Criterion Assessment Examples

ACMG Interpretation of Sequence Variants Guideline 2015

NOTE: Grayed text has been transformed to the sibling data worksheet [DMWG - ACMG Rule Examples Data](https://docs.google.com/spreadsheets/d/1fL3naWSpL_iDxkCN51g1CSLhXU6uAd1vwxZ0m1I4Zeg/edit#gid=1184726012). Please be aware that any changes to Grayed text will NOT be included in the final examples unless explicitly tracked. Notify Larry Babb if you wish to make changes to the Grayed text and wish to have the preserved in the final documentation.

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| PVS1 - Null Variant in known LOF genes | | 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. | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | S Harrison | Reviewer | |  | Modeler | Chris Bizon |
| Variant: NM\_031844.2(HNRNPU):c.2304\_2305del (p.Gly769Glufs\*83)  Variant is frameshift which would be LOF however PVS1 is not applicable because:  a. LOF is not an established disease mechanism for HNRNPU  b. Protein ends at p.826 and this frameshift would create stop at p.852 (769+83) thus NMD would not occur. Instead an elongated protein  CriterionAssessment (entity)  ID: CritAsses1  Allele: CA1  Criterion: PVS1  Outcome: Refuted  Explanation: Variant is not LOF and LOF is not a known mechanism of disease associated with this gene for any condition.  wasGeneratedBy: AssessCriterionActivity1  AssessCriterion (activity)  ID: AssessCriterionActivity1  wasAssociatedWith: SH (agent)  When:..  usedEvidenceStatement: [MolCon001, CondMech002]  usedCriterion: PVS1  usedAllele: CA1  MolecularConsequence (entity - evidenceStatement)  ID: MolCon001  Allele: AI1  Consequence: SO:0001589 (frameshift variant)  LOF: No  Explanation: Protein ends at p.826 and this frameshift would create stop at p.852 (769+83) thus NMD would not occur. Instead an elongated protein    ConditionMechanism (entity - evidenceStatement)  ID: CondMech002  Gene: G1  Mechanism: SO:0002054 (loss of function variant)  Established: False  Explanation: HNRNPU is not associated with any conditions, and therefore LOF is not an established mechanism of any condition for this gene.    Gene  ID: G1  Symbol: HNRNPU  …  CanonicalAllele:  ID: CA1  relatedContextualAllele: AI1  …  ContextualAllele:  ID: AI1  alleleName: NM\_031844.2(HNRNPU):c.2304\_2305del | | | | | | |
| #2 | Author | S Harrison | Reviewer | |  | Modeler | Chris Bizon |
| Variant: NM\_007294.3(BRCA2):c.7762\_7764delinsTT (p.I2588Ffs\*60)  PVS1 is applicable for this variant because  a. BRCA2 - LOF is an established disease mechanism for breast ovarian cancer  b. LOF is predicted to occur as variant is >55bp from penultimate exon  CriterionAssessment  ID: CritAsses2  Allele: CA2  Criterion: PVS1  Outcome: Very Strong Pathogenic  Condition: C1  wasGeneratedBy: AssessCriterionActivity2  AssessCriterion  ID: AssessCriterionActivity2  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [MolCon003, CondMech004]  usedCriterion: PVS1  usedAllele: CA2  MolecularConsequence  ID: MolCon003  Allele: AI2  Consequence: SO:0001589 (frameshift variant)  LOF: Yes  Explanation: LOF is predicted to occur as variant is >55bp from penultimate exon    ConditionMechanism  ID: CondMech004  Gene: G2  Condition: C1  Mechanism: SO:0002054 (loss of function variant)  Established: True  Explanation: This is so well-known that it does not require a citation.    Gene  ID: G2  Symbol: BRCA2  …  Condition:  ID: C1  Name: Breast Ovarian Cancer  CanonicalAllele:  ID: CA2  relatedContextualAllele: AI2  …  ContextualAllele:  ID: AI2  alleleName: NM\_007294.3(BRCA2):c.7762\_7764delinsTT | | | | | | |
| #3 | Author | S Harrison | Reviewer | |  | Modeler | Chris Bizon |
| Variant: NM\_133437.4(TTN):c.10670dupG (p.Leu3558Thrfs)  a. TTN - LOF is an established disease mechanism for dilated cardiomyopathy  b. However variant occurs in an alternate transcript of the TTN gene - this exon is only included in 4% of TTN transcripts  So PVS1 is not applicable    CriterionAssessment  ID: CritAsses3  Allele: CA3  Criterion: PVS1  Outcome: Refuted  Condition: C2  wasGeneratedBy: AssessCriterionActivity3  Explanation: This variant only occurs as LOF in an uncommon transcript (NM\_133437.4). For most transcripts, the variant is intronic.  AssessCriterion  ID: AssessCriterionActivity3  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [MolCon005, MolCon006, CondMech007]  usedCriterion: PVS1  usedAllele: CA3  MolecularConsequence  ID: MolCon005  Allele: AI3  Consequence: SO:0001589 (frameshift variant)  LOF: Yes  Explanation: LOF is predicted but this transcript is rare  MolecularConsequence  ID: MolCon006  Allele: AI4  Consequence: SO:0000191 (interior intron)  LOF: No    ConditionMechanism  ID: CondMech007  Gene: G3  Condition: C2  Mechanism: SO:0002054 (loss of function variant)  Established: True    Gene  ID: G3  Symbol: TTN  …  Condition:  ID: C2  Name: Dilated Cardiomyopathy  CanonicalAllele:  ID: CA3  relatedContextualAllele: [AI3, AI4]  …  ContextualAllele:  ID: AI3  alleleName: NM\_133437.4(TTN):c.10670dupG  ContextualAllele:  ID: AI4  alleleName: LRG\_391t2:c.10303+2691dupG | | | | | | |
| Issues | | The two main concerns for applying this rule is:  a. Is LOF an established disease mechanism?  b. Is LOF actually occurring? | | | | | |

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| PS1 - same as previous pathogenic amino acid change | | Same amino acid change as a previously established pathogenic variant regardless of nucleotide change | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | S Harrison | Reviewer | |  | Modeler | Chris Bizon |
| Assessed variant: PTPN11 - NM\_002834.3:c.855T>G - p.Phe285Leu  If assessing this variant, PS1 could be applied based on PTPN11 variant c.853T>C which also encodes Phe285Leu and is established as Pathogenic  CriterionAssessment  ID: CritAsses4  Allele: CA4  Criterion: PS1  Outcome: Pathogenic Strong  Explanation: The assessed variant produces the same amino acid change (p.Phe285Leu) as known pathogenic variant c.853T>C  wasGeneratedBy: AssessCriterionActivity4  AssessCriterion  ID: AssessCriterionActivity4  wasAssociatedWith: SH  When:..  usedEvidenceStatement: MDVI1  usedCriterion: PS1  usedAllele: [CA4,CA5,CA6]    MendelianDiseaseVariantInterpretation:  ID: MDVI1  Allele: CA5  Condition: C3  clinicalSignificance: Pathogenic  Condition:  ID: C3  Name: Noonan Syndrome 1  CanonicalAllele:  ID: CA4  relatedContextualAllele: AI5  …  ContextualAllele:  ID: AI5  alleleName: NM\_002834.3:c.855T>G  Related: AI7  relatedType: RO: 0003000 (produces)  ...  CanonicalAllele:  ID: CA5  relatedContextualAllele: AI6  ContextualAllele:  ID: AI6  alleleName: NM\_002834.3:c.853T>C  Related: AI7  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA6  relatedContextualAllele: AI7  ContextualAllele:  ID: AI7  alleleName: NP\_002825.3:p.Phe285Leu  Related: AI5  relatedType: RO: 0003001 (produced-by)  Related: AI6  relatedType: RO: 0003001 (produced-by) | | | | | | |
| #2 | Author | S Harrison | Reviewer | |  | Modeler | Chris Bizon |
| Assessed variant: DNAH5 - NM\_001369.2:c.6249G>C - p.Met2083Ile  A different variant creating the same AA change is pathogenic (c.6249G>A - p.Met2083Ile) but that change is pathogenic because it has been shown to disrupt splicing and create a premature stop codon. Prediction tools do not suggest c.6249G>C disrupts splicing so applying PS1 (as met by c.6249G>A) is likely not appropriate.  G>C Predictions:    G>A Predictions:    CriterionAssessment  ID: CritAsses5  Allele: CA7  Condition: C4  Criterion: PS1  Outcome: Insufficient Evidence  Explanation: The assessed variant produces the same amino acid change (p.Met2083Ile) as known pathogenic variant c.6249G>A. But that change is pathogenic because it has been shown to disrupt splicing and create a premature stop codon. Prediction tools do not suggest c.6249G>C disrupts splicing.  wasGeneratedBy: AssessCriterionActivity5  AssessCriterion  ID: AssessCriterionActivity5  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [MDVI2, Splicing evidence?]  usedCriterion: PS1  usedAllele: [CA7,CA8,CA9]    MendelianDiseaseVariantInterpretation:  ID: MDVI2  Allele: CA8  Condition: C4  clinicalSignificance: Pathogenic  Condition:  ID: C4  Name: Primary Ciliary Dyskenisia  CanonicalAllele:  ID: CA7  relatedContextualAllele: AI8  ContextualAllele:  ID: AI8  alleleName: NM\_001369.2:c.6249G>C  Related: AI10  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID: CA8  relatedContextualAllele: AI9  ContextualAllele:  ID: AI9  alleleName: NM\_001369.2:c.6249G>A  Related: AI10  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA9  relatedContextualAllele: AI10  ContextualAllele:  ID: AI10  alleleName: NP\_001360.1:p.Met2083Ile  Related: AI8  relatedType: RO: 0003001 (produced-by)  Related: AI9  relatedType: RO: 0003001 (produced-by) | | | | | | |
| #3 | Author | S Harrison | Reviewer | |  | Modeler | Chris Bizon |
| The Noonan WG decided PS1 was applicable for the same analogous residue positions/regions in highly analogous grouping (HRAS/KRAS/NRAS and MAP2K1/MAP2K2). So HRAS c.173C>T - p.Thr58Ile [g.533883G>A (chr11, GRCh37)] could use KRAS c.173C>T - p.Thr58Ile [g.25380285G>A (chr12, GRCh37)] and vice versa  CriterionAssessment  ID: CritAsses6  Allele: CA10  Condition: C6  Criterion: PS1  Outcome: Strong Pathogenic  Explanation: While the two amino acid changes are not identical, they are the same amino acid change in analogous residues of HRAS and KRAS.  wasGeneratedBy: AssessCriterionActivity6  AssessCriterion  ID: AssessCriterionActivity6  wasAssociatedWith: [SH, NoonanSubgroup]  When:..  usedEvidenceStatement: [MDVI3,MDVI4]  usedCriterion: PS1  usedAllele: [CA10,CA11,CA12,CA13]    MendelianDiseaseVariantInterpretation:  ID: MDVI3  Allele: CA12  Condition: C44  clinicalSignificance: Pathogenic  MendelianDiseaseVariantInterpretation:  ID: MDVI4  Allele: CA12  Condition: C5~~6~~  clinicalSignificance: Pathogenic  Condition:  ID: C5  Name: Costello Syndrome  Condition:  ID: C44  Name: Noonan Syndrome 3  Condition:  ID: C6  Name: Noonan syndrome and Noonan-related syndrome  CanonicalAllele:  ID: CA10  relatedContextualAllele: AI11  ContextualAllele:  ID: AI11  alleleName: NC\_000011.9:g.533883G>A  Related: AI13  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID: CA11  relatedContextualAllele: AI12  ContextualAllele:  ID: AI13  alleleName: NC\_000012.12:g.25227351G>A  Related: AI14  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA12  relatedContextualAllele: AI13  ContextualAllele:  ID: AI13  alleleName: NP\_005334.1:p.Thr58Ile  Related: AI11  relatedType: RO: 0003001 (produced-by)  CanonicalAllele:  ID CA13  relatedContextualAllele: AI14  ContextualAllele:  ID: AI14  alleleName: NP\_004976.2:p.Thr58Ile  Related: AI12  relatedType: RO: 0003001 (produced-by) | | | | | | |
| Issues | | Beware of changes that impact splicing rather than at the amino acid/protein level  ***From CSER paper*: One site erroneously used this to apply prior publication of the same exact variant while this rule, as described in more depth on the ACMG/AMP guideline, only applies when the established pathogenic variant has a different nucleotide change than the variant being interpreted.** | | | | | |

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| PS2 - confirmed de novo no family history | | De novo (both maternity and paternity confirmed) in a patient with the disease and no family history | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | S Harrison | Reviewer | |  | Modeler | Chris Bizon |
| Assessed variant: NM\_000257.2:c.5740G>A (p.Glu1914Lys)  According to PMID:24664454, this variant was identified de novo in an affected (cardiomyopathy) case and  a. There was no family history of the disease  b. The variant was proven to be de novo by showing both parents did not carry the variant  c. Haplotyping confirmed they were the parents  CriterionAssessment  ID: CritAsses7  Allele: CA14  Condition: C7  Criterion: PS2  Outcome: Strong Pathogenic  Explanation: IC1: Individual has condition, FH1: No family history, DNA1: variant is denovo (parentage confirmed)  wasGeneratedBy: AssessCriterionActivity7  AssessCriterion  ID: AssessCriterionActivity7  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [IC1, FH1, DNA1]  usedCriterion: PS2  usedAllele:CA14  IndividualCondition:  ID: IC1  Individual: Ind1  Condition: C7  hasCondition: True  FamilyHistory:  ID: FH1  Proband: Ind1  Condition: C7  familyHasCondition: False  DeNovoAllele:  ID: DNA1  Allele: CA14  Individual: Ind1  maternityConfirmed: True  paternityConfirmed: True  Explanation: parentage confirmed via haplotyping.  Individual:  ID: Ind1  Condition:  ID: C7  Name: Cardiomyopathy    CanonicalAllele:  ID: CA14  relatedContextualAllele: AI15  ContextualAllele:  ID: AI15  alleleName: NM\_000257.2:c.5740G>A | | | | | | |
| #2 | Author | S Harrison | Reviewer | |  | Modeler | Chris Bizon |
| Assessed variant: NM\_001927.3:c.1216C>T (p.Arg406Trp)  According to PMID: 10717012 & 10905661 (same case) this variant was identified de novo in an affected (desmin related myopathy) case and  a. Patient's parents are unaffected  b. Non-paternity was excluded by assessment with microsatellites  c. De novo variant occurred on haplotype inherited from the father    Maternity no explicitly confirmed - but if variant occurred on haplotype inherited from the father, then confirmation of maternity not required.  CriterionAssessment  ID: CritAsses8  Allele: CA15  Condition: C8  Criterion: PS2  Outcome: Strong Pathogenic  Explanation: De novo variant occurred on haplotype inherited from the father. Maternity no explicitly confirmed - but if variant occurred on haplotype inherited from the father, then confirmation of maternity not required.  wasGeneratedBy: AssessCriterionActivity8  AssessCriterion  ID: AssessCriterionActivity8  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [IC2, FH2, DNA2]  usedCriterion: PS2  usedAllele:CA15  IndividualCondition:  ID: IC2  Individual: Ind2  Condition: C8  hasCondition: True  FamilyHistory:  ID: FH2  Proband: Ind2  Condition: C8  familyHasCondition: False  DeNovoAllele:  ID: DNA2  Allele: CA15  Individual: Ind2  maternityConfirmed: False  paternityConfirmed: True  Explanation: Non-paternity was excluded by assessment with microsatellites  Individual:  ID: Ind2  Condition:  ID: C8  Name: desmin related myopathy (myofibrillar myopathy 1)  CanonicalAllele:  ID: CA15  relatedContextualAllele: AI15  ContextualAllele:  ID: AI15  alleleName:NM\_001927.3:c.1216C>T | | | | | | |
| #3 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_000833 (GRIN2A):c.4375T>C (p.Ser1459Gly)    (From CSER bakeoff) - as variant was identified via trio WES, group assumes paternity and maternity were confirmed and thus PS2 is applicable.  Proband affected with epilepsy with mental retardation - both parents unaffected    CriterionAssessment  ID: CritAsses9  Allele: CA16  Condition: C10  Criterion: PS2  Outcome: Strong Pathogenic  Explanation:  wasGeneratedBy: AssessCriterionActivity9  AssessCriterion  ID: AssessCriterionActivity9  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [IC3, FH3, DNA3]  usedCriterion: PS2  usedAllele:CA16  IndividualCondition:  ID: IC3  Individual: Ind3  Condition: C10  hasCondition: True  FamilyHistory:  ID: FH3  Proband: Ind3  Condition: C10  familyHasCondition: False  DeNovoAllele:  ID: DNA3  Allele: CA16  Individual: Ind3  maternityConfirmed: True  paternityConfirmed: True  Explanation: Variant from trio WES, which confirmed patentage  Individual:  ID: Ind3  Condition:  ID: C10  Name: Epilepsy with Mental Retardation  CanonicalAllele:  ID: CA16  relatedContextualAllele: AI16  ContextualAllele:  ID: AI16  alleleName: NM\_000833 (GRIN2A):c.4375T>C | | | | | | |
| Issues | | MYH7 is adding specificity to this rule with  a. No family history = parents have had ECHO and ECG  b. Only paternity confirmation required | | | | | |

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| PS3 - well-established damaging effect functional studies | | Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product | | | | |
| **Evidence Statement Types** | | | | | | |
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| **Examples** | | | | | | |
| #1 | Author | Tasha Strande | Reviewer |  | Modeler |  |
| Assessed variant: NM\_001001431(TNNT2):c.391C>T (p.Arg131Trp)  PMID-17932326: the mutation lowered the Ca2+ affinity of the reconstituted thin filaments; PMID-15923195: reduced Ca2 sensitivity of activation in ATPase and motility assays; PMID-22675533: the mutation desensitized Ca2+ binding to the thin filament.  CSER Bakeoff variant - There was some debate as to whether this rule should be applied or not - 2 sites invoked this rule and one called it weak  CriterionAssessment  ID: CritAsses10  Allele: CA17  Criterion: PS3  Outcome: Strong Pathogenic  Explanation:  wasGeneratedBy: AssessCriterionActivity10  AssessCriterion  ID: AssessCriterionActivity10  wasAssociatedWith: CSER  When:..  usedEvidenceStatement: [F1,F2,F3, F4]  usedCriterion: PS3  usedAllele: CA17, CA18    FunctionalData:  ID: F1  Result:the mutation lowered the Ca2+ affinity of the reconstituted thin filaments;  dataType: Binding assay?  Allele: AI18  Gene:G3  Explanation:  FunctionalData:  ID: F2  Result: reduced Ca2 sensitivity of activation  dataType: ATPase assay?  Allele: AI18  Gene:G3  Explanation:  FunctionalData:  ID: F3  Result: reduced Ca2 sensitivity of activation  dataType: motility assay?  Allele: AI18  Gene:G3  Explanation:  FunctionalData:  ID: F4  Result: desensitized Ca2+ binding to the thin filament.  dataType: bindingAssay  Allele: AI18  Gene: G3  Gene:  ID: G3  Name: TNNT2  CanonicalAllele:  ID: CA17  relatedContextualAllele: AI17  …  ContextualAllele:  ID: AI17  alleleName: NM\_001001431(TNNT2):c.391C>T  Related: AI18  relatedType: RO: 0003000 (produces)  ...  CanonicalAllele:  ID: CA18  relatedContextualAllele: AI18  ContextualAllele:  ID: AI18  alleleName: NP\_001001431.1:p.Arg131Trp  Related: AI17  relatedType: RO: 0003001 (produced-by) | | | | | |
| #2 | Author | Tasha Strande | Reviewer |  | Modeler |  |
| Assessed variant: NM\_003119.23 (SPG7):c.1529C>T (p.Ala510Val)  PMID:20186691- yeast complementation assay  CSER Bakeoff variant - Some sites invoked this rule and others did not → one group bumped this evidence to Moderate  CriterionAssessment  ID: CritAsses11  Allele: CA19  Criterion: PS3  Outcome: Strong Pathogenic  Explanation:  wasGeneratedBy: AssessCriterionActivity11  AssessCriterion  ID: AssessCriterionActivity11  wasAssociatedWith: CSER  When:..  usedEvidenceStatement: F5  usedCriterion: PS3  usedAllele: CA19    FunctionalData:  ID: F5  Result:Functionally Damaging  dataType: Yeast complementation assay  Allele: AI19  Gene:G4  Explanation: Expression of human SPG7 (with this mutation) in yeast with damaged m-AAA protease did not restore respiratory growth or MrpL32 processing, if the function of AFG3L2 was impaired.  Gene:  ID: G4  Name: SPG7  CanonicalAllele:  ID: CA19  relatedContextualAllele: AI19  …  ContextualAllele:  ID: AI19  alleleName: NM\_003119.23 (SPG7):c.1529C>T | | | | | |
| #3 | Author | S Harrison | Reviewer |  | Modeler |  |
| Assessed variant: NM\_004333.4(BRAF):c.1787G>T (p.Gly596Val)  The RASopathy group decided for BRAF both MEK activation and ERK activation assays (in which the variant results in abnormal pattern of pERK/ERK or pMEK/MEK) is a validated assay.  PMID:16439621 shows that Gly596Val results in abnormal/decreased MEK and ERK activiation so PS3 is applicable for this variant    CriterionAssessment  ID: CritAsses12  Allele: CA20  Criterion: PS3  Outcome: Strong Pathogenic  Explanation: The RASopathy group considers MEK and ERK activation assays as well validated.  wasGeneratedBy: AssessCriterionActivity12  AssessCriterion  ID: AssessCriterionActivity12  wasAssociatedWith: RASopathy group  When:..  usedEvidenceStatement: [F6,F7]  usedCriterion: PS3  usedAllele: CA20    FunctionalData:  ID: F5  Result:abnormal/deceased MEK activtation.  dataType: Activation Assay  Allele: AI21  Gene: [G6, G7]  Explanation:  FunctionalData:  ID: F6  Result:abnormal/deceased ERK activtation.  dataType: Activation Assay  Allele: AI21  Gene: [G6, G8]  Explanation:  Gene:  ID: G6  Name: BRAF  Gene  ID: G7  Name: MEK  Gene:  ID: G8  Name: ERK  CanonicalAllele:  ID: CA20  relatedContextualAllele: AI20  …  ContextualAllele:  ID: AI20  alleleName: NM\_004333.4(BRAF):c.1787G>T  Related: AI21  relatedType: RO: 0003000 (produces)  ...  CanonicalAllele:  ID: CA21  relatedContextualAllele: AI21  ContextualAllele:  ID: AI21  alleleName: NP\_004324.2:p.Gly596Val  Related: AI20  relatedType: RO: 0003001 (produced-by) | | | | | |
| Issues | | This particular rule will probably be downgraded often, given that there are not many “Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting” | | | | |

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| PS4 - Prevalence increase in affected v control | | The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls | | | | | | |
| **Evidence Statement Types** | | | | | | | | |
|  | | | **ConditionPrevalance TODO** | | |  | | |
| **Examples** | | | | | | | | |
| #1 | Author | S Harrison | | Reviewer |  | | Modeler | Chris Bizon |
| Assessed variant: SLC26A4 NM\_000441.1:c.1003T>C (p.Phe335Leu)    Across all published data (and LMM data) the c.1003T>C variant has been identified in 32 chromosomes with hearing loss of 4076 total hearing loss chromosomes assayed (32+ ; 4044-). In ExAC (<http://exac.broadinstitute.org/variant/7-107329499-T-C>) this variant is found in 105 / 121336 total chromosomes.    Case control comparison shows statistically significant difference between affects and unaffected (OR 9.1361 99%CI: 6.1425 - 13.5889)  CriterionAssessment  ID: CritAsses13  Allele: CA22  Criterion: PS4  Outcome: Strong Pathogenic  Condition: C10  Explanation: statistically significant difference between affects and unaffecteds  wasGeneratedBy: AssessCriterionActivity13  AssessCriterion  ID: AssessCriterionActivity13  wasAssociatedWith: SH  When:..  usedEvidenceStatement: CC1  usedCriterion: PS4  usedAllele: CA22  usedCondition: C10    CaseControl:  ID: CC1  Allele: CA22  Condition: C10  odssRatio:9.1361  confidenceInterval:99%CI: 6.1425 - 13.5889  caseGroup: GAF1  conrolGroup:: PAF1  Explanation: Treating ExAC data as controls for  GroupAlleleFrequency  ID: GAF1  Ascertainment: Across all published data (and LMM data) with hearing loss.  Allele: AI22  alleleCount: 32  alleleNumber:4076  PopulationAlleleFrequency:  ID: PAF1  Ascertainment: ExAC  Allele: AI22  alleleCount:105  alleleNumber: 121336  ...  Condition:  ID: C10  Name: Hearing Loss (autosomal recessive nonsyndromic deafness)  CanonicalAllele:  ID: CA22  relatedContextualAllele: AI22  …  ContextualAllele:  ID: AI22  alleleName:SLC26A4 NM\_000441.1:c.1003T>C  ... | | | | | | | |
| #2 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Assessed variant: NM\_000540.2 (RYR1):c.1840C>T (p.Arg614Cys)    Across publications, this variant is thought to account for 2%-10% of patients with Malignant hyperthermia. But variant is also only found in 9/66740 Europeans in ExAC (.01%). Based on these differences, groups applied PS4 to this variant classification (without a formal OR analysis).  CriterionAssessment  ID: CritAsses14  Allele: CA23  Criterion: PS4  Outcome: Strong Pathogenic  Condition: C11  Explanation: Across publications, this variant is thought to account for 2%-10% of patients with Malignant hyperthermia. But variant is also only found in 9/66740 Europeans in ExAC (.01%). Based on these differences, groups applied PS4 to this variant classification (without a formal OR analysis).  wasGeneratedBy: AssessCriterionActivity14  AssessCriterion  ID: AssessCriterionActivity14  wasAssociatedWith: SH  When:..  usedEvidenceStatement: CC2  usedCriterion: PS4  usedAllele: CA23  usedCondition: C11  CaseControl:  ID: CC2  Allele: CA23  Condition: C11  caseGroup: GAF2  controlGroup: PAF2  Explanation: Did not calculate formal odds ratio  GroupAlleleFrequency  ID: GAF2  Allele: AI23  Explanation: This allele is thought to account for between 2% and 10% of patients with Malignant hyperthermia.  PopulationAlleleFrequency:  ID: PAF2  Ascertainment: ExAC  Allele: AI23  alleleCount: 9  alleleNumber: 66740  ...  Condition:  ID: C11  Name: Malignant Hyperthermia    CanonicalAllele:  ID: CA23  relatedContextualAllele: AI23  …  ContextualAllele:  ID: AI23  alleleName:NM\_000540.2 (RYR1):c.1840C>T | | | | | | | |
| #3 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Assessed variant: NM\_006208.2 (ENPP1):c.517A>C (p.Lys173Gln)  In a case control study (PMID 23633196), authors compared incidence of C allele (Gln) in individuals affected with abnormal glucose homeostasis vs normal controls. This analysis gave OR 1.06 (95% CI: 0.96-1.18; p value 0.24). With an OR so close to 1.00 and CI that dips below 1.00, different between cases and controls is not significant and thus PS4 is not applicable.    CriterionAssessment  ID: CritAsses15  Allele: CA24  Criterion: PS4  Outcome: Insufficient evidence  Condition:  Phenotype: Phen001  wasGeneratedBy: AssessCriterionActivity15  AssessCriterion  ID: AssessCriterionActivity15  wasAssociatedWith: SH  When:..  usedEvidenceStatement: CC3  usedCriterion: PS4  usedAllele: CA24  usedCondition:  usedPhenotype: Phen001  CaseControl:  ID: CC3  Allele: CA24  Condition:  Phenotype: [Phen001]  caseGroup: GAF3  controlGroup: GA4  oddsRatio: 1.06  confidenceLevel: 0.95  confidenceIntervalLower: 0.96  confidenceIntervalUpper: 1.18  Explanation: Odds ratio is calculated by pooling samples from the six GENIUS T2D cohorts before calculation.  GroupAlleleFrequency  ID: GAF3  Allele: AI24  individualCount:3672  alleleCount: 1198  alleleNumber: 7344  homozygousAlleleIndividualCount: 113  heterozygousAlleleIndividualCount: 972  Ascertainment: Case group is composed of cases from six cohorts from the GENIUS T2D consortium. Case status is defined per-cohort.  raceOrEthnicity: European  GroupAlleleFrequency  ID: GAF4  Allele: AI24  individualCount:2935  alleleCount: 936  alleleNumber: 5870  homozygousAlleleIndividualCount: 77  heterozygousAlleleIndividualCount: 782  Ascertainment: Controls group is composed of controls from six cohorts from the GENIUS T2D consortium. Controls status is defined per-cohort.  raceOrEthnicity: European  Phenotype:  ID: Phen001  Name: Abnormal Glucose Homeostasis    CanonicalAllele:  ID: CA24  relatedContextualAllele: AI24  …  ContextualAllele:  ID: AI24  alleleName:NM\_006208.2:c.517A>C | | | | | | | |
| Issues | |  | | | | | | |

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| PM1 - Located in hot spot w/out benign variation | | Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | Tasha Strande | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_005228.3(EGFR):c.2369C>T(p.Thr790Met)  Variant is located within the kinase domain (where multiple other pathogenic variants are located)  CSER bakeoff - 7/10 sites agreed on this rule  CriterionAssessment  ID: CritAsses16  Allele: CA25  Criterion: PM1  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity16  Explanation: Variant is located within the kinase domain (where multiple other pathogenic variants are located)  AssessCriterion  ID: AssessCriterionActivity16  wasAssociatedWith: TS  When:..  usedEvidenceStatement: RA1, RCA1  usedCriterion: PM1  usedAllele: CA25  RegionContainsAllele:  ID: RCA1  Region: CR1  Allele: AI\_126  Value: True  RegionAnnotation:  ID: RA1  Region: CR1  Value: True  Type: functional  Explanation: This region is the catalytic (kinase) domain.  ContextualRegion:  ID: CR1  Sequence: PS1  Start:704  Stop:1016  ReferenceSequence:  ID: PS1  Identifier: NP\_005219.2  Related: PS2  ReferenceSequence:  ID: PS2  Identifier: NM\_005228.3  Related: PS1  CanonicalAllele:  ID: CA25  relatedContextualAllele: AI25  ContextualAllele:  ID: AI25  alleleName:NM\_005228.3(EGFR):c.2369C>T  Related: AI\_126  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID: CA\_1230  relatedContextualAllele: AI\_126  ContextualAllele:  ID: AI\_126  alleleName: NP\_005219.2:p.Thr790Met  Related: AI25  relatedType: RO: 0003001 (produced-by) | | | | | | |
| #2 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_004333.4(BRAF):c.1789C>G (p.Leu597Val)  RASopathy group decided that exon 15 of BRAF, which encodes the serine-threonine-protein kinase catalytic domain, is an established functional domain for BRAF. Thus, in assessing c.1789C>G, which falls in exon 15, applying PM1 is appropriate  CriterionAssessment  ID: CritAsses17  Allele: CA26  Criterion: PM1  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity17  Explanation: Variant is located in exon 15, which the RASopathy group has decided is an established functional domain for BRAF  AssessCriterion  ID: AssessCriterionActivity17  wasAssociatedWith: SH, RASopathy group  When:..  usedEvidenceStatement: RA2, RCA2  usedCriterion: PM1  usedAllele: CA26  RegionContainsAllele:  ID: RCA2  Region: CR2  Allele: AI26  Value: True  RegionAnnotation:  ID: RA2  Region: CR2  Value: True  Type: functional  Explanation: This region is exon 15 in the transcript, and codes the serine-threonine-protein kinase catalytic domain.  ContextualRegion:  ID: CR2  Sequence: TS2  Start:1803  Stop:1921  ReferenceSequence:  ID: TS2  Identifier: NM\_004333.4  CanonicalAllele:  ID: CA26  relatedContextualAllele: AI26  ContextualAllele:  ID: AI26  alleleName: NM\_004333.4(BRAF):c.1789C>G | | | | | | |
| #3 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Assessed variant: GLA c.902G>C (p.Arg301Pro)  This rule can also be met by a mutational hotspot as the ACMG papers says are “hotspots in less well-characterized regions of genes are reported, in which  pathogenic variants in one or several nearby residues have been observed with greater frequency.”  So for variant GLA c.902G>C (p.Arg301Pro), PM1 is applicable as the following surrounding variants are pathogenic: c.886A>G (p.Met296Val) ; c.890C>T (p.Ser297Phe) ; c.899T>C (p.Leu300Pro) ; c.902G>A (p.Arg301Gln)  CriterionAssessment  ID: CritAsses18  Allele: CA27  Criterion: PM1  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity18  Explanation: The variant is near 4 other pathogenic variants  AssessCriterion  ID: AssessCriterionActivity18  wasAssociatedWith: SH  When:..  usedEvidenceStatement: RA3, MDVI1, MDVI2, MDVI3, MDVI4, RCA1, RCA2, RCA3, RCA4, RCA5,BMVR102  usedCriterion: PM1  usedAllele:CA27  RegionContainsAllele:  ID: RCA1  Region: CR3  Allele: AI27  Value: True  RegionContainsAllele:  ID: RCA2  Region: CR3  Allele: AI28  Value: True  RegionContainsAllele:  ID: RCA3  Region: CR3  Allele: AI29  Value: True  RegionContainsAllele:  ID: RCA4  Region: CR3  Allele: AI30  Value: True  RegionContainsAllele:  ID: RCA5  Region: CR3  Allele: AI31  Value: True  RegionAnnotation:  ID: RA3  Region: CR3  Value: True  Type:mutational hotspot  BenignMissenseVariationRate:  ID: BMVR102  Region: CR3  Value: low  Explanation: ClinVar shows no benign missense variants in (or near) this region. The closest is NM\_000169.2:c.8T>C  ContextualRegion:  ID: CR3  Sequence: TS3  Start:996  Stop:1012  ReferenceSequence:  ID: TS3  Identifier: NM\_000169.2  CanonicalAllele:  ID: CA27  relatedContextualAllele: AI27  ContextualAllele:  ID: AI27  alleleName: NM\_000169.2:c.902G>A  MendelianDiseaseVariantInterpretation:  ID: MDVI1  Allele: CA28  Condition: C22  clinicalSignificance: Pathogenic  MendelianDiseaseVariantInterpretation:  ID: MDVI2  Allele: CA29  Condition: C22  clinicalSignificance: Pathogenic  MendelianDiseaseVariantInterpretation:  ID: MDVI3  Allele: CA30  Condition: C22  clinicalSignificance: Pathogenic  MendelianDiseaseVariantInterpretation:  ID: MDVI4  Allele: CA31  Condition: C22  clinicalSignificance: Pathogenic  Condition:  ID: C22  Condition: Fabry Disease  CanonicalAllele:  ID: CA28  relatedContextualAllele: AI28  ContextualAllele:  ID: AI28  alleleName:NM\_000169.2: c.886A>G  CanonicalAllele:  ID: CA29  relatedContextualAllele: AI29  ContextualAllele:  ID: AI29  alleleName: NM\_000169.2:c.890C>T  CanonicalAllele:  ID: CA30  relatedContextualAllele: AI30  ContextualAllele:  ID: AI30  alleleName:NM\_000169.2:c.899T>C    CanonicalAllele:  ID: CA31  relatedContextualAllele: AI31  ContextualAllele:  ID: AI31  alleleName: NM\_000169.2:c.902G>A | | | | | | |
| Issues | | Suggested that this rule is NOT appropriate for LOF variants  Also, note that when the region of interest is a “well-established functional domain” then it’s not necessary to show through evidence that it is intolerant of benign missense variants. However, when a mutational hotspot is being defined, then showing it does not have benign missense variants is important. | | | | | |

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| PM2 - Absent from controls | | Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Variant assessed: NM\_000257.2 (MYH7) c.5135G>A (p.Arg1712Gln)  Variant is not completely absent from ExAC: <http://exac.broadinstitute.org/variant/14-23884860-C-T>  Variant found in 1/121366 total chr and specifically 1/66706 European chr.    PM2 is applicable as even though variant is not 100% absent, it is practically absent (using rules in Issues below)  CriterionAssessment  ID: CritAsses19  Allele: CA32  Criterion: PM2  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity19  Explanation: PM2 is applicable as even though variant is not 100% absent, it is practically absent (a 95% CI for the population goes from 0.00% to 0.01%)  AssessCriterion  ID: AssessCriterionActivity19  wasAssociatedWith: SH  When:..  usedEvidenceStatement: PAF3, PAF4  usedCriterion: PM2  usedAllele: CA32  PopulationAlleleFrequency  ID:PAF3  Ascertainment: ExAC  raceOrEthnicity: European  alleleNumber:1  alleleCount:66706  alleleFrequency: 1.499e-05  homozygousAlleleIndividualCount: 0  PopulationAlleleFrequency  ID:PAF4  Ascertainment: ExAC  raceOrEthnicity: Combination  alleleNumber:1  alleleCount:121366  alleleFrequency: 8.24e-06  homozygousAlleleIndividualCount: 0  CanonicalAllele:  ID: CA32  relatedContextualAllele: AI32  ContextualAllele:  ID: AI32  alleleName: NM\_000257.2:c.5135G>A | | | | | | |
| #2 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Variant assessed: MUTYH c.64G>A (p,Val22Met)  Variant is very frequent in ExAC:  <http://exac.broadinstitute.org/variant/1-45800156-C-T>  Global MAF 4.9% (5967/121406) and specifically European MAF 7.8% (514/6614) thus variant is too common to be disease causing and PM2 is not applicable  CriterionAssessment  ID: CritAsses20  Allele: CA33  Criterion: PM2  Outcome: Refuted  wasGeneratedBy: AssessCriterionActivity20  AssessCriterion  ID: AssessCriterionActivity20  wasAssociatedWith: SH  When:..  usedEvidenceStatement: PAF5  usedCriterion: PM2  usedAllele: CA33  PopulationAlleleFrequency  ID:PAF5  Ascertainment: ExAC  raceOrEthnicity: Finnish European  alleleNumber:514  alleleCount:6614  alleleFrequency: 0.07771  homozygousAlleleIndividualCount: 21  medianCoverage: 100  CanonicalAllele:  ID: CA33  relatedContextualAllele: AI33  …  ContextualAllele:  ID: AI33  alleleName: NC\_000001.10:g.45800156C>T | | | | | | |
| #3 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Variant assessed: NM\_001399.4 (EDA) c.676C>T (p.Gln226X)  Variant is absent from ExAC (and all other pop databases):  <http://exac.broadinstitute.org/variant/X-69247856-C-T>  However, median coverage at this position is 10X. Typically, for coverage <20X, we don’t say “absent”. So PM2 is not applicable  CriterionAssessment  ID: CritAsses21  Allele: CA34  Criterion: PM2  Outcome: Insufficient Evidence  wasGeneratedBy: AssessCriterionActivity21  Explanation: while the variant is absent from exac, mean coverage (11X) is insufficient to be sure that variant was fully assayed.  AssessCriterion  ID: AssessCriterionActivity21  wasAssociatedWith: SH  When:..  usedEvidenceStatement: PAF6  usedCriterion: PM2  usedAllele: CA34  PopulationAlleleFrequency  ID:PAF6  Ascertainment: ExAC  raceOrEthnicity: Combined  alleleNumber: 0 (how many were tested)  alleleCount: 0 (how many had the specific allele)  alleleFrequency: 0  medianCoverage: 11  CanonicalAllele:  ID: CA34  relatedContextualAllele: AI34  …  ContextualAllele:  ID: AI34  alleleName:NM\_001399.4: c.676C>T | | | | | | |
| #4 | Author | S Harrison | Reviewer | |  | Modeler | L Babb |
| (CSER var#72,classifier#3)  NM\_001369.2(DNAH5):c.7468\_7488del (p.Trp2490\_Leu2496del)  Variant is absent from ExAC (<http://exac.broadinstitute.org/region/5-13810280-13810320>) however:   * Very low coverage across this region (~5X) and usually 20X is used as a cut-off to say “absent” * Variant is 21 nt deletion - databases may not be able to call a variant of that size (I rarely see an indel of this size in ExAC)   So you can’t really say this variant is “absent” (PM2) - so I would argue PM2 is not applicable for this variant  CriterionAssessment  ID: CritAsses31  Allele: CA35  Criterion: PM2  Outcome: Insufficient Evidence  wasGeneratedBy: AssessCriterionActivity21-1  Explanation: while the variant is absent from exac, however  1) very low coverage across this region (~5X) and usually 20X is used as a cut-off to say “absent”  2) variant is 21 nt deletion - databases may not be able to call a variant of that size (I rarely see an indel of this size in ExAC).  AssessCriterion  ID: AssessCriterionActivity31  wasAssociatedWith: SH  When:..  usedEvidenceStatement: PAF6-1  usedCriterion: PM2  usedAllele: CA35  PopulationAlleleFrequency  ID:PAF6-1  Ascertainment: ExAC  raceOrEthnicity: Combined  alleleNumber:  alleleCount:0  alleleFrequency: 0  medianCoverage: 11 | | | | | | |
| #5 | Author | S Harrison | Reviewer | |  | Modeler | C Bizon |
| RECESSIVE EXAMPLE:  NM\_023036.4(DNAI2):c.1304G>A (p.Trp435Ter)  <http://exac.broadinstitute.org/variant/17-72305484-G-A>  Variant occurs in DNAI2 which is known to cause autosomal recessive Primary Ciliary Dyskinesia.  Variant global MAF is 0.01% and 0.02% in European chr (14/66582). Although this variant has been seen in the general population, its frequency is low enough to be consistent with a recessive carrier frequency. So this variant is “nearly” absent enough that PM2 is applicable.  CriterionAssessment  ID: CritAsses51  Allele: CA77  Criterion: PM2  Condition: C004  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity053  Explanation: Although this variant has been seen in the general population, its frequency is low enough to be consistent with a recessive carrier frequency.    AssessCriterion  ID: AssessCriterionActivity053  wasAssociatedWith: SH  When:..  usedEvidenceStatement: PAF157,CondInt156  usedCriterion: PM2  usedAllele: CA77    ConditionInheritance:  ID: CondInt156  Condition: C004  modeOfInheritance:HP:0000007 (Autosomal Recessive)    Condition:  ID: C004  Name: Primary Ciliary Dyskinesia    PopulationAlleleFrequency  ID:PAF157  Ascertainment: ExAC  raceOrEthnicity: Combined  alleleNumber: 121100  alleleCount:14  alleleFrequency: 0.0001158  homozygousAlleleIndividualCount: 0    CanonicalAllele:  ID: CA77  relatedContextualAllele: AI100    ContextualAllele:  ID: AI100  alleleName: NM\_023036.4(DNAI2):c.1304G>A | | | | | | |
| Issues | | Some groups are allowing to rule to be used if even not 100% absent  For instance, Cardio has allowed PM2 to be used if MAF is <0.05% with 95% CI if  CI lower is ~0.00% (at 2 decimal places) AND CI upper is below 0.05%  **Caveat:** Population data for insertions/deletions may be poorly called by next-generation sequencing | | | | | |

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| PM3 - Recessive in trans w/ Path | | For recessive disorders, detected in trans with a pathogenic variant | | | | | | |
| **Evidence Statement Types** | | | | | | | | |
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| **Examples** | | | | | | | | |
| #1 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant assessed: DNAH5 - NM\_001369.2:c.7468\_7488del (p.Trp2490\_Leu2496del)    LMM data - In an affected proband with recessive Primary Ciliary Dyskinesia, the two DNA5 variants were identified:  c.7468\_7488del (p.Trp2490\_Leu2496del)  c.9449delG (p.Gly3150AlafsX24)    This case can be used for PM3 because:  a. Second variant c.9449delG is pathogenic as LOF variants in DNAH5 are associated with autosomal recessive primary ciliary dyskinesia (PCD) with outer dynein arm (ODA) defects (Olbrich 2002, Hornef 2006, Ferkol 2013)  b. Father was het for c.9449delG variant and negative for c.7468\_7488del variant. Mother was het for c.7468\_7488del variant and negative for c.9449delG variant. Thus, variants are in trans in this proband.  CriterionAssessment  ID: CritAsses22  Allele: CA35  Condition: C10  Criterion: PM3  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity22  AssessCriterion  ID: AssessCriterionActivity22  wasAssociatedWith: SH  When:..  usedEvidenceStatement: IndAll1,IC4, MDVI5, CondInt1  usedCriterion: PM3  usedAllele: CA35, CA36  IndividualAllele:  ID: IndAll1  Individual: Ind4  primaryAllele: CA35  secondaryAllele: CA36  primaryZygosity: heterozygous  secondaryZygosity:heterozygous  Phase: trans  Explanation: Father was het for c.9449delG variant and negative for c.7468\_7488del variant. Mother was het for c.7468\_7488del variant and negative for c.9449delG variant. Thus, variants are in trans in this proband.  MendelianDiseaseVariantInterpretation:  ID: MDVI5  Allele: CA36  clinicalSignificance: Pathogenic  Condition: C10  IndividualCondition:  ID: IC4  Condition: C11  Individual: Ind4  hasCondition: True  ConditionInheritance:  ID: ContInt1  Condition: C11  modeOfInheritance: HP:0000007 (Autosomal Recessive)  (source: OMIM - or primary Literature)  Condition:  ID: C10  Name: primary ciliary dyskinesia (PCD) with outer dynein arm (ODA) defects  Condition:  ID: C11  Name: Recessive Primary Ciliary Dyskinesia  Individual:  ID: Ind4    CanonicalAllele:  ID: CA35  relatedContextualAllele: AI35  …  ContextualAllele:  ID: AI35  alleleName:NM\_001369.2:c.7468\_7488del  CanonicalAllele:  ID: CA36  relatedContextualAllele: AI36  ContextualAllele:  ID: AI36  alleleName:NM\_001369.2:c.9449delG | | | | | | | |
| #2 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant assessed: NM\_133261.2 (GIPC3):c.298G>A (p.Asp100Asn)  This variant was found in a case with nonsyndromic hearing loss and who also carried Pathogenic GIPC3 variant c.411+1G>A (called Pathogenic internally at LMM). Based on the NGS data, we are able to determine that c.298G>A and c.411+1G>A are in trans as no NGS reads carry both variants. Thus PM3 is applicable without having to confirm trans by doing parental testing.    CriterionAssessment  ID: CritAsses23  Allele: CA37  Condition: C12  Criterion: PM3  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity23  AssessCriterion  ID: AssessCriterionActivity23  wasAssociatedWith: SH  When:..  usedEvidenceStatement: IndAll2,IC5, MDVI6, CondInt2  usedCriterion: PM3  usedAllele: CA35, CA36  IndividualAllele:  ID: IndAll2  Individual: Ind5  primaryAllele: CA37  secondaryAllele: CA38  primaryZygosity: heterozygous  secondaryZygosity:heterozygous  Phase: trans  Explanation: Based on the NGS data, we are able to determine that c.298G>A and c.411+1G>A are in trans as no NGS reads carry both variants.  IndividualCondition:  ID:IC5  Individual: Ind5  Condition: C12  hasCondition: True  ConditionInheritance:  ID: CondInt2  Condition: C12  Gene: G9  modeOfInheritance: HP:0000007 (Autosomal Recessive)  MendelianDiseaseVariantInterpretation:  ID: MDVI6  Allele: CA38  clinicalSignificance: Pathogenic  Condition: C12  Gene:  ID: G9  Name: GIPC3  Condition:  ID:C12  Name: Nonsyndromic Hearing Loss  Individual:  ID: Ind5  CanonicalAllele:  ID: CA37  relatedContextualAllele: AI37  ContextualAllele:  ID: AI37  alleleName:NM\_133261.2:c.298G>A  CanonicalAllele:  ID: CA38  relatedContextualAllele: AI38  ContextualAllele:  ID: AI38  alleleName:NM\_133261.2:c.411+1G>A | | | | | | | |
| #3 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant assessed: NM\_000391.3(TPP1):c.1678\_1679delCT (p.Leu560ThrfsX47)    From CSER Bakeoff: Two sites applied PM3 to this variant - however variant has only been described in 1 publication in 1 individual with neuronal ceroid lipofuscinosis. However, individual was homozygous for the c.1678\_1679delCT variant. **So applicable of PM3 is incorrect as the variant in trans is not pathogenic (same variant).**    CriterionAssessment  ID: CritAsses24  Allele: CA39  Criterion: PM3  Outcome: Insufficient Evidence  wasGeneratedBy: AssessCriterionActivity24  Explanation: Application of PM3 is incorrect as the variant in trans is know known to be pathogenic (it is the same variant).  AssessCriterion  ID: AssessCriterionActivity24  wasAssociatedWith: SH  When:..  usedEvidenceStatement: IndAll3,IC6,, CondInt3  usedCriterion: PM3  usedAllele: CA39  IndividualAllele:  ID: IndAll3  Individual: Ind6  primaryAllele: CA39  primaryZygosity: homozygous  IndividualCondition:  ID:IC6  Individual: Ind6  Condition: C13  hasCondition: True  ConditionInheritance:  ID: CondInt3  Condition: C13  Gene: G10  modeOfInheritance: HP:0000007 (Autosomal Recessive)  Gene:  ID: G10  Name: TPP1  Condition:  ID:C13  Name: neuronal ceroid lipofuscinosis  Individual:  ID: Ind6  CanonicalAllele:  ID: CA38  relatedContextualAllele: AI39  ContextualAllele:  ID: AI39  alleleName:NM\_000391.3:c.1678\_1679delCT | | | | | | | |
| Issues | | CSER - Laboratories discussed when to modify the strength of PM3, the variant is seen in *trans* with a pathogenic variant for recessive disorders. Published literature may not always explicitly state the phase of variants found in affected individuals which raises a challenge for invoking PM3.  When phase has not been established, some felt that PM3 could be invoked as supporting evidence. Also, if the variant is seen in *trans* with a pathogenic variant in more than one individual it was felt that PM3 can be upgraded to strong. However, sites did not agree on how many additional observations were necessary to call the evidence strong (2 vs. 3) but concluded that such guidance would be useful.    LMM - *INCREASES IN STRENGTH IF VARIANT SEEN IN MULTIPLE COMPOUND HETS WITH DIFFERENT LIKELY PATHOGENIC/PATHOGENIC VARIANTS* | | | | | | |

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| PM4 - Protein length changes or stop-loss | | Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | Tasha Strande | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_001369.2(DNAH5):c.7468\_7488del (p.Trp2490\_Leu2496del)  The Trp2490\_Leu2496del variant in DNAH5 leads to an in-frame deletion of 7 amino acids (not repeat region).    CSER Bakeoff  CriterionAssessment  ID: CritAsses25  Allele: CA40  Criterion: PM4  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity25  Explanation: leads to an in-frame deletion of 7 amino acids (not repeat region).  AssessCriterion  ID: AssessCriterionActivity25  wasAssociatedWith: TS  When:..  usedEvidenceStatement: RA4, MolCon66  usedCriterion: PM4  usedAllele: CA40, CA41  RegionAnnotation:  ID: RA4  Region: CR4  Value: False  Type: repeat  ContextualRegion:  ID: CR4  Sequence: PS4  Start:2490  Stop:2496  ReferenceSequence:  ID: PS4  Identifier: NP\_001360.1  MolecularConsequence  ID: MolCon66  Allele: AI39  Consequence: SO:0001825 (Conservative Inframe Deletion)  CanonicalAllele:  ID: CA40 (CA35)  relatedContextualAllele: AI40  …  ContextualAllele:  ID: AI40 (AI35)  alleleName:NM\_001369.2(DNAH5):c.7468\_7488del  Related: AI40  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA41  relatedContextualAllele: AI41  ContextualAllele:  ID: AI41  alleleName: NP\_001360.1:p.Trp2490\_Leu2496del  Related: AI40  relatedType: RO: 0003001 (produced-by) | | | | | | |
| #2 | Author | Tasha | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_015560(OPA1):c.113\_130del18(p.R38\_S43del)  This variant results in the deletion of 5 amino acids not in repeat region (this variant does however have lower site quality in ExAC)  CSER Bakeoff    CriterionAssessment  ID: CritAsses26  Allele: CA42  Criterion: PM4  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity26  Explanation: leads to an in-frame deletion of 5 amino acids (not repeat region).  AssessCriterion  ID: AssessCriterionActivity26  wasAssociatedWith: TS  When:..  usedEvidenceStatement: RA5, MolCon6  usedCriterion: PM4  usedAllele: CA42, CA43  RegionAnnotation:  ID: RA5  Region: CR5  Value: False  Type: repeat  ContextualRegion:  ID: CR5  Sequence: PS5  Start: 35  Stop: 43  ReferenceSequence:  ID: PS5  Identifier:NP\_056375.2    MolecularConsequence  ID: MolCon6  Allele: AI40  Consequence: SO:0001825 (Conservative Inframe Deletion)  CanonicalAllele:  ID: CA42  relatedContextualAllele: AI42  …  ContextualAllele:  ID: AI42  alleleName: NM\_015560.2:c.113\_130del18  Related: AI43  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA43  relatedContextualAllele: AI43  ContextualAllele:  ID: AI43  alleleName: NP\_056375.2:p.Arg38\_Ser43del  Related: AI42  relatedType: RO: 0003001 (produced-by) | | | | | | |
| #3 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_003924.3(PHOX2B):c.753\_767dup (p.Ala260\_Gly261insAlaAlaAlaAlaAla)  This 15bp duplication variant results in an expansion of a 20 residue polyalanine tract to a 25 residue polyalanine tract. While this variant is well established as pathogenic for central hypoventilation syndrome, PM2 would not be applicable as this rule is for inframe length changing variants in a nonrepeat region    CriterionAssessment  ID: CritAsses27  Allele: CA44  Criterion: PM4  Outcome: Refuted  wasGeneratedBy: AssessCriterionActivity27  Explanation: This 15bp duplication variant results in an expansion of a 20 residue polyalanine tract to a 25 residue polyalanine tract.  AssessCriterion  ID: AssessCriterionActivity27  wasAssociatedWith: SH  When:..  usedEvidenceStatement: RA6, MolCon7  usedCriterion: PM4  usedAllele: CA44, CA45  RegionAnnotation:  ID: RA6  Region: CR6  Value: True  Type: repeat  ContextualRegion:  ID: CR6  Sequence: PS6  Start: 241  Stop: 260  ReferenceSequence:  ID: PS6  Identifier:NP\_003915.2    MolecularConsequence  ID: MolCon7  Allele: AI45  Consequence: SO:0001823 (Conservative Inframe Insertion)    CanonicalAllele:  ID: CA44  relatedContextualAllele: AI44  …  ContextualAllele:  ID: AI44  alleleName: NM\_003924.3:c.753\_767dup  Related: AI45  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA45  relatedContextualAllele: AI45  ContextualAllele:  ID: AI45  alleleName:NP\_003915.2:p.Ala260\_Gly261insAlaAlaAlaAlaAla  Related: AI44  relatedType: RO: 0003001 (produced-by) | | | | | | |
| Issues | | Need to ensure that variant is not located within a repeat region  This rule is also (likely) applicable for frameshift variants that result in extension of the protein as opposed to truncation (similar to stop loss). | | | | | |

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| PM5 - novel missense at same aa residue as known path missense | | Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | Rajarshi Ghosh | Reviewer | |  | Modeler |  |
| Variant assessed: NM\_000363.4(TNNI3):c.485G>A (p.Arg162Gln)  CSER variant. From ClinVar: Doolan et al. (2005) (PMID:15698845) reported mutations at the same residue (R162P) in patients with HCM, supporting the functional importance of this residue in the protein. This rare R162P variant observed multiple times in is Likely Pathogenic in ClinVar with 1 star assertion. Other variants in the same residue R162W has conflicting evidence of pathogenicity(Path by GeneDx : SCV000209174.2 and VUS by LMM:SCV000203864.2). Considered these two only as they have provided assertion criteria.Perhaps requires curation of this variant combining the entire body of evidence before using PM5 .  CriterionAssessment  ID: CritAsses28  Allele: CA46  Criterion: PM5  Condition: C14  Outcome: Supporting Pathogenic  wasGeneratedBy: AssessCriterionActivity28  Explanation: From ClinVar: Doolan et al. (2005) (PMID:15698845) reported mutations at the same residue (R162P) in patients with HCM, supporting the functional importance of this residue in the protein. This rare R162P variant observed multiple times in is Likely Pathogenic in ClinVar with 1 star assertion. Other variants in the same residue R162W has conflicting evidence of pathogenicity(Path by GeneDx : SCV000209174.2 and VUS by LMM:SCV000203864.2). Considered these two only as they have provided assertion criteria.  AssessCriterion  ID: AssessCriterionActivity28  wasAssociatedWith: RG  When:..  usedEvidenceStatement: MDVI6, MDVI7  usedCriterion: PM5  usedAllele: CA46, CA47, CA48, CA49, CA50, CA51  MendelianDiseaseVariantInterpretation:  ID: MDVI6  Allele: CA48  clinicalSignificance: Likely Pathogenic  Condition: C14    Condition:  ID:C14  Name: Primary familial hypertrophic cardiomyopathy  MendelianDiseaseVariantInterpretation:  ID: MDVI7  Allele: CA50  clinicalSignificance: Conflicting  Condition: []  CanonicalAllele:  ID: CA46  relatedContextualAllele: AI46  …  ContextualAllele:  ID: AI46  alleleName: NM\_000363.4(TNNI3):c.485G>A  Related: AI47  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA47  relatedContextualAllele: AI47  ContextualAllele:  ID: AI47  alleleName:NP\_000354.4:p.Arg162Gln  Related: AI46  relatedType: RO: 0003001 (produced-by)  CanonicalAllele:  ID: CA48  relatedContextualAllele: AI48  ContextualAllele:  ID: AI48  alleleName: NM\_000363.4:c.485G>C  Related: AI49  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA49  relatedContextualAllele: AI49  ContextualAllele:  ID: AI49  alleleName:NP\_000354.4:p.Arg162Pro  Related: AI48  relatedType: RO: 0003001 (produced-by)  CanonicalAllele:  ID: CA50  relatedContextualAllele: AI50  ContextualAllele:  ID: AI50  alleleName: NM\_000363.4:c.484C>T  Related: AI51  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA51  relatedContextualAllele: AI51  ContextualAllele:  ID: AI51  alleleName:NP\_000354.4:p.Arg162Trp  Related: AI50  relatedType: RO: 0003001 (produced-by) | | | | | | |
| #2 | Author | Rajarshi Ghosh | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_000531(OTC):c.118C>T(p.R40C) CSER variant  Other pathogenic variants at the same residue per ClinVar : [NM\_000531.5(**OTC**):c.119G>A (p.Arg40His)](http://www.ncbi.nlm.nih.gov/clinvar/variation/11014/) However no assertion criteria is provided for these variants. So pathogenicity needs to be determined by literature search. PMID: 7951259,11768581,11260212  CriterionAssessment  ID: CritAsses29  Allele: CA52  Criterion: PM5  Condition: C15  Outcome: Insufficient Evidence  wasGeneratedBy: AssessCriterionActivity29  Explanation:Other pathogenic variants at the same residue per ClinVar : [NM\_000531.5(**OTC**):c.119G>A (p.Arg40His)](http://www.ncbi.nlm.nih.gov/clinvar/variation/11014/) However no assertion criteria is provided for these variants. So pathogenicity needs to be determined by literature search.  AssessCriterion  ID: AssessCriterionActivity29  wasAssociatedWith: RG  When:..  usedEvidenceStatement: MDVI8  usedCriterion: PM5  usedAllele: CA52, CA53, CA54, CA55  MendelianDiseaseVariantInterpretation:  ID: MDVI8  Allele: CA54  clinicalSignificance: Pathogenic  Condition: C15  Condition:  ID: C15  Name: Ornithine carbamoyltransferase deficiency  CanonicalAllele:  ID: CA52  relatedContextualAllele: AI52  ContextualAllele:  ID: AI52  alleleName: NM\_000531.5(OTC):c.118C>T  Related: AI53  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA53  relatedContextualAllele: AI53  ContextualAllele:  ID: AI53  alleleName:NP\_000522.3:p.Arg40Cys  Related: AI52  relatedType: RO: 0003001 (produced-by)  CanonicalAllele:  ID: CA54  relatedContextualAllele: AI54  ContextualAllele:  ID: AI54  alleleName: NM\_000531.5(OTC):c.119G>A  Related: AI55  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA55  relatedContextualAllele: AI55  ContextualAllele:  ID: AI55  alleleName:NP\_000522.3:p.Arg40His  Related: AI54  relatedType: RO: 0003001 (produced-by) | | | | | | |
| #3 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_007294.3(BRCA1):c.5290C>A (p.Leu1764Ile)  Another variant at this position, but different AA change, has been observed and called pathogenic for breast ovarian cancer by ENIGMA expert panel: NM\_007294.3(BRCA1):c.5291T>C (p.Leu1764Pro)  So PM5 is applicable when assessing p.Leu1764Il  CriterionAssessment  ID: CritAsses30  Allele: CA56  Criterion: PM5  Condition: C1  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity30  Explanation: Another variant at this position, but different AA change, has been observed and called pathogenic for breast ovarian cancer by ENIGMA expert panel:  AssessCriterion  ID: AssessCriterionActivity30  wasAssociatedWith: SH  When:..  usedEvidenceStatement: MDVI9  usedCriterion: PM5  usedAllele: CA56, CA57, CA58, CA59  MendelianDiseaseVariantInterpretation:  ID: MDVI9  Allele: CA58  clinicalSignificance: Pathogenic  Condition: C1  Condition:  ID: C1  Name: Breast Ovarian Cancer  CanonicalAllele:  ID CA56  relatedContextualAllele: AI56  ContextualAllele:  ID: AI56  alleleName: NM\_007294.3(BRCA1):c.5290C>A  Related: AI57  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA57  relatedContextualAllele: AI57  ContextualAllele:  ID: AI57  alleleName:NP\_009225.1:p.Leu1764Ile  Related: AI56  relatedType: RO: 0003001 (produced-by)  CanonicalAllele:  ID: CA58  relatedContextualAllele: AI5  ContextualAllele:  ID: AI58  alleleName: NM\_007294.3(BRCA1):c.5291T>C  Related: AI59  relatedType: RO: 0003000 (produces)  CanonicalAllele:  ID CA59  relatedContextualAllele: AI59  ContextualAllele:  ID: AI59  alleleName:NP\_009225.1:p.Leu1764Pro  Related: AI58  relatedType: RO: 0003001 (produced-by) | | | | | | |
| Issues | | Need to be sure that pathogenic variant exists at the residue in question. Sometimes discrepancy resolution may be required. Some thing like Variantexplorer.org may be useful.  Sometimes no assertion is provided in ClinVar ...may require curation by lit. search. | | | | | |

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| PM6 - assumed de novo | | Assumed de novo, but without confirmation of paternity and maternity | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | Rajarshi Ghosh | Reviewer | |  | Modeler |  |
| Variant assessed:NM\_144997.5(FLCN):c.1285dupC (p.His429Profs) CSER variant.  Evidence obtained via literature search: PMIDs: 12471204,18505456  CriterionAssessment  ID: CritAsses32  Allele: CA60  Condition: CX  Criterion: PM6  Outcome: Moderate Pathogenic  Explanation:Two sporadic cases of BHD contain this allele  wasGeneratedBy: AssessCriterionActivityX  AssessCriterion  ID: AssessCriterionActivity32  wasAssociatedWith: RG  When:..  usedEvidenceStatement: [ICX, FHX, DNAX, ICY, FHY, DNAY]  usedCriterion: PM6  usedAllele:CA60  IndividualCondition:  ID: ICX  Individual: S003  Condition: CX  hasCondition: True  IndividualCondition:  ID: ICY  Individual: F641  Condition: CX  hasCondition: True  FamilyHistory:  ID: FHX  Proband: S003  Condition: CX  familyHasCondition: False  FamilyHistory:  ID: FHY  Proband: F641  Condition: CX  familyHasCondition: False  DeNovoAllele:  ID: DNAX  Allele: CA60  Individual: S003  maternityConfirmed: False  paternityConfirmed: False  Explanation: parentage confirmation not mentioned in the source article  DeNovoAllele:  ID: DNAY  Allele: CA60  Individual: F641  maternityConfirmed: False  paternityConfirmed: False  Explanation: parentage confirmation not mentioned in the source article  Individual:  ID: S003  Individual:  ID: F641  Condition:  ID: CX  Name: Birt-Hogg-Dube Syndrome  CanonicalAllele:  ID CA60  relatedContextualAllele: AI60  ContextualAllele:  ID: AI60  alleleName: NM\_144997.5(FLCN):c.1285dupC  legacyName: c.1733insC | | | | | | |
| #2 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_002755.3(MAP2K1):c.158T>C (p.Phe53Ser)  From PMID:16439621, affected child with CFC was found to carry c.158T>C. Both parents are unaffected and from Sanger seq neither parent was found to carry c.158T>C variant - so authors conclude variant occurred de novo. Since there was no mention of parental confirmation, PS2 cannot be used but PM6 is applicable.  CriterionAssessment  ID: CritAsses33  Allele: CA61  Condition: CX  Criterion: PM6  Outcome: Moderate Pathogenic  Explanation:affected child with CFC was found to carry c.158T>C. Both parents are unaffected and from Sanger seq neither parent was found to carry c.158T>C variant - so authors conclude variant occurred de novo. Since there was no mention of parental confirmation, PS2 cannot be used but PM6 is applicable  wasGeneratedBy: AssessCriterionActivityX  AssessCriterion  ID: AssessCriterionActivity33  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [ICX, FHX, DNAX]  usedCriterion: PM6  usedAllele:CA61  IndividualCondition:  ID: ICX  Individual: IndX  Condition: CX  hasCondition: True  FamilyHistory:  ID: FHX  Proband: IndX  Condition: CX  familyHasCondition: False  DeNovoAllele:  ID: DNAX  Allele: CA61  Individual: IndX  maternityConfirmed: False  paternityConfirmed: False  Explanation: parentage confirmation not mentioned in the source article  Individual:  ID: IndX  Condition:  ID: CX  Name: Cardio-facio-cutaneous (CFC) syndrome  CanonicalAllele:  ID CA61  relatedContextualAllele: AI61  ContextualAllele:  ID: AI61  alleleName: NM\_002755.3(MAP2K1):c.158T>C | | | | | | |
| #3 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_004333.4(BRAF):c.1741A>G (p.Asn581Asp)  From 2 publications (PMID 22876591 & 16439621), 3 unrelated probands with CFC were found to carry c.1741A>G variant. There is no family history of CFC in any family and all three sets of parents were confirmed to not have the variant. Since there was no mention of parental confirmation, PS2 cannot be used but PM6 is applicable. However, since there are 3 de novo observations, many groups (including Noonan and MYH7) would allow PM6 to be upgraded to Strong (PM6\_S)  CriterionAssessment  ID: CritAsses34  Allele: CA62  Condition: CX  Criterion: PM6  Outcome: Strong Pathogenic  Explanation: 3 unrelated probands with CFC were found to carry c.1741A>G variant. There is no family history of CFC in any family and all three sets of parents were confirmed to not have the variant. Since there was no mention of parental confirmation, PS2 cannot be used but PM6 is applicable. However, since there are 3 de novo observations, many groups (including Noonan and MYH7) would allow PM6 to be upgraded to Strong (PM6\_S)  wasGeneratedBy: AssessCriterionActivity34  AssessCriterion  ID: AssessCriterionActivity34  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [ICX, FHX, DNAX, ICY,FHY,DNAY, ICZ, FHZ, DNAZ]  usedCriterion: PM6  usedAllele:CA62  IndividualCondition:  ID: ICX  Individual: IndX  Condition: CX  hasCondition: True  IndividualCondition:  ID: ICY  Individual: IndY  Condition: CX  hasCondition: True  IndividualCondition:  ID: ICZ  Individual: IndZ  Condition: CX  hasCondition: True  FamilyHistory:  ID: FHX  Proband: IndX  Condition: CX  familyHasCondition: False  FamilyHistory:  ID: FHY  Proband: IndY  Condition: CX  familyHasCondition: False  FamilyHistory:  ID: FHZ  Proband: IndZ  Condition: CX  familyHasCondition: False  DeNovoAllele:  ID: DNAX  Allele: CA62  Individual: IndX  maternityConfirmed: False  paternityConfirmed: False  DeNovoAllele:  ID: DNAY  Allele: CA62  Individual: IndY  maternityConfirmed: False  paternityConfirmed: False  DeNovoAllele:  ID: DNAZ  Allele: CA62  Individual: IndZ  maternityConfirmed: False  paternityConfirmed: False  Individual:  ID: IndX  Individual:  ID: IndY  Individual:  ID:IndZ  Condition:  ID: CX  Name: Cardio-facio-cutaneous (CFC) syndrome  CanonicalAllele:  ID CA62  relatedContextualAllele: AI62  ContextualAllele:  ID: AI62  alleleName: NM\_004333.4(BRAF):c.1741A>G | | | | | | |
| Issues | |  | | | | | |

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| PP1 - segregates with disease in affected family members | | Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease | | | | | | |
| **Evidence Statement Types** | | | | | | | | |
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| **Examples** | | | | | | | | |
| #1 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant assessed: MYBPC3 c.1351+1G>A  In PMID:17081393, variant c.1351+1G>A was identified in 1 individual with HCM. Segregation analysis determined 5 other members of the family also carried this variant. However, only 3 of those family members had HCM (aged 48-75). The 2 other family members were young and asymptomatic. So from this paper, only count **3 segregations**    In LMM internal family, c.1351+1G>A was identified in 1 individual with HCM. Variant also found in individual’s sister - who also had clinical dx of HCM. So **1 segregation from this family**  Can add segregations together - so in total have 4 segregations from 2 families  MYBPC1 well established as causal for HCM (dominant)  CriterionAssessment  ID: CritAsses035  Allele: CA063  Condition: C023  Criterion: PP1  Outcome: Supporting Pathogenic  Explanation: In total, 4 segs from 2 families  wasGeneratedBy: AssessCriterionActivity037  AssessCriterion  ID: AssessCriterionActivity037  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [CI106,ASD105]  usedCriterion: PP1  usedAllele:CA063    ConditionInheritance:  ID: CI106  Condition: C023  modeOfInheritance:: HP:0000006 (Autosomal Dominant)  AggregateSegregationData:  ID: ASD105  Condition: C023  Allele: CA063  FamilyData: FSD103, FSD104  totalNumberOfSegregations: 4  FamilySegregationData:  ID:FSD103  Condition:C023  Allele: CA063  Family: F1  phenotypePositiveAllelePositive: 3  phenotypeNegativeAllelePositive: 2  Explanation: identified in 1 individual with HCM. Segregation analysis determined 5 other members of the family also carried this variant. However, only 3 of those family members had HCM (aged 48-75). The 2 other family members were young and asymptomatic. So from this paper, only count **3 segregations.** The two apparently refuting segregations can be ignored due to the age-dependent penetrance of HCM.  FamilySegregationData:  ID:FSD104  Condition:C023  Allele: CA063  Family: F2  phenotypePositiveAllelePositive: 1  Explanation: In LMM internal family, c.1351+1G>A was identified in 1 individual with HCM. Variant also found in individual’s sister - who also had clinical dx of HCM.  Family:  ID: F1  Family:  ID: F2  Condition :  ID: C023  Name: Hypertrophic Cardiomyopathy  CanonicalAllele:  ID CA63  relatedContextualAllele: AI  ContextualAllele:  ID: AI  alleleName: NM\_000256.3(MYBPC3)c.1351+1G>A | | | | | | | |
| #2 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant assessed: USH2A c.1000C>T (Arg334Trp)  Family 1: c.1000C>T identified in the homozygous state in 1 individual with Usher syndrome. 5 affected family members also homozygous for the variant. **So 5 segs**  Family 2: c.1000C>T identified in het state in 1 individual with Usher. Individual compound het with Gly1840Val USH2A variant - confirmed in trans. Gly1840Val called Pathogenic. Individual has 3 affected sibs all of whom are compound het with 1000C>T & Gly1840Val. One additional sib only carried 1000C>T and is unaffected. **So 3 segs**    In total, 8 segs from 2 families (can combine hom segs and compound het segs). Most groups would allow this to be shifted up the Moderate (PP1\_M)    USH2A well established as causal for Usher (recessive)  CriterionAssessment  ID: CritAsses036  Allele: CA63  Condition: CX  Criterion: PP1  Outcome: Moderate Pathogenic  Explanation: In total, 8 segs from 2 families (can combine hom segs and compound het segs). Most groups would allow this to be shifted up the Moderate (PP1\_M)  wasGeneratedBy: AssessCriterionActivityX    AssessCriterion  ID: AssessCriterionActivity038  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [CI110,ASD109]  usedCriterion: PP1  usedAllele:CA62    ConditionInheritance:  ID: CI110  Condition: C021  modeOfInheritance:: HP:0000007 (Autosomal Recessive)  AggregateSegregationData:  ID: ASD109  Condition: C021  Allele: CA62  FamilyData: FSD107, FSD108  totalNumberOfSegregations: 8  FamilySegregationData:  ID:FSD107  Condition:CX  Allele: CA62  Family: F3  phenotypePositiveAllelePositive: 5  Explanation: proband and 5 affected family members all homozygous for [NM\_206933.2(**USH2A**):c.1000C>G (p.Arg334Gly)](http://www.ncbi.nlm.nih.gov/clinvar/variation/48342/).  FamilySegregationData:  ID:FSD108  Condition:CX  Allele: CA62  Family: F4  Explanation: c.1000C>T identified in het state in 1 individual with Usher. Individual compound het with Gly1840Val USH2A variant - confirmed in trans. Gly1840Val called Pathogenic. Individual has 3 affected sibs all of whom are compound het with 1000C>T & Gly1840Val. One additional sib only carried 1000C>T and is unaffected.  Family:  ID: F3 (was F1)  Family:  ID: F4 (was F2)  Condition :  ID: CX  Name: Usher syndrome  CanonicalAllele:  ID CA62  relatedContextualAllele: AI63  ContextualAllele:  ID: AI62  alleleName: [NM\_206933.2(**USH2A**):c.1000C>G (p.Arg334Gly)](http://www.ncbi.nlm.nih.gov/clinvar/variation/48342/) | | | | | | | |
| #3 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Assessed variant: NM\_000059.3(BRCA2):c.1832C>G (p.Ser611X)  From PMID 24156927, variant was found in patient with breast ovarian cancer. Variant was also found in one relative also affected with breast ovarian cancer. Relationship to the proband unknown. BRCA2 is well established for breast ovarian cancer (dominant), however as PP1 is for “Co-segregation with disease in **multiple** affected family members” PP1 is not applicable for this variant because it’s only a single segregation.  CriterionAssessment  ID: CritAsses037  Allele: CA64  Condition: C001  Criterion: PP1  Outcome: Insufficient Evidence  Explanation: PP1 is for “Co-segregation with disease in **multiple** affected family members” PP1 is not applicable for this variant because it’s only a single segregation.  wasGeneratedBy: AssessCriterionActivityX  AssessCriterion  ID: AssessCriterionActivity039  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [CI112,FSD111]  usedCriterion: PP1  usedAllele:CA64    ConditionInheritance:  ID: CI112  Condition: C001  modeOfInheritance::HP:0000006 (Autosomal Dominant)    FamilySegregationData:  ID:FSD111  Condition:C001  Allele: CA64  Family: F5  phenotypePositiveAllelePositive: 2  Explanation: variant was found in patient with breast ovarian cancer. Variant was also found in one relative also affected with breast ovarian cancer. Relationship to the proband unknown.    Family:  ID: F5    Condition :  ID: C001  Name: Breast Ovarian Cancer    CanonicalAllele:  ID CA64  relatedContextualAllele: AI64    ContextualAllele:  ID: AI64  alleleName: NM\_000059.3(BRCA2):c.1832C>G | | | | | | | |
| #4 | Assessed Variant: NM\_001001430.2(TNNT2):c.629\_631delAGA  2 families with segregation of this variant (PMID 20031601)  CriterionAssessment  ID: CritAsses038  Allele: CA65  Condition: C002  Criterion: PP1  Outcome: Moderate Pathogenic  wasGeneratedBy: AssessCriterionActivity040    AssessCriterion  ID: AssessCriterionActivity40  wasAssociatedWith: CB  When:..  usedEvidenceStatement: [CI116,ASD115]  usedCriterion: PP1  usedAllele:CA65  ConditionInheritance:  ID: CI116  Condition: C002  modeOfInheritance::HP:0000006 (Autosomal Dominant)  AggregateSegregationData:  ID: ASD115  Condition: C002  Allele: CA65  FamilyData: FSD113, FSD114  totalNumberOfSegregations: 9  FamilySegregationData:  ID:FSD113  Condition:C002  Allele: CA65  Family: F006  phenotypePositiveAllelePositive: 1  phenotypeNegativeAlleleNegative: 1  columns=[“Family”,”Individual”,”Father”,”Mother”,”Sex”,”Affected”,”Genotype”]  pedigree=[[F006,F006.1,0,0,1,2,0/0],  [F006,F006.2,0,0,2,1,0/0],  [F006,F006.3,F006.1,F006.2,2,3,0/0],  [F006,F006.4,F006.1,F006.2,1,2,1/2],  [F006,F006.5,F006.1,F006.2,2,1,1/1],  [F006,F006.6,F006.1,F006.2,1,2,1/2]]  affectedValues={1: “Unaffected”, 2: “Affected”, 3: “Any Cardiovascular Abnormality”}  genotypeValues={0: “Unknown”,1:”Reference”, 2: CA65}  FamilySegregationData:  ID:FSD114  Condition:C002  Allele: CA65  Family: F007  phenotypePositiveAllelePositive: 2  phenotypeNegativeAlleleNegative:3  columns=[“Family”,”Individual”,”Father”,”Mother”,”Sex”,”Affected”,”Genotype”,”ObligateGenotype”]  affectedValues={1: “Unaffected”, 2: “Affected”, 3: “Any Cardiovascular Abnormality”}  genotypeValues={0: “Unknown”,1:”Reference”, 2: CA65}  pedigree=[[F007, F007.1, 0, 0, 1, 3, 0/0, 0],  [F007, F007.2, 0, 0, 2, 1, 0/0, 0],  [F007, F007.3, 0, 0, 1, 3, 0/0, 0],  [F007, F007.4, 0, 0, 2, 1, 0/0, 0],  [F007, F007.5, 0, 0, 1, 3, 0/0, 0],  [F007, F007.6, F007.1, F007.2, 2, 3, 1/2, 1],  [F007, F007.7,0, 0, 1, 1, 0/0, 0],  [F007, F007.8, F007.1, F007.2, 2, 1, 1/2, 1],  [F007, F007.9,F007.3, F007.4, 1, 1, 0/0, 0],  [F007, F007.10, 0, 0, 1, 1, 0/0, 0],  [F007, F007.11, F007.5, F007.6, 2, 2, 1/2, 0],  [F007, F007.12, F007.5, F007.6, 1,2, 0/0, 0],  [F007, F007.13, F007.5, F007.6, 2,1, 1/1,0],  [F007, F007.14, F007.5, F007.6, 1, 1, 1/1, 0],  [F007, F007.15, F007.7, F007.8, 1, 2, 1/2, 0],  [F007, F007.16, F007.8, F007.9, 1, 2, 1/2, 0],  [F007, F007.17, F007.8, F007.9, 1, 2, 0/0,0],  [F007, F007.18, F007.8, F007.9, 1, 2, 0/0, 0],  [F007, F007.19, F007.10, F007.11, 1, 2, 0/0,0],  [F007.20,F007.10, F007.11, 1,1,1/1,0]]  Family:  ID:F6  Family:  ID: F7  Condition:  ID:C002  Name: Dilated Cardiomyopathy  CanonicalAllele:  ID CA65  relatedContextualAllele: AI65    ContextualAllele:  ID: AI65  alleleName: NM\_001001430.2(TNNT2):c.629\_631delAGA | | | | | | | |

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| PP2 - missense with pathogenic missense common | | 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 | | | | | | |
| **Evidence Statement Types** | | | | | | | | |
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| **Examples** | | | | | | | | |
| #1 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| MYH7 example  MYH7 has a missense Z score of 6.54 - which is in the top 0.2% of missense Z scores - suggesting it is intolerant of missense changes  NM\_000257.3(MYH7):c.5401G>A (p.Glu1801Lys)  http://www.ncbi.nlm.nih.gov/clinvar/variation/43069/    CriterionAssessment  ID: CritAsses039  Allele: CA066  Criterion: PP2  Outcome: Supporting Pathogenic  wasGeneratedBy: AssessCriterionActivity041    AssessCriterion  ID: AssessCriterionActivity041  wasAssociatedWith: SH  When:..  usedEvidenceStatement: MC, BMVR, CondMech  usedCriterion: PP2  BenignMissenseVariationRate:  ID: BMVR119  Gene: G8  Value: low  Explanation: Based on the gene size, ExAC expects 740 missense variants to be observed, but actually observes only 420, giving a z score of 6.5, which is in the top 0.02% of genes.  ConditionMechanism  ID: CondMech118  Gene: G8  Condition:  Mechanism: SO:0001583 (missense variant)  Common: True  Explanation: Of 178 pathogenic alleles in ClinVar, 149 are missense.  MolecularConsequence:  ID: MC117  Allele: AI070  Consequence: SO:0001583 (missense variant)  LOF: No  Gene:  ID: G8  Name: MHY7  CanonicalAllele:  ID CA066  relatedContextualAllele: AIY    ContextualAllele:  ID: AI070  alleleName: NM\_000257.3(MYH7):c.5401G>A | | | | | | | |
| #2 | Author | Rajarshi Ghosh | | Reviewer |  | | Modeler |  |
| TODO | | | | | | | |
| #3 | Author |  | | Reviewer |  | | Modeler |  |
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| Issues | | **Seems no one has established firm rules for when this rule is applicable so not sure how great any examples will be**  Agree with the lack of firm rules. Seems like one criteria may be observed missense variants over 5% allele frequency in ExAC. If this seems reasonable then I could generate a couple of examples. | | | | | | |

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| PP3 - computational evidence supports deleterious effect | | Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.) | | | | |
| **Evidence Statement Types** | | | | | | |
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| **Examples** | | | | | | |
| #1 | Author | Rajarshi Ghosh | Reviewer |  | Modeler |  |
| Assessed variant: NM\_005228.3(EGFR):c.2369C>T (p.Thr790Met) /[rs121434569](http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/?chr=7&from=55249071&to=55249071&gts=rs121434569&mk=55249071:55249071%7Crs121434569)  CSER variant.  SIFT: Damaging, Polyphen: Probably damaging, FATHMM: Tolerated, LRT: Damaging, MetaSVM:Tolerated, Mutation assessor:Tolerated, MutationTaster: Disease-causing,Provean: Damaging and Condel :deleterious  Predictions obtained via dbNSFP and VEP. with transcript NM\_005228.3  *Note: Does not quite fit the strict ACMG guideline of complete concordance.*  CriterionAssessment  ID: CritAsses040  Allele: CA67  Criterion: PP3  Outcome: Supporting Pathogenic  wasGeneratedBy: AssessCriterionActivity042    AssessCriterion  ID: AssessCriterionActivity042  wasAssociatedWith: RG, CSER  When:..  usedEvidenceStatement: [ISP1,ISP2, ISP3, ISP4, ISp5, ISP6, ISP7, ISP98, ISP9]  usedCriterion: PP3  usedAllele:AI67  InSilicoPrediction:  ID: ISP1  Allele: AI67  predictionType: Missense Effect  Tool: SIFT  Value: Damaging  InSilicoPrediction:  ID: ISP2  Allele: AI67  predictionType: Missense Effect  Tool: Polyphen  Value: Probably damaging  InSilicoPrediction:  ID: ISP3  Allele: AI67  predictionType: Missense Effect  Tool: FATHMM  Value: Tolerated  InSilicoPrediction:  ID: ISP4  Allele: AI67  predictionType: Missense Effect  Tool: LRT  Value: Damaging  InSilicoPrediction:  ID: ISP5  Allele: AI67  predictionType: Missense Effect  Tool: MetaSVM  Value: Tolerated  InSilicoPrediction:  ID: ISP6  Allele: AI67  predictionType: Missense Effect  Tool: Mutation assessor  Value: Tolerated  InSilicoPrediction:  ID: ISP7  Allele: AI67  predictionType: Missense Effect  Tool: Mutation taster  Value: Disease-causing  InSilicoPrediction:  ID: ISP8  Allele: AI67  predictionType: Missense Effect  Tool: Provean  Value: Damaging  InSilicoPrediction:  ISP9  Allele: AI67  predictionType: Missense Effect  Tool: Condel  Value: deleterious  CanonicalAllele:  ID CA67  relatedContextualAllele: AI67    ContextualAllele:  ID: AI67  alleleName: NM\_005228.3(EGFR):c.2369C>T | | | | | |
| #2 | Author | Rajarshi Ghosh | Reviewer |  | Modeler |  |
| Assessed variant [rs61755320](http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/?chr=16&from=89613145&to=89613145&gts=rs61755320&mk=89613145:89613145%7Crs61755320)  SIFT: Deleterious, Polyphen: Probably damaging, FATHMM: Damaging, LRT: Damaging, MetaSVM:Damaging, Mutation assessor: Medium functional impact, MutationTaster: Disease-causing,Provean: Damaging and Condel :Deleterious  Predictions obtained via dbNSFP and VEP on [ENST00000566221](http://useast.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;t=ENST00000566221;tl=MoxioTNLCoDEazEy-1744945).  CriterionAssessment  ID: CritAsses041  Allele: CA68  Criterion: PP3  Outcome: Supporting Pathogenic  wasGeneratedBy: AssessCriterionActivity043    AssessCriterion  ID: AssessCriterionActivity043  wasAssociatedWith: RG  When:..  usedEvidenceStatement: [ISP1,ISP2, ISP3, ISP4, ISp5, ISP6, ISP7, ISP98, ISP9]  usedCriterion: PP3  usedAllele:AI70  InSilicoPrediction:  ID: ISP1  Allele: AI70  predictionType: Missense Effect  Tool: SIFT  Value: Deleterious    InSilicoPrediction:  ID: ISP2  Allele: AI70  predictionType: Missense Effect  Tool: Polyphen  Value: Probably damaging    InSilicoPrediction:  ID: ISP3  Allele: AI70  predictionType: Missense Effect  Tool: FATHMM  Value: Damaging    InSilicoPrediction:  ID: ISP4  Allele: AI70  predictionType: Missense Effect  Tool: LRT  Value: Damaging    InSilicoPrediction:  ID: ISP5  Allele: AI70  predictionType: Missense Effect  Tool: MetaSVM  Value: Tolerated    InSilicoPrediction:  ID: ISP6  Allele: AI70  predictionType: Missense Effect  Tool: Mutation assessor  Value: Medium functional impact    InSilicoPrediction:  ID: ISP7  Allele: AI70  predictionType: Missense Effect  Tool: Mutation taster  Value: Disease-causing    InSilicoPrediction:  ID: ISP8  Allele: AI70  predictionType: Missense Effect  Tool: Provean  Value: Damaging    InSilicoPrediction:  ISP9  Allele: AI70  predictionType: Missense Effect  Tool: Condel  Value: deleterious    CanonicalAllele:  ID CA68  relatedContextualAllele: AI67    ContextualAllele:  ID: AI68  alleleName: NM\_003119.3(SPG7):c.1529C>T    ContextualAllele:  ID: AI69  alleleName: [ENST00000268704](http://useast.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;source=dbSNP;t=ENST00000268704.6;v=rs61755320;vdb=variation;vf=12256745).6:c.1529C>T    ContextualAllele:  ID: AI70  alleleName: [ENST00000566221](http://useast.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;source=dbSNP;t=ENST00000566221.5;v=rs61755320;vdb=variation;vf=12256745).5:c.127C>T | | | | | |
| #3 | Author | S Harrison | Reviewer |  | Modeler |  |
| (Wanted to include a splicing example too)  Variant: NM\_000363.4(TNNI3):c.592C>G (p.Leu198Val)  Overall the in silico predictions do not predict an impact:  AlignGVGD: C0 (benign)  PolyPhen2: benign  MAPP: good (benign)  SIFT: Tolerated  MutationTaster: disease-causing    However, nucleotide is somewhat conserved:   * G nucleotide at this position not observed in any species * pyloP: 1.255 * PhastCons: 0.992   Variant is predicted to create a new donor splice site at position c.591  All 5 splicing tools used predict an impact      So even though the computational tools for AA changes are NOT predicting an impact, splicing tools are predicting an impact - so PP3 is applicable  CriterionAssessment  ID: CritAsses042  Allele: CA69  Condition: CX  Criterion: PP3  Outcome: Supporting Pathogenic  wasGeneratedBy: AssessCriterionActivity044  Explanation: even though the computational tools for AA changes are NOT predicting an impact, splicing tools are predicting an impact - so PP3 is applicable  AssessCriterion  ID: AssessCriterionActivity044  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [ISP1,ISP2, ISP3, ISP4, ISp5, ISP6, ISP7, ISP98, ISP9, ISP10]  usedCriterion: PP3  usedAllele:AI71  InSilicoPrediction:  ID: ISP1  Allele: AI71  predictionType: Missense Effect  Tool: SIFT  Value: Tolerated    InSilicoPrediction:  ID: ISP2  Allele: AI71  predictionType: Missense Effect  Tool: Polyphen2  Value: benign    InSilicoPrediction:  ID: ISP3  Allele: AI71  predictionType: Missense Effect  Tool: AlignGVGD  Value: C0 (benign)    InSilicoPrediction:  ID: ISP4  Allele: AI71  predictionType: Missense Effect  Tool: MAPP  Value: good (benign)    InSilicoPrediction:  ID: ISP5  Allele: AI71  predictionType: Missense Effect  Tool: MutationTaster  Value: disease-causing    InSilicoPrediction:  ID: ISP6  Allele: AI71  predictionType: Splicing Prediction  Tool: SSF  Value: 71.53    InSilicoPrediction:  ID: ISP7  Allele: AI71  predictionType: Splicing Prediction  Tool: MaxEnt  Value: 7.39    InSilicoPrediction:  ID: ISP8  Allele: AI71  predictionType: Splicing Prediction  Tool: NNSPLICE  Value: 0.87    InsilicoPrediction:  ISP9  Allele: AI71  predictionType:Splicing Prediction  Tool: GeneSplicer  Value: 7.75    InsilicoPrediction:  ISP10  Allele: AI71  predictionType:Splicing Prediction  Tool: HSF  Value: 81.75  CanonicalAllele:  ID CA069  relatedContextualAllele: AI71    ContextualAllele:  ID: AI71  alleleName: NM\_000363.4(TNNI3):c.592C>G | | | | | |
| Issues | | Don’t know if there is a definition of “multiple” . If >1 then the algorithms chosen are at the discretion of the curator/lab. There is a difference between lines of evidence and number of implementations. | | | | |

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| PP4 - disease specific phenotype and family history | | Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology | | | | | | |
| **Evidence Statement Types** | | | | | | | | |
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| **Examples** | | | | | | | | |
| #1 | Author | Rajarshi Ghosh | | Reviewer |  | | Modeler |  |
| Assessed variant: NM\_000060.3(BTD):c.1330G>C (p.Asp444His)  PMIDs: 10206677; 9654207  To be completed | | | | | | | |
| #2 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant: NM\_001114753.1(ENG):c.818C>T (p.Thr273Ile)  Variant found in a case with clinical symptoms matching Hereditary Hemorrhagic Telangiectasia (HHT) Type 1. HHT type 1 is only known to be caused by variants in ENG - so PP4 is applicable  (CSER example)  CriterionAssessment  ID: CritAsses044  Allele: CA70  Condition: C024  Criterion: PP4  Outcome: Supporting Pathogenic  wasGeneratedBy: AssessCriterionActivity046    AssessCriterion  ID: AssessCriterionActivity046  wasAssociatedWith: SH, CSER  When:..  usedEvidenceStatement: CE1  usedCriterion: PP4  usedAllele:AI70  ConditionEitiology:  ID: CE1  Condition: C024  Gene: G35  Specificity: complete  Explanation: . HHT type 1 is only known to be caused by variants in ENG -  Gene:  ID: G35  Name: ENG  Condition: :  ID: C024  Name: Hereditary Hemorrhagic Telangiectasia Type 1  CanonicalAllele:  ID CA70  relatedContextualAllele: AI70    ContextualAllele:  ID: AI70  alleleName: NM\_001114753.1(ENG):c.818C>T | | | | | | | |
| #3 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant: NM\_000257.2(MYH7):c.1822T>G (Leu608Val)  Variant found in a case with clinical diagnosis of hypertrophic cardiomyopathy. However….   1. Only ~50% of HCM is genetic (can be caused by environment) 2. More than 10 genes can cause HCM (specifically 9 sarcomere genes)   <http://omim.org/phenotypicSeries/PS192600>  PP4 not applicable for this variant/condition because HCM does not have a single genetic etiology  CriterionAssessment  ID: CritAsses045  Allele: CA71  Condition: C23  Criterion: PP4  Outcome: Refuted  wasGeneratedBy: AssessCriterionActivity047    AssessCriterion  ID: AssessCriterionActivity047  wasAssociatedWith: SH  When:..  usedEvidenceStatement: CE149  usedCriterion: PP4  usedAllele:AI71  ConditionEitiology:  ID: CE149  Condition: C23  Gene: G8  Specificity: low  Explanation: 1.Only ~50% of HCM is genetic (can be caused by environment) 2. More than 10 genes can cause HCM (specifically 9 sarcomere genes)  Gene:  ID: G8  Name: MYH7  Condition: :  ID: C23  Name: Hypertrophic Cardiomyopathy  CanonicalAllele:  ID CA71  relatedContextualAllele: AI71    ContextualAllele:  ID: AI71  alleleName: NM\_000257.2(MYH7):c.1822T>G | | | | | | | |
| Issues | |  | | | | | | |

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| PP5 - <need shorthand desc> | | Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation | | | | | | |
| **Evidence Statement Types** | | | | | | | | |
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| **Examples** | | | | | | | | |
| #1 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant: NM\_000059.3(BRCA2):c.3G>T (p.Met1Ile)  Variant has been classified as “Pathogenic” by Sharing Clinical Reports Project (SCV000054057.3): <http://www.ncbi.nlm.nih.gov/clinvar/variation/37871/>  Sharing Clinical Reports Project are reports from Myriad - who are considered experts in this field but do not share their evidence - only their interpretation.  (Note: this is the exact scenarios this rule was written for - but people are being very liberal in applying this rule)  CriterionAssessment  ID: CritAsses046  Allele: CA72  Condition: C25  Criterion: PP5  Outcome: Supporting Pathogenic  wasGeneratedBy: AssessCriterionActivity048    AssessCriterion  ID: AssessCriterionActivity048  wasAssociatedWith: SH  When:..  usedEvidenceStatement: MDVI\_A  usedCriterion: PP5  usedAllele:AI72    MendelianDiseaseVariantIntpretation:  ID: MDVI\_A  Allele: CA72  Condition: C25  ClinicalSignificance: Pathogenic  Explanation: Variant has been classified as “Pathogenic” by Sharing Clinical Reports Project (SCV000054057.3)    Condition: :  ID: C25  Name: Breast-ovarian cancer, familial 2    CanonicalAllele:  ID CA72  relatedContextualAllele: AI72    ContextualAllele:  ID: AI72  alleleName: NM\_000059.3(BRCA2):c.3G>T | | | | | | | |
| #2 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant: NM\_001458.4(FLNC):c.577G>A (p.Ala193Thr)  Variant has been classified as “Pathogenic” by OMIM (SCV000043718.1):  <http://www.ncbi.nlm.nih.gov/clinvar/variation/29592/>  PP5 is not applicable for this variant because   1. OMIM is not considered an expert or reputable source for variant interpretations 2. OMIM’s evidence for this pathogenic call is available (the publication link - PMID:21620354 - and free text summary) so people should use the evidence from OMIM in their assessment NOT OMIM’s interpretation     CriterionAssessment  ID: CritAsses047  Allele: CA73  Condition: C27  Criterion: PP5  Outcome: Insufficient Evidence  wasGeneratedBy: AssessCriterionActivity049  Explanation: OMIM is not considered an expert or reputable source for variant interpretations.  Further, OMIM’s evidence for this pathogenic call is available (the publication link - PMID:21620354 - and free text summary) so people should use the evidence from OMIM in their assessment NOT OMIM’s interpretation. This is marked as Insufficient Evidence rather than refuted because the OMIM entry does not rule out a later application of PP5 based on eg. an expert panel.    AssessCriterion  ID: AssessCriterionActivity049  wasAssociatedWith: SH  When:..  usedEvidenceStatement: MDVI\_A  usedCriterion: PP5  usedAllele:AI73    MendelianDiseaseVariantIntpretation:  ID: MDVI\_A  Allele: CA73  Condition: C27  ClinicalSignificance: Pathogenic  Explanation: Variant has been classified as “Pathogenic” by OMIM (SCV000043718.1)    Condition: :  ID: C27  Name: Myopathy, distal, 4    CanonicalAllele:  ID CA73  relatedContextualAllele: AI73    ContextualAllele:  ID: AI73  alleleName: NM\_001458.4(FLNC):c.577G>A | | | | | | | |
| #3 | Author | S Harrison | | Reviewer |  | | Modeler |  |
| Variant: NM\_000492.3(CFTR):c.2053dupC (p.Gln685Profs)  Variant has been called Pathogenic by Expert panel CFTR (SCV000245981.1)  <http://www.ncbi.nlm.nih.gov/clinvar/variation/209066/>  Many groups/labs are letting expert panel pathogenic interpretations count as “reputable sources” - so PP5 is met    CriterionAssessment  ID: CritAsses048  Allele: CA74  Condition: C26  Criterion: PP5  Outcome: Supporting Pathogenic  wasGeneratedBy: AssessCriterionActivity050    AssessCriterion  ID: AssessCriterionActivity050  wasAssociatedWith: SH  When:..  usedEvidenceStatement: MDVI\_A  usedCriterion: PP5  usedAllele:AI74    MendelianDiseaseVariantIntpretation:  ID: MDVI\_A  Allele: CA74  Condition: C26  ClinicalSignificance: Pathogenic  Explanation: Variant has been classified as “Pathogenic” by Expert panel CFTR (SCV000245981.1)    Condition: :  ID: C26  Name: Cystic Fibrosis    CanonicalAllele:  ID CA74  relatedContextualAllele: AI74    ContextualAllele:  ID: AI74  alleleName: NM\_000492.3(CFTR):c.2053dupC | | | | | | | |
| Issues | |  | | | | | | |

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| BA1 - present in population databases at greater than 5% | | Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium | | | | |
| **Evidence Statement Types** | | | | | | |
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| **Examples** | | | | | | |
| #1 | Author | Rajarshi Ghosh | Reviewer |  | Modeler |  |
| Variant assessed: NM\_000057.2 (BLM):c.2603C>T (p.P868L )  **Population Frequencies from ExAc**   |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Population** | **Allele Count** | **Allele Number** | **Number of Homozygotes** | **Allele Frequency** | | European (Finnish) | 671 | 6580 | 31 | 0.102 | | African | 704 | 10296 | 14 | 0.06838 | | European (Non-Finnish) | 4239 | 66162 | 150 | 0.06407 | | Other | 49 | 902 | 0 | 0.05432 | | South Asian | 707 | 16414 | 23 | 0.04307 | | Latino | 357 | 11440 | 5 | 0.03121 | | East Asian | 5 | 8608 | 0 | 0.0005809 | | **Total** | **6732** | **120402** | **223** | **0.05591** |   CriterionAssessment  ID: CritAsses049  Allele: CA67  Criterion: BA1  Outcome: Stand-alone Benign  wasGeneratedBy: AssessCriterionActivity051    AssessCriterion  ID: AssessCriterionActivity051  wasAssociatedWith: RG  When:..  usedEvidenceStatement: PAF153  usedCriterion: BA1  usedAllele:AI69    PopulationAlleleFrequency:  ID: PAF153  Ascertainment: ExAC  Allele: AI70  alleleCount: 6732  alleleNumber: 120402  homozygousAlleleIndividualCount: 223  alleleFrequenecy: 0.05591  population: Combined    CanonicalAllele:  ID CA70  relatedContextualAllele: AI70    ContextualAllele:  ID: AI70  alleleName: NM\_000057.2 (BLM):c.2603C>T | | | | | |
| #2 | Author | S Harrison | Reviewer |  | Modeler |  |
| Variant: NM\_004004.5(GJB2):c.79G>A (p.Val27Ile)  http://exac.broadinstitute.org/variant/13-20763642-C-T  Globally, this variant has a MAF of 0.0454 (4.5%) which would put it under the 5% rule and thus BA1 would not be applicable.  However, variant is enriched in East Asian population - 29% MAF - so way over the 5% cut-off. So now BA1 is applicable  CriterionAssessment  ID: CritAsses050  Allele: CA75  Criterion: BA1  Outcome: Stand-alone Benign  wasGeneratedBy: AssessCriterionActivity052  Explanation: Globally, this variant has a MAF of 0.0454 (4.5%) which would put it under the 5% rule and thus BA1 would not be applicable. However, variant is enriched in East Asian population - 29% MAF - so way over the 5% cut-off. So now BA1 is applicable    AssessCriterion  ID: AssessCriterionActivity052  wasAssociatedWith: RG  When:..  usedEvidenceStatement: PAF154,PAF155  usedCriterion: BA1  usedAllele:AI75    PopulationAlleleFrequency:  ID: PAF154  Ascertainment: ExAC  Allele: AI75  alleleCount: 5507  alleleNumber: 121350  homozygousAlleleIndividualCount: 768  alleleFrequenecy: 0.04538  population: Combined    PopulationAlleleFrequency:  ID: PAF155  Ascertainment: ExAC  Allele: AI75  alleleCount: 2518  alleleNumber: 8632  homozygousAlleleIndividualCount: 405  alleleFrequenecy: 0.2917  population: East Asian    CanonicalAllele:  ID CA75  relatedContextualAllele: AI75    ContextualAllele:  ID: AI75  alleleName: NM\_004004.5(GJB2):c.79G>A | | | | | |
| #3 | Author |  | Reviewer |  | Modeler |  |
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| Issues | | Several groups have lowered the BA1 threshold (e.g. PTEN-1%). Also the sequence variant interpretation group is working on a modified version of this guideline including the minimum # of alleles that need to be examined and the population stratification effects, if any. | | | | |

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| BS1 - MAF too high for disorder | | Allele frequency is greater than expected for disorder | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | Rajarshi Ghosh | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_000138.4(FBN1):c.8176C>T (p.Arg2726Trp)  Max Allele frequency: 0.2% (ExAC/Latino) 0.07%(ExAC/overall) WITH 1 HOMOZYGOTE.  Condition prevalence: Marfan prevalence 0.0007 – 0.00017  PMID:25637381 Allele frequency was less than a disease-specific maximum frequency.  CriterionAssessment  ID: CritAsses052  Allele: CAZ  Condition: C28  Criterion: BS1  Outcome: Strong Benign  wasGeneratedBy: AssessCriterionActivity054    AssessCriterion  ID: AssessCriterionActivity054  wasAssociatedWith:RG  When:..  usedEvidenceStatement: [PAF158, CPrev159, CPA160, CIA161]  usedCriterion: BS1  PopulationAlleleFrequency:  ID: PAF158  Ascertainment: ExAC  Allele: AIZ  alleleCount: 89  alleleNumber: 121410  homozygousAlleleIndividualCount: 1  alleleFrequency: 0.0007  population: Combined  ConditionPrevalence:  ID: CPrev159  Condition: C28  Minimum: 0.0017  Maximum: 0.0007  Population: Overall  ConditionPenetrance:  ID: CPA160  Condition C28  Penetrance: High    ConditionInheritance:  ID CIA161  Condition: C28  modeOfInheritance::HP:0000006 (Autosomal Dominant)    Condition:  ID: C28  Name: Marfan Syndrome    CanonicalAllele:  ID CAZ  relatedContextualAllele: AIZ    ContextualAllele:  ID: AIZ  alleleName: NM\_000138.4(FBN1):c.8176C>T | | | | | | |
| #2 | Author | Rajarshi Ghosh | Reviewer | |  | Modeler |  |
| CSER variant : NM\_004541.3(NDUFA1):c.94G>C (p.Gly32Arg)  Max Allele frequency: 0.9% (ExAC/NFE) 0.6%(ExAC/overall) (with 1 homozygote with the same amino acid change but different codon G>A).  NDUFA1 is associated with Mitochondrial complex 1 deficiency as obtained from Medgen search. http://www.ncbi.nlm.nih.gov/medgen/?term=NDUFA1  Condition prevalence: prevalence of all mitochondrial disease per Gene Reviews, 2014 is estimated around 1 in 8500  CriterionAssessment  ID: CritAsses053  Allele: CAZ  Condition: C29  Criterion: BS1  Outcome: Strong Benign  wasGeneratedBy: AssessCriterionActivity055    AssessCriterion  ID: AssessCriterionActivity055  wasAssociatedWith:RG,CSER  When:..  usedEvidenceStatement: [PAF162, CPrev163, CPA164, CIA165]  usedCriterion: BS1  PopulationAlleleFrequency:  ID: PAF162  Ascertainment: ExAC  Allele: AIZ  alleleCount: 545  alleleNumber: 87696  homozygousAlleleIndividualCount: 1  hemizygousAlleleIndividualCount: 206  alleleFrequency: 0.00625  population: Combined  ConditionPrevalence:  ID: CPrev163  Condition: C29  Value: 0.00012  Population: Overall  ConditionPenetrance:  ID: CPA164  Condition C29  Penetrance: High    ConditionInheritance:  ID CIA165  Condition: C29  modeOfInheritance: HP:0001423 (X-linked dominant inheritance)  Condition:  ID: C29  Name: Mitochondrial complex 1 deficiency    CanonicalAllele:  ID CAZ  relatedContextualAllele: AIZ    ContextualAllele:  ID: AIZ  alleleName: NM\_004541.3(NDUFA1):c.94G>C | | | | | | |
| #3 | Author | M. DiStefano | Reviewer | |  | Modeler |  |
| Variant: NM\_000414.3(HSD17B4):c.56C>G (p.Ala19Gly) BS1 does not apply    Allele frequency in ExAC:    Variants in HSD17B4 are associated with Perrault syndrome. However, BS1 does not apply because this disease is so rare that it does not have an accurate prevalence. Only 100 cases have ever been reported. See the “prevalence” section in GeneReviews: <http://www.ncbi.nlm.nih.gov/books/NBK242617/>  CriterionAssessment  ID: CritAsses054  Allele: CAZ  Condition: C30  Criterion: BS1  Outcome: Insufficient Evidence  wasGeneratedBy: AssessCriterionActivity055  Explanation: Perrault syndrome may be underreported due to misdiagnosis. If the prevalence estimates rise, this assessment may need to be revisited.    AssessCriterion  ID: AssessCriterionActivity055  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: [PAF166, CPrev167, CPA168, CIA169]  usedCriterion: BS1  PopulationAlleleFrequency:  ID: PAF166  Ascertainment: ExAC  Allele: AIZ  alleleCount: 3  alleleNumber: 120870  alleleFrequency:2.4e-5  population: Combined  ConditionPrevalence:  ID: CPrev167  Condition: C30  Maximum: 1e-6  Population: Overall  ConditionPenetrance:  ID: CPA168  Condition C30  Penetrance: High    ConditionInheritance:  ID CIA169  Condition: C30  modeOfInheritance::HP:0000007 (Autosomal Recessive)    Condition:  ID: C30  Name: Perrault Syndrome    CanonicalAllele:  ID CAZ  relatedContextualAllele: AIZ    ContextualAllele:  ID: AIZ  alleleName:NM\_000414.3(HSD17B4):c.56C>G | | | | | | |
| Issues | |  | | | | | |

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| BS2 - control frequency higher than penetrance predicts | | 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 | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | S Harrison | Reviewer | |  | Modeler |  |
| Variant: NM\_001197104(KMT2A):c.6572G>A(p.R2191Q)  KMT2A is associated with Wiedemann-Steiner syndrome which is a dominant disorder  According to Baylor internal data on this variant, it has been observed multiple times in asymptomatic parents internally and in controls and observed once as a homozygotes internally in unaffected  CriterionAssessment  ID: CritAsses55  Allele: CA81  Condition: C31  Criterion: BS2  Outcome: Strong Benign  wasGeneratedBy: AssessCriterionActivity57  Explanation: According to Baylor internal data on this variant, it has been observed multiple times in asymptomatic parents internally and in controls and observed once as a homozygotes internally in unaffected. Although the evidence for full penetrance is weak, the fact that there are multiple asymptomatic occurrences helps the case.    AssessCriterion  ID: AssessCriterionActivity57  wasAssociatedWith: SH  When:..  usedEvidenceStatement: [CPA, CIA, IA1, IC1, IA2, IC2]  usedCriterion: BS2  usedAllele: CA81    ConditionPenetrance:  ID: CPA(170)  Condition C31  Penetrance: Complete  Explanation: The evidence for full penetrance is not great, due to the rarity of the disease, but the fact that it's diagnosed at a young age and majority of cases are de novo is suggestive    ConditionInheritance:  ID CIA(171)  Condition: C31  modeOfInheritance::HP:0000006 (Autosomal Dominant)    IndividualAllele:  ID: IA1 (172)  Individual: Ind13  primaryAllele: CA81  primaryZygosity: heterozygous    IndividualAllele:  ID: IA2 (173)  Individual: Ind14  primaryAllele: CA81  primaryZygosity: homozygous    IndividualCondition:  ID: IC1 (174)  Individual: Ind13  Condition: C31  hasCondition: False    IndividualCondition:  ID: IC2 (175)  Individual: Ind14  Condition: C31  hasCondition: False    Individual:  ID: Ind13    Individual:  ID: Ind14    Condition:  ID: C31  Name: Wiedemann-Steiner syndrome    CanonicalAllele:  ID CA81  relatedContextualAllele: AI76    ContextualAllele:  ID: AI76  alleleName: NM\_001197104(KMT2A):c.6572G>A | | | | | | |
| #2 | Author | M DiStefano | Reviewer | |  | Modeler |  |
| Variant: NM\_000038.5(APC ):c.3386T>C(p.Leu1129Ser)  APC is associated with Familial Adenomatous Polyposis, which is a dominant disorder.  This variant was observed in unaffected healthy control chromosomes. PMIDs: 15122587, 18199528, 22703879, 24728327  Note: CSER Variant  CriterionAssessment  ID: CritAsses56  Allele: CA82  Condition: C32  Criterion: BS2  Outcome: Strong Benign  wasGeneratedBy: AssessCriterionActivity58  Explanation: This variant was observed in unaffected healthy control chromosomes. PMIDs: 15122587, 18199528, 22703879, 24728327    AssessCriterion  ID: AssessCriterionActivity58  wasAssociatedWith: MDS  When:..  usedEvidenceStatement: [CPA, CIA, IA1, IC1, IA2, IC2, IA3, IC3, IA4, IC4]  usedCriterion: BS2  usedAllele:CA82    ConditionPenetrance:  ID: CPA (176)  Condition C32  Penetrance: Complete  Explanation: the disease manifests as hundreds of colorectal ademonas during adolescence. This can progress to colorectal cancer later in life, but the adenomas manifest early.    ConditionInheritance:  ID CIA (177)  Condition: C32  modeOfInheritance::HP:0000006 (Autosomal Dominant)    IndividualAllele:  ID: IA1 (178)  Individual: Ind15  primaryAllele: CA82  primaryZygosity: heterozygous    IndividualAllele:  ID: IA2 (179)  Individual: Ind16  primaryAllele: CA82  primaryZygosity: heterozygous    IndividualAllele:  ID: IA3 (180)  Individual: Ind17  primaryAllele: CA82  primaryZygosity: heterozygous    IndividualAllele:  ID: IA4 (181)  Individual: Ind18  primaryAllele: CA82  primaryZygosity: heterozygous    IndividualCondition:  ID: IC1 (182)  Individual: Ind15  Condition: C32  hasCondition: False    IndividualCondition:  ID: IC2 (183)  Individual: Ind16  Condition: C32  hasCondition: False    IndividualCondition:  ID: IC3 (184)  Individual: Ind17  Condition: C32  hasCondition: False    IndividualCondition:  ID: IC4 (185)  Individual: Ind18  Condition: C32  hasCondition: False    Individual:  ID: Ind15    Individual:  ID: Ind16    Individual:  ID: Ind17    Individual:  ID: Ind18    Condition:  ID: C32  Name: Familial Adenomatous Polyposis    CanonicalAllele:  ID CA82  relatedContextualAllele: AI77    ContextualAllele:  ID: AI77  alleleName: NM\_000038.5(APC ):c.3386T>C | | | | | | |
| #3 | Author | M DiStefano | Reviewer | |  | Modeler |  |
| Variant: NM\_000138.4(FBN1 ):c.2956G>A (p.Ala986Thr)  FBN1 is associated with Marfan Syndrome which is a dominant disorder. Although this variant was found in 5 individuals with Marfan Syndrome, it was identified in 2 unaffected relatives from two separate families. PMID: 12402346  Note: CSER Variant  CriterionAssessment  ID: CritAsses57  Allele: CA83  Condition: C28  Criterion: BS2  Outcome: Strong Benign  wasGeneratedBy: AssessCriterionActivity59  Explanation: Although this variant was found in 5 individuals with Marfan Syndrome, it was identified in 2 unaffected relatives from two separate families. PMID: 12402346    AssessCriterion  ID: AssessCriterionActivity59  wasAssociatedWith: MDS  When:..  usedEvidenceStatement: [CPA, CIA, IA1, IC1, IA2, IC2]  usedCriterion: BS2  usedAllele:CA83    ConditionPenetrance:  ID: CPA  Condition C28  Penetrance: Complete  Explanation:Marfan syndrome involves a range of skeletal features that should be identifiable at birth, if not during adolescence. The ocular and cardiovascular aspects may manifest later. 1/4 of mutations are de novo.    ConditionInheritance:  ID CIA  Condition: C28  modeOfInheritance::HP:0000006 (Autosomal Dominant)    IndividualAllele:  ID: IA1  Individual: Ind19  primaryAllele: CA83  primaryZygosity: heterozygous    IndividualAllele:  ID: IA2  Individual: Ind20  primaryAllele: CA83  primaryZygosity: heterozygous    IndividualCondition:  ID: IC1  Individual: Ind19  Condition: C28  hasCondition: False    IndividualCondition:  ID: IC2  Individual: Ind20  Condition: C28  hasCondition: False    Individual:  ID: Ind19    Individual:  ID: Ind20    Condition:  ID: C28  Name: Marfan Syndrome    CanonicalAllele:  ID CA15  relatedContextualAllele: AI78    ContextualAllele:  ID: AI78  alleleName: NM\_000138.4(FBN1 ):c.2956G>A | | | | | | |
| Issues | | This rule should not be used for diseases with reduced penetrance or late/variable age of onset (such as certain cardiomyopathies). | | | | | |

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| BS3 - well-established no damaging effect functional studies | | Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing | | | | |
| **Evidence Statement Types** | | | | | | |
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| **Examples** | | | | | | |
| #1 | Author | S Harrison | Reviewer |  | Modeler |  |
| Variant: NM\_000218.2(KCNQ1):c.1179G>T (p.Lys393Asn)  (TODO) | | | | | |
| #2 | Author | M. DiStefano | Reviewer |  | Modeler |  |
| Variant: NM\_000441.1(SLC26A4): c.349C>T (p.Leu117Phe)  *SLC26A4* encodes Pendrin, an anion transporter. Variants in SLC26A4 are associated with Pendred syndrome. A common functional assay for transporters is to measure channel function *in vitro*. It is also common to measure cellular localization of the transporter, as it will not function properly if it’s mislocalized. When this variant was expressed as a GFP-tagged construct in HEK 293 cells and imaged, it localized to the apical membrane as wild type protein does. Additionally, iodide efflux, a speculated function of the channel, was unchanged in cells expressing the channel variant. The PMID for this paper is 1193231.  CriterionAssessment  ID: CritAsses59  Allele: CA300  Criterion: BS3  Outcome: Strong Benign  Explanation:  wasGeneratedBy: AssessCriterionActivity61  AssessCriterion  ID: AssessCriterionActivity61  wasAssociatedWith: MDS  When:..  usedEvidenceStatement: [F192,F193]  usedCriterion: BS3    FunctionalData:  ID: F192  Result:Unchanged from reference  dataType: iodide efflux  Allele: AI301  Gene:G16  Explanation:  FunctionalData:  ID: F193  Result: localized to apical membrane  dataType: cellular localization  Allele: AI301  Gene:G16  Explanation: Both wild type and NP\_000432..1:p.Leu117Phe localize to the same location  Gene:  ID: G16  Name: SLC26A4  CanonicalAllele:  ID: CA85  relatedContextualAllele: AI300  …  ContextualAllele:  ID: AI300  alleleName: NM\_000441.1(SLC26A4): c.349C>T  Related: AI301  relatedType: RO: 0003000 (produces)  ...  CanonicalAllele:  ID: CA301  relatedContextualAllele: AI301  ContextualAllele:  ID: AI301  alleleName: NP\_000432..1:p.Leu117Phe  Related: AI300  relatedType: RO: 0003000 (produced-by) | | | | | |
| #3 | Author | M. DiStefano | Reviewer |  | Modeler |  |
| Variant: NM\_152594.2(SPRED1): c.587C>T (p.Thr196Ile)  Variants in SPRED1 are associated with café au lait macules (CALMs), axillary freckling, and macrocephaly. Research has demonstrated that the protein is phosphorylated and inhibits differentiation in neurons by inhibiting Map Kinase. 1) A construct with the variant was transfected into PC12 cells and they were treated with nerve growth factor to stimulated neurite outgrowth. The variant did not reduce or alter neurite outgrowth. 2) Elk1 phosphorylation, a downstream readout of Map kinase activation, was also measured by a luciferase assay and found to be unchanged. The PMID for this is: 19920235.  CriterionAssessment  ID: CritAsses60  Allele: CA87  Criterion: BS3  Outcome: Strong Benign  Explanation:  wasGeneratedBy: AssessCriterionActivity62  AssessCriterion  ID: AssessCriterionActivity62  wasAssociatedWith: MDS  When:..  usedEvidenceStatement: [F194,F195]  usedCriterion: BS3    FunctionalData:  ID: F194  Result:Unchanged from reference  dataType:neurite outgrowth  Allele: AI300  Gene:G45  Explanation:  FunctionalData:  ID: F195  Result: localized to apical membrane  dataType: Elk1 pohsphorylation via luciferase assay  Allele: AI300  Gene:G45  Gene:  ID: G45  Name: SPRED1  CanonicalAllele:  ID: CA87  relatedContextualAllele: AI300  ContextualAllele:  ID: AI300  alleleName: NM\_152594.2(SPRED1): c.587C>T | | | | | |
| Issues | | This will all depend on what is “established”, which could end up being defined by the different clinical domain working groups. | | | | |

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| BS4 - does not segregate with disease | | Lack of segregation in affected members of a family | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | S Harrison | Reviewer | |  | Modeler |  |
| NM\_00527.4(LDLR):c.313+2T>C  PMID:26036859  Variant 313+2T>C was observed in one case diagnosed with Familial Hypercholesterolemia, characterized by elevated LDLC levels. Two additional family members also have elevated LDLC levels but neither carry the 313+2T>C variant.  BS4 would be applicable for this variant    CriterionAssessment  ID:CritAsses61  Allele:CA88  Condition:C33  Criterion:BS4  Outcome: Strong Benign  wasGeneratedBy: AssessCriterionActivity63    AssessCriterion  ID: AssessCriterionActivity63  wasAssociatedWith: SH  When:..  usedEvidenceStatement:FSD1  usedCriterion:BS4  usedAllele:CA88    FamilySegregationData:  ID:FSD196  Condition:C33  Allele: CA88  Family: F8  phenotypePositiveAllelePositiveIndividuals: 0  anyInconsistentSegregations: true  phenotypePositiveAlleleNegativeIndividuals: 2  Explanation. observed in one case diagnosed with Familial Hypercholesterolemia, characterized by elevated LDLC levels. Two siblings of the proband also have elevated LDLC levels but neither carry the 313+2T>C variant.    Family:  ID: F8    Condition :  ID: C33  Name: Familial Hypercholesterolemia    CanonicalAllele:  ID CA88  relatedContextualAllele: AI71    ContextualAllele:  ID: AI71  alleleName: NM\_00527.4(LDLR):c.313+2T>C | | | | | | |
| #2 | Author | S Harrison | Reviewer | |  | Modeler |  |
| NM\_000256.3(MYBPC3):c.1624+4A>T  Internal Data: Variant 1624+4A>T was found in one individual with HCM and segregated with disease in 3 additional relatives. The variant was not found in one additional family member who also had HCM. However, BS4 would not be applicable for this family because this non-segregation is likely to be a phenocopy explained by environmental factors (obese, alcohol).  CriterionAssessment  ID:CritAsses62  Allele:CA89  Condition:C23  Criterion:BS4  Outcome: Insufficient Evidence  wasGeneratedBy:AssessCriterionActivity64  Explanation: The inconsistent segregation data is reasonably explainable as a phenocopy, and therefore does not provide evidence for the benignity of this allele.    AssessCriterion  ID:AssessCriterionActivity64  wasAssociatedWith:SH  When:..  usedEvidenceStatement:FSD197  usedCriterion:BS4  usedAllele:CA89    FamilySegregationData:  ID:FSD197  Condition:C23  Allele:CA89  Family: F9  phenotypePositiveAllelePositiveIndividuals:3  anyInconsistentSegregations:true  phenotypePositiveAlleleNegativeIndividuals:1  Explanation.The individual with HCM but without the allele is likely a phenocopy explained by environmental factors (obese, alcohol)  Family:  ID: F9  Condition :  ID: C23  Name: Hypertrophic Cardiomyopathy  CanonicalAllele:  ID CA89  relatedContextualAllele:AI102  ContextualAllele:  ID: AI102  alleleName: NM\_000256.3(MYBPC3):c.1624+4A>T | | | | | | |
| #3 | Author | S Harrison | Reviewer | |  | Modeler |  |
| NM\_001103.3(ACTN2):c.1484C>T (p.Thr495Met)  In a family tested by the LMM, the c.1484C>T variant was identified in a proband with clinical diagnosis of HCM. This proband has two relatives that also have clinical diagnosis of HCM however never relative carries the c.1484C>T variant.  BS4 is applicable for this variant - especially since there are two non-segregations in this family  (As a sidenote, this variant would be a good one to use in the interface or to test rule application, because there is segregation in another family - so theoretically PP1 and BS4 could be used) | | | | | | |
| Issues | | This rule is for “lack of segregation” meaning phenotype positive / genotype negative (and not pheno neg / geno pos). Also need to be careful of phenocopies. Also need rules for how many cases are needed in use this rule | | | | | |

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| BP1 - missense variant when truncating is pathogenic | | Missense variant in a gene for which primarily truncating variants are known to cause disease | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1a | Author | Rajarshi Ghosh | Reviewer | |  | Modeler |  |
| Assessed variant: NM\_000059.3(BRCA2) :c.4779A>C (p.Glu1593Asp)  Monoallelic truncating mutations in BRCA2 confers high risk of HBOC and biallellic truncating mutations are largely responsible for Fanconi Anemia. This was identified through literature search.  http://hmg.oxfordjournals.org/content/16/R1/R60.full  *Note : would be nice to have a list of genes where primarily truncating mutations results in disease.*  CriterionAssessment  ID:CritAsses64  Allele:CA91  Condition:C34  Criterion:BP1  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity66    AssessCriterion  ID: AssessCriterionActivity66  wasAssociatedWith:RG  When:..  usedEvidenceStatement:,MC198, CM199  usedCriterion:BP1  usedAllele:CA91    MolecularConsequence:  ID: MC198  Allele: AI110  Consequence: SO:0001583 (missense variant)  LOF: No    ConditionMechanism:  ID: CM199  Condition: C34  Gene: G2  Mechanism: LOF  Primary: true  Explanation: ClinVar shows 421 variants in BRCA2 that are pathogenic for HBOC. 402 of these (~95%) are frameshift, nonsense, or splice-site variants.    Gene:  ID: G2  Name: BRCA2  Condition :  ID: C34  Name:Hereditary Breast and Ovarian Cancer  CanonicalAllele:  ID CA91  relatedContextualAllele:AI110  ContextualAllele:  ID: AI110  alleleName: NM\_000059.3(BRCA2) :c.4779A>C | | | | | | |
| #1b | Author | Rajarshi Ghosh | Reviewer | |  | Modeler |  |
| (same as previous - this is a compound example)  Assessed variant: NM\_000059.3(BRCA2) :c.4779A>C (p.Glu1593Asp)  Monoallelic truncating mutations in BRCA2 confers high risk of HBOC and biallellic truncating mutations are largely responsible for Fanconi Anemia. This was identified through literature search.  http://hmg.oxfordjournals.org/content/16/R1/R60.full  *Note : would be nice to have a list of genes where primarily truncating mutations results in disease.*  CriterionAssessment  ID:CritAsses65  Allele:CA91  Condition:C35  Criterion:BP1  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity67    AssessCriterion  ID: AssessCriterionActivity67  wasAssociatedWith: RG  When:..  usedEvidenceStatement:, MC198, CM200  usedCriterion:BP1  usedAllele:CA91    MolecularConsequence: (same as previous example)  ID: MC198  Allele: AI110  Consequence: SO:0001583 (missense variant)  LOF: No  ConditionMechanism:  ID: CM200  Condition: C35  Gene: G2  Mechanism: LOF  Primary: true  Explanation: ClinVar shows 61 variants in BRCA2 that are pathogenic for Fanconi Anemia. 55 of these (~90%) are frameshift, nonsense, or splice-site variants.  Gene:  ID: G2  Name: BRCA2  Condition :  ID: C35  Name: Fanconi Anemia  CanonicalAllele: (same as previous example)  ID CA91  relatedContextualAllele:AI110  ContextualAllele:  ID: AI110  alleleName: NM\_000059.3(BRCA2) :c.4779A>C | | | | | | |
| #2 | Author | M DiStefano | Reviewer | |  | Modeler |  |
| NM\_144612.6(LOXHD1 ): c.1028G>A (p.Arg343His)  Homozygous truncating variants in LOXHD1 are associated with autosomal recessive hearing loss. This was demonstrated by both identification in human patients and a mouse model. (I used the OMIM entry as a source, but you could also directly use the papers.)  <http://omim.org/entry/613072?search=LOXHD1&highlight=loxhd1>  Note: CSER variant  CriterionAssessment  ID:CritAsses66  Allele:CA105  Condition:C10  Criterion:BP1  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity68    AssessCriterion  ID: AssessCriterionActivity68  wasAssociatedWith: MDS  When:..  usedEvidenceStatement:AI98, CM202, MC201  usedCriterion:BP1  usedAllele:CA105    MolecularConsequence:  ID: MC201  Allele: AI98  Consequence: SO:0001583 (missense variant)  LOF: No    ConditionMechanism:  ID: CM202  Condition: C10  Gene: G48  Mechanism: Loss of function  Primary: true  Explanation: shown in both humans and mouse models    Gene:  ID: G48  Name: LOXHD1    Condition :  ID: C10  Name: Hearing Loss  CanonicalAllele:  ID CA92  relatedContextualAllele: AI98  ContextualAllele:  ID: AI98  alleleName: NM\_144612.6(LOXHD1 ): c.1028G>A | | | | | | |
| #3 | Author | M DiStefano | Reviewer | |  | Modeler |  |
| NM\_1333378.4(TTN): c.2926T>C (p.Trp976Arg) Example where rule does not apply.  Truncating variants in TTN are associated with DCM. However, if missense variants are located in the A-band, impact the A-band, or are in a highly expressed region, they can also be associated with DCM. This variant is pathogenic for DCM. (It also segregates with disease in patients).  Source for TTN truncations/the A-band rule: PMID 22335739  CriterionAssessment  ID:CritAsses67  Allele:CA93  Condition:C2  Criterion:BP1  Outcome: Refuted  wasGeneratedBy: AssessCriterionActivity69  Explanation: While truncations are usually the cause of TTN related DCM, missense variants in certain regions can also cause disease.    AssessCriterion  ID: AssessCriterionActivity69  wasAssociatedWith:MDS  When:..  usedEvidenceStatement:CM204, CM205, MC203  usedCriterion:BP1  usedAllele:CA93    MolecularConsequence:  ID: MC203  Allele: AI99  Consequence: SO:0001583 (missense variant)  LOF: No    ConditionMechanism:  ID: CM204  Condition: C2  Gene: G3  Mechanism: Loss of function  Primary: true  Explanation:    ConditionMechanism:  ID: CM205  Condition: C2  Gene: G3  Mechanism: Missense  Primary: false  Established: true  Explanation: Truncating variants in TTN are associated with DCM. However, if missense variants are located in the A-band, impact the A-band, or are in a highly expressed region, they can also be associated with DCM.  Gene:  ID: G3  Name: TTN    Condition :  ID: C2  Name:Dilated Cardiomyopathy  CanonicalAllele:  ID CA93  relatedContextualAllele:AI106  ContextualAllele:  ID: AI99  alleleName: NM\_1333378.4(TTN): c.2926T>C | | | | | | |
| Issues | | Is there any guidance on the proportion of pathogenic variants that are LOF to apply this rule? 99%? 90%? 75%? | | | | | |

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| BP2 - in trans with dominant OR in cis with path variant | | 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 | | | | | | |
| **Evidence Statement Types** | | | | | | | | |
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| **Examples** | | | | | | | | |
| #1 | Author | M. DiStefano | | Reviewer |  | | Modeler |  |
| Variant: NM\_206933.2 (USH2A): c.6721C>T (p.Pro2241Ser)  Variants in USH2A can be associated with Retinitis Pigmentosa, which is a recessive disease. BP2 can be applied because this variant was observed in cis with a very common pathogenic variant in USH2A (Cys759Phe) in 2 individuals (LMM internal data). A publication supporting the claim that the Cys759Phe variant is a common pathogenic variant can be found here: 10775529  CriterionAssessment  ID:CritAsses68  Allele:CA94  Condition:C36  Criterion:BP2  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity70    AssessCriterion  ID: AssessCriterionActivity70  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: IA206, IA207, MDV210, IC208, IC209  usedCriterion:BP2  usedAllele:CA94    IndividualCondition:  ID: IC208  Individual: I21  Condition: C36  hasCondition: True    IndividualCondition:  ID: IC209  Individual: I22  Condition: C36  hasCondition: True  IndividualAllele:  ID: IA206  Individual: I21  primaryAllele: CA94  secondaryAllele: CA95  phase: cis    IndividualAllele:  ID: IA207  Individual: I22  primaryAllele: CA94  secondaryAllele: CA95  phase: cis    Individual:  ID: I22    Individual:  ID: I21    Gene:  ID: G33  Name: USH2A    MendelianDiseaseVariantInterpretation:  ID: MDV210  Condition: C36  Allele: CA95  clinicalSignificance: Pathogenic  Condition :  ID: C36  Name: Retinitis Pigmentosa  CanonicalAllele:  ID CA94  relatedContextualAllele:AI100  ContextualAllele:  ID: AI100  alleleName: NM\_206933.2 (USH2A): c.6721C>T  CanonicalAllele:  ID CA95  relatedContextualAllele:AI101  ContextualAllele:  ID: AI101  alleleName: NM\_206933.2(USH2A):c.2276G>T | | | | | | | |
| #2 | Author | M. DiStefano | | Reviewer |  | | Modeler |  |
| Variant: NM\_000492.3(CFTR): c.2002C>T (p.Arg668Cys)  Variants in CFTR are associated with a range of recessive conditions, including Cystic Fibrosis, CBAVD, and ideopathic pancreatisis. This variant has been identified in cis with pathogenic variants in patients with CBAVD (PMID: 9272157), supporting the interpretation that this variant is benign for CBAVD. The using a different patient, the same argument could be made for cystic fibrosis (PMID:22678879) but this is not modeled here..  CriterionAssessment  ID:CritAsses69  Allele:CA96  Condition:C37  Criterion:BP2  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity71    AssessCriterion  ID: AssessCriterionActivity71  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: IA211, MDV213, IC212  usedCriterion:BP2  usedAllele:CA96    IndividualCondition:  ID: IC213  Individual: I23  Condition: C37  hasCondition: True  IndividualAllele:  ID: IA211  Individual: I23  primaryAllele: CA96  secondaryAllele: CA97  phase: cis    Individual:  ID: I23    MendelianDiseaseVariantInterpretation:  ID: MDV213  Condition: C37  Allele: CA97  clinicalSignificance: Pathogenic  Condition :  ID: C37  Name: Congenital Absence of the Vas Deferens  CanonicalAllele:  ID CA96  relatedContextualAllele:AI102  ContextualAllele:  ID: AI102  alleleName: NM\_000492.3(CFTR): c.2002C>T  CanonicalAllele:  ID CA97  relatedContextualAllele: AI103  ContextualAllele:  ID: AI103  alleleName:NP\_000483.3:p.Gly576Ala | | | | | | | |
| #3 | Author | M. DiStefano | | Reviewer |  | | Modeler |  |
| Variant: NM\_000257.2(MYH7): c.2559G>A (p.Glu867Lys)  Internal LMM data: BP2 does not apply because, although this variant was found in trans with another variant in the same gene, that variant is VUS and not an established pathogenic variant and cardiomyopathies can be variable in penetrance. The trans variant is c.2537A>G (p.Glu846Gly).  CriterionAssessment  ID:CritAsses70  Allele:CA98  Condition:C38  Criterion:BP2  Outcome: Insufficient Evidence  wasGeneratedBy: AssessCriterionActivity72  Explanation: although this variant was found in trans with another variant in the same gene, that variant is VUS and not an established pathogenic variant and cardiomyopathies can be variable in penetrance.    AssessCriterion  ID: AssessCriterionActivity72  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: CP218, IC, IA, CI, MDVI  usedCriterion:BP2  usedAllele:CA98  ConditionPenetrance:  ID: CP218  Condition: C38  Penetrance: Incomplete  IndividualConditon:  ID: IC215  Individual: I24  Condition C38  hasCondition: true  IndividualAllele:  ID: IA214  Individual: I24  primaryAllele: CA98  secondaryAllele: CA99  Phase: trans  ConditionInheritance:  ID: CI217  Condition: C38  modeOfInheritance::HP:0000006 (Autosomal Dominant)  Individual:  ID: I24  MendelianDiseaseVariantInterpretation:  ID: MDVI216  Allele: CA99  Condition: C38  clnicalSingificance:Uncertain Significance  Condition:  ID: C38  Name: Cardiomyopathy?  CanonicalAllele:  ID CA98  relatedContextualAllele:AI120  ContextualAllele:  ID: AI120  alleleName: NM\_000257.2(MYH7): c.2559G>A  CanonicalAllele:  ID CA99  relatedContextualAllele: AI121  ContextualAllele:  ID: AI121  alleleName:NM\_000257.2(MYH7): c.2537A>G | | | | | | | |
| Issues | |  | | | | | | |

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| BP3 - in-frame indel unknown function | | In-frame deletions/insertions in a repetitive region without a known function | | | | | |
| **Evidence Statement Types** | | | | | | | |
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| **Examples** | | | | | | | |
| #1 | Author | M. DiStefano | Reviewer | |  | Modeler |  |
| Variant: NM\_001271208.1(NEB):c.169\_183delCTGGCACAGCCAGCA  This variant is an in-frame deletion of 15 bp. EGL has classified it in ClinVar as likely benign. The ClinVar entry can be found here: <http://www.ncbi.nlm.nih.gov/clinvar/variation/95115/#clinical-assertions>  NEB is a gene with a lot of repetitive regions with unknown function. There are two parts in the gene that are 99% identical and thought to be duplications. Used the OMIM gene entry as a source (<http://omim.org/entry/161650?search=NEB&highlight=neb>) and the paper for the gene description: Donner 2004 PMID: 15266303  Here is a screenshot from Alamut to show that this is a repeat region (deletion marked in green rectangle):      CriterionAssessment  ID:CritAsses71  Allele:CA100  Criterion:BP3  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity82    AssessCriterion  ID: AssessCriterionActivity82  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: RA220, RA221, MC223, RCA222  usedCriterion:BP3  usedAllele:CA100  RegionContainsAllele: (E42)  ID: RCA222  Region: CR219  Allele: AI106  Value: True  RegionAnnotation: (E24)  ID: RA220  Region: CR219  type: repeating  Value: true  RegionAnnotation: (E24)  ID: RA221  Region: CR219  type: functional  Value: false  Explanation: region does not have a known function  ContextualRegion: (E25)  ID: CR219  Sequence: RS08  Start:154  Stop:183  ReferenceSequence:  ID: RS08  Identifier: NM\_001271208.1  MolecularConsequence (E02)  ID: MolCon223  Allele: AI106  Consequence: SO:0001825 (Conservative Inframe Deletion)  CanonicalAllele:  ID CA100  relatedContextualAllele:AI106  ContextualAllele:  ID: AI106  alleleName: NM\_001271208.1(NEB):c.169\_183delCTGGCACAGCCAGCA | | | | | | |
| #2 | Author | M. DiStefano | Reviewer | |  | Modeler |  |
| NM\_133378.4 (TTN): c.26752\_26761delACGGCAGAGC (p.Thr8918CysfsX3)  This variant is classified as likely pathogenic by LMM for DCM. The ClinVar entry can be found here:  http://www.ncbi.nlm.nih.gov/clinvar/RCV000155429/  BP3 does not apply for this variant because it is not an in-frame deletion (it's a deletion of 10 bp) and, although the I Band is a repetitive region that contains tandem arrays of immunoglobulin domains, these folds may be an important component for TTN's elasticity. Source for I-band information: PMID: 10573426.  CriterionAssessment  ID:CritAsses72  Allele:CA101  Criterion:BP3  Outcome: Refuted  wasGeneratedBy: AssessCriterionActivity83  Explanation: BP3 does not apply for this variant because it is not an in-frame deletion (it's a deletion of 10 bp) and, although the I Band is a repetitive region that contains tandem arrays of immunoglobulin domains, these folds may be an important component for TTN's elasticity.  AssessCriterion  ID: AssessCriterionActivity83  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: RA225, MolCon227, RCA226  usedCriterion:BP3  usedAllele:CA101  RegionContainsAllele:  ID: RCA226  Region: CR224  Allele: AI107  Value: True  RegionAnnotation:  ID: RA225  Region: CR224  type: repeating  Value: true  Explanation: The I-Band is a large region of repetitive Igg domains.  ContextualRegion:  ID: CR224  Sequence: RS009  Start:6509  Stop:39262  ReferenceSequence:  ID: RS009  Identifier: NM\_133378.4  MolecularConsequence  ID: MolCon227  Allele: AI107  Consequence: SO:0001589 (frameshift variant)  CanonicalAllele:  ID CA101  relatedContextualAllele:AI107  ContextualAllele:  ID: AI107  alleleName: NM\_133378.4 (TTN): c.26752\_26761delACGGCAGAGC | | | | | | |
| #3 | Author | M. DiStefano | Reviewer | |  | Modeler |  |
| Variant: NM\_147196.2(TMIE):c.391\_393dupAAG (p.Lys131\_Asp132insLys)  This variant is an in-frame duplication (3bp) that results in the addition of a Lysine in a repetitive region of 9 lysines. The ClinVar entry can be found here: <http://www.ncbi.nlm.nih.gov/clinvar/variation/179307/>  I’ve also included the Alamut screenshot below to demonstrate the repetitive region. The duplication is the small green dot in the bottom right corner.  CriterionAssessment  ID:CritAsses73  Allele:CA102  Criterion:BP3  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity84    AssessCriterion  ID: AssessCriterionActivity84  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: RA229, MolCon231, RCA230  usedCriterion:BP3  usedAllele:CA102  RegionContainsAllele:  ID: RCA230  Region: CR228  Allele: AI108  Value: True  RegionAnnotation:  ID: RA229  Region: CR228  type: repeating  Value: true  ContextualRegion:  ID: CR228  Sequence: RS010  Start: 522  Stop:548  ReferenceSequence:  ID: RS010  Identifier: NM\_147196.2  MolecularConsequence  ID: MolCon231  Allele: AI108  Consequence: SO:0001823 (Conservative inframe insertion)  CanonicalAllele:  ID CA102  relatedContextualAllele:AI108  ContextualAllele:  ID: AI108  alleleName: NM\_147196.2(TMIE):c.391\_393dupAAG | | | | | | |
| Issues | |  | | | | | |

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| BP4 - computational evidence for no gene impact | | Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.) | | | | |
| **Evidence Statement Types** | | | | | | |
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| **Examples** | | | | | | |
| #1 | Author | Rajarshi Ghosh | Reviewer |  | Modeler |  |
| Assessed variant: NM\_000059.3(BRCA2):c.4779A>C (p.Glu1593Asp)  CSER variant.  All (SIFT,FATHMM,LRT,MetaSVM,MutationAssessor,Mutationtaster,Provean) but Polyphen and Condel predicts benign.  Predictions obtained via dbNSFP and VEP on rs80358703.  CriterionAssessment  ID: CritAsses74  Allele: CA103  Criterion: BP4  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity85  Explanation: Seven of the nine predictions found this variant to be benign, which we consider sufficient to support.    AssessCriterion  ID: AssessCriterionActivity85  wasAssociatedWith: RG  When:..  usedEvidenceStatement: [ISP1,ISP2, ISP3, ISP4, ISp5, ISP6, ISP7, ISP98, ISP9]  usedCriterion: BP4  usedAllele:CA103  InSilicoPrediction: (E47)  ID: ISP1  Allele: AI109  predictionType: Missense Effect  Tool: SIFT  Value: Benign    InSilicoPrediction:  ID: ISP2  Allele: AI109  predictionType: Missense Effect  Tool: Polyphen  Value: Possibly damaging    InSilicoPrediction:  ID: ISP3  Allele: AI109  predictionType: Missense Effect  Tool: FATHMM  Value: Benign    InSilicoPrediction:  ID: ISP4  Allele: AI109  predictionType: Missense Effect  Tool: LRT  Value: Benign    InSilicoPrediction:  ID: ISP5  Allele: AI109  predictionType: Missense Effect  Tool: MetaSVM  Value: Benign    InSilicoPrediction:  ID: ISP6  Allele: AI109  predictionType: Missense Effect  Tool: Mutation assessor  Value: Benign    InSilicoPrediction:  ID: ISP7  Allele: AI109  predictionType: Missense Effect  Tool: Mutation taster  Value: Benign    InSilicoPrediction:  ID: ISP8  Allele: AI109  predictionType: Missense Effect  Tool: Provean  Value: Benign    InsilicoPrediction:  ISP9  Allele: AI109  predictionType: Missense Effect  Tool: Condel  Value: deleterious    CanonicalAllele:  ID CA103  relatedContextualAllele: AI109    ContextualAllele:  ID: AI109  alleleName: NM\_000059.3(BRCA2):c.4779A>C | | | | | |
| #2 | Author | Rajarshi Ghosh | Reviewer |  | Modeler |  |
| Variant assessed: NM\_017636.3(TRPM4):c.2531G>A (p.Gly844Asp)  All (SIFT,FATHMM,MetaSVM,MutationAssessor,Mutationtaster,Provean) but Polyphen and Condel predicts benign.  Predictions obtained via dbNSFP and VEP on rs200038418.  TODO - Excluding this example - no additional value from above  CanonicalAllele:  ID CA104  relatedContextualAllele: AI110    ContextualAllele:  ID: AI110  alleleName: NM\_017636.3(TRPM4):c.2531G>A | | | | | |
| #3 | Author | M. DiStefano | Reviewer |  | Modeler |  |
| Variant:NM\_133379.3(TTN) : c.15283T>C (p.Tyr5095His) BP4 does not apply  BP4 does not apply for this variant because it is located in exon 45A of TTN, which is difficult to sequence and in silico tools do not offer accurate predictions for it.  AlignGVGD, MAPP, SIFT, and MutationTaster do not have predictions for this variant. However, PolyPhen-2 HumVar predicts that it is “possibly damaging” and HumDiv predicts that it is “Probably damaging”. These predictions cannot be trusted because of the sequencing difficulties. SIFT gives a prediction of “tolerated”.    TODO - chris will do.  Yes, want something that would highlight which transcript is relevant.  CanonicalAllele:  ID CA105  relatedContextualAllele: AI111    ContextualAllele:  ID: AI111  alleleName: NM\_133379.3(TTN) : c.15283T>C | | | | | |
| Issues | | Same issues as PP3 | | | | |

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| BP5 - alternate cause of disease | | Variant found in a case with an alternate molecular basis for disease | | | | | | |
| **Evidence Statement Types** | | | | | | | | |
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| **Examples** | | | | | | | | |
| #1 | Author | M. DiStefano | | Reviewer |  | | Modeler |  |
| Variant: NM\_000256.3(MYBPC3): c.2497G>A (p.Ala833Thr)  This variant was identified in multiple individuals with pathogenic variants in other genes. This was observed in internal LMM data (>5 patients have likely pathogenic/pathogenic alleles in other genes) and in the literature in Van Driest et al (PMID: 15519027) The proband with HCM in this article had a likely pathogenic variant in TNNT2.  <http://www.ncbi.nlm.nih.gov/clinvar/variation/43677/> (TNNT2 variant)  CriterionAssessment  ID:CritAsses77  Allele:CA106  Criterion:BP5  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity88  Explanation: The evidence here only explicitly describes a single individual with a likely pathogenic variant in TNNT2, but there are numerous other examples that have been observed in internal LMM data.    AssessCriterion  ID: AssessCriterionActivity88  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: IC242, IA241, MDVI243  usedCriterion:BP5  usedAllele:CA106  MendelianDiseaseVariantInterpretation:  ID: MDVI243  Allele: CA107  Condition: C23  clinicalSignificance: Likely Pathogenic  IndividualCondition:  ID: IC242  Individual: I25  Condition: C23  hasCondition: true  Condition:  ID: C23  Name: Hypertrophic Cardiomyopathy  IndividualAllele:  ID: IA241  primaryAllele: CA106  secondaryAllele: CA107  Individual:  ID: I25  CanonicalAllele:  ID CA106  relatedContextualAllele:AI112  ContextualAllele:  ID: AI112  alleleName: NM\_000256.3(MYBPC3): c.2497G>A    CanonicalAllele:  ID CA107  relatedContextualAllele:AI113  ContextualAllele:  ID: AI113  alleleName: NM\_000364.3(TNNT2):c.878G>A | | | | | | | |
| #2 | Author | M. DiStefano | | Reviewer |  | | Modeler |  |
| Variant: NM\_022124.5(CDH23):c.5660C>T (p.Thr1887Ile)  Pathogenic variants in CDH23 are associated with Usher Syndrome Type 1, which is recessive.  This variant was identified in multiple individuals with pathogenic variants in other genes. Internal LMM data: The variant was identified in 10 probands and 2 of those probands had other pathogenic variants. One proband was had a (trans) pair of GJB2 (c.71G>A (p.Trp24x) ; c.231G>A (p.Trp77x) )  truncation variants and the other was homozygous for DFNB31 (now WHRN) (c.643delG (p.Val215fs)) truncating variants.  The proband with the GJB2 variants just had profound sensorineural hearing loss. The proband with the DFNB31/WHRN variants did have Usher Syndrome. WHRN is associated with Type 2D.  2a)  CriterionAssessment  ID:CritAsses78  Allele:CA108  Criterion:BP5  Condition: C15  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity89    AssessCriterion  ID: AssessCriterionActivity89  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: MDVI247, MDVI248, IC246, IA244, IA245  usedCriterion:BP5  MendelianDiseaseVariantInterpretation:  ID: MDVI247  Allele: CA109  Condition: C15  clinicalSignificance: Likely Pathogenic  MendelianDiseaseVariantInterpretation:  ID: MDVI248  Allele: CA110  Condition: C15  clinicalSignificance: Likely Pathogenic  IndividualCondition:  ID: IC246  Individual: I26  Condition: C15  hasCondition: true  IndividualAllele:  ID: IA245  primaryAllele: CA108  IndividualAllele:  ID: IA244  primaryAllele: CA109  primaryZygosity: heterozygous  secondaryAllele: CA110  secondaryZygosity: heterozygous  Phase: trans  Individual:  ID: I26  Condition:  ID: C15  Name: Non-syndromic genetic deafness  2b)  CriterionAssessment  ID:CritAsses79  Allele:CA108  Criterion:BP5  Condition: C21  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity90  AssessCriterion  ID: AssessCriterionActivity90  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: MDVI251, IC250, IA249  usedCriterion:BP5  IndividualAllele:  ID: IA249  primaryAllele: CA108  secondaryAllele: CA111  secondaryZygosity: homozygous  MendelianDiseaseVariantInterpretation:  ID: MDVI251  Allele: CA111  Condition: C21  clinicalSignificance: Pathogenic  IndividualCondition:  ID: IC250  Individual: I2  Condition: C21  hasCondition: true  Individual:  ID: I27  Condition:  ID: C21  Name: Usher Syndrome  CanonicalAllele:  ID CA108  relatedContextualAllele:AI114  ContextualAllele:  ID: AI114  alleleName: NM\_022124.5(CDH23):c.5660C>T    CanonicalAllele:  ID CA109  relatedContextualAllele:AI115  ContextualAllele:  ID: AI115  alleleName: NM\_004004.5(GJB2):c.71G>A  CanonicalAllele:  ID CA110  relatedContextualAllele:AI116  ContextualAllele:  ID: AI116  alleleName:NM\_004004.5(GJB2):c.230G>A  CanonicalAllele:  ID CA111  relatedContextualAllele:AI117  ContextualAllele:  ID: AI117  alleleName:NM\_015404.3(WHRN):c.643delG | | | | | | | |
| #3 | Author | M. DiStefano | | Reviewer |  | | Modeler |  |
| Variant: NM\_001844.4(COL2A1):c.4316C>T (p.Thr1439Met) BP5 does not apply  Variants in COL2A1 are associated with dominant spondyloepiphyseal dysplasia congenita. This variant was identified in a proband with another pathogenic variant: NM\_000095.2(COMP):c.1042T>C (p.Cys348Arg). Variants in COMP are associated with dominant Pseudoachondroplasia. However, BP5 does not apply because the combination of these two variants results in a more severe phenotype with a different condition name (Pseudoachondroplastic spondyloepiphyseal dysplasia syndrome). The pubmed ID for the reference is: 11746045  CriterionAssessment  ID:CritAsses80  Allele:CA112  Criterion:BP5  Condition: C39  Outcome: Insufficient evidence  wasGeneratedBy: AssessCriterionActivity91  Explanation :Variants in COL2A1 are associated with dominant spondyloepiphyseal dysplasia congenita (a primary bone dysplasia). CA112 was identified in a proband with CA113, which is pathogenic for another primary bone dysplasia, dominant Pseudoachondroplasia. However, this does not provide supporting evidence for CA112 being benign because the combination of a COL2A1 and COMP variant are known to produce a more severe phenotype, Pseudoachondroplastic spondyloepiphyseal dysplasia syndrome, which is the phenotype of the proband.Because the combination of alleles may produce a qualitatively different phenotype, the bone dysplasia caused by the COMP variant does not provide evidence that the COL2A1 variant is benign for any bone dysplasias in general.  AssessCriterion  ID: AssessCriterionActivity91  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: MDVI254, IndAll253, IC252, MDVI270  usedCriterion:BP5  usedAllele:CA112  MendelianDiseaseVariantInterpretation:  ID: MDVI270  Allele: CA113  Condition: C41  clinicalSignificance: VUS  Explanation: Pseudoachondroplastic spondyloepiphyseal dysplasia syndrome is caused by a combination of a pathogenic variant in COL2A1 and another in COMP. So CA113 may be involved, but is not the sole cause.  MendelianDiseaseVariantInterpretation:  ID: MDVI254  Allele: CA113  Condition: C40  clinicalSignificance: pathogenic  IndividualAllele:  ID: IndAll253  Individual: I28  primaryAllele: CA112  secondaryAllele: CA113  IndividualCondition:  ID: IC252  Individual: I28  Condition: C41  hasCondition: True  Condition:  ID: C39  Name: Primary bone dysplasia  Condition:  ID: C41  Name: Pseudoachondroplastic spondyloepiphyseal dysplasia syndrome  Condition:  ID: C40  Name: Pseudoachondroplasia  Individual:  ID: I28  CanonicalAllele:  ID CA112  relatedContextualAllele:AI118  ContextualAllele:  ID: AI118  alleleName:NM\_001844.4(COL2A1):c.4316C>T  CanonicalAllele:  ID CA113  relatedContextualAllele:AI119  ContextualAllele:  ID: AI119  alleleName:NM\_000095.2(COMP):c.1042T>C | | | | | | | |
| Issues | |  | | | | | | |

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| BP6 - benign from reputable source without shared data | | Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation | | | | |
| **Evidence Statement Types** | | | | | | |
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| **Examples** | | | | | | |
| #1 | Author | M. DiStefano | Reviewer |  | Modeler |  |
| Variant: NM\_007294.3(BRCA1):c.6207C>T  Pathogenic variants in BRCA1 are associated with hereditary breast and ovarian cancer. This variant is classified as benign by ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles), which is a ClinVar expert panel. Many labs are using the classifications of ClinGen expert panels for their variant interpretations, so BP6 is met.  (SCV000244500.1)  <http://www.ncbi.nlm.nih.gov/clinvar/variation/209219/>  CriterionAssessment  ID:CritAsses81  Allele:CA114  Criterion:BP6  Condition: C34  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity92  AssessCriterion  ID: AssessCriterionActivity92  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: MDVI255  usedCriterion:BP6  usedAllele:CA114  MendelianDiseaseVariantInterpretation:  ID: MDVI255  Allele: CA114  Condition: C34  clinicalSignificance: benign  Explanation: classified by ENIGMA.  Condition:  ID: C34  Name: Hereditary breast and ovarian cancer  CanonicalAllele:  ID CA114  relatedContextualAllele:AI150  ContextualAllele:  ID: AI120  alleleName: NM\_007294.3(BRCA1):c.6207C>T | | | | | |
| #2 | Author | M. DiStefano | Reviewer |  | Modeler |  |
| Variant: NM\_000059.3(BRCA2):c.-26G>A  Variant has been classified as “Benign” by Sharing Clinical Reports Project (SCV000189291.1)  <http://www.ncbi.nlm.nih.gov/clinvar/variation/125965/#clinical-assertions>  Sharing Clinical Reports Project are reports from Myriad - who are considered experts in this field but do not share their evidence - only their interpretation.  (Note: this is the exact scenarios this rule was written for - but people are being very liberal in applying this rule)  CriterionAssessment  ID:CritAsses82  Allele:CA115  Criterion:BP6  Condition: C42  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity93    AssessCriterion  ID: AssessCriterionActivity93  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: MDVI256  usedCriterion:BP6  usedAllele:CA115  MendelianDiseaseVariantInterpretation:  ID: MDVI256  Allele: CA115  Condition: C42  clinicalSignificance: benign  Explanation: classified by Sharing Clinical Reports Project.  Condition:  ID: C42  Name: Familial Cancer of Breast  CanonicalAllele:  ID CA115  relatedContextualAllele:AI160  ContextualAllele:  ID: AI121  alleleName: NM\_000059.3(BRCA2):c.-26G>A | | | | | |
| #3 | Author | M. DiStefano | Reviewer |  | Modeler |  |
| Variant: NM\_000274.3(OAT):c.1311G>C (p.Leu437Phe)  Variant has been classified as “Benign” by by OMIM (SCV000020324.2)  http://www.ncbi.nlm.nih.gov/clinvar/variation/158/    BP6 is not applicable for this variant because   1. OMIM is not considered an expert or reputable source for variant interpretations 2. OMIM’s evidence for this benign call is available (the publication link - PMID:1737786- and free text summary) so people should use the evidence from OMIM in their assessment NOT OMIM’s interpretation.   CriterionAssessment  ID:CritAsses83  Allele:CA116  Criterion:BP6  Condition: C43  Outcome: Insufficient Evidence  wasGeneratedBy: AssessCriterionActivity94  AssessCriterion  ID: AssessCriterionActivity94  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: MDVI257  usedCriterion:BP6  usedAllele:CA116  MendelianDiseaseVariantInterpretation:  ID: MDVI257  Allele: CA116  Condition: C43  clinicalSignificance: benign  Explanation: classified by OMIM  Condition:  ID: C43  Name: Gyrate atrophy of choroid and retina  CanonicalAllele:  ID CA116  relatedContextualAllele:AI170  ContextualAllele:  ID: AI122  alleleName: NM\_000274.3(OAT):c.1311G>C | | | | | |
| Issues | |  | | | | |

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| BP7 - silent variant no splicing impact and low conservation | | 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 | | | | | | |
| **Evidence Statement Types** | | | | | | | | |
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| **Examples** | | | | | | | | |
| #1 | Author | M. DiStefano | | Reviewer |  | | Modeler |  |
| Variant: NM\_138691.2 (TMC1): c.684C>T (p.Thr228Thr)  This is a synonymous variant with little conservation and no predicted impact on splicing. Conservation and splicing algorithm values are listed below.  Conservation:  phyloP: -2.297  PhastCons: 0.039  Splicing:      CriterionAssessment  ID:CritAsses84  Allele:CA117  Criterion:BP7  Outcome: Supporting Benign  wasGeneratedBy: AssessCriterionActivity95  AssessCriterion  ID: AssessCriterionActivity95  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: InSilico258, Cons259, Cons260, MC261  usedCriterion:BP7  usedAllele:CA117  InSilicoPrediction:  ID: InSilico258  Value: no impact  Type: Splicing Prediction  Allele: AI123  Conservation:  ID: Cons259  Allele: CA117  isConserved: false  Method: phyloP:  Score: -2.297  Conservation:  ID: Cons260  Allele: CA117  isConserved: false  Method: PhastCons  Score: 0.039  MolecularConsequence:  ID: MC261  Allele: AI123  Consequence: SO:0001819 (Synonomous Variant)  CanonicalAllele:  ID CA117  relatedContextualAllele:AI123  ContextualAllele:  ID: AI123  alleleName: NM\_138691.2 (TMC1): c.684C>T | | | | | | | |
| #2 | Author | M. DiStefano | | Reviewer |  | | Modeler |  |
| Variant: NM\_000257.2(MYH7):c.327C>T (p.Tyr109Tyr) Note: CSER Bakeoff Variant    This is a synonymous variant with little conservation and no predicted impact on splicing. Conservation and splicing algorithm values are listed below.    Conservation:  phyloP: -0.76  PhastCons: 0.63      CriterionAssessment  ID:CritAsses85  Allele:CA118  Criterion:BP7  Outcome: Supportive Benign  wasGeneratedBy: AssessCriterionActivity96  AssessCriterion  ID: AssessCriterionActivity96  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: InSilico262, Cons263, Cons264, MC265  usedCriterion:BP7  usedAllele:CA118  InSilicoPrediction:  ID: InSilico262  Value: no impact  Type: Splicing Prediction  Allele: AI124  Explanation: Provenance would show that this comes from Alamut  Conservation:  ID: Cons263  Allele: CA118  isConserved: false  Method: phyloP:  Score: -0.76  Conservation:  ID: Cons264  Allele: CA118  isConserved: false  Method: PhastCons  Score: 0.63  MolecularConsequence:  ID: MC265  Allele: AI124  Consequence: SO:0001819 (Synonomous Variant)  CanonicalAllele:  ID CA118  relatedContextualAllele:AI124  ContextualAllele:  ID: AI124  alleleName: NM\_000257.2(MYH7):c.327C>T | | | | | | | |
| #3 | Author | M. DiStefano | | Reviewer |  | | Modeler |  |
| Variant: NM\_002880.3(RAF1):c.321T>C (p.Gly107Gly) BP7 does not apply  This variant is a silent variant that does not result in an amino acid change. It is not very conserved and splicing tools suggest no change. However, BP7 does not apply because the variant is located in the first base of exon 4 of RAF1, which is part of the 3’ splice region of the exon. For that reason, we can’t fully trust computational tools. Functional assays would have to confirm that this variant truly does not alter splicing.  Convervation:  PhyloP: 0.45  PhastCons: 1.00    CriterionAssessment  ID:CritAsses86  Allele:CA119  Criterion:BP7  Outcome: Insufficient Evidence  wasGeneratedBy: AssessCriterionActivity97  Explanation: This variant is a silent variant that does not result in an amino acid change. It is not very conserved and splicing tools suggest no change. However, BP7 does not apply because the variant is located in the first base of exon 4 of RAF1, which is part of the 3’ splice region of the exon. For that reason, we can’t fully trust computational tools. Functional assays would have to confirm that this variant truly does not alter splicing.  AssessCriterion  ID: AssessCriterionActivity97  wasAssociatedWith:MDS  When:..  usedEvidenceStatement: InSilico266, Cons267, Cons268, MC269  usedCriterion:BP7  usedAllele:CA119  InSilicoPrediction:  ID: InSilico266  Value: no impact  Type: Splicing Prediction  Allele: AI125  Explanation: Provenance would show that this comes from Alamut  Conservation:  ID: Cons267  Allele: CA119  isConserved: false  Method: phyloP  Score: 0.45  Conservation:  ID: Cons268  Allele: CA119  isConserved: false  Method: PhastCons  Score: 1.00  MolecularConsequence:  ID: MC269  Allele: AI125  Consequence: SO:0001819 (Synonomous Variant)  CanonicalAllele:  ID CA119  relatedContextualAllele:AI125  ContextualAllele:  ID: AI125  alleleName: NM\_002880.3(RAF1):c.321T>C | | | | | | | |
| Issues | |  | | | | | | |