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Variable population prevalence estimates of germline *TP53* variants: a gnomAD-based analysis

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Abstract

Reports of variable cancer penetrance in Li-Fraumeni syndrome (LFS) have raised questions regarding the prevalence of pathogenic germline *TP53* variants. We previously reported higher-than-expected population prevalence estimates in sequencing databases composed of individuals unselected for cancer history. This study aimed to expand and further evaluate the prevalence of pathogenic and likely pathogenic germline *TP53* variants in the gnomAD dataset (n = 138,632). Variants were selected and classified based on our previously published algorithm and compared with alternative estimates based on three different classification databases: ClinVar, HGMD, and the UMD_*TP53* website. Conservative prevalence estimates of pathogenic and likely pathogenic *TP53* variants were within the range of one carrier in 3,555–5,476 individuals. Less stringent classification increased the approximate prevalence to one carrier in every 400–865 individuals, mainly due to the inclusion of the controvertible p.N235S, p.V31I, and p.R290H variants. This study shows a higher-than-expected population prevalence of pathogenic and likely pathogenic germline *TP53* variants even with the most conservative estimates. However, these estimates may not necessarily reflect the prevalence of the classical LFS phenotype which is based upon cancer family history. Comprehensive approaches are needed to better understand the interplay of

germline *TP53* variant classification, prevalence estimates, cancer penetrance, and LFS-associated phenotype.

Keywords

gnomAD; Li-Fraumeni syndrome; pathogenic variant; prevalence; *TP53*; variant classification

INTRODUCTION

Germline *TP53* variants are the primary genetic cause of Li-Fraumeni syndrome (LFS), a hereditary cancer-predisposition disorder associated with a high lifetime cancer risk. The diagnosis of LFS is made based on clinical criteria that account for both personal and family history of cancer (Bougeard et al., 2015). The core LFS-associated tumor spectrum includes pre-menopausal breast cancer, soft-tissue and bone sarcomas, brain tumors, and adrenocortical carcinomas (Bougeard et al., 2015; Mai et al., 2016; Amadou, Waddington Achatz, & Hainaut, 2017; Guha & Malkin, 2017). LFS has been typically associated with a high cancer penetrance. The overall cumulative cancer incidence has been suggested to be nearly 100% by the age of 70 years (Bougeard et al., 2015; Mai et al., 2016) with variable patterns mainly attributed to different classes of germline *TP53* variants (Olivier, Hollstein, & Hainaut, 2010; Bougeard et al., 2015). However, more recent studies have proposed changes in this paradigm. An updated analysis of the germline dataset of the International Agency for Research on Cancer (IARC) has suggested that the cancer penetrance may be, in fact, incomplete. According to this review, carriers of germline *TP53* variants detected in the clinical setting of LFS, have been reported to have a cancer penetrance of 80% by the age of 70 years (Amadou et al., 2017). Another study identified that carriers of germline *TP53* variants, ascertained through multigene panel testing, were significantly older at cancer diagnosis and less likely to meet classic clinical criteria for LFS than those ascertained through more traditional single gene-based testing (Rana et al., 2018).

Previous estimates for population prevalence of pathogenic germline *TP53* variants have ranged from 1:5,000 in a breast cancer cohort (Lalloo et al., 2003) to 1:20,000 in a clinical-based cohort (Gonzalez et al., 2009). With recent studies suggesting an overestimation of the cancer penetrance in LFS and a more variable phenotypic spectrum than previously described, it follows to question whether we have subsequently underestimated the prevalence of germline *TP53* variants. To provide more accurate prevalence estimates of germline *TP53* variants, we previously investigated sequencing data from 63,983 unrelated individuals unselected for cancer history (de Andrade et al., 2017). Based on our non-clinical classification criteria, we reported a higher-than expected prevalence of pathogenic and likely pathogenic germline *TP53* variants that may be, by some estimates, up to 10 times higher than originally described (Lalloo et al., 2003; Gonzalez et al., 2009). Of note, two controvertible variants, p.N235S and p.R290H, classified as likely pathogenic by our non-clinical classification criteria were found to be responsible for more than 60% of the total prevalence. The Genome Aggregation Database (gnomAD) is a larger population sequencing dataset (Lek et al., 2016) which compiles germline variation data on 138,632 unrelated individuals, a number over twice as large as our previous report. Therefore, our

aim was to further investigate and validate the prevalence of pathogenic and likely pathogenic germline *TP53* variants in the gnomAD dataset, and to consider alternative prevalence estimates based on additional variant classification databases.

METHODS

Variant annotation, selection, and classification

We evaluated the prevalence of germline *TP53* variants in the gnomAD database (file downloaded on March 30th, 2018) composed of 138,632 individuals (median age = 54 years old) who had either their exome or genome sequenced as part of several studies (Lek et al., 2016). Variant annotation, selection, and classification were performed according to our previously published pipeline (de Andrade et al., 2017). Importantly, these non-clinical classification criteria are independent of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) guidelines for variant classification (Richards et al., 2015). Therefore, these criteria are not necessarily representative of the more stringent methods utilized when determining the pathogenicity of a variant for clinical use. Variants were classified as either pathogenic (P), likely pathogenic (LP), possibly pathogenic (PP), likely benign (LB), or variants of uncertain significance (VUS). The classification algorithm combines functional data results from a yeast-based assay (Kato et al., 2003), bioinformatics pathogenicity predictions provided by the Rare Exome Variant Ensemble Learner (REVEL) (Ioannidis et al., 2016), and clinical classifications provided by both the ClinVar (Landrum et al., 2016) and the Human Gene Mutation Database (HGMD) (Stenson et al., 2014). Complete description and specifications can be found in our recent investigation (de Andrade et al., 2017). After exclusion of two variants (p.P72R and p.P47S) with minor allele frequency (MAF) greater than 0.01 (1%) in any gnomAD population, and one potential technical artifact/low confidence call (i.e., p.K291RfsTer18), a total of 199 unique *TP53* variants were further evaluated.

International Agency for Research on Cancer (IARC) germline database

The numbers of individuals found to have germline *TP53* variants in gnomAD were compared with the germline dataset of LFS patients provided by the International Agency for Research on Cancer (IARC - version R19). For the purpose of our analyses, we only considered unique individual code entries for either confirmed or obligatory germline *TP53* variant carriers (Bouaoun et al., 2016).

Creation of a presumed gnomAD “non-TCGA” dataset

The current release of the gnomAD dataset includes 7,208 samples from the Cancer Genome Atlas (TCGA) project, a cancer-based cohort. To date, an official non-TCGA version of gnomAD has not been released. In order to provide population prevalence estimates excluding cancer-affected individuals, we created a dataset of what we presume comprise the gnomAD “non-TCGA” dataset (n = 131,424) using the following method: sequencing data on germline *TP53* variants detected in 32 cancer types from the TCGA project were downloaded from the Genomic Data Commons (GDC) Application Programming Interface (API). Samples from the acute myeloid leukemia (LAML) project were not included due to the possibility of contamination with circulating tumor cells (Weitzel et al., 2017).

Sequencing data from a total of 8,991 individuals with a blood-derived normal sample were analyzed (data accessed on March 21st, 2018). Germline variant calling was performed jointly for each cancer type using three different callers: Genome Analysis Toolkit (GATK) UnifiedGenotyper, GATK HaplotypeCaller, and FreeBayes. Sequencing data was assembled using bcbio-nextgen (<https://github.com/chapmanb/bcbio-nextgen>). Variants were included in the analysis if they were reported by at least two of these callers and if they had alternate allele fraction greater than 0.2 (threshold used in the latest gnomAD release). Variants found in common among gnomAD and TCGA were evaluated further to exclude potential individuals from TCGA carrying a germline *TP53* variant. Thus, our gnomAD “non-TCGA” dataset excluded individuals harboring germline *TP53* variants that were detected in TCGA with an allele fraction > 0.2 and assumed to be part of gnomAD.

ClinVar, HGMD, and the Universal Mutation *TP53* classification databases

All germline *TP53* variants identified in gnomAD also had their respective classification queried from three databases and analyzed separately. The ClinVar database (Landrum et al., 2016), which includes variant classifications provided by clinical laboratories, was consulted to collect submitter-level classifications for *TP53* variants reported as of germline origin (data last accessed in May 4th, 2018). Variant classification was also collected as it is from the HGMD® professional 2018.2 database, which compiles research-based evidence on genetic variants (Stenson et al., 2014). We also collected germline *TP53* classification from the Seshat web-tool, which is a classification algorithm integrated with the Universal Mutation Database_ *TP53* website (UMD_ *TP53*, current release: 2017_R2, <https://p53.fr/>), curated and maintained by scientific experts on the *TP53* gene (Soussi, Kato, Levy, & Ishioka, 2005; Leroy et al., 2013; Leroy, Anderson, & Soussi, 2014).

Variant classification categories stratification

Different variant classification categories are provided by each of the databases included. For the purpose of the analyses, we stratified all the variant into two groups. The combined “P/LP” category refers to *TP53* variants that were classified as P or LP by our classification criteria or by at least one of the three other classifications (ClinVar, HGMD, UMD_ *TP53*). Variants were considered as part of this group if they matched any one of the following: classified as P or LP by our classification criteria; as P or LP by the UMD_ *TP53* database; denoted P or LP by at least one submitter level classification entry in ClinVar; or classified as a “disease causing mutation” (DM) by HGMD.

The combined “PP, LB/B, VUS” category corresponds to the remaining variants that did not receive a P or LP classification either by our classification criteria or by any of the three databases. Variants were considered as part of this group if they matched any of the following: classified as either PP, LB, or VUS by our published criteria; as PP, B, or VUS by the UMD_ *TP53* database; submitter level classification as either LB, B, or VUS in ClinVar (i.e., no P/LP entry); classified as either “disease causing mutation?” (DM?) or as *in vitro* or *in vivo* functional polymorphism (FP) by HGMD.

RESULTS

A total of 64 unique *TP53* variants ($n = 399$ carrier individuals) were classified as P/LP either by our classification criteria or by at least one of the three classification databases (Table 1 and Table 2). Fourteen variants were suggested to be P/LP by all four classifications, 14 others by three, 20 by two, and 16 by one classification used (Table 2).

Germline *TP53* variants classified as P/LP by our classification criteria

Twenty-one unique variants classified as pathogenic (P) by our criteria were identified in 39 individuals ($39/138,632 = 0.028\%$, Table 1). The p.R283H variant was relatively common in this dataset, being detected in 10 individuals ($10/39 = 25.6\%$) and reported once in the IARC database. These 21 variants are currently represented in a total of 306 patients in the IARC database, particularly due to the high prevalence of the p.R248W, p.R175H, and p.R248Q variants (Table 2). Considering only the *TP53* variants classified as pathogenic by our classification criteria, the population prevalence estimate is one carrier in every 3,555 individuals (Table 1).

Twenty-six unique *TP53* variants were classified as likely pathogenic (LP) by our criteria. These 26 variants were identified in 278 individuals ($278/138,632 = 0.201\%$, Table 1). Overall, the IARC database reported these 26 variants in 303 LFS patients, with a significant contribution of the p.R337H variant in 188 individuals (Table 2). Three variants, namely p.N235S, p.V31I, and p.R290H, were respectively detected in 58, 57 (including one homozygous), and 42 individuals; jointly accounting for 157 individuals ($157/278 = 56\%$). In comparison, these three variants account for only 33 individuals in IARC (Table 2). The prevalence of all germline *TP53* variants classified as LP by our criteria is estimated to be approximately one carrier in every 500 individuals (Table 1).

Germline *TP53* variants classified as P/LP in classification databases

Variant classification by different clinical databases were analyzed separately and yielded similar population prevalence estimates. A total of 28 unique germline *TP53* variants had at least one submitter level entry of P/LP in ClinVar, corresponding to a prevalence estimate of one carrier in every 779 individuals (0.128% , Table 1 and Table 2). This high estimate may be accounted for by the presence of the p.V31I and p.R290H variants since both are currently classified as LP by at least one clinical laboratory (Table 2). The HGMD database suggests higher prevalence estimates of approximate one variant carrier in every 400 individuals (0.25% , Table 1) due to its broader inclusion of 53 variants currently classified as DM, including the p.N235S and p.V31I (Table 1 and Table 2). The classification provided by the UMD_*TP53* database includes a total of 26 germline P/LP *TP53* variants (Table 1 and Table 2); however, it does not include any of the variants highly represented and present in the other classification databases (Table 2). This conservative UMD_*TP53* database approach reflects a prevalence estimate of one carrier in every 4,077 individuals (0.025% , Table 1).

Germline *TP53* variants not classified as P/LP

A total of 135 unique variants included in the combined PP, LB/B, or VUS group were detected in 442 individuals (0.319%, Table 1). Of these, the three most predominant variants were the p.G360A, p.A83V, and p.Y107H, which were present in 121 individuals (121/442 = 27.3%, Table 1 and Supp. Table S1). Of all the 135 variants, the IARC database has report on only ten variants, while the HGMD and ClinVar databases have no report for 110 and 48 out of these variants, respectively (Supp. Table S1).

Population prevalence of germline *TP53* variants in the presumed gnomAD “non-TCGA” dataset

A total of 38 individuals with P/LP germline *TP53* variants in gnomAD were assumed to be selected from the TCGA project (Table 2). After exclusion of these 38 individuals from the total count (n=399), there are 361 carrier individuals in the adjusted gnomAD “non-TCGA” dataset (Table 1). Adjusted P/LP prevalence estimates, which account for the exclusion of these individuals in the gnomAD “non-TCGA” dataset (n=131,424), are within the approximate range of one carrier in every 423–5,476 individuals (0.018–0.237%, Table 1).

Similarly, 41 individuals assumed to be part of TCGA harbored 31 variants from the combined group of variants classified as PP, LB/B, and VUS (Supp. Table S1). After exclusion of these individuals, the adjusted prevalence of PP, LB/B, and VUS variants remained essentially constant and around 0.305% (Table 1).

DISCUSSION

Based on our analyses of germline *TP53* variants identified in the gnomAD dataset, we validated our previous report that population prevalence estimates of pathogenic or likely pathogenic germline *TP53* variants is higher than previously reported even when considering conservative estimates (one carrier in every 3,555–5,476 individuals). However, prevalence estimates are highly variable especially due to differing interpretations of germline *TP53* variants that may be associated with reduced cancer penetrance. Whereas publicly available sequencing databases have allowed the identification of germline *TP53* variants in different populations, these estimates may not necessarily reflect the disease prevalence of LFS. The estimation of the true family-based prevalence of LFS is a challenging task complicated by its wide phenotypic spectrum and variable cancer penetrance.

Conservative estimates including only variants classified as pathogenic according to our non-clinical classification criteria or based on the UMD_*TP53* database suggest an approximate population prevalence of one carrier in every 3,555–5,476 individuals. Our previous analysis showed a prevalence of pathogenic variants in the range of 0.06% (~ one in every 1,560 individuals) in the ExAC non-TCGA subset (de Andrade et al., 2017). The lower prevalence detected in this new analysis (0.018–0.028%) may be partially accounted for by the minimal increase of five carriers of pathogenic germline *TP53* variants detected in gnomAD, a database over twice as large as our previous study. In total, eight variants classified as pathogenic by our criteria in the previous analysis of the ExAC non-TCGA subset were not identified in gnomAD (these include p.E285V, p.C277Y, p.R273H, p.R273S,

p.V272M, p.S241C, p.C238Y, and p.P152L). One of the possible explanations is the use of different quality control parameters for both variant and sample inclusion in gnomAD. We can mention the establishment of an important threshold for alternate allele fraction greater than 0.2 in order to avoid potential inclusion of somatic variants. Multi-gene panel testing has increased the complexity of genetic testing for cancer predisposition genes due to the considerable detection of somatic *TP53* variants (Weitzel et al., 2017). These somatic *TP53* variants still have an undefined accurate specification for the alternate allele fraction. Consequently, the threshold employed by gnomAD minimizes, but still may not fully exclude, potential false positive variants that may have originated due to clonal hematopoiesis and that could have been interpreted as of germline origin (Jacobs et al., 2012; Genovese et al., 2014; Coffee et al., 2017).

Less stringent variant classifications suggest that the overall prevalence of P/LP germline *TP53* variants may be as high as one carrier in every 400 individuals (range 0.116–0.25%). Three *TP53* variants are considerably overrepresented in these estimates: the p.N235S, p.R290H, and p.V31I. The unclear clinical significance of the variants p.N235S and p.R290H has also been discussed in our previous study (de Andrade et al., 2017).

The variant p.N235S is currently classified as DM by HGMD, but has tolerant *in silico* analyses scores, and lacks evidence of segregation with disease (van Hest et al., 2007) and of functional consequences (Kato et al., 2003; Boutell, Hart, Godber, Kozlowski, & Blackburn, 2004). It also has a minor allele frequency in gnomAD that may be considered too high for the disorder by current ACMG-AMP clinical guidelines (0.054% in the Finnish population; 14 alleles). Consequently, p.N235S is described as LB or VUS by most clinical pipelines. This variant remains within our exploratory dataset as we only restricted to variants with a MAF less than 1% to allow for broad capture of potentially deleterious variants.

The p.R290H variant is often considered as a VUS in clinical practice primarily due to the lack of LFS phenotypic evidence (Arcand et al., 2008), its “supertrans” classification in yeast functional assays (Kato et al., 2003), *in vitro* studies showing apparently normal functional activity (Wang, Niu, Lam, Xiao, & Ren, 2014; Zerdoumi et al., 2017), and evidence of potential lack of segregation with disease (Quesnel et al., 1999). However, its presence in some LFS families reported in IARC, and its current LP classification by some clinical laboratories, warrant further evaluation to determine its true pathogenic potential.

The p.V31I variant currently has four different classification entries in ClinVar. It has been identified exclusively in individuals of East-Asian ancestry in the gnomAD dataset (0.3%), with one Japanese report (Yamada et al., 2007; Bouaoun et al., 2016) and two cases suggestive of LFS from South Korea (Park, Choi, Suh, Ki, & Kim, 2016). The high representation of the p.V31I variant is a factor that might indicate that this variant may be a potential moderate-risk allele or a specific genetic variant in individuals of East-Asian ancestry. A founder effect may also be hypothesized as illustrated by the known pathogenic p.R337H variant present in 0.3% of the population of certain regions from Brazil (Achatz & Zambetti, 2016).

The under-reporting of LFS in most parts of Asia makes it difficult to estimate the association of specific variants with disease in this geographical area.

The fact that these three variants have been observed at a frequency that could be considered too prevalent for the disorder may be deemed by some as strong evidence supporting a more benign classification by current guidelines. Disregarding the p.N235S, p.R290H, and p.V31I variants - altogether identified in 150 carrier individuals in the gnomAD “non-TCGA” dataset - from the likely pathogenic category according to our classification criteria, the adjusted respective prevalence would be 0.078% (~ one carrier in every 1,276 individuals). These three variants speak towards the somewhat subjective nature of current variant classification methods as well as the complications in classifying variants that may be associated with reduced penetrance. In these cases, the rules are designed in such a way that these variants are likely to obtain a VUS classification and thus, these high estimates should be interpreted with caution.

Part of the current criteria for classifying a variant is the evaluation of the observed allele frequency of the variant compared with the expected prevalence of the disease. Based on the current American College of Medical Genetics and Genomics (ACMG) guidelines, the excess of individuals with a specific variant relative to the disease prevalence in the population is used as evidence that the variant may not be pathogenic (Richards et al., 2015; Amendola et al., 2016). The optimal utilization of this rule in the setting of LFS may be confounded by recent studies that have suggested a reduced cancer penetrance and broader phenotypic spectrum than previously appreciated (Amadou et al., 2017; Rana et al., 2018). Furthermore, our current analyses demonstrate that different variant classifications may drastically alter what is potentially disease prevalence, creating a cycle of misclassifications and imprecise estimates. Estimating the true population prevalence of LFS is further hindered by the lack of a gold standard of “known pathogenic” *TP53* variants.

To begin to address this issue, we assessed *TP53* variants by consulting three major variant classification databases in addition to our own non-clinical criteria. It is important to note that there are discordances even among major clinical laboratories with similar schema based on the ACMG-AMP standard guidelines for variant classification (Richards et al., 2015; Amendola et al., 2016). Clinical or research-based databases, such as those utilized in our analyses, have been shown to contain discordant and sometimes inaccurate interpretations of variant pathogenicity due to non-standardized approaches (Dorschner et al., 2013; Yang et al., 2017; Shah et al., 2018). Ultimately, variant misclassifications may lead to inflated genetic risk estimates and elevated disease prevalence estimates (Shah et al., 2018). Current literature suggests that many of the variants in current databases are re-classified to be less pathogenic as they are re-assessed (Macklin, Durand, Atwal, & Hines, 2018; Shah et al., 2018). It is also interesting to note that up to 0.3% of the gnomAD population harbors germline *TP53* variants without a current classification suggestive of P/LP by our stratified approach. However, a minority of these variants may eventually be found to have important implications with regard to disease prevalence and for recommendations for cancer screening and clinical follow-up. Accurate variant classification is a challenging and evolving effort that will benefit from additional bioinformatics prediction tools, functional assays, and collaborations to encourage data sharing (Amendola

et al., 2016; Harrison et al., 2017). A specialized Clinical Domain working group of the Clinical Genome Resource (ClinGen) (Rehm et al., 2015), composed of a curation expert panel on LFS and the *TP53* gene, is currently refining classification guidelines specifically for germline *TP53* variants. This effort, among others, will increase concordance and accuracy in variant curation and help address the uncertainties surrounding the true prevalence and phenotype of LFS.

This study may be underestimating the population prevalence of germline *TP53* variants due to some limitations. Our current analyses are limited to missense and nonsense variants affecting the canonical transcript only. We acknowledge that insertions and deletions are described in 10–15% of all the LFS families reported in IARC (Bouaoun et al., 2016), and microdeletion/duplications may be found in up to 11% of the families (Smith et al., 2016). These structural changes are likely underrepresented in publicly available databases due to challenges in identifying them by conventional sequencing platforms. Non-coding germline variants (Macedo et al., 2016), and synonymous variants (Varley et al., 2001) in *TP53* have also been reported to contribute to the LFS phenotype. Due to limited prediction tools and literature for further interpretation, such variants were not included. The exclusion of individuals who have had severe pediatric disorders from the current gnomAD release (Lek et al., 2016) further impacts prevalence estimates of *TP53* variants and, ultimately, LFS, in which childhood cancers are a main part of the phenotype (Amadou et al., 2017). Since not all individuals from TCGA is part of gnomAD, and it is not documented who these individuals are, our creation of a presumed gnomAD “non-TCGA” dataset is not absolute and may have excluded carrier individuals from other non-TCGA projects. The non-TCGA estimates may also be impacted by differences in variant calling. Variants in gnomAD were reported only based on the GATK HaplotypeCaller pipeline (Lek et al., 2016), whereas we used a combination of three variant callers. As with any population sequencing dataset, there may be additional hidden biases for selection of individuals to be sequenced that are not completely elucidated.

Our analyses on a larger population sequencing database reinforce the concept that the estimated population prevalence of pathogenic and likely pathogenic germline *TP53* variants is higher than originally estimated (conservative estimates: one carrier in every 3,555–5,476 individuals). These estimates are considerably wide based on different variant classifications used and due to potential inflated genetic risk of some *TP53* variants. While we do not report on disease prevalence of LFS, our observations in regard to germline *TP53* variants illustrate the importance of understanding cancer penetrance and ascertainment bias of the patients and families with the most striking cancer histories. Our study also highlights some of the current challenges in variant interpretation – particularly for variants with potentially reduced penetrance or variable phenotype for which current variant interpretation guidelines were not designed – and the discordance in some variant calls due to the subjective application of classification rules. Future prospective population-based studies are encouraged to better determine genotype-phenotype correlations and to potentially provide additional genetic parameters to the current clinical criteria for LFS diagnosis. Pathogenic and likely pathogenic germline *TP53* variants found in atypical clinical settings should be comprehensively evaluated based on individual cancer risk and management strategies for LFS only indicated after thorough discussion with specialists. Comprehensive, collaborative

inter-disciplinary approaches are integral to the further understanding of population prevalence estimates, LFS-associated cancer penetrance, and improved concordance of germline *TP53* variant classification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.
Prevalence of *TP53* variants in the gnomAD dataset.

Variant distribution of a total of 199 unique *TP53* variants identified in the gnomAD database (n = 138,632 individuals) according to different classifications. Prevalence distribution separated by pathogenic, likely pathogenic, and P/LP (reflecting the sum of pathogenic and likely pathogenic classifications) according to our previously published criteria (de Andrade et al., 2017). Other classification databases were also used, such as ClinVar, HGDM, and the UMD_TP53 website. A total of 64 unique *TP53* variants were classified as P or LP by either our criteria or by any of the three databases (n = 399 carrier individuals). The remaining 135 unique variants had only PP, LB/B, or VUS classifications. For ClinVar, variants were included in the P/LP category if they had at least one P or LP submitter-level classification for *TP53* variants reported as of germline origin. For HGMD, the P/LP category included all of those variants currently classified as DM. The prevalence estimates for the gnomAD “non-TCGA” dataset (n = 131,424 individuals) were calculated after the exclusion of carrier individuals assumed to be from TCGA (n = 38 carrier individuals). Abbreviations: gnomAD, Genome Aggregation Database; UMD, Universal Mutation Database; TCGA, The Cancer Genome Atlas; HGMD, Human Gene Mutation Database; P, pathogenic; LP, likely pathogenic; PP, possibly pathogenic; LB, likely benign; B, benign; VUS, variant of uncertain significance; DM, disease causing mutation.

		Variants (n)	Total individuals (n)	Prevalence (%)	One carrier in every (n)
Complete gnomAD (n=138,632 individuals)					
64 unique variants with at least one P/LP classification (n=399 carrier individuals)					
Our classification	Pathogenic	21	39	0.028	3,555
	Likely pathogenic	26	278	0.201	499
	P/LP	47	317	0.229	437
Other classification databases	ClinVar (P/LP)	28	178	0.128	779
	HGMD (DM)	53	346	0.250	401
	UMD_TP53 (P/LP)	26	34	0.025	4,077
135 unique variants with no P/LP classification					
PP, LB/B, VUS		135	442	0.319	314
gnomAD “non-TCGA” (n=131,424 individuals)					
64 unique variants with at least one P/LP classification (n=361 carrier individuals)					
Our classification	Pathogenic	21	30	0.023	4,381
	Likely pathogenic	26	253	0.193	519
	P/LP	47	283	0.215	464
Other classification databases	ClinVar (P/LP)	28	152	0.116	865
	HGMD (DM)	53	311	0.237	423
	UMD_TP53 (P/LP)	26	24	0.018	5,476
135 unique variants with no P/LP classification					
PP, LB/B, VUS		135	401	0.305	328

Table 2.
Complete description of the 64 P/LP germline *TP53* variants detected in the gnomAD dataset.

A total of 64 unique germline *TP53* variants detected in gnomAD were classified as P or LP by either our criteria (de Andrade et al., 2017) or by any of the three classification databases. Variant distribution according to the respective classifications based on our previously published criteria(de Andrade et al., 2017), HGMD, ClinVar, and the UMD_TP53 databases. Variants are first ordered by the total number of concordant classifications as P/LP and then by the total number of individuals detected in gnomAD. Transcriptional activity described as reported (Kato et al., 2003) and compiled in the IARC database. (†) denotes that the total number of individuals harboring the variant p.V31I includes one homozygous individual. The TCGA numbers represent the assumed count of individuals from the TCGA project that may have been included in the gnomAD dataset. Max. MAF calculated to represent the maximum population frequency across the ancestries included in gnomAD. Data extracted from the IARC germline *TP53* database (version R19) include the number of unique entries for confirmed or obligatory carriers only. For ClinVar, numbers between parentheses correspond to the number of submitter-level entries for each classification considering only germline origin. Variants reported based on the canonical transcript NM_000546.5 (nucleotide numbering for cDNA uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1). Abbreviations: TA, transcriptional activity; gnomAD, Genome Aggregation Database; TCGA, The Cancer Genome Atlas; Max. MAF, maximum minor allele frequency; IARC, International Agency for Research on Cancer; REVEL, Rare Exome Variant Ensemble Learner; HGMD, Human Gene Mutation Database; UMD, Universal Mutation Database; NF, non-functional; PF, partially functional; F, functional; S, supertrans; DM, disease causing mutation; P, pathogenic; LP, likely pathogenic; PP, possibly pathogenic; LB, likely benign; B, benign; VUS, variant of uncertain significance.

cDNA change	Aminoacid Change	TA class	gnomAD (n)	TCGA (n)	Max. MAF (%)	IARC (n)	REVEL score	HGMD	ClinVar	UMD TP53	Our classification
P/LP by all four classifications (n=14)											
c.743G>A	p.R248Q	NF	5	1	0.006	53	0.934	DM	P (10), LP (1)	P	P
c.817C>T	p.R273C	NF	3	1	0.006	17	0.901	DM	P (6), LP (1)	P	P
c.659A>G	p.Y220C	NF	2	1	0.002	23	0.936	DM	P (5)	P	P
c.844C>T	p.R282W	NF	1	1	0.001	38	0.896	DM	P (10), LB (1)	P	P
c.818G>T	p.R273L	NF	1	1	0.001	1	0.921	DM	P (1)	P	P
c.772G>A	p.E258K	NF	1	.	0.001	2	0.961	DM	P (3), VUS(1)	P	P
c.742C>T	p.R248W	NF	1	1	0.001	67	0.927	DM	P (5), LP (1)	P	P
c.734G>T	p.G245V	NF	1	1	0.006	2	0.972	DM	LP (1)	P	P
c.722C>T	p.S241F	NF	1	.	0.001	3	0.958	DM	P (2), LP (2)	P	P
c.711G>A	p.M237I	NF	1	.	0.006	2	0.923	DM	LP (1), VUS (2)	P	P
c.524G>A	p.R175H	NF	1	.	0.001	64	0.922	DM	P (7)	P	P
c.473G>A	p.R158H	NF	1	1	0.010	23	0.844	DM	P (4), LP (2)	P	P
c.394A>G	p.K132E	NF	1	.	0.010	2	0.968	DM	LP (1), VUS (1)	LP	P

cDNA change	Aminoacid Change	TA class	gnomAD (n)	TCGA (n)	Max. MAF (%)	IARC (n)	REVEL score	HGMD	ClinVar	UMD TP53	Our classification
c.374C>T	p.T125M	NF	1	.	0.011	1	0.925	DM	LP (5), VUS (1)	LP	P
P/LP by three classifications (n=14)											
c.91G>A	p.V31I	F	57 ⁺	4	0.309	3	0.569	DM	LP (1), LB (3), B (1), VUS (2)	B	LP
c.847C>T	p.R283C	F	24	8	0.016	9	0.74	DM	LP (1), VUS (8)	PP	LP
c.848G>A	p.R283H	NF	10	1	0.010	1	0.836	DM	LP (5), VUS (2)	PP	P
c.566C>T	p.A189V	F	6	.	0.029	4	0.79	DM	P (1), VUS (4)	PP	LP
c.467G>A	p.R156H	PF	5	1	0.006	13	0.627	DM	LP (1), LB (1), VUS (3)	PP	LP
c.542G>A	p.R181H	PF	3	.	0.003	5	0.797	DM	P (1), LP (4)	PP	LP
c.1010G>A	p.R337H	PF	2	2	0.006	188	0.693	DM	P (8)	PP	LP
c.845G>A	p.R282Q	PF	2	.	0.004	2	0.887	DM	P (2), LP (2), VUS (1)	PP	LP
c.472C>T	p.R158C	PF	2	1	0.002	2	0.835	DM	LP (2), VUS (2)	PP	LP
c.974G>T	p.G325V	F	1	.	0.001	3	0.68	DM	P (2), VUS (3)	VUS	LP
c.841G>A	p.D281N	NF	1	.	0.007	2	0.943	DM	.	P	P
c.655C>T	p.P219S	NF	1	.	0.001	2	0.96	DM	LP (2), VUS (1)	PP	P
c.586C>T	p.R196*	.	1	.	0.001	22	.	DM	P (6)	P	PP
c.527G>T	p.C176F	PF	1	.	0.001	.	0.951	DM	.	P	LP
P/LP by two classifications (n=20)											
c.704A>G	p.N235S	F	58	2	0.054	20	0.593	DM	LB (4), VUS (3)	PP	LP
c.869G>A	p.R290H	S	42	1	0.046	10	0.584	DM?	LP (1), VUS (5)	PP	LP
c.1096T>G	p.S366A	F	14	1	0.037	2	0.585	DM	LB (3), VUS (1)	VUS	LP
c.329G>A	p.R110H	PF	13	1	0.016	1	0.592	DM	VUS (5)	PP	LP
c.509C>T	p.T170M	PF	12	.	0.008	1	0.868	DM	VUS (4)	PP	LP
c.997C>T	p.R333C	F	6	.	0.018	2	0.862	DM	VUS (4)	VUS	LP
c.523C>T	p.R175C	PF	6	2	0.015	1	0.833	DM	VUS (2)	PP	LP
c.665C>T	p.P222L	F	5	.	0.003	2	0.585	DM	LB (1), VUS (4)	PP	LP
c.245C>T	p.P82L	F	5	.	0.007	9	0.57	DM	LB (1), VUS (2)	VUS	LP
c.970G>C	p.D324H	S	3	1	0.009	1	0.634	DM	VUS (3)	VUS	LP
c.877G>T	p.G293W	F	3	.	0.002	2	0.675	DM	VUS (5)	VUS	LP
c.800G>A	p.R267Q	PF	3	1	0.003	12	0.902	DM	VUS (4)	PP	LP
c.784G>A	p.G262S	NF	3	.	0.002	1	0.876	DM	VUS (5)	PP	P

cDNA change	Aminoacid Change	TA class	gnomAD (n)	TCGA (n)	Max. MAF (%)	IARC (n)	REVEL score	HGMD	ClinVar	UMD TP53	Our classification
c.685T>C	p.C229R	PF	2	.	0.002	6	0.754	DM	VUS (2)	VUS	LP
c.1093C>T	p.H365Y	F	1	.	0.001	2	0.714	DM	VUS (1)	VUS	LP
c.917G>A	p.R306Q	NF	1	.	0.003	1	0.764	DM	VUS (3)	PP	P
c.672G>T	p.E224D	F	1	.	0.007	1	0.697	DM	.	PP	LP
c.587G>A	p.R196Q	PF	1	.	0.001	2	0.888	DM	VUS (3)	PP	LP
c.401T>G	p.F134C	NF	1	.	0.001	.	0.949	.	LP (1)	PP	P
c.107C>T	p.P36L	NF	1	.	0.001	1	0.542	DM	.	VUS	P
P/LP by one classification (n=16)											
c.787A>G	p.N263D	PF	28	1	0.092	.	0.315	DM	VUS (3)	VUS	PP
c.1015G>A	p.E339K	F	18	.	0.037	1	0.469	DM	LB (2), VUS (2)	VUS	PP
c.469G>A	p.V157I	PF	11	1	0.012	6	0.425	DM	VUS (3)	VUS	PP
c.604C>T	p.R202C	F	7	.	0.015	3	0.498	DM	LB (1), VUS (2)	PP	PP
c.1073A>T	p.E358V	F	5	.	0.029	4	0.481	DM	LB (1), VUS (2)	VUS	PP
c.376–2dupA	p.?	.	2	1	0.002	.	.	.	VUS (5)	LP	VUS
c.1136G>A	p.R379H	F	1	.	0.011	1	0.338	DM	VUS (3)	VUS	PP
c.830G>T	p.C277F	NF	1	1	0.001	.	0.932	.	VUS (1)	P	VUS
c.725G>C	p.C242S	NF	1	.	0.003	.	0.947	.	.	LP	VUS
c.661G>T	p.E221*	.	1	.	0.006	.	.	.	.	P	VUS
c.581T>G	p.L194R	NF	1	.	0.010	.	0.933	.	VUS (2)	P	VUS
c.536A>G	p.H179R	NF	1	.	0.010	.	0.935	.	VUS (1)	P	VUS
c.530C>T	p.P177L	NF	1	.	0.001	.	0.865	.	VUS (1)	LP	VUS
c.466C>T	p.R156C	PF	1	.	0.003	3	0.493	DM	VUS (4)	PP	PP
c.454C>T	p.P152S	NF	1	.	0.001	.	0.863	DM?	VUS (1)	LP	VUS
c.376–2A>C	p.?	.	1	.	0.003	.	.	.	.	LP	VUS