# Developing optimized ACMG-AMP criteria for classification of germline variants in *TP53*

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## Introduction

Germline pathogenic variants in *TP53* cause Li-Fraumeni Syndrome (LFS), an autosomal dominant cancer predisposition syndrome associated with high risk of malignancy, including soft tissue sarcoma, osteosarcoma, pre-menopausal breast cancer, brain tumors, adrenocortical carcinoma, and leukemia. Individuals with germline *TP53* pathogenic variants often develop LFS-associated cancers in early childhood or early adulthood and are at increased risk for multiple primary cancers. It is estimated that up to 80% of individuals meeting classic clinical criteria for LFS have a detectable *TP53* pathogenic variant, most of which are missense variants. In addition, the frequency of de novo *TP53* mutations in LFS is around 7-20%. At the somatic level, mutations in *TP53* are frequent in the majority of cancers. Germline pathogenic variants in *TP53* are clinically actionable prompting the National Comprehensive Cancer Network to publish guidelines for screening recommendations and counseling about risk-reduction strategies for individuals with *TP53* germline mutations and their at-risk relatives. Accurate and consistent classification of variants in *TP53* across clinical and research laboratories are therefore very important for patient care.

The ClinGen *TP53* expert panel was formed under the umbrella of the Hereditary Cancer Domain and tasked with the goal to optimize the 2015 ACMG-AMP Variant Interpretation Guidelines for clinical interpretation of variants identified in *TP53*. Members of the panel consist of clinicians, researchers, genetic counselors, statisticians, structural biologists and diagnostic laboratory members with expertise and experience in *TP53*-associated pathology and *TP53* variant classification.

Here we present the draft optimized pathogenic and benign evidence criteria. These criteria will be validated on a set of established benign and pathogenic variants as an initial test of these optimizations.

## Proposed Li-Fraumeni Syndrome (LFS) Associated Cancer Categories

Strong Associated LFS Cancers

- Breast cancer (IDC and DCIS) <31 years of age
- Choroid plexus carcinoma
- Adrenocortical adenoma or carcinoma <18 years of age
- Rhabdomyosarcoma <46 years of age
- Osteosarcoma <46 years of age

Moderate Associated LFS Cancers

- Breast cancer >30 (upper age limit to be determined)
- Malignant brain tumors <46 years of age (excludes optic pathway gliomas)
- Primary lung cancer <46 years of age
- Adrenocortical adenoma or carcinoma >18 years of age
- Osteosarcoma >45 years of age
- Other sarcomas (malignant phyllodes tumor), with the exception of dermatofibrosarcoma & Ewing sarcoma
- Low-hypodiploid ALL

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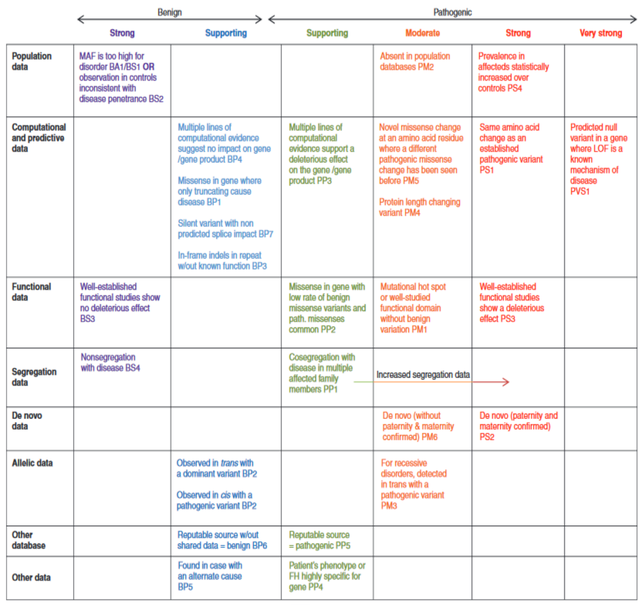
## Modified ACMG Classification criteria

The group was tasked with developing *TP53*-specific variant classification criteria, using the 2015 ACMG/AMP Variant Interpretation Guidelines [1] as a starting point.

Three working groups were formed to review and present current knowledge related to *TP53* surrounding each ACMG/AMP criteria evidence type.




- Computational/Predictive working group
- Phenotype/Segregation/*De novo* working group
- Functional Data working group

## Draft *TP53* Benign Criteria

Benign criteria are presented below. Rules to combine criteria were kept as defined by ACMG/AMP.

| Benign Criteria | | |
|---|---|---|
| Criteria | Description | Modification |
| **STAND ALONE** | | |
| BA1 | Allele frequency is greater than expected for disorder | Minor allele frequency cutoff of 0.1% minimum of 5 alleles present in the population |
| **STRONG** | | |
| BS1 | Allele frequency is greater than expected for the disorder | Frequency cutoff of 0.06%; minimum of 5 alleles present in the population |
| BS2 | Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age | This rule does not apply since the expected penetrance in population cohorts is not known |
| BS3 | Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing | **Strong evidence** - transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) shows retained activity + other assays including colony formation assays and knock-in mouse models<br>**Supporting evidence** - transactivation assays in yeast (IARC classification based on data from Kato et al, 2003) shows retained activity* |
| BS4 | Lack of segregation in affected members of a family | Variant segregates to opposite side of the family who meets LFS criteria OR Variant present in >3 living unaffected individuals above age 46 years |
| **SUPPORTING** | | |
| BP1 | Missense variant in a gene for which primarily truncating variants are known to cause disease | Does not apply |
| BP2 | Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance | Under review |
| BP3 | In-frame deletions/insertions in a repetitive region without a known function | Does not apply |
| BP4 | Multiple lines of computational evidence suggest no impact on gene/gene product | Concordance of 2 predictors: REVEL and AGVGD (zebrafish). Consider Provean for indels |
| BP5 | Variant found in a case with an alternate molecular basis for disease | Under review |
| BP6 | Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation | Do not plan to use |
| BP7 | A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved | Concordance of MaxEntScan and HSF; If a new alternate site is predicted, compare strength to native site in interpretation |

## Draft *TP53* Pathogenic Criteria

Draft pathogenic criteria were presented to the Genomic Variant Working Group in May 2017. Rules to combine criteria were kept as defined by ACMG/AMP.

| Pathogenic Criteria | | |
|---|---|---|
| Criteria | Description | Modification |
| **VERY STRONG** | | |
| PVS1 | Null variant in a gene where LOF is a known mechanism of disease | Includes truncating variants (except in the NMD resistant 3' end – 55 nucleotides before penultimate) and splicing variants |
| **STRONG** | | |
| PS1 | Same amino acid change as a previously established pathogenic variant regardless of nucleotide change | Must confirm there is no difference in splicing<br>- **Strong evidence** if using RNA data<br>- **Moderate evidence** if *in silico* modeling is used * |
| PS2 | *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history | Strength of evidence dependent on tumor type (see LFS Cancer Categories):<br>- **Very strong evidence** – synchronous/metachronous LFS assoc. tumors in strong category *<br>- **Strong evidence** – synchronous/metachronous LFS assoc. tumors in a moderate category (or mixed strong/moderate categories) OR a single tumor in strong category<br>- **Moderate evidence** – a tumor in moderate category * |
| PS3 | Well-established *in vitro* or *in vivo*, functional studies supportive of a damaging effect on the gene or gene product | **Strong evidence** -Transactivation assays in yeast shows loss of activity (categorized as non-functional according to IARC classification based on data from Kato et al, 2003) + other assays including colony formation assays and knock-in mouse models<br>**Moderate evidence** - Transactivation assays in yeast shows loss of activity (categorized as non-functional according to IARC classification based on data from Kato et al, 2003) OR other assays including other transactivation assays, colony formation assays and knock-in mouse models show complete loss of |
| PS4 | The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls | *TP53* deleterious alterations are rare and would require large scale case control studies with sufficient power at the variant level. Relative Risk (RR) or Odds Ratios (OR) should be greater than 2 and the lower bound of the confidence interval should be greater than 1 |
| **MODERATE** | | |
| PM1 | Located in a mutational hot spot and/or critical and well-established functional domain without benign variation | This rule can be applied to variants in hot spots, but not to variants within functional domains. |
| PM2 | Absent in population databases (or at extremely low frequency if recessive) | **Supporting evidence** - Needs to be absent from controls (gnomAD and other large relevant population cohorts) |
| PM3 | For recessive disorders, detected in trans with a pathogenic variant | Does not apply |
| PM4 | Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants | This rule should not be used at this time due to limited data |
| PM5 | Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before | **Supporting evidence** - a matrix such as grantham or blossom should be used to compare the variants; new variant must be equal or worse than known mutation; rule out splicing as a mechanism of mutation using *in silico* tools*<br>**Moderate evidence** - multiple known pathogenic variants (>2) at that residue using the same requirements as above (excluding hot spots) |
| PM6 | Assumed *de novo*, but without confirmation of paternity and maternity | Strength of evidence would be dependent on tumor type (see LFS Cancer Categories):<br>- **Strong evidence** – synchronous/metachronous LFS assoc. tumors in strong pathogenic category *<br>- **Moderate evidence** – synchronous/metachronous LFS assoc. tumors in a moderate pathogenic category (or mixed strong/moderate categories) OR a single tumor in strong category<br>- **Supporting evidence** – a single tumor in moderate category * |
| **SUPPORTING** | | |
| PP1 | Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease | Strength of evidence would be dependent on the # of meiosis within a family:<br>- **Strong evidence** - ≥6 meiosis in >1 family *<br>- **Moderate evidence** – 4-5 meiosis in family *<br>- **Supporting evidence** – 3 meiosis in 1 family |
| PP2 | Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease | This rule should not be used due to the frequency of benign missense variants |
| PP3 | Multiple lines of computational evidence support a deleterious effect on the gene or gene product | Concordance of two predictors: REVEL and AGVGD (Zebrafish). Splicing: Concordance of MaxEntScan and HSF. Consider using Provean for indels. |
| PP4 | Patient's family history is highly specific for a disease with a single genetic etiology | Moderate evidence – family history meets Classic LFS criteria *<br>Supporting evidence – family history meets Chompret criteria |
| PP5 | Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation. | N/A |

* Would require code change

## Future Directions

- 10-15 variants classified as pathogenic or likely pathogenic and 10-15 variants classified as benign or likely benign by multiple ClinVar submitters will be tested.
- Following final edits to the criteria and curation process, an application will be submitted to ClinGen for formal Expert Panel status.

## References

1. Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May 17(5):405-24.