody>
</table>

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ClinGen *PAH* Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: *PAH*


<table>
<thead>
<tr>
<th><strong>PP5</strong></th>
<th><strong>PM3_Supporting</strong></th>
<th><strong>N/A</strong></th>
</tr>
</thead>
</table>
| **Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation** | **Detected *in trans* with another variant:**  
• 2 compound heterozygotes (with VUS *in trans*)  
• 2 homozygotes (allele drop out excluded)** | **Strength** |

**BENIGN CRITERIA**

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<thead>
<tr>
<th>Criteria</th>
<th>Criteria Description</th>
<th>Specification</th>
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</thead>
<tbody>
<tr>
<td><strong>STAND ALONE CRITERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA1</td>
<td>Allele frequency above 0.015 (1.5%)</td>
<td>Disease-Specific</td>
</tr>
<tr>
<td><strong>STRONG CRITERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BS1</td>
<td>Allele frequency greater than expected for disease (&gt;0.002, 0.2%)</td>
<td>Disease-Specific</td>
</tr>
<tr>
<td>BS2</td>
<td>Observed in the homozygous state in a healthy adult</td>
<td>None</td>
</tr>
<tr>
<td>BS3</td>
<td><em>Well-established in vitro or in vivo functional studies shows no damaging effect on protein function</em></td>
<td>N/A</td>
</tr>
<tr>
<td>BS4</td>
<td>Lack of segregation in affected members of a family.</td>
<td>None</td>
</tr>
<tr>
<td><strong>SUPPORTING CRITERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP1</td>
<td>Missense variant in gene where only LOF causes disease</td>
<td>N/A</td>
</tr>
<tr>
<td>BP2</td>
<td><em>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.</em></td>
<td>N/A</td>
</tr>
<tr>
<td>BP3</td>
<td>In-frame deletions/insertions in a repetitive region without a known function</td>
<td>N/A</td>
</tr>
<tr>
<td>BP4</td>
<td>Multiple lines of computational evidence suggest no impact on gene or gene product</td>
<td>None</td>
</tr>
<tr>
<td>BP5</td>
<td>Variant found in a case with an alternate molecular basis for disease</td>
<td>None</td>
</tr>
<tr>
<td>BP6</td>
<td><em>Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation</em></td>
<td>N/A</td>
</tr>
<tr>
<td>BP7</td>
<td>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.</td>
<td>None</td>
</tr>
<tr>
<td>BS3_Supporting</td>
<td><em>Well-established in vitro or in vivo functional studies shows no damaging effect on protein function</em></td>
<td>Strength; Disease-Specific</td>
</tr>
<tr>
<td></td>
<td>• Enzyme activity &gt;85%</td>
<td></td>
</tr>
</tbody>
</table>

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This version specified for the following genes: PAH


Key: Disease-Specific: Disease-specific modifications based on what is known about PAH; Strength: Increasing or decreasing strength of criteria based on the amount of evidence; N/A: not applicable for PAH; None: no changes made to existing criteria definitions.

VERY 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

Caveats:
- Use caution interpreting LOF variants at the extreme 3’ end of a gene
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact

PAH: PVS1 is applicable as described

PM3_VeryStrong Detected in trans with a pathogenic variant

PAH:
- 4 Compound heterozygotes with 3 different pathogenic/likely pathogenic variants
- 2 Compound heterozygotes with 2 different pathogenic/likely pathogenic variant & 4 homozygotes
- 3 Compound heterozygotes with 2 different pathogenic/likely pathogenic variant & 2 homozygotes

STRONG EVIDENCE OF PATHOGENICITY

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

PAH: PS1 is applicable as described

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

PAH: Only applicable when proband has a known pathogenic variant in trans with the de novo variant

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

**PAH:**
- *In vitro* enzyme activity <50% compared to wild type control.
  - Expression systems placing the mutant (and wild-type) cDNAs into plasmid vectors and introducing these into human or other mammalian host cells, which is the closest available approximation to the *in vivo* situation. (e.g. COS cells) (Trunzo, et al. Gene. 2016 594:138-143. PMID: 27620137).
- RT-PCR evidence of missplicing
  - For non-canonical splicing variants, RT-PCR evidence demonstrating transcripts of alternative length or specific intron or exon inclusion/exclusion. These studies can be performed in patient derived cells, or by placing the mutant genomic DNA into plasmid vectors and introducing these into human or other mammalian host cells. Assays should demonstrate defective splicing with RT-PCR analysis or RNA sequencing to confirm alternative transcripts.

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

**PAH:** This criterion is not applicable for PAH. For proband counting, use PM3 criterion

**PM3_Strong**
Detected in *trans* with a pathogenic variant

**PAH:** Use SVI thresholds
- Compound heterozygous with 2 different pathogenic/likely pathogenic variants
- Compound heterozygous with pathogenic/likely pathogenic variant & 2 homozygotes

**PP1_Strong**
Co-segregation with disease in multiple affected family members

**PAH:**
- 3 affected segregations + 0 unaffected segregations
- 2 affected segregations + 3 unaffected segregations
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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

**PAH:** Not applicable

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

**PAH threshold:** <0.0002 (0.02%). The 0.0002 cutoff is based on disease frequency of 1:12,000 and the most common PAH pathogenic variant, R408W, the ExAC frequency is 0.0006594 (ExAC MAF: 0.001109 74/66718 European Non-Finnish) and gnomAD overall: 0.0009056 (gnomAD MAF: 0.001728 219/126,700 European Non-Finnish)

PM3 For recessive disorders, detected in trans with a pathogenic variant

Note: This requires testing of parents (or offspring) to determine phase

**PAH:** PM3 is applicable as described

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

**PAH:** PM4 is applicable as described

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

**PAH:** PM5 is applicable as described

PM6 Assumed de novo, but without confirmation of paternity and maternity

**PAH:** Not applicable

PP4_Moderate Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology

**PAH:**

- A plasma phenylalanine concentration persistently above 120μmol/L (2mg/dL), and either normal urine pterins and normal DHPR activity, or sequencing of genes in the BH4 cofactor metabolism pathway to exclude a defect of BH4 cofactor metabolism.

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**PP1_Moderate**  
Co-segregation with disease in multiple affected family members

**PAH:**  
- 2 affected segregations + 0 unaffected segregations

**SUPPORTING EVIDENCE OF PATHOGENICITY**

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

**PAH:**  
- 1 affected segregation + 3 unaffected segregations

**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

**PAH:** Not applicable

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

**PAH:** PP3 is applicable as described

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

**PAH:**  
- A plasma phenylalanine concentration persistently above 120μmol/L (2mg/dL) without analysis of urine pterins, DHPR activity, or sequencing to exclude defects of BH4 cofactor metabolism.

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

**PAH:** Not applicable

**PM3_Supporting** Detected in trans with a pathogenic variant

**PAH:** Use SVI thresholds

- 2 compound heterozygotes (with a VUS in trans)
- 2 homozygotes (with allele drop out excluded)

**STAND ALONE EVIDENCE OF BENIGN IMPACT**

**BA1**  
Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes, or ExAC

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**PAH**: An allele frequency ≥0.015 (1.5%), which is calculated with genetic heterogeneity of 90% to account for defects of BH4 metabolism, and penetrance of 80% to account for individuals who come to attention after becoming clinically symptomatic.

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**STRONG EVIDENCE OF BENIGN IMPACT**

**BS1**  
Allele frequency is greater than expected for disorder

**PAH**: An allele frequency ≥0.002 (0.2%)

**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.

**PAH**: Only to be used when variant is observed in the homozygous state in a healthy adult

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

**PAH**: See BS3_Supporting

**BS4**  
Lack of segregation in affected members of a family

**PAH**: BS4 applicable as described

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**SUPPORTING EVIDENCE FOR BENIGN IMPACT**

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

**PAH**: 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

**PAH**: Not applicable

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

**PAH**: Not applicable

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

**PAH**: BP4 applicable as described

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<table>
<thead>
<tr>
<th>BP5</th>
<th>Variant found in a case with an alternate molecular basis for disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAH:</td>
<td>BP5 applicable as described</td>
</tr>
</tbody>
</table>

| BP6  | Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation |
| PAH: | 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 |
| PAH: | BP7 applicable as described                                           |

| BS3_Supporting | Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing. |
| PAH:           | • In vitro enzyme activity >85% compared to wild type.               |
|                |   o Expression systems: placing the mutant (and wildtype) cDNA into plasmid vectors and introducing these into host cells. Transiently transfected human or other mammalian host cells are the closest available approximation to the in vivo situation. (e.g. COS cells) (Trunzo, et al. Gene. 2016 594:138-143) |

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RULES FOR COMBINING PATHOGENIC CRITERIA

Pathogenic

1. 1 Very Strong (PVS1, PM3_VeryStrong) AND
   a. ≥1 Strong (PS1-PS4, PM3_Strong, PP1_Strong) OR
   b. ≥2 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) OR
   c. 1 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) and 1 Supporting (PP1-PP5, PM3_Supporting) OR
   d. ≥2 Supporting (PP1-PP5, PM3_Supporting)

2. ≥2 Strong (PS1-PS4, PM3_Strong, PP1_Strong) OR

3. 1 Strong (PS1-PS4, PM3_Strong, PP1_Strong) AND
   a. ≥3 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) OR
   b. 2 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) AND ≥2 Supporting (PP1-PP5, PM3_Supporting) OR
   c. 1 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) AND ≥4 Supporting (PP1-PP5, PM3_Supporting)

Likely Pathogenic

1. 1 Very Strong (PVS1, PM3_VeryStrong) AND 1 Moderate (PP1-PP5, PM3_Supporting) OR

2. 1 Strong (PS1-PS4, PM3_Strong, PP1_Strong) AND 1-2 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) OR

3. 1 Strong (PS1-PS4, PM3_Strong, PP1_Strong) AND ≥2 Supporting (PP1-PP5, PM3_Supporting) OR

4. ≥3 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) OR

5. 2 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) AND ≥2 Supporting (PP1-PP5, PM3_Supporting) OR

6. 1 Moderate (PM1-PM6, PP4_Moderate, PP1_Moderate) AND ≥4 Supporting (PP1-PP5, PM3_Supporting)

RULES FOR COMBINING BENIGN CRITERIA

Benign

1. 1 Stand-Alone (BA1) OR

2. ≥2 Strong (BS1-BS4)

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Likely Benign

1. 1 Strong (BS1-BS4) and 1 Supporting (BP1-BP7, BS3_Supporting) OR
2. ≥2 Supporting (BP1–BP7, BS3_Supporting)

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