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Review

Emerging insights into ethnic-specific *TP53* germline variants

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Abstract

The recent expansion of human genomics repositories has facilitated the discovery of novel *TP53* variants in populations of different ethnic origins. Interpreting *TP53* variants is a major clinical challenge because they are functionally diverse, confer highly variable predisposition to cancer (including elusive low-penetrance alleles), and interact with genetic modifiers that alter tumor susceptibility. Here, we discuss how a cancer risk continuum may relate to germline *TP53* mutations on the basis of our current review of genotype–phenotype studies and an integrative analysis combining functional and sequencing datasets. Our study reveals that each ancestry contains a distinct *TP53* variant landscape defined by enriched ethnic-specific alleles. In particular, the discovery and characterization of suspected low-penetrance ethnic-specific variants with unique functional consequences, including P47S (African), G334R (Ashkenazi Jewish), and rs78378222 (Icelandic), may provide new insights in terms of managing cancer risk and the efficacy of therapy. Additionally, our analysis highlights infrequent variants linked to milder cancer phenotypes in various published reports that may be underdiagnosed and require further investigation, including D49H in East Asians and R181H in Europeans. Overall, the sequencing and projected functions of *TP53* variants arising within ethnic populations and their interplay with modifiers, as well as the emergence of CRISPR screens and AI tools, are now rapidly improving our understanding of the cancer susceptibility spectrum, leading toward more accurate and personalized cancer risk assessments.

More than 40 years of extensive research on p53 has established its role as a tumor suppressor and revealed the pervasiveness of aberrations linked to this protein in cancer. Normally, p53 is activated by cellular stress and operates as a transcription factor to induce cell signaling networks that prevent tumors from developing. Spontaneous mutations in the *TP53* gene that encodes p53 are the most common alterations in tumors, occurring at a rate of at least 50% across all cancer types (1). Inherited or germline *TP53* mutations predispose carriers to a multitude of different childhood- and adult-onset cancers in Li-Fraumeni Syndrome (LFS), and they are associated with hereditary breast cancer risk (2). Estimates of germline *TP53* mutations in early-onset breast cancers range between 2% and 8% in families that often do not fulfill criteria for LFS (3-6). The lifetime risk of developing cancer for individuals diagnosed with LFS is nearly 90%, where the median age at first cancer is 34 years for women and 45 years for men (7). However, a large degree of phenotypic heterogeneity is observed among germline *TP53* mutation carriers, including highly variable disease penetrance, age of onset, and tumor types (8). The differences in disease severity between germline carriers have been linked to the uneven functional effects of different *TP53* mutations. Despite this knowledge, detailed studies assessing the effects of *TP53* mutations have been largely limited to the hotspots that account for approximately 30% of cancer-associated mutations, whereas relatively little has been studied of the remaining 70% of rare and infrequent mutations. Increased DNA sequencing in the clinical setting and greater research efforts to sequence the general population have uncovered novel *TP53* variants, many of which are variants of uncertain significance (VUS) expected to have little to no cancer-associated risk. However, pathogenic variants have been detected at a higher frequency than expected (9). Importantly, differences in germline *TP53* variant frequencies have been discovered across various ethnicities. Here, we examine ethnic-specific *TP53* variants and cancer-related germline *TP53* mutational landscapes in diverse populations using analytical tools combining several functional datasets and sequencing databases.

TP53 mutational landscapes have identified functionally diverse variants in cancer and the general population

The increased application of next-generation sequencing technology has contributed to the identification of hundreds of novel germline variances in *TP53*. To date, more than 500 different germline mutations and at least 4500 somatic mutations have been linked to cancer, spanning the entire *TP53* gene (10). Meanwhile, this expansion of sequencing data has also led to the

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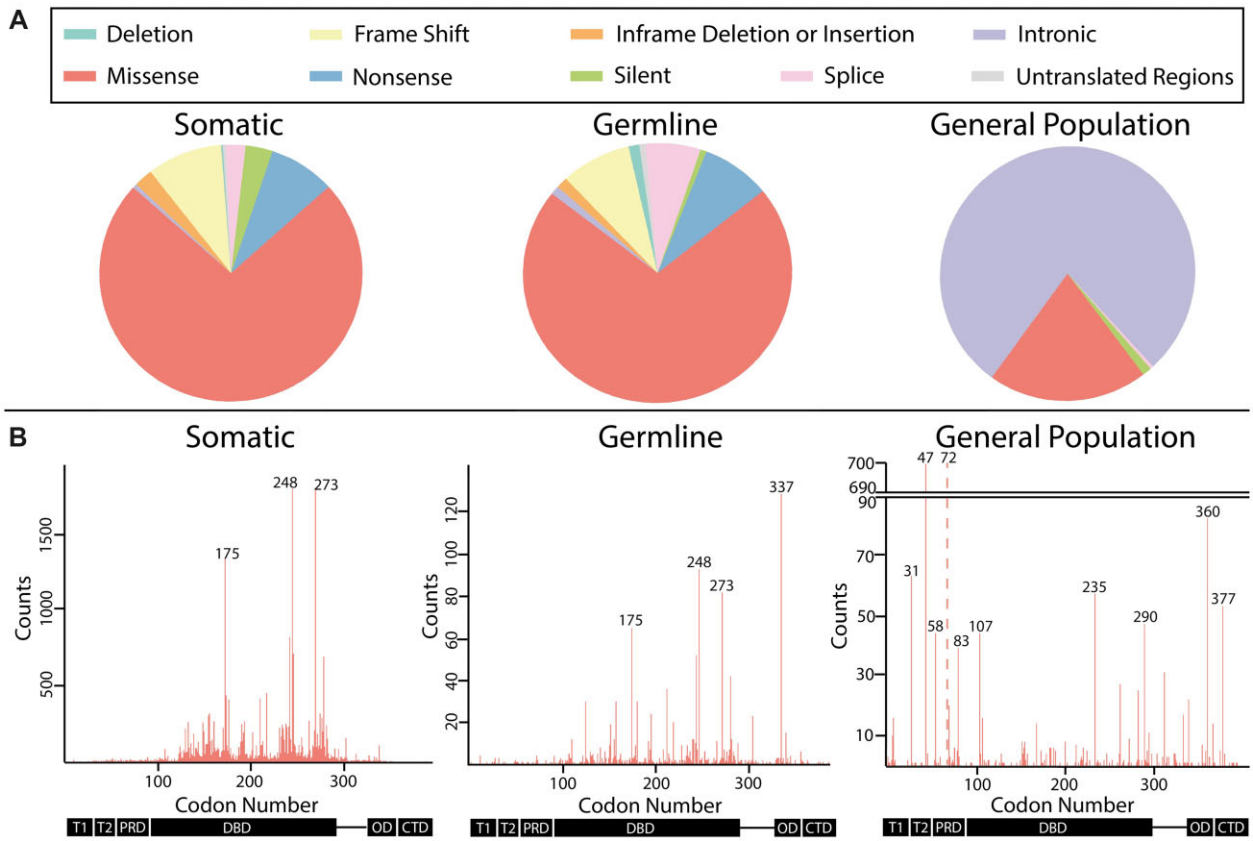

Figure 1. The landscape of *TP53* mutations in cancer and variants in the general population. **A**) The proportion of each type of mutation is represented within the following populations: tumors with somatic *TP53* mutations (27 217 tumors; data from The *TP53* Database), carriers of germline *TP53* mutations (1384 carriers; data from The *TP53* Database), and *TP53* variants in the general population without cancer history (310 081 variants from 76 156 genome samples; data from gnomAD version 3 noncancer selection mapped against the canonical transcript [Ensembl transcript ID ENST00000269305.9]). **B**) The occurrence of nonsynonymous mutations affecting the coding region. CTD = C-terminal domain; DBD = DNA-binding domain; OD = oligomerization domain; PRD = proline-rich domain; T1/T2 = transactivation domain 1 and 2. In these populations, data are from the noncancer dataset of gnomAD version 3 for Africans and version 2 for the rest.

identification of *TP53* VUS within the general population. As a result, interpreting the clinical significance of a *TP53* variant remains an ongoing challenge. We initially compared the mutational landscapes of *TP53* variants found in the general population and tumors by plotting the types and locations of *TP53* alterations (Figure 1). The datasets for somatic and germline mutations associated with cancer were obtained from The *TP53* Database (version R20) (10), and data for the general population were acquired from the Genome Aggregation Database (gnomAD) (11) noncancer dataset mapped against the canonical transcript (Ensembl transcript ID ENST00000269305.9). Figure 1 shows that, unlike other tumor suppressor genes that often acquire deletions or truncating mutations, more than 70% of *TP53* mutations in cancer are missense mutations that tend to cluster around the DNA-binding domain (DBD). It has been suggested that this clustering phenomenon may be due to a historical sequencing bias between exons 5 and 8 (12), although mutational selection processes also provide an explanation for this enrichment and the prevalence of a few "hotspot" mutations in this region (13,14). More recently, the immunogenicity of hotspot mutations was found to be a contributing factor toward their selection and prevalence in cancer (15).

In contrast to cancer-associated mutations, *TP53* variants in the general population without cancer history are more commonly found in the intronic regions. Interestingly, a considerable number of missense substitutions are also observed in the general population that evenly span most of the p53 coding region, as opposed to the clustering phenomenon of cancer-associated mutations. Of note, codon 72 of p53 is either proline (72P) or arginine (72R), and their prevalence varies between ethnicities (discussed below). Although some reports indicate certain functional discrepancies, both 72P and 72R are considered wild-type alleles (16) that typically serve as normal healthy controls for research studies.

Importantly, both datasets used in the present analysis have limitations and sampling biases to consider for their interpretation. First, The *TP53* Database is largely a repository of family-based association studies collected from select cancer centers and is enriched for highly pathogenic variants. As a result, there are sampling biases because of the inclusion of several family members. To address the familial sampling biases, we have included only 1 representative member from each family in The *TP53* Database. Next, the gnomAD "noncancer" dataset does not necessarily represent a completely cancer-free population. Samples were ascertained from individuals who were not specifically selected for having cancer in a cancer study; however, individuals recruited for other noncancer projects may have developed cancer. In addition, individuals recruited with severe pediatric diseases as well as their first-degree relatives are excluded from gnomAD in an effort to represent the general population. Therefore, this dataset may contain low-penetrance and adult-onset cancer predisposition alleles present in the general

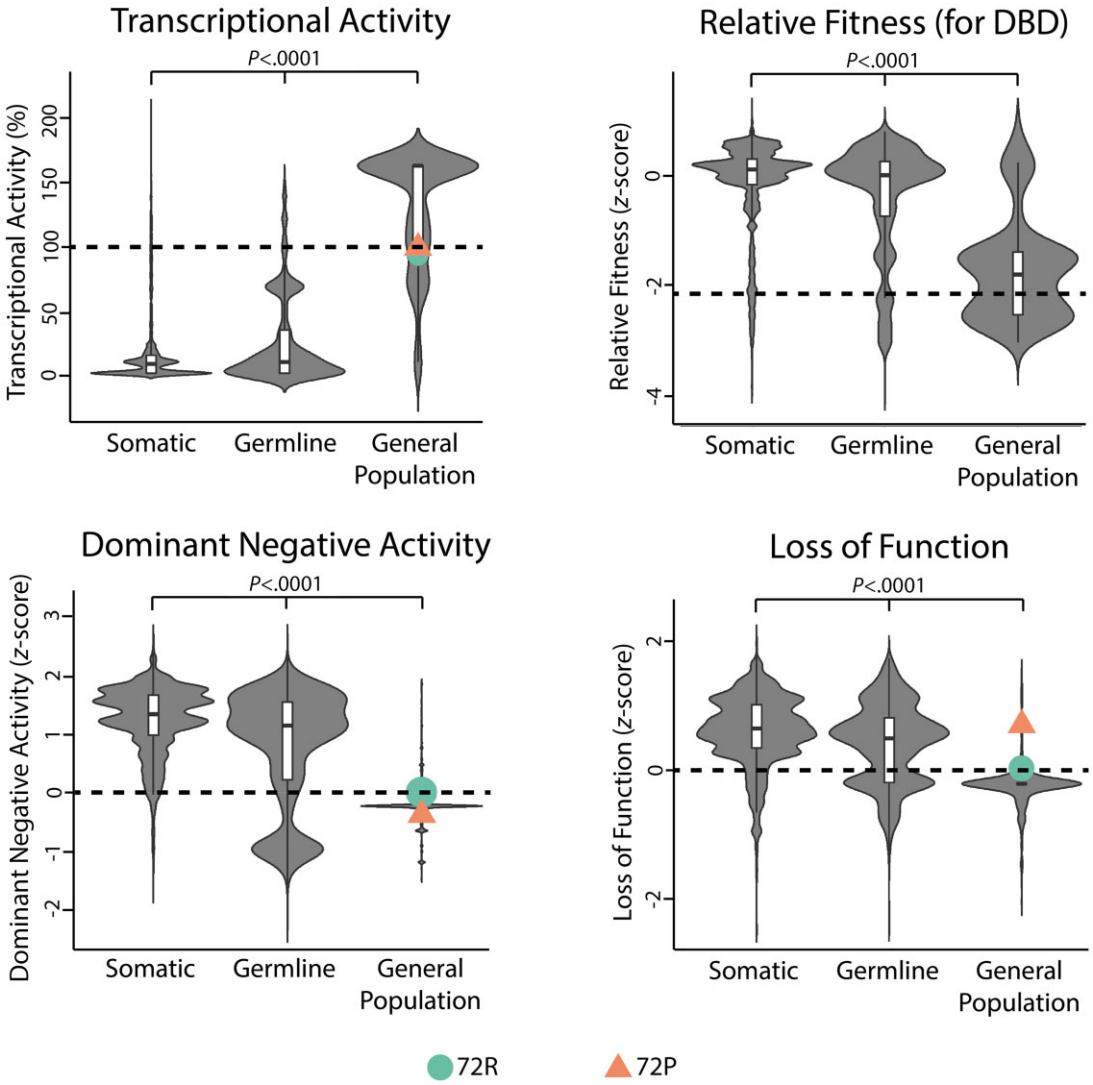

Figure 2. The functional diversity of *TP53* mutations in cancer and in variants found in the general population without cancer history. Violin plots with overlaid box plots display the functional distributions of the variants observed in Figure 1, B in terms of transcriptional activity (ability to transactivate p53-responsive promoter elements) (17), dominant-negative activity (ability to reduce the sensitivity of wild-type p53 to nutlin-3 inhibition) (13), loss-of-function (loss of sensitivity to nutlin-3 inhibition) (13), and relative fitness (selective advantage in a competitive growth culture; only measured for alterations in the DNA-binding domain [DBD]) (14). Each data point represents a different variant and its frequency within the population. Values of wild-type p53 are shown as horizontal dashed lines. Carriers of the predominant single nucleotide polymorphisms at codon 72 (72P and 72R; both considered wild-type alleles) were excluded from the plots but represented as colored symbols. *P*-values were obtained using the Kruskal-Wallis test.

population that are difficult to identify through family-based association studies and thus may also be underrepresented in The *TP53* Database. Overall, the inherent sampling biases of these databases emphasize a knowledge gap with respect to low-frequency, low-penetrance, and adult-onset variants that are overlooked in the current classification system. This issue prompted our further analysis to compare and contrast the functionality of variants identified in The *TP53* Database relative to the gnomAD dataset.

Functional studies involving large p53 mutant libraries have uncovered a broad spectrum of consequences that accompany this wide mutational landscape (13,14,17). These functional consequences range from the retention of wild-type-like behavior to a complete loss of tumor suppression to the gain of aggressive oncogenic functions (18). Moreover, certain p53 mutants can impart a dominant negative (DN) effect to inactivate wild-type p53 through the formation of mixed mutant and wild-type heterotetramers via interactions through the oligomerization domain (19). This information is relevant because most individuals with a germline *TP53* mutation are heterozygous carriers. In Figure 2, we combined 4 comprehensive functional datasets with clinical genomics datasets to display the wide-ranging impact of p53 variants found in association with cancer (*TP53* Database) or in the general population (gnomAD). These functional assessments included transcriptional activity (TA) (17), DN activity (13), loss-of-function (LOF) (13), and relative fitness (14). An inverse gradient is observed when comparing the functionality of tumor-associated variants with those occurring in the general population without cancer history. Importantly, a limited number of variants found in the gnomAD noncancer dataset have functional impairment equivalent to mutations linked to cancer, despite the elimination of individuals with cancer history in the dataset. Cancer-associated variants present in gnomAD are more likely to be variants that predispose to reduced penetrance or adult-onset cancers; moreover, they could interact with additional genetic and environmental modifying factors that can

influence variant pathogenicity and cancer susceptibility. The presence of potentially pathogenic *TP53* mutations in the general population raises questions around the different cancer risk levels associated with individual variants.

Pathogenic germline *TP53* mutations, LFS, and a spectrum of cancer susceptibility

A diagnosis of LFS is determined by an evolving set of clinical classification criteria based on personal and family histories of cancer (20). The increasingly routine sequencing of *TP53* in the clinic has aided the identification of *TP53* variants associated with cancer. It has also led to the identification of carriers of germline variants that do not present classical LFS phenotypes. Previously, updated clinical definitions were made to include patients who do not meet the strict criteria of classic LFS and to encompass the different clinical presentations associated with *TP53* mutations, referred to as the Chompret criteria (21). More recently, LFS was redefined as a spectrum disorder to reflect the high clinical variability based on accumulating evidence from genotype–phenotype association studies (20). Such studies have highlighted the differential impact of distinct features or classes of mutations in germline carriers and demonstrate the wide clinical spectrum that includes high- and low-penetrance alleles with childhood- and adult-onset cancers (21-25). For example, the degree to which germline variants affect the oligomeric stability of p53 was found to influence cancer penetrance and overall cancer survival status, where mutations leading to a complete disruption of p53 oligomerization (monomeric) were associated with more severe outcomes (23,26). It is therefore reasonable to assume that a cancer risk continuum extends beyond the LFS spectrum to low- and incomplete-penetrance variants that may be found in the general population.

Population prevalence of pathogenic germline TP53 variants

Population prevalence estimates of pathogenic germline *TP53* mutations remain a major topic of interest. Early estimates were based on observational studies of individuals selected based on personal or familial cancer history, which ranged from 1 carrier in 5000 (27) to 1 in 20 000 individuals (28). A recent estimate used population sequencing data from the gnomAD database and a bioinformatic algorithm to predict pathogenic variants in 63 986 unrelated individuals without cancer history. The authors provided a conservative prevalence estimate of pathogenic and likely pathogenic *TP53* variants in the range of 1 in 3555 to 5476 individuals, and, using a less stringent classification of pathogenicity inclusion criteria, this estimate increased to 1 in 400 to 865 individuals (9). This study underscores the importance of variant interpretation on the accuracy of prevalence estimates with clinical significance. Defining pathogenicity has major implications for cancer risk with considerable psychological stress and financial burden associated with the diagnosis of a pathogenic variant and cancer surveillance (29). For these reasons, variants are thoroughly evaluated to avoid misclassification. *TP53* mutagenesis studies serve as important clinical tools for the assessment of variant pathogenicity. However, most of the research has been conducted with the hotspot and highly pathogenic variants. As a result, lower frequency and often lower penetrance variants are overlooked in the current classification system. Moreover, functional studies often use artificial scenarios of overexpression and transient expression in tumor cell lines and yeast cells. Considering the advancements in CRISPR technology, functional studies using CRISPR-based knock-in models should be used to clarify the contribution of rare variants to cancer under more physiologically relevant conditions.

Prevalence of pathogenic variants across ethnicities

It was previously reported that, using the Exome Aggregation Consortium database, pathogenic *TP53* variants are reported more frequently in the European descent population than other ancestries combined (9). Using the gnomAD database, a larger and updated version of Exome Aggregation Consortium, we find comparable differences in prevalence rates of *TP53* pathogenic/likely pathogenic (P/LP) variants between ethnicities (European [28/77 165], African [9/20 744], East Asian [4/9977], South Asian [1/15 308], Other [7/26 519]; $P = .3176$, χ^2 test) or when pooled (European [28/77 165] vs non-European [21/72 548]; $P = .4765$, Fisher exact test). In our analysis, *TP53* pathogenic variants are observed most frequently in individuals of Ashkenazi Jewish descent (4/5185; 0.08%). Populations of Non-Finnish European individuals (29/64 603; 0.04%), African persons (9/20 744; 0.04%), and East Asian people (4/9977; 0.04%) have similar prevalence rates, followed by Finnish people (2/12 562; 0.02%) and Latino/admixed American persons (2/17 720; 0.01%). South Asian people have the lowest prevalence rate (1/15 308; 0.006%). These prevalence rates vary widely, and considerably larger population sample sizes will be required to determine statistically meaningful interpretations, particularly to confirm the higher prevalence in the Ashkenazi Jewish population and the considerably lower prevalence observed in South Asian people.

In The *TP53* Database's repository of germline variants identified in affected individuals, patient ethnicity is not reported. However, mutational landscapes can be compared based on world regions for some insight. By delineating variants in this way, we observe overall similar pathogenic hotspot mutation rates (which occur more frequently in the DBD) across these geographical populations, except for African and Latino populations, where data are particularly lacking (Figure 3). Notably, mutations at residues R273 and R248 are common in the 3 largest population cohorts (European, East Asian, and South Asian), and the common hotspot at residue R175 is reported less frequently in the South Asian population.

Emerging ethnic-specific *TP53* variant landscapes

The majority of genomics data has originated from individuals of European descent. However, mounting efforts to study population genetics in different ethnicities are now providing diversified datasets where *TP53* mutation patterns can be examined. Some variants in the *TP53* gene are found to be enriched in specific ethnic groups. In some cases, this enrichment has been suggested to be due to the adaptation of selective functionality, although the biological relevance of most such variants and their impact on cancer susceptibility remain unclear (16). Given the recent evidence that *TP53* mutation frequencies in cancer are influenced by immune selective pressure (15), it will be important to explore whether the action of similar processes shaped by HLA subtypes can explain the differing variant landscapes across ethnicities (Figure 3). In this section, we outline the distribution of *TP53* variants in various ethnicities and highlight those of interest.

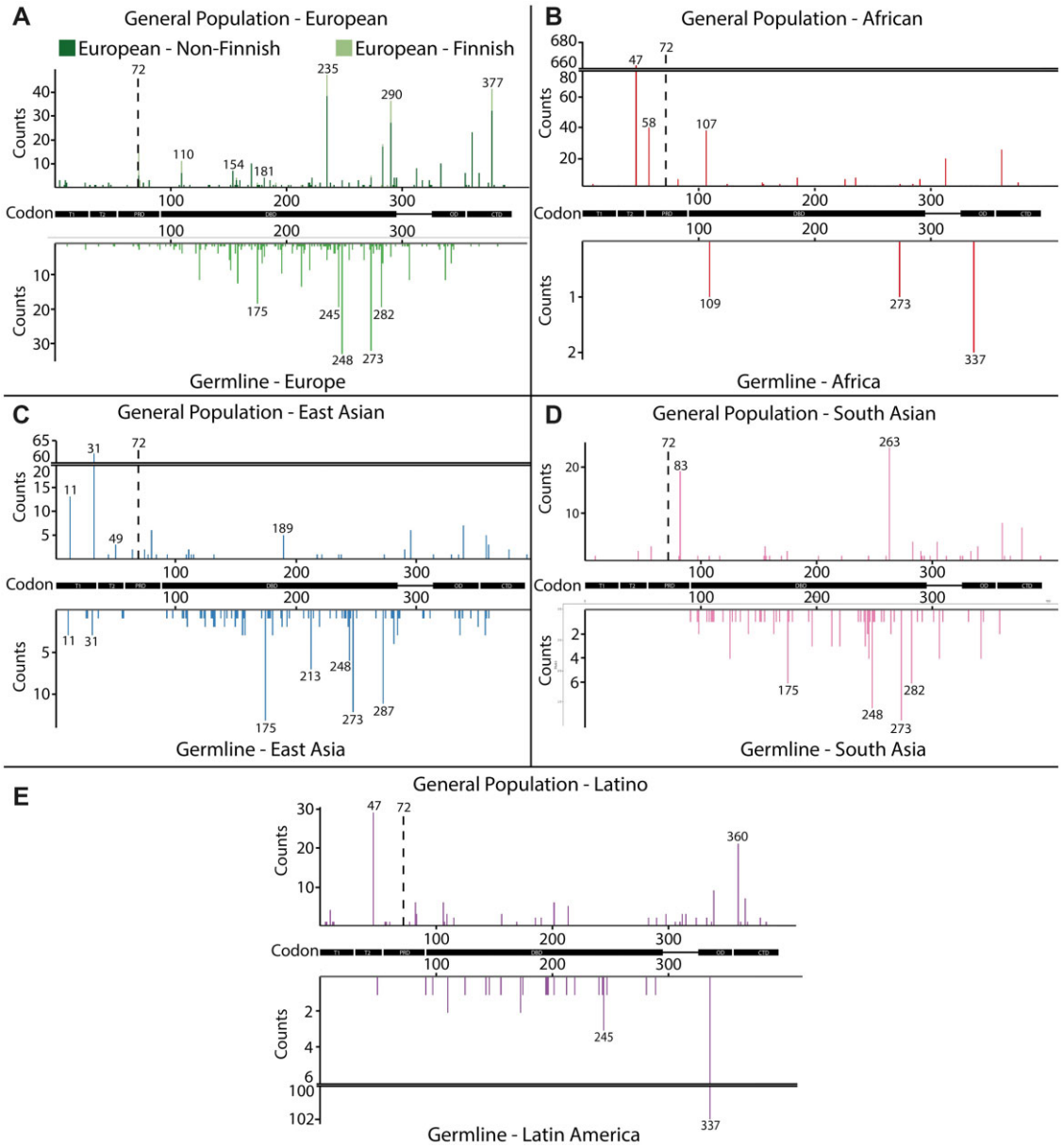

Figure 3. The landscapes of *TP53* exonic variants across different ethnic groups and world regions. The top upwards-facing histograms compare *TP53* variant allele counts in 20 744 Africans, 456 Amish, 5185 Ashkenazi Jewish, 9977 East Asians, 64 603 European non-Finnish, 12 562 Finnish, 17 720 Latino/admixed Americans, 158 Middle Eastern, 15 308 South Asians, and 3614 Others (data from the noncancer dataset of gnomAD version 3 for Africans and version 2 for the rest). The down-facing histograms below compare verified pathogenic germline *TP53* mutations within populations of the indicated world regions (data from The *TP53* Database).

P72R *TP53* polymorphism

The single nucleotide polymorphism (SNP) that results in a change from the proline ancestral allele (P72) to arginine (R72) is thought to have occurred during the human migration out of Africa (30). Now, R72 is common in all world populations, although its frequency shows considerable ethnic bias. P72 is the predominant allele in African populations today, and only 7.5% of the population is homozygous for the R72 allele. In contrast, homozygous carriers of R72 represent 27% of European-descent populations. Interestingly, the R72 allele is more common in populations of increasing latitude and colder climates (30,31). It has been suggested that this polymorphism may have provided a selective advantage for environmental adaptations such as famine and cold exposure because the R72 allele results in a higher rate of obesity in mice fed a high-fat diet (32). Although R72 has been shown to affect metabolism and cell fate decisions between growth arrest and cell death (33,34), numerous cancer association studies investigating this SNP have produced inconsistent and conflicting conclusions (35). Furthermore, genome-wide association studies have not established an association between R72 or P72 (rs1042522) and cancer risk (https://www.ebi.ac.uk/gwas/variants/rs1042522). Thus, the lack of a consensus on the most studied *TP53* SNP suggests that its impact on cancer risk is likely modest.

TP53 variant landscape in Europeans

The European cohorts contain the largest collections of variant data among all ethnicities, providing the greatest resolution of population variant frequencies. A wide range of rare and infrequent variants span the *TP53* coding region in the European-

descent population with and without cancer history (Figure 3, A). Some variants are observed at relatively higher frequencies in the European gnomAD noncancer dataset, such as N235S (allele frequency [AF] = 0.03%, allele count [AC] = 47), T377P (AF = 0.03%, AC = 41), and R290H (AF = 0.02%, AC = 36), although these are not unique to the European population and appear in various other ethnicities. Among these, N235S and R290H are found in The *TP53* Database, linking them to individuals affected by cancer. Still, they are both considered benign by ClinVar, and functional studies report no considerable loss of activity (Table 1). Some rare variants are found exclusively in non-Finnish European individuals, including G154S (AF = 0.005%, AC = 6) and R181H (AF = 0.003%, AC = 4). The apparent specificity to Europeans may be attributed to the increased resolution provided by the largest sample size, although G154S is markedly enriched in Estonian persons (AF = 0.1%, AC = 5) as well as T377P in Bulgarian people (AF = 0.3%, AC = 7). Nonetheless, the presence of R181H with 4 counts in the gnomAD noncancer dataset is surprising because most reports suggest it is P/LP, indicating that it could act as a low-penetrance or late-onset allele. Indeed, R181H as well as G154S exhibit reduced TA and moderate DN activity (Table 1). Currently 19 carriers of R181H with various tumor types are documented in The *TP53* Database from 9 separate families. Interestingly, the median age at first tumor onset among these R181H carriers was 40.5 years (n = 10), which supports the notion that it may predispose to adult-onset tumors.

Unexpectedly, we identified R110C as a variant that is present only in the Finnish population (AF = 0.02%, AC = 5). This variant is reported just once in the literature by Kharaziha et al. (36) in a Swedish patient from a family with hereditary breast cancer. The authors found 1 other count of this variant at that time in a Finnish individual in gnomAD. After a functional evaluation using 2 different p53-null cell lines, Kharaziha et al. concluded that R110C is partially functional, in agreement with previous measurements of R110C in p53 functional mutagenesis studies (Table 1). Their characterization of R110C suggested that it may confer a milder phenotype of adult-onset breast cancer and should be classified as LP. However, it remains classified in ClinVar as a VUS because more clinical evidence is required to verify this assessment by demonstrating causality. R110C is not in the germline dataset of The *TP53* Database, although it has been identified in the context of a sporadically occurring somatic variant in 13 separate tumor samples (germline testing not conducted). Coincidentally, 5 of 13 of these tumor samples were acquired from Finland (ethnicities unknown). Ideally, a cancer association study could confirm the prevalence and pathogenicity of this Finnish variant. However, given its rarity, a large-scale clinical study is likely not feasible. In such cases, CRISPR knock-in studies may provide important new insights into the contribution of rare germline-encoded *TP53* variants to cellular transformation and cancer risk.

P47S and the African *TP53* variant landscape

A recent update to gnomAD with the substantial addition of samples from individuals of African descent (12 487-20 744 samples) allows for the deeper interrogation of low-frequency variants in this population. The *TP53* variant landscape in the African population is distinct from other ethnic-specific landscapes (Figure 3, B), consisting of several missense variants that are found to be enriched (Table 1). Among these, P47S was the first African-specific variant to be described (37) that is present in 1.6% of African Americans (gnomAD), with higher frequencies in certain African populations. Early work with this variant reported only subtle defects in p53 function. However, more recent investigations, including the generation of a mouse model, show that the P47S variant is associated with a mild impairment of apoptosis following DNA damage and an increased cancer risk in mice and humans (38,39). Specifically, the P47S variant has been linked to premenopausal breast cancer in African American women (39), and P47S mice are predisposed to developing spontaneous cancers, particularly hepatocellular carcinoma (38). A follow-up study will be necessary to verify whether this SNP contributes to increased cancer risk in individuals of African descent because the results were marginally statistically significant. A large population-based study will be required to provide sufficient power to determine the cancer risk associated with an allele of low frequency. Mechanistically, the phosphorylation of p53 at serine 46 promotes the transcription of proapoptotic target genes. This phosphorylation event is reduced in the context of a P47S mutation and may explain the reduction in apoptosis (40). Although P47S tumor cells display decreased sensitivity to some chemotherapeutics, cisplatin and BET (bromodomain and extra-terminal motif) inhibitors result in superior efficacy in treating P47S tumors, demonstrating how therapy can be tailored based on *TP53* genotype (41).

The P47S variant also has a marked defect in its ability to induce ferroptosis, an iron-mediated form of cell death. This mechanism of apoptosis results in the accumulation of iron in mouse and human cells and is associated with "iron overload" disorder in individuals of African descent (42). Interestingly, this ferroptotic defect causes high iron content in murine macrophages that alters their cytokine profiles, which leads to protection against the malaria toxin hemozoin. The altered macrophage phenotype may confer a survival advantage because the P47S variant is most prevalent in malaria-endemic regions of Africa (42). Another interesting observation from P47S mice is the increased glutathione accumulation that causes elevated mTOR (mammalian target of rapamycin) activity and oxidative metabolism (43). Mice carrying P47S have improved metabolic efficiency and are larger in size, again suggesting that this variant may have been a selective advantage (43). Our evaluation of gnomAD identified another rare African-specific VUS at codon 47, P47T (AF = 0.01%, AC = 4), which has near wild-type function from previous assessments (Figure 2; Table 1). Because serine (S) and threonine (T) both represent mutations involving the replacement of the imino side chain of proline with a hydroxyl side chain at position 47, one should investigate the effect of P47T on serine 46 phosphorylation, ferroptosis, and metabolism.

Other African-centric variants have been described, including Y107H (AF = 0.09%, AC = 38) and P58R (AF = 0.1%, AC = 40). P58R has conflicting interpretations of pathogenicity (although most suggest it is likely benign in ClinVar), and it exhibits wild-type activity in functional assays. Y107H appears in 3 unrelated individuals in The *TP53* Database (country of origin or ethnicity unknown) and is partially functional, having reduced TA (Table 1) particularly at the *MDM2* gene promoter element, where it exhibited only 35% residual TA compared with wild-type p53 (17). However, it is currently considered benign (ClinVar) because it does not have strong evidence for causality and is observed in multiple women older than 70 years who have never had cancer (FLOSSIES database). Overall, there are few documented cases and little is known about cancer-associated *TP53* germline mutations and LFS in the African population (44) (Figure 3).

Table 1. *TP53* variants enriched in different ethnic populations

Variant	rsIDs	Ethnicity[a]	Counts/ total counts[a]	Population prevalence (%)[a]	ClinVar clinical significance	TA (Median[b]) (17)[c]	LOF (z score) (13)[d]	DN (z score) (13)[e]	RFS (z score) (14)[f]
G154S	rs137852789	European non-Finnish	6/6	0.005	Uncertain significance	22.8	0.01	0.77	−2.46
R181H	rs397514495	European non-Finnish	4/4	0.003	Conflicting interpretations	42.15	−0.25	0.91	−2.11
N235S	rs144340710	European (including Finnish)	47/52	0.03	Benign	123.2	−0.10	0.17	−2.63
R290H	rs55819519	European (including Finnish)	36/42	0.02	Benign	140.9	−0.34	0.47	−1.54
T377P	rs774269719	European (including Finnish)	41/59	0.03	Uncertain significance	75.65	−0.97	−0.65	N/A
R110C	rs587781371	Finnish	5/5	0.02	Uncertain significance	19.35	−0.86	0.42	−1.84
PAS	rs78378222	European (including Finnish)	1033/1170	1.4	Conflicting interpretations	N/A	N/A	N/A	N/A
G334R	rs730882028	Ashkenazi Jewish	1/1	0.03	Uncertain significance	86.55	0.02	−0.88	N/A
P47S	rs1800371[T]	African	666/695	1.6	Benign	165.45	−0.23	−0.23	N/A
P47T	rs1800371[A]	African	4/4	0.01	Conflicting interpretations	165	−0.62	−0.01	N/A
P58R	rs144386518	African	40/41	0.1	Conflicting interpretations	109.2	0.04	−0.64	N/A
Y107H	rs368771578	African	38/40	0.09	Benign	64.95	−0.11	0.10	−1.37
E11Q	rs201382018	East Asian	13/14	0.07	Conflicting interpretations	128.9	−0.60	−0.91	N/A
V31I	rs201753350	East Asian	63/64	0.3	Conflicting interpretations	108.75	−0.52	−0.27	N/A
D49H	rs587780728	East Asian	1/1	0.006	Likely benign	18.6	0.57	−0.35	N/A
A189V	rs121912665	Korean	5/6	0.1	Conflicting interpretations	83.5	−0.98	0.04	−2.68
A83V	rs201717599	South Asian	19/35	0.06	Conflicting interpretations	100.5	−0.55	−0.13	N/A
N263D	rs72661119	South Asian	24/27	0.08	Conflicting interpretations	65.3	−0.37	0.57	−1.77
G360A	rs35993958	Latino/admixed American	18/53	0.05	Likely benign	74.95	−0.41	−1.19	N/A

[a] Data from the gnomAD noncancer dataset (version 3 for Africans and version 2 for other ethnicities) mapped against the canonical transcript.
[b] Median measurement across 8 different p53 promoter elements.
[c] Transcriptional activity.
[d] Loss-of-function.
[e] Dominant negative activity.
[f] Relative fitness score.

TP53 variant landscape in East Asian populations

Several variants have been found specifically in Asian populations (45). V31I stands out as the most predominant variant in the East Asian population (AF = 0.3%, AC = 63), followed by E11Q (AF = 0.07%, AC = 13) (Figure 3, C). Both variants have conflicting interpretations of pathogenicity in ClinVar, although the majority of reports on V31I support that it is likely benign. V31I and E11Q are found in individuals and families affected by cancer in The *TP53* Database, although there is insufficient evidence to classify them as pathogenic. In fact, these variants behave similarly to wild-type p53 in most functional studies (Table 1). Still, in a series of 89 Korean LFS patients, 2 carried a copy of V31I, and the authors of this study noted that it was identified in patients with adult-onset tumors (46). In a larger cohort of 1685 Japanese patients with cancer from Project HOPE, the prevalence of V31I was 1.7% (29/1685), which is consistent with the expected prevalence in the Japanese population based on the gnomAD noncancer dataset, suggesting it is benign (AF = 1.7%, AC = 2) (47). In contrast, E11Q and D49H had higher incidences in this cancer cohort (1.0% and 0.06%, respectively) compared with the general East Asian population (0.07% and 0.006%, respectively), and the authors postulated that D49H is a low-penetrance variant. Of the 6 Japanese patients with cancer identified with germline D49H variants, 1 carrier and their family fulfilled Chompret criteria for the diagnosis of LFS, although this pedigree also carried a second *TP53* variant A159V. More recently, another report of a family affected by LFS carrying D49H supported the pathogenicity of this variant, and the family is catalogued in The *TP53* Database (48). Structurally, D49 is located in transactivation domain 2 and is involved in a salt bridge with an arginine residue in the CRB, a p53 transcriptional coactivator (49). A change from a negatively charged aspartic acid (D) side chain to a positively charged histidine (H) would be expected to diminish or abolish this interaction. Based on a yeast transcriptional reporter assay, D49H only has 18.6% TA compared with wild-type p53 (Table 1). Overall, the accumulation of D49H in a cancer dataset, and its functional impairment is suggestive of a low-penetrance variant; however, the current clinical evidence is lacking.

Last, a partially functional variant A189V that is enriched in Korean people (AF = 0.1%, AC = 5) was reported in a Korean family with a history of late-onset cancers (Table 1) (50). The pedigree had incomplete LFS features, but the proband's breast tumor underwent loss of heterozygosity to gain another mutant allele, which is a defining feature of LFS tumor evolution (51). Additionally, A189V was linked to late-onset cancer in a separate case report, where the proband manifested 6 primary colon tumors followed by a lung squamous cell carcinoma despite being a nonsmoker (52). In general, rare variants having potentially lower penetrance and milder phenotypes with adult-onset are difficult to assess. Population genetics datasets are not yet large enough to establish the risk associated with infrequent variants. In addition, population-based cancer association studies may not be feasible. As such, CRISPR knock-in approaches could be used to clarify their contribution to cancer risk.

TP53 variant landscape in South Asian populations

Two variants have emerged at relatively higher frequencies in the South Asian population, N263D and A83V (Figure 3, D, Table 1). The gnomAD general population has 27 counts of N263D: 24 originating from the South Asian population (AF = 0.08%) and 3 counts in "Other" unidentified ancestry. A83V has 35 counts in gnomAD and is enriched in the South Asian population (AF = 0.06%, AC = 19). Both variants have conflicting reports of pathogenicity based on ClinVar assessments. We examined the functional experiments conducted with these variants and found that both retain near wild-type properties that suggest they are both likely benign. However, N263D has partial TA with remarkably low TA of the MDM2 promoter (1.4%) (17) and exhibits some DN tendency (13). The possible effects of intracellular p53 accumulation from reduced MDM2 transactivation should be investigated to determine any clinical significance.

TP53 variant landscape in Latino/Admixed American populations

There are no variants found specifically in the Latino/admixed American population, likely because of the mixed ancestries in this population. The African-centric variant P47S is the most prominent variant present in the Latino/admixed American population (AF = 0.08%, AC = 29), followed by G360A (AF = 0.05%, AC = 18), which retains near wild-type function and is considered likely benign (ClinVar) (Figure 3, E; Table 1).

R337H: the pH-dependent "Brazilian mutation"

The R337H mutation was identified in association with a high incidence of adrenocortical carcinomas in populations in Brazil (53). A large population of R337H carriers has been found in southern Brazil with a population frequency of 0.3%, an alarmingly high frequency that arose from a genetic founder effect of Caucasian origin (European/Portuguese-Iberic) (54,55). As a result, R337H is one of the most commonly reported germline *TP53* variants to date. Codon 337 is located in the oligomerization domain of the p53 protein, which participates in a salt bridge with aspartic acid 352 at the dimer–dimer interface to stabilize the domain (56). Structural studies demonstrate that the oligomeric stability of R337H is pH dependent (56). In contrast to other common LOF alleles, R337H often exhibits wild-type–like activity *in vitro*, although its unique pH-dependent stability has been shown to affect its TA (57).

R337H is considered a reduced-penetrance allele (58). However, studies of R337H carriers show variable patterns of individual and familial risk, ranging from aggressive phenotypes that meet LFS criteria to asymptomatic carriers with no cancer history that were detected by population screening (20). Recently, a nonsense variant in the putative tumor suppressor *XAF1* (E134*; rs146752602) was discovered in a subset of R337H carriers that are associated with more aggressive cancer phenotypes compared with carriers of either variant alone (58). Normally, wild-type *XAF1* promotes p53-mediated apoptosis. However, *XAF1*-E134* is an inactive variant that can lead to a more aggressive cancer phenotype in carriers of a *TP53*-R337H mutation. Importantly, a promoter-reporter assay demonstrated that *XAF1*, but not *XAF1*-134*, could stimulate the transactivation function of wild-type p53 and hypomorphic (partially functional) *TP53* variants in addition to R337H, including G334R, T125M, R175L, and R290H (58). Conversely, *XAF1* could not rescue the transactivation ability of transcriptionally inactive p53 variants such as R175H. Therefore, it was proposed that *XAF1*-E134* acts as a modifier for carriers of hypomorphic variants (58). In this first report, the compound haplotype (*XAF1*-134* and *TP53*-R337H) was identified in 2 Spanish families and a subset of Brazilian carriers, but not in the French or Portuguese families studied. In the gnomAD noncancer dataset, we found that the frequency of the *XAF1*-134* variant ranges widely between ethnic groups, being highest in Amish people (6.6%; 61/912 counts) and European persons (non-Finnish) (0.59%; 380/64 812 counts), lowest in African individuals (0.085%; 35/41 118 counts), and is not present in individuals of East Asian (0 of 4956 counts), Middle Eastern (0/304 counts), or Ashkenazi Jewish (0/3300 counts) descent. Further investigations will be necessary to determine the extent to which *XAF1*-134* can alter cancer susceptibility of other hypomorphic *TP53* variants and to establish whether the frequency of compound haplotypes vary across ethnicities.

The generation of a knock-in *TP53*-R334H mouse model (the human R337H homolog) has also provided important insight into the understanding of this variant. Two separate groups have generated this mouse model. First, Park et al. (59) reported no statistically significant impact on tumor development or lifespan, although they found increased susceptibility to liver tumorigenesis in R334H mice treated with the genotoxic chemical diethylnitrosamine. Using a considerably larger cohort of animals in an updated trial, Jeffers et al. observed incomplete penetrance and the development of tumors with long latency in R334H homozygous mice, which is consistent with many human carriers (60). The high incidence of pediatric adrenocortical carcinomas and early-onset breast cancers in association with human R337H carriers is not recapitulated in this mouse model. These studies demonstrate a low but elevated cancer risk for R337H carriers and suggest that additional genetic alterations may be involved in driving tissue-specific tumorigenesis and the aggressive phenotypes observed in LFS. Further research analyzing the effects of modifier genes such as *XAF1*-E143* in this mouse model is anticipated.

G334R: an Ashkenazi Jewish-predominant mutation

G334R is a rare *TP53* variant that was recently linked to individuals with Ashkenazi Jewish descent and found to cause low cancer penetrance (61). Although it is not identified in the gnomAD noncancer dataset version 2 that contains more Ashkenazi Jewish samples (n = 5185), 1 count was identified in the smaller dataset of genomes in version 3 (n = 1736; AF = 0.03%). This variant is also located in the oligomerization domain, structurally serving as a hinge residue in the short hairpin loop that connects the β sheet and α-helix. As a result, G334R exhibits slightly reduced stability. Functionally, this variant displays mild defects in p53 function in H1299 lung cancer cells (23,61), although it maintained near wild-type transcriptional activity in a yeast-based assay (17) and no apparent DN behavior or LOF in an analysis conducted with A549 lung cancer cells (13) (Table 1). Clinically, carriers have a similar incidence of pediatric adrenocortical carcinomas compared with classic LFS families, although a statistically significantly later age of onset is observed for other LFS-component tumor types (61). Overall, it is suggested that G334R leads to a lower penetrance LFS phenotype because of the mild impairment of p53 function as a result of its decreased thermal stability (61).

A SNP within the *TP53* polyadenylation signal (PAS; rs78378222) in the Icelandic population

There is increasing evidence that noncoding variants can also affect cancer susceptibility. In a genome-wide association study aimed at identifying new risk variants for cutaneous basal cell carcinoma, an SNP was identified within the 3' untranslated region of *TP53* that alters the AATAAA polyadenylation signal to AATACA, with an observed frequency of nearly 2% in the Icelandic population. This SNP (rs78378222), referred to as "PAS," impairs the proper termination and polyadenylation of the *TP53* transcript and leads to a lower cellular abundance of *TP53* mRNA. The PAS allele was also identified in non-Icelandic samples from Denmark, eastern Europe, and Spain, with frequencies that decline with each population's distance from Iceland (62). Based on the gnomAD noncancer dataset, the frequency of PAS in the European population is 1.4% (Table 1). The functional significance of this polymorphism was demonstrated by an independent group using H1299 p53-null cells (63). Cells expressing the PAS allele had reduced p53 expression and apoptosis compared with cells expressing wild-type p53. Carriers of the PAS SNP

were found to have an elevated risk for cutaneous basal cell carcinoma in addition to prostate cancer, glioma, and colorectal adenoma (62). Since its discovery in 2011, the PAS allele has been the subject of several independent studies reporting its cancer risk associations, including with glioma, neuroblastoma, and esophageal squamous cell carcinoma (64-67). Moreover, this allele was linked to cancer predisposition after being identified at a high frequency (7/129; 5.4%) in a cohort of patients diagnosed with LFS or LFS-like syndrome carrying no *TP53* germline alteration in the coding regions (68). Given the abundance of noncoding *TP53* variants in the general population (Figure 1, A) and their potential for cancer susceptibility, further interrogation of noncoding variants is warranted.

Improving *TP53* variant classifications

The first major leap toward classifying all *TP53* variants used a yeast-based transcription reporter assay (17). In this work, Kato and colleagues (17) generated a mutant p53 library using site-directed mutagenesis and measured transactivation at 8 different p53-responsive promoter elements. Two saturation mutagenesis analyses were subsequently performed more than a decade later in human cells using lentiviral delivery strategies. Specifically, Giacomelli et al. (13) reported the creation of isogenic *TP53*-wild-type and -null A549 human lung carcinoma cell lines and delivered a p53 mutant library by lentivirus. Using nutlin-3 and etoposide treatments to modulate and induce p53 activity in the isogenic cell line pair, this study provided a comprehensive dataset on mutant p53 cellular functionality including DN capability. Concomitantly, Kotler and coworkers (14) used HEK 293 T immortalized human embryonic kidney cells to measure the relative fitness of p53 variants in a competitive growth assay. Although this p53 mutant library was limited to the DBD, they performed an additional in vivo assay in mice that demonstrated a selective advantage of hotspot mutants, which the authors postulated was due to a context-dependent gain-of-function. Functional studies such as these saturation mutagenesis experiments have been important to advancing our understanding of p53; however, they often use artificial systems that can be improved. Redman-Rivera et al. (69) used a CRISPR knock-in strategy to achieve endogenous expression of hotspot mutants (R175H and R273H) under the control of the native p53 promoter in nontransformed epithelial cells and explored their gain-of-function molecular mechanisms. This type of genetic approach can provide greater insight into cellular transformation in the context of germline variants. Scalable methods will also be required to characterize the wide array of p53 variants found in humans. Currently, the vast majority of *in vitro* screens have been focused on the hotspot and highly pathogenic *TP53* variants, whereas far less is known of the impact of infrequent and hypomorphic variants. CRISPR saturation prime editing has already been applied for high-throughput variant classification of *NPC1* (Niemann-Pick disease type C) and *BRCA2* genes (70) and could be applied to *TP53* under physiologically relevant conditions and drug treatments.

In terms of clinical approaches, diagnostic tests are under development that show the potential to provide personalized cancer risk assessments. Recent investigations demonstrated that *TP53* variant carriers can be discriminated from wild-type p53 carriers by measuring functional and molecular characteristics such as p53 target gene expression patterns or DNA-binding capacity (71-73). Raad and coworkers (71) developed a blood-based assay where peripheral blood mononuclear cells are cultured, activated, treated with doxorubicin, and probed for the expression of p53 and its target genes. They discovered distinct functional profiles when comparing 8 P/LP variant carriers with 51 wild-type individuals, and, importantly, this strategy could be applied to VUS and low penetrance alleles. Such measurements could provide clinically applicable tests that inform of patient-specific cancer risk. Finally, AI-based methods have already leveraged p53-related measurements as predictive markers in different contexts (74-76). The generation of diverse large-scale datasets will fuel the use of AI-based methods as cancer predictive tools.

Conclusion: p53 on a path toward precision oncology

The broad functional and clinical gradient associated with *TP53* alterations presents a challenge for variant interpretations that have major clinical implications. Germline *TP53* variants are functionally diverse, confer highly variable predisposition to cancer, interact with modifying factors that can alter cancer susceptibility, and are found in varying frequencies across different ethnicities. Our integrative analysis combining functional and sequencing datasets suggests that a cancer risk continuum related to the *TP53* variant functional gradient may extend beyond LFS to lower-risk variants found in the general population. A growing number of studies have identified ethnic-specific *TP53* variants with unique cancer associations, placing certain populations at increased risk. However, many of these variants are rare and largely uncharacterized compared with highly pathogenic cancer hotspot variants. Currently, variant interpretation relies on functional evaluations that have been conducted in transformed cell lines or yeast with transient overexpression (13,14,17). Advances in high-throughput assays, CRISPR technology, patient-specific diagnostic tests, and AI-based methods may lead to personalized quantifiable cancer risk assessments. We envision tailored treatment and surveillance strategies based on an individual's *TP53* genotype. For example, the discovery of variant-specific sensitivities to pharmaceuticals as observed for the P47S variant can lead to more efficacious treatment strategies. Alternatively, the identification of *XAF1*-134* as a modifier of R337H (and potentially other hypomorphic variants) can lead to improved cancer risk assessments, and the specific associations between variants and cancer types can enhance screening strategies. Furthermore, the recent developments of diagnostic functional tests demonstrate the exciting potential to more accurately determine an individual's cancer risk. Overall, understanding the genetic risk factors associated with cancer susceptibility across ethnicities such as germline *TP53* variants will help to identify and better serve at-risk populations.

Data availability

No new data were generated in support of this research. The analyses underlying this article are available in the article.

Author contributions

Nicholas W. Fischer, PhD (Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing—original draft; Writing—review and editing), Yu-Heng Vivian Ma, PhD (Conceptualization; Data curation; Formal analysis; Investigation; Validation; Visualization; Writing—original draft; Writing—review and editing), Jean

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Conflicts of interest

There are no missing disclosures or conflicts of interest associated with this work.

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Please note, while this report was in review, a paper was published (77) that demonstrated the p53 variant Y107H confers increased cancer risk in mice and identified a tumor suppressive mechanism which has implications for the efficacy of immunotherapy and may be applicable to other hypomorphic variants.

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