



Published in final edited form as:

*Hum Mutat.* 2021 March ; 42(3): 223–236. doi:10.1002/humu.24152.

## Specifications of the ACMG/AMP variant interpretation guidelines for germline *TP53* variants

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### CONFLICTS OF INTEREST

The following authors have no conflicts of interest to disclose: K.L., M.O., K.C.A., S.B., A.K.M.F., M.F., B.A.S., L.W., L.Z. The following authors have made extensive contributions to the *TP53* literature and have previously published assertions on *TP53* variants: C.F., P.L.M., L.D.A., D.G.E., P.J., K.M., T.P.S., A.B.S., S.A.S. The following authors are an employee, trainee or consultant for a commercial laboratory that offers genetic testing for *TP53*: T.P., R.H., K.M., S.E.P. J.M. is an employee of GeneDx/BioReference Laboratories, Inc./OPKO Health and has a salary as the only disclosure. The PERCH software, for which B.J.F. is the inventor, has been non-exclusively licensed to Ambry Genetics Corporation for their clinical genetic testing services and research. B.J.F. also reports funding and sponsorship to his institution on his behalf from Pfizer Inc. and Regeneron Genetics Center LLC.

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## Abstract

Germline pathogenic variants in *TP53* are associated with Li-Fraumeni syndrome (LFS), a cancer predisposition disorder inherited in an autosomal dominant pattern associated with high risk of malignancy, including early onset breast cancers, sarcomas, adrenocortical carcinomas, and brain tumors. Intense cancer surveillance for individuals with *TP53* germline pathogenic variants is associated with reduced cancer-related mortality. Accurate and consistent classification of germline variants across clinical and research laboratories is important to ensure appropriate cancer surveillance recommendations. Here, we describe the work performed by the Clinical Genome Resource *TP53* Variant Curation Expert Panel (ClinGen *TP53* VCEP) focused on specifying the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines for germline variant classification to the *TP53* gene. Specifications were developed for twenty ACMG/AMP criteria while nine were deemed not applicable. The original strength level for ten criteria was also adjusted due to current evidence. Use of *TP53*-specific guidelines and sharing of clinical data amongst experts and clinical laboratories led to a decrease in variants of uncertain significance from 28% to 12% compared with the original guidelines. The ClinGen *TP53* VCEP recommends the use of these *TP53*-specific ACMG/AMP guidelines as the standard strategy for *TP53* germline variant classification.

## Keywords

*TP53*; variant curation; pathogenic variant; cancer

## INTRODUCTION

The *TP53* gene encodes p53, a protein with essential roles in genome stability and key cellular functions such as cell cycle, metabolism, apoptosis, senescence and differentiation (Lane, 1992; Zerdoumi et al., 2017). Germline pathogenic variants in *TP53* occur in ~70% of individuals with Li-Fraumeni syndrome (LFS) (Varley, 2003), defined by patterns of personal and family history of certain early onset cancers, mainly pre-menopausal breast cancer, bone and soft tissue sarcomas, adrenocortical carcinomas and brain tumors. The cumulative incidence of cancer in LFS families is known to be high, with breast cancer risk reported to be higher than for *BRCA1/2* carriers (Shin et al., 2020), and up to 100% risk of any cancer by age 70y for both males and females (Mai et al., 2016). This high penetrance and severe consequences of undiagnosed LFS has led to recommendations for intensive cancer surveillance and other clinical management strategies (Ballinger et al., 2017; Evans, Birch, Ramsden, Sharif, & Baser, 2006; Frebourg, Bajalica Lagercrantz, Oliveira, Magenheimer, & Evans, 2020; Hanson et al., 2020; Kratz et al., 2017; Schon & Tischkowitz, 2018; Villani et al., 2016). Reported prevalence estimates range from 1:5,000 to 1:20,000 (Gonzalez, Noltner, et al., 2009; Lalloo et al., 2006), but recent studies suggest the frequency of germline *TP53* suspected pathogenic variants may be higher (Amendola et al., 2015; de Andrade et al., 2019; de Andrade et al., 2017).

Germline *TP53* genetic testing was initially recommended for individuals meeting Classic LFS (Li et al., 1988) and/or Chompret criteria, most recently revised in 2015 (Bougeard et al., 2015). Advances in sequencing technologies have greatly expanded the use of multi-gene panel testing, and even exome and genome sequencing, in the clinical setting. This has led to an increased number of variants of uncertain significance identified in *TP53*, including in cancer patients who do not meet LFS criteria, and individuals without cancer (Bittar et al., 2019). Given the significant, not only clinical but also emotional, challenges (Peters et al., 2016; Young et al., 2019) that come with an LFS diagnosis, it is essential to correctly assign *TP53* germline variant pathogenicity.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) variant curation guidelines are a series of generic criteria with varying levels of strength for and against pathogenicity, incorporating evidence from multiple data sources (Richards et al., 2015). As part of the directive of the Clinical Genome consortium (ClinGen: <https://clinicalgenome.org>), specifications of these guidelines for specific gene/disease pairs are developed and documented by a Variant Curation Expert Panel (VCEP), and have been completed for hereditary cancer genes including *PTEN* (Mester et al., 2018), *CDHI* (Lee et al., 2018) and *RUNXI* (Luo et al., 2019).

Herein, we present the scientific rationale and recommendations of the ClinGen *TP53* VCEP to adapt the ACMG/AMP guidelines for the classification of *TP53* germline variants and present results from pilot testing of the finalized guidelines.

## METHODS

The *TP53* VCEP followed ClinGen standard operating procedures (see [https://clinicalgenome.org/site/assets/files/3677/clingen\\_variant-curation\\_sopv1.pdf](https://clinicalgenome.org/site/assets/files/3677/clingen_variant-curation_sopv1.pdf)). The VCEP formed in 2015 by recruiting international *TP53* experts knowledgeable in phenotype, molecular diagnosis and functional processes. Twenty-four members contributed to at least one of three different evidence type working groups: Population/Computational, Functional, and Clinical. Each group reviewed the ACMG/AMP *TP53*-specifications in detail, incorporated this with critical review of the relevant literature and analyses of relevant data to inform evidence weights, and came to consensus for each specification.

The VCEP members nominated variants for pilot testing the *TP53*-specific ACMG/AMP guidelines. Specifically, 23 variants were chosen to cover varying molecular effects and the availability of data to assess the usability of different rule codes. Seven variants from the International Agency on Research in Cancer (IARC) *TP53* Database (Bouaoun et al., 2016) were included for their rich phenotypic information, but had no prior variant classifications. Thirteen variants from the ClinVar database (Landrum et al., 2018) were used to balance the spectrum of suspected classifications (with annotation at time of study initiation ranging from benign to pathogenic). In addition to evidence available from public databases, case level evidence available from clinical laboratory databases was provided by relevant VCEP members to the biocurators, including information regarding cancer type(s) and family history, familial variant segregation, and *de novo* observations. Variant classifications (pathogenic (P), likely pathogenic (LP), variant of uncertain significance

(VUS), likely benign (LB) and benign (B)) were also provided by the nominating VCEP member, which are referred to as prior expert assertions. Of note, variants sourced from ClinVar had assertions submitted by laboratories with representation on our VCEP, so that laboratory's assertion in 2017 was considered as the prior expert assertion. Each variant was independently curated by two of the collaborating five biocurators using the original ACMG/AMP guidelines and the *TP53* specifications to test user interpretability. Only one of the five biocurators had prior experience with the rule specifications during development. The criteria combinations for a given classification tier were followed as originally proposed (Richards et al., 2015), with the additional combination of very strong plus supporting criteria reaching LP for the *TP53*-specific guidelines supported by the Tavtigian et al. Bayesian rule combination calculator (Tavtigian et al., 2018). The resulting classifications were compared between biocurators, against prior assertions by the nominating expert(s) or contributing laboratories, and with ClinVar assertions (Landrum et al., 2018) when possible. During this phase, the *TP53*-specific guidelines were refined, and the final draft and results of the application of the evidence codes to the pilot variants was presented to the ClinGen Sequence Variant Interpretation (SVI) Committee for approval.

## RESULTS

### *TP53*-specific variant curation criteria

Final *TP53*-specific ACMG/AMP guidelines are summarized in Table 1. Of the 28 original criteria, nine (PM3, PM4, PP2, PP4, PP5, BP1, BP3, BP5, and BP6) were excluded due to either limited data to support use of a rule code, irrelevance to *TP53*, or to avoid redundancy with another criterion specification. Additional details are shown in Supplementary Table S1. The remaining 19 criteria were modified by detailing the content and/or changing the strength level. Rationale for criteria specification are explained below.

### Population/Computational Working Group

**Population data**—BS1 and BA1 are criteria against pathogenicity based on the frequency of a germline variant in healthy individuals. To define the *TP53* variant frequency cutoff for BS1, we calculated the maximum credible population allele frequency as reported by Whiffin et al. (Whiffin et al., 2017). In order not to over-estimate the prevalence of LFS, we used values at the lower end of published estimates of germline pathogenic *TP53* variants, namely 1 in 5,000 individuals (Lalloo et al., 2006). Similarly, 30% cancer risk was selected for penetrance in order to allow for the inclusion of hypomorphic alleles, based on the reported penetrance of the Brazilian founder c.1010G>A (p.R337H) variant for LFS malignancies (Achatz & Zambetti, 2016). Genetic heterogeneity was set at 1.0 as *TP53* is the only LFS-associated gene. This resulted in BS1 at 0.03% (0.0003). BA1 was then defined as 0.1% (0.001) based on a lifetime penetrance estimate of 70%, holding allelic and genetic heterogeneity at 1.0, and then increasing the derived maximum credible population allele frequency of 0.01% (0.0001) by an order of magnitude to arrive at a cutoff of 0.1% (0.001). For both criteria, a minimum of five alleles in a given population was required to ensure a comparable cohort. The *TP53* VCEP specifically recommends the use of the most up-to-date non-cancer dataset of the gnomAD database (Karczewski et al., 2019), as this is the largest control database currently publicly available, and to ignore frequencies in Ashkenazi Jewish

and Finnish populations due to founder effects (Kaariainen, Muilu, Perola, & Kristiansson, 2017; Shi et al., 2017).

The PM2 criterion uses absence in controls as evidence towards pathogenicity. Due to the overall rarity of *TP53* germline variants (benign or pathogenic), the *TP53* VCEP downgraded this criterion to supporting strength level. This criterion would not be applied to rare variants that do not fill additional pathogenic rule criteria and would otherwise meet a classification as a benign or likely benign variant.

PS4 requires case-control analyses, considered impractical for *TP53* due to variant rarity and limited number of published studies. We instead developed a proband counting system to assign pathogenicity, based on the number of variant carriers meeting each of the existing clinical criteria for LFS; this is also the recommendation of the ClinGen SVI Committee. A likelihood ratio (LR) towards pathogenicity was calculated by dividing the proportion of carriers meeting classic LFS or Chompret 2015 criteria by the proportion of non-carriers meeting the same criteria, using data from individuals undergoing multigene panel testing at Ambry Genetics (Supplementary Figure S1). Results indicated that one proband with a variant meeting classic LFS or Chompret 2015 criteria would provide enough evidence to reach moderate or supporting (LRs=15.47 or 3.37 as per our analyses, respectively) strength level, respectively (Tavtigian et al., 2018). However, given the width of the confidence intervals, more reduced evidence weights were assigned using a point system based on the number of probands meeting classic LFS or Chompret 2015 (Table 2). Additionally, it was decided that PS4 should not be applied if the variant also meets the population rules BS1 or BA1, to avoid coincidental accumulation of proband counts for common variants, following the approach previously developed for *PTEN* (Mester et al., 2018).

The BS2 criterion uses the presence of a variant in unaffected adults as evidence against pathogenicity. Two independent datasets (Ambry Genetics and GeneDx) were used to calculate how many observations of cancer-free adults at age 60 equated to strong evidence against pathogenicity. The LR towards pathogenicity was calculated by comparing the proportion of these individuals with a known *TP53* pathogenic variant versus individuals without a *TP53* pathogenic variant (Supplementary Table S2). The LRs estimated for observation of a variant in one healthy individual were 0.66 (Ambry dataset) and 0.28 (GeneDx dataset). Selecting the more conservative LR of 0.66, two to seven healthy individuals were considered necessary to apply BS2\_Supporting (LRs ranging from 0.44 to 0.06), and eight or more to apply BS2 (LRs lower than 0.04) (Tavtigian et al., 2018). Given that the dataset used for this analysis included mostly females, and the associated risk of pre-menopausal breast cancer (Mai et al., 2016) for female *TP53* carriers elevates their cancer risk compared with male *TP53* carriers, it was stipulated that this criterion should be applied to female carriers only.

**Computational and predictive data**—PP3 and BP4 are commonly used criteria related to predictions using bioinformatic tools. Based on previous findings (Fortuno, James, Young, et al., 2018), an optimized version of Align-GVGD (Mathe et al., 2006) combined with BayesDel (Feng, 2017) (both included in the IARC *TP53* Database R20 (Bouaoun et al., 2016)) were selected for bioinformatic prediction of *TP53* missense variant pathogenicity.

To assess prediction of effects on splicing, we suggest the use of a metapredictor, such as SpliceAI (Jaganathan et al., 2019) or VarSEAK (<https://varseak.bio/>).

The BP7 criterion for silent variants was expanded to specify the use of a metapredictor to exclude the use of this code for variants predicted to have any effects on splicing.

The strength level for PM5, which relies on previous observations of pathogenic variants at a given location as evidence of pathogenicity for other variants at the same location, was assessed as follows: the 493 non-functional variants from yeast transactivation assays (Kato et al., 2003) were assessed for the number of additional variants at the same amino acid residue that were also reported as non-functional, and compared to the number of functional or super trans variants (N=1239) at that position to generate a LR towards pathogenicity (Supplementary Table S3). Results indicated PM5 was applicable if two other pathogenic variants have been seen at a given amino acid residue (LR=6.46), but should be downgraded to supporting strength level if only one other pathogenic variant has been seen at the same residue (LR=2.90) (Tavtigian et al., 2018). Further, the following restrictions were added: (i) known P/LP variants must be based on classifications using the *TP53*-specific guidelines; (ii) variants using this rule must have equal or higher Grantham score (Grantham, 1974) than at least one pathogenic variant observed at that codon; (iii) this criterion cannot be used for any variant for which PM1 has been applied; (iv) splicing effects are excluded based on bioinformatic evidence from a metapredictor.

PS1 may be applied as strong strength if effects on splicing due to the nucleotide change are excluded using splicing assay data, or downgraded to PS1\_Moderate if only bioinformatic predictions are available as evidence against aberrant splicing. The comparative variant must have been classified as P/LP using the *TP53*-specific guidelines.

PVS1 is the only original criterion with a very strong strength level for pathogenicity, which applies to loss of function variants. The ClinGen SVI Committee has published further guidance on this rule (Abou Tayoun et al., 2018), which the *TP53* VCEP has agreed to follow, including applying relative strengths for different types of null variants based on characteristics, such as variant type and location.

### Functional Working Group

**Functional data**—There are multiple mechanisms by which p53 function may be altered. To date, there are only three published systematic functional studies of p53 missense variants, which measure relevant disease mechanisms: transactivation activity (Kato et al., 2003), loss of function (LOF) (Giacomelli et al., 2018; Kotler et al., 2018) and dominant-negative effect (DNE) (Giacomelli et al., 2018). To elaborate the specifications of the functional-related criteria PS3 and BS3, and in agreement with SVI recommendations for application of the functional criteria (Brnich et al., 2019), we first assessed the relative performance of these assays as positive and negative predictors of variant pathogenicity (i.e., clinical calibration) using the Matthews correlation coefficient; clinical reference sets were assumed pathogenic missense variants (present in classic LFS probands in the IARC *TP53* Database R19 and absent in controls, N=52) and assumed benign missense variants (in controls from non-cancer gnomAD v2.1.1 or FLOSSIES (<https://whi.color.com/>) at

a frequency higher than 0.0001 and not found in patients, N=31) (Supplementary Table S4). In addition, we also calculated LRs towards pathogenicity for different combinations of functional results to assist application of strength levels (Supplementary Table S5). Following these results, a conservative decision tree considering availability and relative performance of the different assays is shown in Figure 1. Of note, this decision tree also considers other non-systematic functional assays, especially when those show conflicting evidence, as noted.

**Hotspot data**—The second part of the PM1 criterion related to protein regions was considered not applicable, as there is no known functional domain without benign variation in *TP53* given the evidence available. However, there are several well-described hotspots in *TP53*, occurring at amino acid positions 175, 245, 248, 249, 273, and 282 (Bouaoun et al., 2016; Fortuno, Pesaran, et al., 2019), for which PM1 is applicable. Additionally, analyses of tumor DNA sequencing have identified a large number of *TP53* variants as somatic hotspots, with information available at [cancerhotspots.org](http://cancerhotspots.org) (Chang et al., 2016; Chang et al., 2018). Following published recommendations from the ClinGen Germline/Somatic Variant Curation Subcommittee (Walsh et al., 2018), it was specified that the PM1 criterion can also apply to somatically detected hotspots with 10 occurrences in [cancerhotspots.org](http://cancerhotspots.org). This information is annotated in the IARC *TP53* Database R20 (Bouaoun et al., 2016).

### Clinical Working Group

**Segregation data**—The original ACMG/AMP criteria use segregation as supporting strength for pathogenicity (PP1), allowing for stronger evidence if there is more segregation data, or a strong strength of evidence against pathogenicity when there is lack of segregation (BS4). Given the wide spectrum of cancer types that have been reported for *TP53* carriers (Caron et al., 2016; Kratz et al., 2017; Olivier, Hollstein, & Hainaut, 2010), the criteria were specified based only on the number of meioses of any LFS-associated cancer type, and number of families reported. Based on the gradations created previously for *PTEN*, the resulting *TP53* specifications for PP1 are detailed in Table 1.

For BS4, which uses lack of segregation as evidence against pathogenicity, we considered potential issues due to the high *de novo* rate reported for *TP53* (Gonzalez, Buzin, et al., 2009), and specified use under these two scenarios: the variant segregates to the side of the family that does not meet LFS criteria, or the variant is present in three or more living unaffected individuals (where at least two of three are female) above 55 years of age (age specification consistent with Chompret 2015 criteria).

**De novo data**—The *TP53* VCEP provides guidance for assigning the strength for the original *de novo* PS2 and PM6 criteria that should be based on the type of cancer and its relevance to the *TP53* spectrum. This was accomplished using a point system incorporating parentage and proband cancer type which also allows for combination of points if there are multiple *de novo* reports of the same variant (Table 3).

**Cis/trans testing data**—The use of BP2, which uses observation with another pathogenic variant as evidence against pathogenicity, was specified as follows: variant is observed

*in trans* with a *TP53* pathogenic variant (phase confirmed), or there are three or more observations with a *TP53* pathogenic variant when phase is unknown. In this scenario, the variant must be seen with at least two different *TP53* pathogenic variants (as specified by the *TP53* VCEP).

### Testing of the *TP53* specifications on a pilot set of variants

There were 43 variants used for pilot testing. Classifications were compared between biocurators using the original ACMG/AMP and the *TP53*-specific guidelines, the existing assertions in ClinVar, and prior assertions by experts (Table 4). Of the 42 pilot *TP53* variants recorded in ClinVar in September 2019, 16 (38%) were annotated as conflicting (see Table 4). Between-biocurator consistency of variant classification was high: 72.1% when using the original ACMG/AMP guidelines, and 81.4% using *TP53*-specific guidelines (Table 4). There were fewer VUS using *TP53*-specific guidelines compared with the original guidelines (5/43 (12%) vs 12/43 (28%)) (Figure 2). Using the *TP53*-specific guidelines and sharing clinical data amongst experts and clinical laboratories, the majority of VUS were downgraded to LB (13/16; 81%), two moved to LP (2/16; 12.5%), and one remained at VUS (1/16; 6%).

## DISCUSSION

The task of the *TP53* VCEP was to specify the ACMG/AMP guidelines to assist with the clinical classification of germline variants in the *TP53* gene. There is a rapidly growing number of individuals without personal or family history consistent with LFS who have presumed germline *TP53* pathogenic variants (Batalini et al., 2019; Fortuno, James, & Spurdle, 2018), and cancer surveillance for people with *TP53* pathogenic germline variants is time intensive and emotionally stressful. Given that *TP53* is also on the ACMG Secondary Findings medically actionable list for return of results (Kalia et al., 2017), this work will also be important to help streamline variant curation and hopefully decrease variant classification discrepancies between laboratories.

Our pilot study demonstrated that the *TP53*-specific guidelines decreased the number of VUS compared with the original guidelines and increased the number of variants classified as not clinically relevant. Our study also showed strong intra-biocurator consistency, suggesting that the criteria appear to be straightforward to interpret; this should allow for fewer conflicting variant calls between laboratories. For example, the criteria related to population data can be applied more often, bioinformatic and functional data can now be applied more broadly.

One of the benefits of curating *TP53* variants was the wealth of existing knowledge, including data readily available in public databases. The IARC *TP53* database (Bouaoun et al., 2016) is a rich source of data that was helpful in assessing pathogenicity codes, including PS1, PS2, PS4, PM5, PM6, and PP1. The existing data on p53 mouse models searchable by knockout variant of interest was also useful for adapting functional rule codes. Additionally, the *TP53* VCEP benefited from data sharing amongst participating researchers, clinicians, and clinical laboratories. VCEP members added clinical information pertaining to additional probands to those identified in the literature, to increase the number of pathogenic



codes applicable to certain variants and to shift classifications from VUS to LP. Laboratory members also shared data from their large hereditary cancer panels that were helpful in defining and using the benign codes BS2 and BP2.

Consistent with the ClinGen VCEP process, all of the *TP53* classified variants have been submitted to the ClinVar database (Landrum et al., 2018) with a summary of the classification process. More details about the evidence used to classify each variant is available through a link to the public access ClinGen Evidence Repository (<https://erepo.clinicalgenome.org/evrepo/>). The *TP53* VCEP will continue to meet monthly to curate additional variants and submit them to ClinVar. Variants previously classified as LP or VUS will be reviewed at least every two years in the event new evidence has emerged, while variants classified as LB will be reviewed when new evidence is available or when requested by the public via the ClinGen website ([www.clinicalgenome.org](http://www.clinicalgenome.org)). It is also anticipated that these specifications will be updated and reviewed as needed. For example, in the first iteration of these specifications, we suggested the use of MaxEntScan (Yeo & Burge, 2004) and Human Splicing Finder (HSF) (Desmet et al., 2009), to predict variant spliceogenicity. However, HSF is no longer freely available. As a consequence, we conducted comparisons of predictions using other tools and now suggest the use of a metapredictor that captures multiple spliceogenic mechanisms. This follows advice of the SVI Committee. More definitive studies are warranted to calibrate the strength of predictions of splicing algorithms, and so future iterations of these specifications may provide further details on the use of a specific splicing predictor. The specifications may also consider additional evidence types to improve *TP53* variant classification, such as the relationship between somatic and germline counts reported to be positively correlated only for pathogenic variants (Fortuno, Cipponi, et al., 2019) or HER2+ breast tumor pathology as a positive predictor of *TP53* variant pathogenicity (Fortuno et al., 2020). The most up to date version of the ClinGen *TP53* VCEP specifications is available at <https://clinicalgenome.org/affiliation/50013/>.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGEMENTS

The ClinGen *TP53* Variant Curation Expert Panel thanks the ClinGen *PTEN* Variant Curation Expert Panel and the Executive Committee of the Hereditary Cancer Clinical Domain Working Group for sharing their experience with developing criteria specifications. We also thank Steven Harrison, Leslie Biesecker and the remainder of the ClinGen Sequence Variant Interpretation Working Group for their guidance. Finally, thank present and past members of the *TP53* VCEP, Maria Isabel Achatz, Rebecca Bassett, Jeffrey Bissonnette, David Goldgar, Sharisse Jimenez, Chimene Kesserwan, Deb Ritter, Leighton Telling, and Mackenzie Trapp.

## FUNDING

This Expert Panel is funded by National Human Genome Research & National Cancer Institutes (1U41HG006834, 1U01HG007437, 1U01HG007436, HHSN261200800001E, U41HG009650, 1U41HG00964). The work of C.F. was supported by a University of Queensland (UQ) International Scholarship from the UQ School of Medicine. The work of M.F., K.C.A., and S.A.S. was supported by the intramural research program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health. The work of A.B.S. was supported by Australian National Health and Medical Research funding (ID1061779, ID1161589). The work of

D.G.E. was supported by the NIHR Manchester Biomedical Research Centre (IS-BRC-1215-20007). The work of T.P.S. was supported by NIH-NCI K08CA234394 and R01CA242218.

## DATA AVAILABILITY STATEMENT

Most of the data that support the findings of this study are openly available in the IARC TP53 Database at <https://p53.iarc.fr> (version R20, July 2019). Other data supporting the findings of this study are not publicly available due to private or ethical restrictions. The publicly available Clinical Genome Resource (ClinGen) Evidence Repository contains all of the curated evidence for the variants submitted to ClinVar (e.g., <https://erepo.clinicalgenome.org/evrepo/ui/interpretation/7b4332f9-03a6-43f1-afde-508d91bd92d5>).

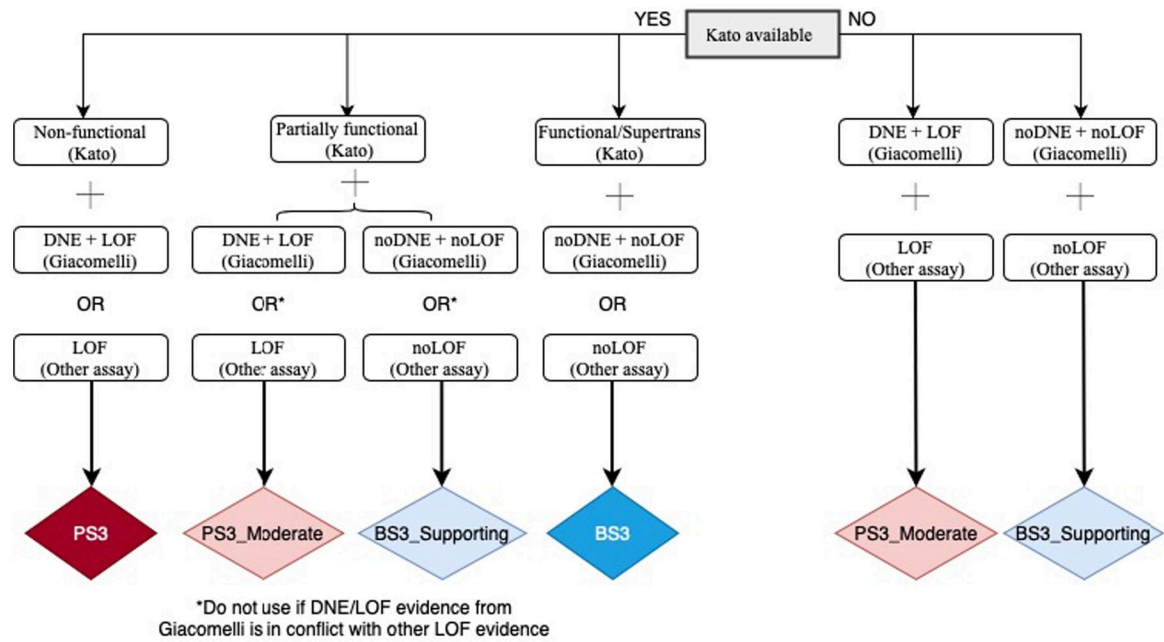
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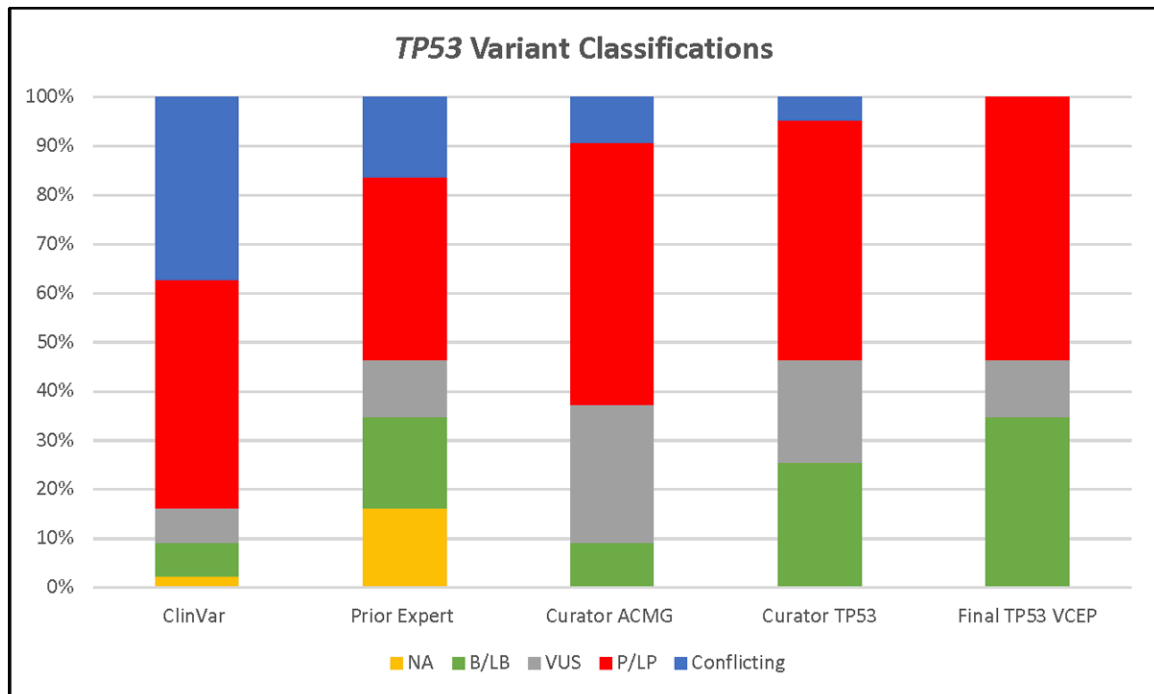
**Figure 1.**

Flow chart for the specifications of PS3 and BS3 criteria.

Non-functional (Kato) = median transactivation activity  $\leq 20\%$ ; Partially functional (Kato) = median transactivation activity  $>20$  and  $\leq 75\%$ ; Functional/Supertrans (Kato) = median transactivation activity  $>75\%$ ; DNE+LOF (Giacomelli) = p53WTNutlin3 Z-score  $\geq 0.61$  and Etoposide Z-score  $\leq -0.21$ ; noDNE+noLOF (Giacomelli) = p53WTNutlin3 Z-score  $< 0.61$  and Etoposide Z-score  $> -0.21$ . Other assays are available in IARC TP53 Database or original publications, and include in vitro growth assays in H1299 human cells from Kotler et al., (2018) with RFS score  $> -1.0$  for LOF and RFS score  $< -1.0$  for noLOF; or colony formation assays, growth suppression assays, apoptosis assays, tetramer assays, knock-in mouse models.

\* If a variant does not match any of the possibilities shown, it is considered to have “no evidence to review” and no functional criterion can be applied.

Abbreviations: DNE = Dominant-negative effect; LOF = Loss-of-function.



**Figure 2.** Variant classifications for 43 pilot TP53 variants in ClinVar, from the nominating expert(s), biocurators using the original ACMG/AMP guidelines, and the TP53-specific guidelines. Abbreviations: B = Benign, LB = Likely benign, VUS = Variant of uncertain significance, LP = Likely pathogenic, P = Pathogenic, Conflicting = Clinically relevant conflicting interpretations of pathogenicity, and NA = Not Available.

**Table 1.** Summary of the *TP53*-specific ACMG/AMP criteria developed by the ClinGen *TP53* Variant Curation Expert Panel

Original ACMG/AMP guidelines		<i>TP53</i> specifications
Rule code	Criteria description	
<b>PVS1</b>	Null variant (nonsense, frameshift, canonical $\pm 1$ or 2 splice sites, initiation codon, single or mult exon deletion) in a gene where LOF is a known mechanism of disease	Use SVI-approved decision tree to determine the strength of this criterion (refer to Abou Tayoun et al. for more details).
<b>PS1</b>	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	Use original description with the following additions: <b>PS1:</b> - Must confirm there is no difference in splicing using RNA data. - Can only be used to compare to variants classified as Pathogenic or Likely Pathogenic by the <i>TP53</i> VCEP (see ClinVar for VCEP classifications).  <b>PS1_Moderate:</b> - Must confirm there is no difference in splicing using a metapredictor. - Can only be used to compare to variants classified as Pathogenic or Likely Pathogenic by the <i>TP53</i> VCEP (see ClinVar).
<b>PS2</b>	<i>De novo</i> (both maternity and paternity confirmed) in a patient with the disease and no family history	Use SVI-approved scoring system to determine the strength of this criterion (refer to Table 2 for more details)
<b>PS3</b>	Well-established <i>in vitro</i> or <i>in vivo</i> functional studies supportive of a damaging effect on the gene or gene product	<b>PS3:</b> transactivation assays in yeast demonstrate a low functioning allele (< 20% activity) <b>AND</b> there is evidence of dominant negative effect and loss-of-function <b>OR</b> there is a second assay showing low function (colony formation assays, apoptosis assays, tetramer assays, knock-in mouse models and growth suppression assays). <b>PS3_Moderate:</b> transactivation assays in yeast demonstrate a partially functioning allele (>20% and < 75% activity) <b>AND</b> there is evidence of dominant negative effect and loss-of-function <b>OR</b> there is a second assay showing low function (colony formation assays, apoptosis assays, tetramer assays, knock-in mouse models and growth suppression assays). <b>PS3_Moderate:</b> there is no data available from transactivation assays in yeast <b>BUT</b> there is evidence of dominant negative effect and loss-of-function <b>AND</b> there is a second assay showing low function (colony formation assays, apoptosis assays, tetramer assays, knock-in mouse models and growth suppression assays). Refer to Figure 1 for more details.
<b>PS4</b>	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	Use SVI-approved scoring system to determine the strength of this criterion (refer to Table 3 for more details). This criterion cannot be applied when a variant also meets BA1 or BS1. Refrain from considering probands who have another pathogenic variant(s) in a highly penetrant cancer gene(s) that is a logical cause for presentation. Caveat: Please be mindful of the risk of clonal hematopoiesis of indeterminate potential with <i>TP53</i> variants (Coffee et al., 2017; Weitzel et al., 2017). One should take care to ensure that probands have germline and not mosaic somatic <i>TP53</i> variants.
<b>PM1</b>	Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation	Located in a mutational hot-spot defined as: - Variants within the following codons on protein NP_000537.3: 175, 273, 245, 248, 282, 249. - Variants seen in <a href="https://cancerhotspots.org">cancerhotspots.org</a> (v2) with >10 somatic occurrences (recommendation from the ClinGen Germline/Somatic Variant Curation Subcommittee).



Original ACMG/AMP guidelines		TP53 specifications
Rule code	Criteria description	
PM2	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium	PM2 Supporting: absent from population databases (gnomAD (most up-to-date non-cancer dataset) is the preferred population database at this time <a href="http://gnomad.broadinstitute.org">http://gnomad.broadinstitute.org</a> ).
PM3	For recessive disorders, detected <i>in trans</i> with a pathogenic variant	Excluded (refer to Supplementary Table S1 for more details).
PM4	Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants	Excluded (refer to Supplementary Table S1 for more details).
PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	PM5: novel missense change at an amino acid residue where at least two other different missense changes determined to be pathogenic by the TP53 VCEP have been seen before. PM5 Supporting: novel missense change at an amino acid residue where a different missense change determined to be pathogenic by the TP53 VCEP has been seen before. Both criteria require the following additions: - Grantham should be used to compare the variants, and the variant being evaluated must have equal to or higher score than the observed pathogenic variants. - Splicing should be ruled out using a metapredictor. - This criterion cannot be applied when a variant also meets PM1.
PM6	Assumed <i>de novo</i> , but without confirmation of paternity and maternity	Use SVI-approved scoring system to determine the strength of this criterion (refer to Table 2 for more details).
PP1	Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	PP1: co-segregation with disease is observed in 3–4 meioses in one family. PP1_Moderate: co-segregation with disease is observed in 5–6 meioses in one family. PP1_Strong: co-segregation with disease is observed >7 meioses 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	Excluded (refer to Supplementary Table S1 for more details).
PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)	PP3: Use original description with the following additions: - For missense variants, use a combination of BayesDel ( 0.16) and optimised Align-GVGD (C55-C25). - For splicing variants, use a metapredictor.
PP4	Patient's phenotype or family history is highly specific for a disease with a single genetic etiology	PP3_Moderate: for missense variants, use a combination of BayesDel ( 0.16) and optimized Align-GVGD (C65).
PP5	Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation	Excluded (refer to Supplementary Table S1 for more details).
BA1	Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium	Allele frequency is 0.1% in a non-founder population with a minimum of five alleles (gnomAD (most up-to-date non-cancer dataset)) is the preferred population database at this time <a href="http://gnomad.broadinstitute.org">http://gnomad.broadinstitute.org</a> ).

Original ACMG/AMP guidelines		TP53 specifications
Rule code	Criteria description	
<b>BS1</b>	Allele frequency is greater than expected for disorder	Allele frequency is 0.03% and <0.1% in a non-founder population with a minimum of five alleles (gnomAD (most up-to-date non-cancer dataset) is the preferred population database at this time <a href="http://gnomad.broadinstitute.org">http://gnomad.broadinstitute.org</a> ).
<b>BS2</b>	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	BS2: observed in a single dataset in 8 females, who have reached at least 60 years of age without cancer (i.e. cancer diagnoses after age 60 are ignored). BS2 Supporting: observed in a single dataset in 2-7 females, who have reached at least 60 years of age without cancer. Caveat: Be mindful of the risk of clonal hematopoiesis of indeterminate potential with TP53 variants (Coffee et al., 2017; Weitzel et al., 2017). Individuals with mosaic somatic TP53 variants should not be included as evidence for BS2.
<b>BS3</b>	Well-established <i>in vitro</i> or <i>in vivo</i> functional studies show no damaging effect on protein function or splicing	- BS3: transactivation assays in yeast demonstrate a functional allele or super-transactivation (>75% activity) AND there is no evidence of dominant negative effect and loss-of-function OR there is a second assay showing retained function (colony formation assays, apoptosis assays, tetramer assays, knock-in mouse models and growth suppression assays). - BS3 Supporting: transactivation assays in yeast demonstrate a partially functioning allele (>20% and 75% activity) AND there is no evidence of dominant negative effect and loss-of-function OR there is a second assay showing retained function (colony formation assays, apoptosis assays, tetramer assays, knock-in mouse models and growth suppression assays). - BS3 Supporting: there is no data available from transactivation assays in yeast BUT there is no evidence of dominant negative effect and loss-of-function AND there is a second assay showing retained function (colony formation assays, apoptosis assays, tetramer assays, knock-in mouse models and growth suppression assays). Refer to Figure 1 for more details.
<b>BS4</b>	Lack of segregation in affected members of a family	The variant segregates to opposite side of the family meeting LFS criteria, or the variant is present in >3 living unaffected individuals (at least two of three should be female) above 55 years of age.
<b>BP1</b>	Missense variant in a gene for which primarily truncating variants are known to cause disease	Excluded (refer to Supplementary Table S1 for more details).
<b>BP2</b>	Observed <i>in trans</i> with a pathogenic variant for a fully penetrant dominant gene/disorder or observed <i>in cis</i> with a pathogenic variant in any inheritance pattern	Variant is observed <i>in trans</i> with a TP53 pathogenic variant (phase confirmed), or there are three or more observations with a TP53 pathogenic variant when phase is unknown (at least two different TP53 pathogenic variants). The other observed pathogenic variants must have been classified using the TP53-specific guidelines.
<b>BP3</b>	In-frame deletions/insertions in a repetitive region without a known function	Excluded (refer to Supplementary Table S1 for more details).
<b>BP4</b>	Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	Same rule description with the following additions: - For missense variants, use a combination of BayesDel (<0.16) and optimized Align-GVGD (C15-C0). - For splicing variants, use a metapredictor.
<b>BP5</b>	Variant found in a case with an alternate molecular basis for disease	Excluded (refer to Supplementary Table S1 for more details).
<b>BP6</b>	Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	Excluded (refer to Supplementary Table S1 for more details).
<b>BP7</b>	A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice	Same description with the following additions:

<b>Original ACMG/AMP guidelines</b>		<b>TP53 specifications</b>
<b>Rule code</b>	<b>Criteria description</b>	
	consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved	<ul style="list-style-type: none"> <li>- Splicing should be ruled out using a metapredictor.</li> <li>- If a new alternate site is predicted, compare strength to native site in interpretation.</li> </ul>

Abbreviations: SVI = Sequence Variant Interpretation; VCEP = Variant Curation Expert Panel

**Table 2.**

Point system created for the specifications of the PS4 rule \*

Classic LFS	PS4 Evidence Strength	# of Points Required
• 1 point for each proband	PS4	4 or more points
<b>Chompret 2015</b>	PS4_Moderate	2–3 points
• 0.5 point for each proband	PS4_Supporting	1 point

\* Not to be used if a variant also meets BS1 or BA1 rules.

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**Table 3.**

Point systems created for the specifications of the PS2 and PM6 criteria and *TP53*-associated cancers with different strength levels\*

<p><b>Strong LFS criteria</b></p> <ul style="list-style-type: none"> <li>• 2 points for each cancer in <i>de novo</i> individual when maternity and paternity are confirmed</li> <li>• 1 point for each cancer when parental testing is not available</li> </ul>	<ul style="list-style-type: none"> <li>• Breast cancer (IDC &amp; DCIS) &lt;31 years of age</li> <li>• Choroid plexus carcinoma</li> <li>• Adrenocortical adenoma or carcinoma &lt;18 years of age</li> <li>• Rhabdomyosarcoma &lt;46 years of age</li> <li>• Osteosarcoma &lt;46 years of age</li> <li>• Hypodiploid ALL (Specifically low-hypodiploid 32–39 chromosomes)</li> <li>• Sonic Hedgehog medulloblastoma</li> </ul>		
<p><b>Moderate LFS criteria</b></p> <ul style="list-style-type: none"> <li>• 1 point for each cancer in <i>de novo</i> individual when maternity and paternity are confirmed</li> <li>• 0.5 point for each cancer when parental testing is not available</li> </ul>	<ul style="list-style-type: none"> <li>• Breast cancer &gt;30 and &lt;50 years of age</li> <li>• Malignant brain tumors (excluding optic gliomas) &lt;46 years of age</li> <li>• Primary lung cancer &lt;46 years of age</li> <li>• Adrenocortical adenoma or carcinoma 18 and &lt;50 years of age</li> <li>• Rhabdomyosarcoma or osteosarcoma &gt;45 years of age</li> <li>• Other sarcomas (<i>e.g.</i>, malignant phyllodes tumor, leiomyosarcoma, liposarcoma) &lt;60 years of age           <ul style="list-style-type: none"> <li>– Exclude dermatofibrosarcoma &amp; Ewing sarcoma</li> </ul> </li> </ul>		
Total points required to assign the following rule codes			
<b>PM6_Supporting</b>	<b>PS2_Moderate or PM6</b>	<b>PS2 or PM6_Strong</b>	<b>PS2_VeryStrong or PM6_VeryStrong</b>
0.5	1	2	4

\* If there are multiple reports of *de novo* probands, the points for each *de novo* observation can be summed. If the proband has multiple cancers, only the strongest associated LFS cancer should be used.

**Table 4.**  
**Variant classified during the pilot testing phase using the *TP53*-specific ACMG/AMP guidelines.**

All variants were annotated in relation to the transcript NM\_000546.5 and protein NP\_000537.3.

<i>TP53</i> variant	ClinVar ID	ClinVar Classifications (September, 2019)*	Prior Variant Classifications <sup>^</sup>	Dual Biocurator Classifications		<i>TP53</i> -specific ACMG/AMP Final Classifications	Final Criteria Applied
				Original ACMG/AMP Guidelines <sup>#</sup>	<i>TP53</i> -specific ACMG/AMP Guidelines <sup>%</sup>		
c.1079G>C p. (Gly360Ala)	142003	B/LB	LB	B/VUS	LB	LB	BS1, BS3_Supporting, BP4
c.883C>T p. (Pro295Ser)	428862	LB	LB	VUS	LB	VUS	BP4
c.206C>G p. (Ala69Gly)	230112	LB	LB	VUS	VUS	VUS	PM2_Supporting, BP4
c.1093C>T p. (His365Tyr)	80708	VUS	VUS	VUS	LB	LB	BS3, BP4
c.403T>G p. (Cys135Gly)	376563	VUS	VUS	LP	VUS	LP	PS3, PM2_Supporting, PP3_Moderate
c.1000G>C p. (Gly334Arg)	182969	VUS	VUS	VUS	VUS	VUS	BS3, PP3, PS4_Supporting
c.532C>G p. (His178Asp)	482223	LP	NA	LP/P	LP/P	LP	PS3, PM2_supporting, PM6, PP3_Moderate
c.538G>A p. (Glu180Lys)	245711	LP	P	P	VUS	LP	PM2_Supporting, PP3, PS4_Supporting, PM6, PS3_Moderate
c.431A>T p. (Gln144Leu)	376647	LP	NA	VUS	VUS	VUS	PM2_Supporting, PS4_Supporting, BS3_Supporting
c.578A>C p. (His193Pro)	376612	LP	NA	P	P	P	PS3, PM6, PM2_Supporting, PP3_Moderate, PS4_Supporting
c.743G>T p. (Arg248Leu)	230253	LP	P	P/LP	LP	P	PS3, PM1, PM2_Supporting, PP3_Moderate, PS4_Supporting
c.97-1G>A	638853	LP	P	P	LP/P	P	PVS1_Strong, PM2_Supporting, PS4_Supporting
c.537T>A p. (His179Gln)	406578	P/LP	NA	P	P	LP	PM6, PS3, PM2_Supporting, PP3
c.517G>A p. (Val173Met)	233951	P/LP	LP	P	LP/P	P	PS2, PS3, PM2_Supporting, PS4_Supporting, PP3

TP53 variant	ClinVar ID	ClinVar Classifications (September, 2019)*	Prior Variant Classifications <sup>^</sup>	Dual Biocurator Classifications		TP53-specific ACMG/AMP Final Classifications	Final Criteria Applied
				Original ACMG/AMP Guidelines <sup>#</sup>	TP53-specific ACMG/AMP Guidelines <sup>%</sup>		
c.743G>A p. (Arg248Gln)	12356	P/LP	P	P	LP	P	PS3, PM1, PP3, PS4, PS2
c.818G>A p. (Arg273His)	12366	P/LP	P	P	P	P	PS3, PS2, PS4, PM1, PP3
c.1031T>C p. (Leu344Pro)	12375	P	LP	P/LP	LP	P	PS3, PM2_Supporting, PP3_Moderate, PS4_Moderate
c.659A>G p. (Tyr220Cys)	127819	P	P	P	P	P	PS3, PP1_strong, PM6, PP3_Moderate, PS4_Moderate
c.742C>T p. (Arg248Trp)	12347	P	P	P	P	P	PS2, PS3, PP1_Strong, PM1, PS4, PP3_Moderate
c.993+1delG	428898	P	P	P	P	P	PVS1, PM2_Supporting, PS4_Supporting, PP1_Moderate
c.892G>T p. (Glu298*)	93323	P	P	P	P	P	PVS1, PM2_Supporting, PS4_Supporting
c.372C>A p. (Cys124*)	458537	P	NA	P	P	P	PVS1, PM6, PM2_Supporting
c.488A>G p. (Tyr163Cys)	127814	P	NA	LP/P	P	P	PS3, PS2_Moderate, PM2_Supporting, PP3_Moderate, PS4_Supporting
c.455C>T p. (Pro152Leu)	142766	P	P	P	LP/P	P	PS3, PP3_Moderate, PS4, PP1
c.919+1G>A	633606	P	P	P	P	P	PVS1_Strong, PM6_Supporting, PM2_Supporting
c.733G>A p. (Gly245Ser)	12365	P	P	P	P	P	PS3, PM1, PP3, PS4
c.869G>A p. (Arg290His)	127825	Conflicting	VUS	VUS	VUS	B	BS3, BP4, BS1, BS2_Supporting
c.847C>T p. (Arg283Cys)	127824	Conflicting	VUS	VUS	VUS	VUS	BS3, PP3
c.1040C>A p. (Ala347Asp)	43587	Conflicting	P	P	LP/P	LP	PS3, PM2_Supporting, PP1_Moderate, PS4_Supporting
c.1120G>C p. (Gly374Arg)	230269	Conflicting	LB/VUS	LB/VUS	LB	LB	BS3, BP4
c.1096T>G p. (Ser366Ala)	135360	Conflicting	LB/VUS	LB/VUS	LB	LB	BS3, BP4

TP53 variant	ClinVar ID	ClinVar Classifications (September, 2019)*	Prior Variant Classifications <sup>^</sup>	Dual Biocurator Classifications		TP53-specific ACMG/AMP Final Classifications	Final Criteria Applied
				Original ACMG/AMP Guidelines <sup>#</sup>	TP53-specific ACMG/AMP Guidelines <sup>%</sup>		
c.935C>G p. (Thr312Ser)	141102	Conflicting	LB/VUS	B/LB	B/LB	B	BS1, BS3, BP4
c.892G>A p. (Glu298Lys)	141483	Conflicting	LB	LB	LB	LB	BP4, BS3
c.704A>G p. (Asn235Ser)	127821	Conflicting	LB/VUS	VUS	B	B	BS1, BS3, BS4, BP4, BP2, BS2_Supporting
c.245C>T p. (Pro82Leu)	182946	Conflicting	LB/VUS	VUS	LB	LB	BS3, BP4
c.28G>A p. (Val10Ile)	127806	Conflicting	LB/VUS	LB	LB/VUS	LB	BS3, BP4
c.21T>A p. (Asp7Glu)	140782	Conflicting	LB	VUS	VUS	LB	PM2_Supporting, BS3, BP4
c.145G>A p. (Asp49Asn)	186363	Conflicting	LB	VUS	LB	LB	BP4, BS3
c.641A>G p. (His214Arg)	376615	Conflicting	LP	LP/P	LP	LP	PS3, PM2_Supporting, PS4_Supporting
c.217G>A p. (Val73Met)	142386	Conflicting	LB	VUS	LB/VUS	B	BS1, BS3, BP4
c.139C>T p. (Pro47Ser)	43588	Conflicting	B	B/LB	B	B	BA1, BS3, BP4, BS2
c.319T>C p. (Tyr107His)	140786	Conflicting	LB/VUS	LB/VUS	B	B	BA1, BP4, BS3_Supporting, BS2_Supporting
c.428T>C p. (Val143Ala)	792574	NA	NA	LP/P	LP	LP	PS3, PM6, PM2_Supporting, PP3

Abbreviations: B = Benign, LB = Likely benign, VUS = Variant of uncertain significance, LP = Likely pathogenic, P = Pathogenic, Conflicting = Conflicting interpretations of pathogenicity, NA = Not Available.

\* ClinVar assertions were documented in September 2019. Conflicting variants were those that spanned different clinically relevant classifications.

<sup>^</sup> Assertions provided by VCEP members with variant nominations. Some variants were nominated by multiple members; some with conflicting assertions. Variants listed as "NA" were not nominated by a VCEP member.

<sup>#</sup> Variants were assessed by two curators using the original ACMG/AMP guidelines and any curation assertions conflicts are noted.

<sup>%</sup> Additional rule specifications after pilot testing are largely responsible for differences in classifications between the Biocurator TP53-specified Classifications and the Final TP53 VCEP Classifications.