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ARTICLE

Characteristics predicting reduced penetrance variants in the high-risk cancer predisposition gene *TP53*

Cristina Fortuno,[1] Marcy E. Richardson,[2] Tina Pesaran,[2] Kelly McGoldrick,[2] Paul A. James,[3,4] and Amanda B. Spurdle[1,5,6,*]

Summary

Disease-causing variants with penetrance that is lower than the average expected for a given gene complicate classification, even when using gene-specific guidelines. For *TP53*, a gene associated with some of the highest cancer risks, even reduced penetrance disease-predisposition variants remain clinically actionable. We conducted a review of ClinVar submissions to identify *TP53* variants flagged as having reduced penetrance by genetic testing laboratories and analyzed functional, bioinformatic, immunogenicity, frequency, and clinical features of these variants compared with standard pathogenic and benign variants. Our findings show that reported reduced penetrance *TP53* variants are more likely to exhibit intermediate functional activity in multiple assays and are predicted as deleterious with bioinformatic tools, though with lower scores than pathogenic variants. These variants also have a higher population frequency than pathogenic variants, and heterozygotes tend to manifest disease later in life, suggesting a need for refined clinical criteria to better capture attenuated Li-Fraumeni syndrome phenotypes. Finally, by applying a random forest prediction model to all *TP53* uncertain or conflicting variants in ClinVar, we identified 106 additional variants with potential reduced penetrance.

Introduction

The American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) germline variant classification guidelines are inherently dichotomous, distinguishing between benign and pathogenic variants for genes associated with Mendelian diseases.[1] For hereditary cancer predisposition genes associated with high risk of cancer, such as *BRCA1, BRCA2, CDH1, PTEN,* and *TP53,* Clinical Genome (ClinGen) Variant Curation Expert Panels (VCEPs) have helped refine these general guidelines by incorporating gene-specific considerations,[2–5] such as establishing expectations based on the standard penetrance associated with a gene of interest.

Disease-causing variants with atypical penetrance, particularly those with reduced penetrance compared with the standard penetrance expected for that gene, pose challenges in variant classification, including when following gene-specific VCEP guidelines. These challenges may include incorrect assumptions about clinical presentation, conflicting or intermediate functional results, and unusual variant frequencies in cases and controls. As a result, these variants are at risk of being classified as variants of uncertain significance (VUS) due to conflicting evidence, or perhaps misclassified as benign/likely benign due to the strong weight placed on evidence types that lack sensitivity to distinguish them as reduced penetrance, such as functional data and observation in unaffected individuals. Misclassification as benign/likely benign is particularly concerning in clinical settings, as these variants are less likely to be revisited or further investigated compared with VUS simply because of resource constraints. The problem is so significant that ClinGen has set up a Low Penetrance/Risk Allele working group to establish a standardized framework and terminology for categorizing risk alleles and low penetrance variants in any disease gene (https://www.clinicalgenome.org/site/assets/files/4531/clingenrisk_terminology_recomendations-final-02_18_20.pdf) and the Cancer Variant Interpretation Group-UK has developed a classification framework for cancer risk genes specifically.[6] While these efforts mark significant progress in the field, there is still a need for more practical evidence-based guidance.

A good example for investigating variants associated with reduced penetrance is the gene *TP53* (HGNC: 11998), in which pathogenic germline variants cause Li-Fraumeni syndrome (LFS) (MONDO:0018875), characterized by increased risk of various cancers, including the LFS core cancers such as early-onset breast cancer, brain cancer, adrenocortical carcinoma, and sarcomas.[7] *TP53* pathogenic germline variants are known to have a very strong association with high cancer risks at early ages, and estimated "standard penetrance" is high; even after strict penetrance analysis adjusting for ascertainment bias, the lifetime cancers risks associated with *TP53* variants is close to 100%.[8] As a result, carriers are

[1]Population Health Program, QIMR Berghofer, Herston, QLD 4006, Australia; [2]Ambry Genetics, Aliso Viejo, CA, USA; [3]Parkville Familial Cancer Centre, Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, VIC, Australia; [4]Sir Peter MacCallum Department of Oncology, University of Melbourne, Melbourne, VIC, Australia; [5]Faculty of Medicine, The University of Queensland, Brisbane, QLD 4006, Australia
[6]Lead contact
*Correspondence: amanda.spurdle@qimrb.edu.au
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recommended to initiate annual whole-body magnetic resonance imaging (MRI) and brain MRI from a young age.[9,10] This expected clinical presentation, in addition to abundant functional data,[11–14] and the existence of *TP53* VCEP specifications,[5] make *TP53* an ideal candidate for investigating features that can better predict variants associated with reduced or atypical cancer penetrance.

Multigene panel testing is increasing the identification of *TP53* germline variants in patients who do not meet the traditional clinical criteria for testing, i.e., Classic LFS and Chompret 2015.[7,15] This may reflect that the spectrum of LFS cancers, and/or their age at presentation, is broader than previously recognized.[16,17] However, it is also possible that the proportion of *TP53* variants exhibiting reduced penetrance (compared with the average penetrance expected from historical studies) is higher than is currently appreciated. At this time, the only firmly established reduced penetrance *TP53* variant is NM_000546.6(TP53):c.1010G>A (p.R337H), a Brazilian founder variant.[18] Although several other variants have been proposed as suspected reduced penetrance based on clinical presentation and/or functional results, their age-specific cancer risks remain unconfirmed. Accurate identification of reduced penetrance *TP53* variants, accompanied by age-specific risks, is critical to ensure appropriate patient care.[19,20]

The aim of this work was to assess the functional, bioinformatic, immunogenicity, frequency, and clinical characteristics of a subset of *TP53* pathogenic germline variants reported to exhibit reduced penetrance, to identify attributes that can distinguish them from both *TP53* pathogenic variants with standard penetrance and from well-established benign variants that are not clinically actionable. We demonstrate that this approach can yield information useful to build future variant classification models capable of separating pathogenic variants with standard penetrance from those associated with reduced penetrance.

Material and methods

Definitions

In this study, we used the following terminology to categorize variants: benign variants—unlikely to be clinically actionable for *TP53*-related diseases using current specifications; reduced penetrance pathogenic variants—disease-causing but known or suspected to exhibit lower, moderate, or atypical penetrance compared with standard pathogenic *TP53* variants; and pathogenic variants—assumed to have standard penetrance for *TP53*.

ClinVar review

To identify potential reduced penetrance *TP53* variants, in addition to information available in the literature, we downloaded ClinVar[21] submissions for *TP53* variants (as at March 14, 2025), and identified evidence summaries that included the terms "reduced," "moderate," or "lower penetrance/risk." At that time, and even at this date (May 7, 2025), there are no ClinVar submissions for *TP53* variants formally categorized as "pathogenic, low penetrance" or "established risk allele." Variants identified in this step formed our reduced penetrance variant group.

Statistical analyses

Distribution of functional results, predictive components, and allele frequency for predefined variant sets

We defined reference sets of *TP53* variants based on ClinVar classifications (as at April 24, 2024), based on VCEP or multiple nonconflicting submissions, as follows: benign reference set, 62 unique missense variants classified as benign/likely benign; and pathogenic reference set, 113 unique missense variants classified as pathogenic/likely pathogenic. Reduced penetrance variants were excluded from these reference sets and grouped separately regardless of their ClinVar classification.

We then investigated the distribution of functional, bioinformatic, immunogenicity, and frequency data for missense variants across the three groups of variants (benign and pathogenic reference sets, and the reduced penetrance variant group as defined above). For functional scores, we used data from currently available systematic assays used by the *TP53* VCEP (specifications v2.3.0), namely Kato et al.,[11] Giacomelli et al.,[12] Kotler et al.,[13] and Funk et al.[14] Score cutoffs used to assign loss of function (LoF) or noLoF were as defined by the original studies, and currently used by the *TP53* VCEP. We also analyzed the Kato data using published data that converted the results into four classes with different levels of functional disruption (A–D), using a hierarchical Ward's clustering method.[22] For bioinformatic scores, we used predictions from BayesDel[23] and aGVGD,[24,25] which are currently used by the *TP53* VCEP,[5] and for which we applied the VCEP-defined cutoffs for predicting deleteriousness (where relevant), as well as the more recent predictor AlphaMissense,[26] using the cutoffs defined in the original study. Immunogenicity scores (immune fitness) were analyzed separately, for which we used predictions derived from a mathematical framework that quantifies a variant's susceptibility to the immune system, which is proportional to its probability of being presented on HLA-I.[27] Although these scores are not currently used in *TP53* variant classification, they provide an independent type of evidence not currently considered in existing guidelines and are available for all missense variants. Additionally, we investigated the distribution of the total allele frequency across all genetic ancestry groups for all variants using gnomAD v4.1.[28] Sensitivity analyses were performed to investigate the distribution of all scores above, after excluding variants flagged as reduced penetrance by only a single diagnostic laboratory.

We conducted Kruskal-Wallis tests with post hoc pairwise comparisons using Wilcoxon tests, and chi-square tests to analyze statistical differences between all variant groups in RStudio (R version 4.4.1).

Personal cancer history analysis of clinical presentation for pathogenic vs. reduced penetrance variants

We used previously established methods[29] to conduct personal history analyses using binary logistic regression in SPSS (version 23). Proband data were derived from multigene panel testing hereditary cancer results between 2011 and 2020 from Ambry Genetics, as previously used.[30] This project was approved by the QIMR Berghofer Human Research Ethics Committee (Project 1051). After excluding individuals identified to have a *TP53* variant with variant allele fraction $\leq$35% (to remove potential mosaic or somatic cases), the Ambry dataset included 256,868 probands negative for pathogenic/likely pathogenic or uncertain

Table 1. *TP53* variants included in the reduced penetrance group

Transcript (NM_000546.6)	Protein (NP_000537.3)	ClinVar classification (as at March 14, 2025)
c.1101-1G>A	?	Pathogenic/Likely pathogenic
c.1010G>A	p.R337H	Pathogenic/Likely pathogenic
c.1000G>C	p.G334R	Uncertain significance
c.848G>A	p.R283H	Uncertain significance
c.845G>A	p.R282Q	Conflicting classifications of pathogenicity
c.799C>T	p.R267W	Pathogenic/Likely pathogenic
c.766A>G	p.T256A	Uncertain significance
c.541C>T	p.R181C	Conflicting classifications of pathogenicity
c.542G>A	p.R181H	Pathogenic/Likely pathogenic
c.467G>A	p.R156H	Uncertain significance
c.405C>G	p.C135W	Conflicting classifications of pathogenicity

variants in all genes included in multigene panel tests (as classified by Ambry Genetics), 203 probands with *TP53* reduced penetrance variants (as described above), and 505 probands with *TP53* pathogenic/likely pathogenic variants (as classified by Ambry Genetics, excluding those falling in the reduced penetrance group) (Figure S1). Of the 189 unique variants observed in the pathogenic group, 59 overlapped with the ClinVar pathogenic reference set.

For this study we conducted two independent analyses, comparing clinical presentation in the baseline group of individuals without a known *TP53* pathogenic variant to (1) features of individuals with a pathogenic variant and (2) features of individuals with a reduced penetrance variant.

The phenotypic predictors included in the analysis were the LFS core cancers across different age ranges at first diagnosis, as follows: breast cancer <31 years, breast cancer 31–35 years, breast cancer 36–60 years, brain tumor ≤45 years, brain tumor 46–60 years, sarcoma ≤45 years, sarcoma 46–60 years, and adrenocortical carcinoma ≤60 years.

Significant differences in the proportion of individuals affected, and their age at first cancer diagnosis, among the three groups were analyzed with chi-square and Kruskal-Wallis tests, respectively.

Random forest predictive model

We built a random forest classification model to categorize the missense variant class (pathogenic, benign, or reduced penetrance) based on scores from the four different functional assays (Kato, Giacomelli, Kotler, Funk), and the four computational predictors (BayesDel, AlphaMissense, aGVGD, Immune fitness) analyzed in this study, as well as the total allele frequency. The model was trained using data from the ClinVar reference set variants as well as the reduced penetrance group (excluding c.1101-1G>A), where the missing values in the Kotler and Funk scores were imputed by replacing them with the median value within each variant class. A random seed of 123 was set to ensure reproducibility. We used 500 trees in the random forest, and model performance was evaluated using a confusion matrix. The feature importance of each score was also assessed to understand which variables contributed most to the model predictions, using Mean Decrease in Gini values.

We applied this model to the remaining variants in ClinVar (as at March 14, 2025) in order to identify new potential reduced penetrance *TP53* variants based on their unique functional, predictive, and allele frequency characteristics. After excluding variants for which both Kotler and Funk scores were missing, the ClinVar classifications for these variants were as follows: 12 benign/likely benign, 26 pathogenic/likely pathogenic missense variants in ClinVar classified by single submitters only (and therefore not included in the original ClinVar reference sets), and 590 *TP53* missense variants classified as uncertain significance or with conflicting classifications in ClinVar.

Results

Suspected reduced penetrance variants

In addition to the known Brazilian founder variant NM_000546.6(TP53):c.1010G>A (p.R337H),[18] we identified 10 more variants (nine missense, one splice site) listed in ClinVar meeting our search criteria for designation as (suspected) reduced penetrance (Table 1). An additional variant, NM_000546.6(TP53):c.467G>A (p.R156H), was included due to being flagged as suspected reduced penetrance by Couch et al.[31] and Behoray et al.[32] Only four of the 11 variants were classified as pathogenic/likely pathogenic in ClinVar, one of these being the Brazilian founder variant. Rationale for inclusion of these variants, including ClinVar evidence summaries and/or literature is detailed in Table S1. Six of the identified variants (c.405C>G (p.C135W), c.542G>A (p.R181H), c.766A>G (p.T256A), c.845G>A (p.R282Q), c.848G>A (p.R283H), and c.1101-1G>A) had only been flagged as reduced penetrance by Ambry Genetics, and therefore excluded as part of the sensitivity analyses.

Comparison of functional impact for missense variants

Kato and Giacomelli assay results were available for all 185 missense variants across the three variant groups. Kotler assay data were available for 137 variants (including eight with reduced penetrance) and Funk assay data for 134 variants (including eight with reduced penetrance), due to

Table 2. Median functional scores for all variant groups and number of variants in each functional category per variant group

Variant group		Kato	Giacomelli	Kotler	Funk
Total variants assessed		185	185	137	134
Median functional score (*n* noLoF, *n* partial function, *n* LoF)	Benign	93.70 (49, 12, 1)	0.54 (61, na, 1)	−2.09 (20, na, 0)	−0.86 (18, na, 1)
	Reduced	40.30 (1, 7, 2)	0.19 (9, na, 1)	−1.82 (6, na, 2)	0.66 (2, na, 6)
	Pathogenic	6.60 (0, 4, 109)	−1.00 (9, na, 104)	0.05 (5, na, 104)	0.93 (1, na, 106)
p value[a]		1.67×10^{-28}	7.20×10^{-29}	4.51×10^{-13}	3.11×10^{-12}
Cutoff for noLoF		>75	>−0.21	≤−1	<0
Partial function range		20–75	na	na	na
Cutoff for LoF		≤20	≤−0.21	>−1	≥0

LoF, loss of function; na, not applicable.
[a]Kruskal-Wallis test comparing differences across the three groups of variants.

both of these assays being restricted to specific exons. The vast majority (>85%) of variants in the benign or pathogenic reference sets exhibited functional impact corresponding to noLoF or LoF, respectively (Table 2). Of the four functional assays, only Kato defined a score range indicating intermediate function; seven out of 10 (70%) reduced penetrance missense variants fell within this range, with LoF category assigned to two of the three remaining variants in this group. For the remaining assays, LoF was assigned for one (Giacomelli), two (Kotler), and six (Funk) of the reduced penetrance variants.

Differences in the distribution of functional assay scores across all three variant groups were highly significant ($p < 0.001$) (Table 2). Overall, the median distribution of functional assay scores for the reduced penetrance variants fell between that of benign and pathogenic variants (Figure 1). The distribution of Kato and Giacomelli scores differed highly significantly between pairwise comparisons of the variant groups ($p < 0.001$). Regarding the Kotler scores, significant differences were observed between the benign vs. pathogenic and reduced penetrance vs. pathogenic groups ($p < 0.001$), but no significant difference was found between the benign vs. reduced penetrance groups ($p > 0.05$). For the Funk assay, differences in scores were highly significant comparing benign vs. pathogenic or reduced penetrance ($p < 0.001$), with somewhat smaller differences between the reduced penetrance vs. pathogenic group ($p < 0.01$).

Significant differences in the distribution of the Kato clustering classes were observed across the three variant groups ($p = 1.41 \times 10^{-35}$). The benign group was predominantly represented by class D (42 of 62, 68%), the reduced penetrance group by class C (7 of 10, 70%), and the pathogenic group by class A (93 of 113, 82%) (Figure 2).

Predictive components

Bioinformatic analysis of missense variants

Bioinformatic predictions were available for all 185 missense variants in all three variant groups. Both the BayesDel and AlphaMissense tools performed well in predicting deleteriousness for benign and pathogenic variants, with median scores typically below or above the selected cutoffs, respectively (Table 3). All variants in the reduced penetrance and pathogenic groups were predicted as deleterious by BayesDel, while 54 of the 62 (87%) benign variants were predicted neutral. For AlphaMissense, which includes an ambiguous category, five out of 62 (8%) benign variants, seven out of 10 (70%) reduced penetrance variants, and 110 out of 113 (97%) pathogenic variants were predicted deleterious. Two variants in each group were predicted to have an intermediate effect. Only one variant in both the reduced penetrance and pathogenic groups was predicted as neutral, compared with 55 (89%) in the benign group.

Differences in the distribution of bioinformatic results were highly significant across all three variant groups ($p < 0.001$) (Table 3). Similar to observations for the functional assays, the median distribution of BayesDel and AlphaMissense scores for reduced penetrance variants appeared to be positioned between that of benign and pathogenic variants (Figure 3). For both tools, pairwise comparisons showed that the differences between reduced penetrance and pathogenic variants were not as marked ($p < 0.05$ for BayesDel and $p < 0.01$ for AlphaMissense) as for the remaining comparisons ($p < 0.001$).

The distribution of the aGVGD categories was significantly different across the three variant groups ($p = 5.31 \times 10^{-22}$). The benign group was predominantly represented by class C0 (54 of 62, 87%), the pathogenic group by class C65 (65 of 113, 57%), while the reduced penetrance group had a higher diversity of categories (Figure 4). In particular, three reduced penetrance variants were C0 (predicted neutral) and three were C65 (predicted most deleterious), while the other four fell into categories between.

Immunogenicity analysis of missense variants

Immune fitness predictions were available for all 185 missense variants across the three variant groups. While

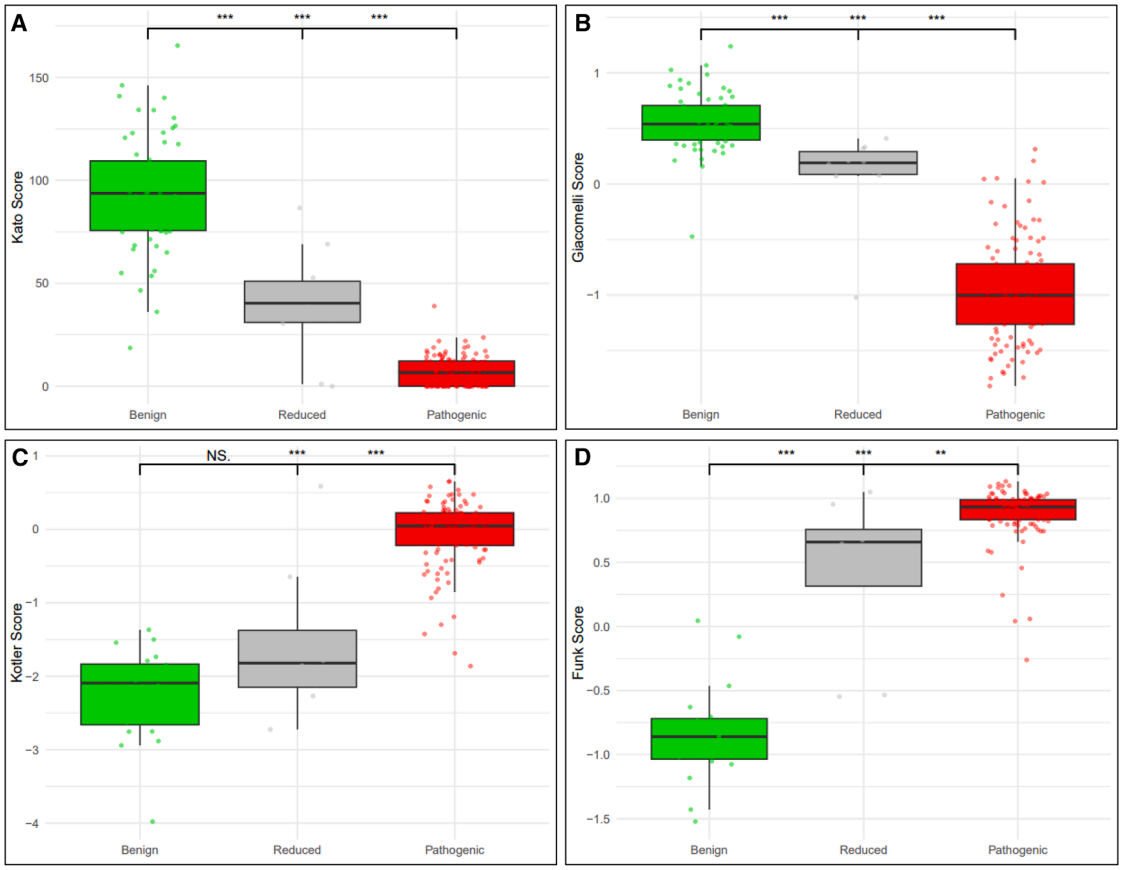

Figure 1. Distribution of functional assay scores across *TP53* benign (green), reduced penetrance (gray), and pathogenic (red) variants

(A) Kato et al.[11]; (B) Giacomelli et al.[12]; (C) Kotler et al.,[13]; and (D) Funk et al.[14] p values refer to pairwise comparisons with Wilcoxon tests, where *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Statistical comparisons noted in the center are for benign vs. pathogenic variants.

there are no published cutoffs to define different levels of immune fitness, the distribution scores differed significantly across all three variant groups ($p = 9.40 \times 10^{-21}$). Benign variants had the highest immune fitness (median −0.41), followed by reduced penetrance variants (median −0.55) and pathogenic variants (median −0.68). Pairwise comparisons showed that the differences between the reduced penetrance and pathogenic variants were somewhat less pronounced ($p < 0.01$) than the differences observed in comparisons involving benign variants (Figure 5).

Differences in allele frequency across groups

The distribution of allele frequency differed significantly across all three variant groups ($p = 3.48 \times 10^{-20}$) (Table 4). All 113 pathogenic variants met PM2_Supporting (as defined by the *TP53* VCEP specifications v2.3.0), as opposed to nine of 11 (82%) reduced penetrance variants and 40 of 62 (64%) benign variants. Of note, six of the variants in the benign group met BS1 based on the filtering allele frequency (as defined by the *TP53* VCEP specifications v2.3.0), as opposed to none in the reduced penetrance groups. As expected, benign variants exhibited the highest allele frequency, pathogenic variants the lowest, and reduced penetrance variants fell in-between (Figure 6). The differences between benign and pathogenic variants, as well as between reduced penetrance and pathogenic variants, were both highly significant ($p < 0.001$); however, the allele frequency distribution between benign and reduced penetrance variants did not differ significantly ($p > 0.05$).

Sensitivity analyses

After excluding variants with only ClinVar submissions from Ambry Genetics suggesting reduced penetrance (five missense and one splice site), the differences in the distributions of scores remained similar; the reduced penetrance variants continued to have scores distributed in the middle between benign and pathogenic variants (Figures S2–S7). Three-way distribution comparisons for all functional, predictive, and allele frequency components remained significantly different ($p < 0.01$), although overall differences in the pairwise comparisons against reduced penetrance variants became less pronounced, as expected given the smaller sample set for reduced penetrance variants.

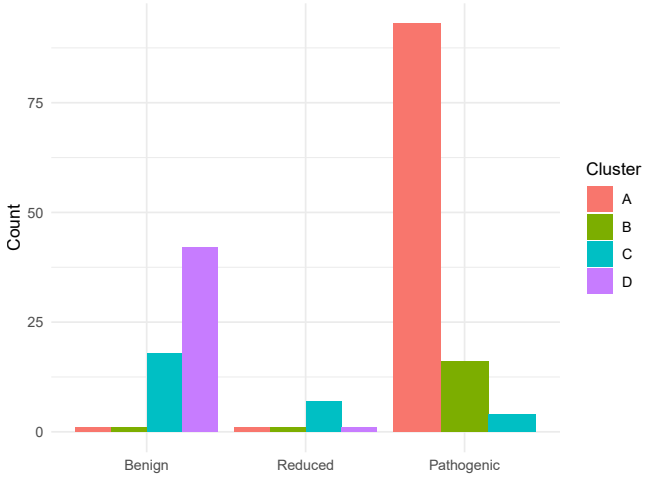

Figure 2. Distribution of Kato clustering classes across *TP53* benign, reduced penetrance, and pathogenic variants ($p = 1.41 \times 10^{-35}$)

Classes represent a gradient of yeast-based transcriptional activity, from lowest (A) to highest (D) as per Montellier et al.[22]

Phenotypic predictors associated with reduced penetrance variants

The Ambry dataset used for the personal history analysis had proband data for carriers of all reduced penetrance variants included in this study. The variants with the highest number of probands were c.542G>A (p.R181H) and c.467G>A (p.R156H), with 46 probands each, while c.405C>G (p.C135W) and c.1101-1G>A had only one proband each. The number of individuals with the remaining reduced penetrance variants ranged from two to 28. For this analysis, the control group included individuals without a known pathogenic/likely pathogenic or uncertain variant.

The proportion of individuals affected with any cancer for the control, reduced penetrance, and pathogenic groups was 40%, 43%, and 48%, respectively ($p < 0.001$), with average age at first cancer diagnosis being 50.3 years, 47.7 years, and 36.6 years, respectively ($p < 0.001$).

In the phenotypic prediction analysis, odds ratios (ORs) represent enrichment of the phenotype within the cohort of tested individuals, and not cancer risk relative to baseline population risk. The ORs indicated significant enrichment of the following phenotypes in the pathogenic variant group relative to individuals without a pathogenic variant in this cohort (Table 5): breast cancer <31 years, breast cancer 31–35 years, brain tumor ≤45 years, sarcoma ≤45 years, sarcoma 46–60 years, and adrenocortical carcinoma ≤60 years. In the reduced penetrance group, only breast cancer <31 years and sarcoma ≤45 years showed significant enrichment compared with individuals without a pathogenic variant, but with much lower ORs compared with the pathogenic variant group (OR 3.05 vs. 16.07 for breast cancer <31 years, significantly different based on the confidence intervals; OR 7.09 vs. 27.85 for sarcoma ≤45 years, not significantly different). The reduced penetrance group lacked enough data for sarcoma 46–60 years and adrenocortical carcinoma ≤60 years.

Identification of additional potential reduced penetrance *TP53* missense variants

The random forest model was trained to categorize variants into three classes (pathogenic, benign, and reduced penetrance), based on nine components (Kato, Giacomelli, Kotler, Funk, BayesDel, AlphaMissense, aGVGD, immune fitness, and total allele frequency). The model performed well in predicting benign (1.6% classification error) and pathogenic variants (0.9% classification error) but showed a higher error rate for reduced penetrance variants (30%), as shown by the confusion matrix (Table S2).

The feature importance analysis of the random forest model revealed that the Kato score was the most influential feature in categorizing variants into the three groups, with the highest Mean Decrease in Gini value of 22.2, closely followed by Funk (20.8) (Figure S8). Other influential features were Giacomelli (18.3) and Kotler (16.0). Allele frequency and Immune fitness had the lowest importance in this model, with a Mean Decrease in Gini value of 1.1.

When this model was applied to additional variants in ClinVar (that is, variants not in any of the reference groups), the model categorization agreed with classifications for all 12 benign/likely benign variants and all 36 pathogenic/likely pathogenic single submissions in ClinVar. Of the remaining 590 uncertain significance or conflicting *TP53* missense variants to which this model was applied, 106 were categorized as reduced penetrance, of which 85 were uncertain and 21 conflicting in ClinVar (Table S3). In the Ambry Genetics dataset, 121 individuals carried one of these variants. The average age at first cancer diagnosis was 44.8 years, and 45% were affected by any cancer; proportions that were not significantly different from those observed in the original reduced penetrance variant group ($p > 0.05$).

Discussion

For most cancer genes, current variant classification models are not designed to classify reduced penetrance variants. For genes like *TP53*, where pathogenic variants are linked to an extremely high cancer risk[8] and have associated intensive surveillance strategies,[33] even variants with a lower-than-average penetrance warrant clinical action. Further, for variants that are already classified as pathogenic, it is important to confirm which of these have reduced penetrance, as this could lead to different clinical management strategies for carriers, as it is the case for R337H carriers.[20]

Our study enhances understanding of the functional, bioinformatic, immunogenicity, frequency, and clinical characteristics of reported reduced penetrance cancer gene variants, using *TP53* as an example, and

Table 3. Median bioinformatic scores for all variant groups and number of variants in each bioinformatic category per variant group

Variant group		BayesDel	AlphaMissense
Total variants assessed		185	185
Median bioinformatic score (*n* predicted neutral, *n* intermediate, *n* deleterious)	Benign	−0.05 (54, na, 8)	0.08 (55, 2, 5)
	Reduced	0.47 (0, na, 10)	0.82 (1, 2, 7)
	Pathogenic	0.55 (0, na, 113)	0.99 (1, 2, 110)
p value[a]		2.45×10^{-27}	1.58×10^{-27}
Cutoff for neutral		<0.16	<0.34
Intermediate range		na	0.564–0.34
Cutoff for deleterious		≥0.16	>0.564

na, not applicable.
[a]Kruskal-Wallis test comparing differences across the three groups of variants.

demonstrates how these features can be used to provide a foundation for prediction models to better identify reduced penetrance disease-causing variants as being distinct from benign or standard penetrance pathogenic variants. Importantly, based on the distribution of these features, we developed a preliminary predictive model that identified 106 potential reduced penetrance *TP53* variants, currently in ClinVar as VUS or conflicting. These variants are provided in Table S3 as candidates for future *TP53*-specific analyses aimed at confirming their reduced penetrance, including formal penetrance studies and modeling approaches designed to assess their clinical impact. While we highlight the preliminary nature of this predictive model, based on a small reference set of reported (suspected) reduced penetrance variants, we note that the model correctly identified all variants with single submission assertions as benign/likely benign or pathogenic/likely pathogenic.

From examination of the different data types, we highlight several interesting observations that collectively suggest that existing ClinGen VCEP approaches could be extended to better identify reduced penetrance variants, by incorporating more precise functional ranges, recalibrated bioinformatic tools, and descriptions of attenuated LFS phenotypes that can better distinguish between reduced and standard penetrance variants.

First, reduced penetrance variants were more likely to exhibit intermediate functional activity, as demonstrated by four independent functional studies.[11–14] Among existing assays, only Kato explicitly includes a "partial function" category, where most reduced penetrance variants clustered in this study. While this could have served as a potential reason for them being flagged as potential reduced penetrance by the ClinVar submitters, similar distributions were also observed for the other functional assays. Notably, the Kato clustering classes,[22] offering greater granularity than the original three Kato categories, could be leveraged for improved specificity, in line with a recent cancer risk study that found that carriers of cluster B or C variants were less likely to meet clinical testing criteria for LFS.[34] Interestingly, in our random effects model all four functional assays had the highest influence to predict reduced penetrance, in comparison with predictive components and allele frequency. Ideally, new functional assays should be developed to better capture intermediate functions or alternative disease mechanisms, but our findings suggest that existing assays could be recalibrated for finer granularity to identify suspected reduced penetrance *TP53* variants. Further, current assays differ slightly in how they analyze functional impact, and our study revealed distinct differences in the distribution of results across the three variant groups, indicating that integrating multiple assays into a combined model may be more informative than relying on a single assay, as has been suggested in the context of multiplexed assays of variant effect.[35]

Second, reduced penetrance variants tended to be predicted as deleterious by multiple bioinformatics tools, although with lower scores than pathogenic variants on average. In addition, aGVGD[24,25] displayed a greater diversity of results for reduced penetrance variants compared with the other groups. Although AlphaMissense[26] currently includes a score range for intermediate function, most reduced penetrance variants were predicted deleterious following the cutoffs recommended in the original publication, suggesting that recalibration of this tool to introduce more categories could enhance its value to separate reduced penetrance from standard penetrance pathogenic variants. Similarly, recalibration to define additional categories for BayesDel[23] could also improve its utility for identifying variants with reduced penetrance. Analysis of the predicted immune fitness also showed that reduced penetrance variants have an intermediate fitness, with lower immune susceptibility than pathogenic variants, in agreement with a potential lower oncogenic potential.[27]

Third, the total allele frequency distribution of reduced penetrance variants more closely resembled that of benign variants than pathogenic variants. However, most still met the PM2_Supporting criterion, suggesting that their somewhat higher frequency would not impact the application of pathogenic clinical codes (i.e., PS4) that require PM2_Supporting to be met in order to be considered.

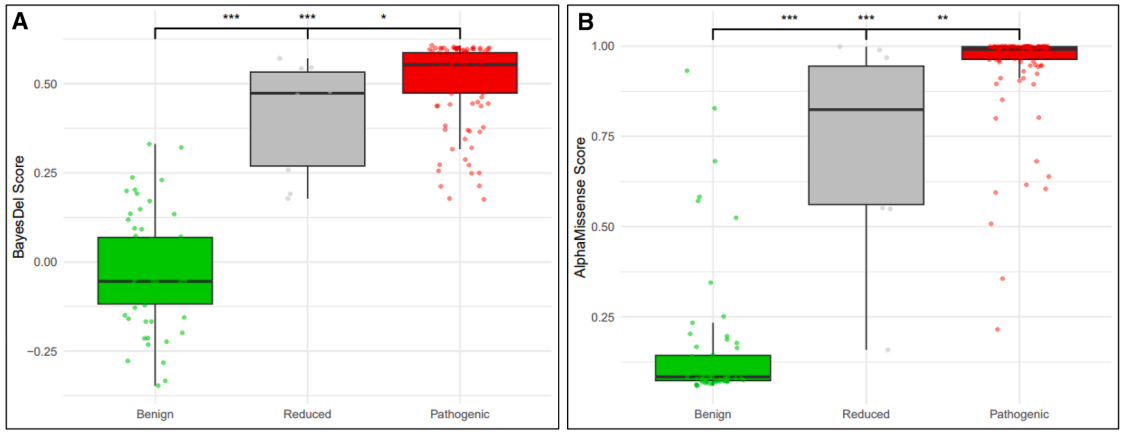

Figure 3. Distribution of bioinformatic scores across *TP53* benign (green), reduced penetrance (gray), and pathogenic (red) variants

(A) BayesDel (Feng et al.[23]); (B) AlphaMissense (Cheng et al.[26]). *p* values refer to pairwise comparisons with Wilcoxon tests, where $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. Statistical comparisons noted in the center are for benign vs. pathogenic variants.

Last, as we would expect, in comparison to standard pathogenic variants, for reduced penetrance variants we observed later average age at first cancer diagnosis, and the core LFS cancers currently used as clinical evidence of pathogenicity were not as highly associated. However, it is notable that the most characteristic *TP53*-associated cancers, particularly early-onset breast cancer and sarcomas, remained significantly associated with reduced penetrance variants, although with lower level of enrichment than for the group with standard pathogenic variants. While these results do not suggest that the identified significant phenotypes can serve as predictors of reduced penetrance variants, they do provide a way to analyze potential differences relative to standard high-penetrance pathogenic variants. This all suggests that more attenuated LFS phenotypes should be considered for application of clinical evidence in favor of pathogenicity to facilitate identification of *TP53* reduced penetrance variants.[16]

We recognize the potential for circularity in our study. Our reference sets of pathogenic and benign variants were derived from ClinVar, where some of the predictive components analyzed in this study would have contributed to their classifications. However, no single component alone would have been decisive in classifications made by expert panels or through multiple non-conflicting submissions, as these typically rely on a combination of evidence types. Additionally, we incorporated newer functional assays[14] and bioinformatic tools[26] that were

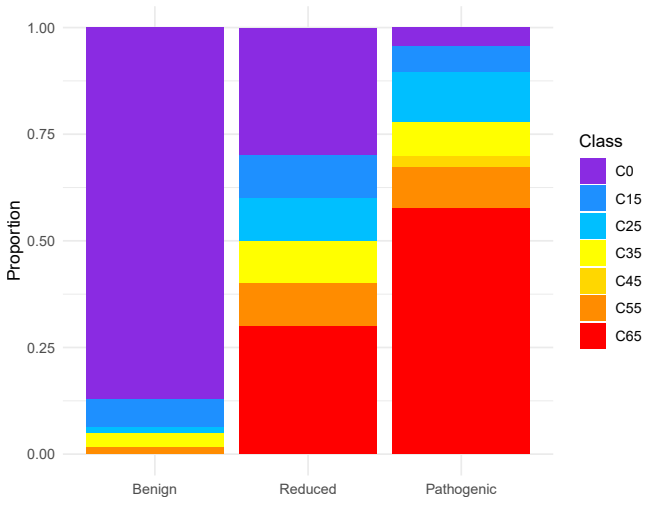

Figure 4. Distribution of aGVGD categories across *TP53* benign, reduced penetrance, and pathogenic variants ($p = 5.31 \times 10^{-22}$)

C65 is predicted most likely to interfere with function, and C0 least likely, based on the biochemical variation at each position in a multiple sequence alignment of orthologous sequences as per Tavtigian et al.[24]

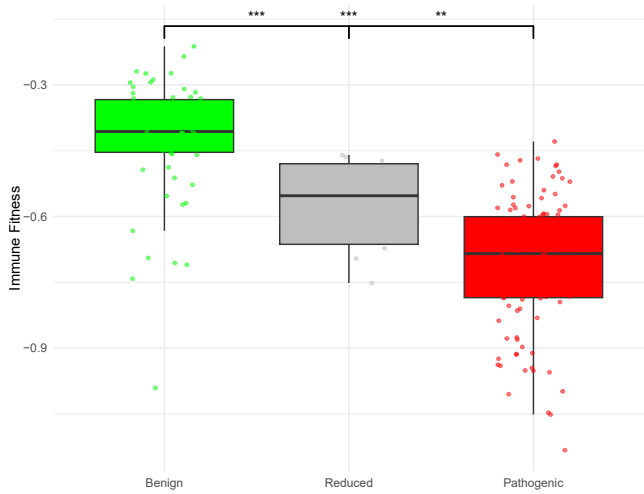

Figure 5. Distribution of immune fitness across *TP53* benign (green), reduced penetrance (gray), and pathogenic (red) variant groups (Hoyos et al.[27])

p values refer to pairwise comparisons with Wilcoxon tests, where $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. Statistical comparisons noted in the center are for benign vs. pathogenic variants.

Table 4. Total allele frequency from gnomAD v4.1 for all variant groups ($n = 186$ total variants) and number of variants meeting population frequency codes per variant group

Variant group	Allele frequency median	*N* meeting PM2_Supporting	*N* meeting any benign code (BA1 or BS1)
Benign	0.0000068	40	6 (BS1)
Reduced	0.0000019	9	0
Pathogenic	0	113	0
p value[a]	3.48×10^{-20}		
Cutoff for PM2_Supporting	Total allele frequency <0.00003 (and <0.00004 for any single genetic ancestry group with multiple alleles in non-founder populations)		
Cutoff for BS1	Filtering allele frequency in non-founder populations ≥0.0003		

[a]Kruskal-Wallis test comparing differences across the three groups of variants.

published after our reference sets were established, ensuring that these classifications were not directly influenced by these newer methodologies. In addition, as previously noted, some components used in this study, such as Kato results, may have contributed to flagging certain variants as potentially reduced penetrance. However, the similar distribution observed across other functional studies suggests that this is not solely driven by the Kato results. Suspicions of reduced penetrance are typically supported by internal clinical data from laboratories, rather than relying on functional results alone, as indicated by the evidence summaries in ClinVar (Table S1). Additionally, the fact that our model predictions aligned correctly with all single pathogenic and benign submissions in ClinVar is not unexpected, given that components of the model likely influenced the original classification of these variants. Nevertheless, this performance demonstrates the model's ability to align with these classifications and to prioritize other potential reduced penetrance variants for research purposes.

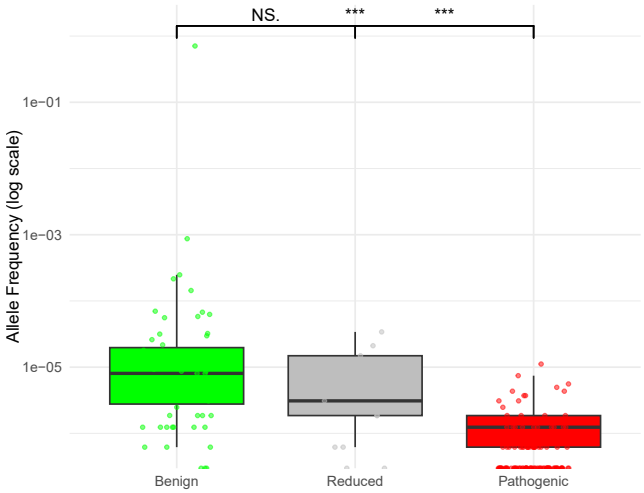

Figure 6. Distribution of total allele frequency in gnomAD v4.1 across *TP53* benign (green), reduced penetrance (gray), and pathogenic (red) variant groups ($p = 3.48 \times 10^{-20}$)
p values refer to pairwise comparisons with Wilcoxon tests, where *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Statistical comparisons noted in the center are for benign vs. pathogenic variants.

Another substantial limitation is the small sample size for the reduced penetrance group, particularly given that most of these variants are suspected, with only one being officially confirmed.[18] It is possible that some of these variants may not truly exhibit reduced penetrance. It is also conceivable that some variants included in the pathogenic and benign reference sets could be associated with reduced penetrance. In the end, both the low number of reduced penetrance variants and uncertainty in the reference panels reflect the difficulty of identifying these variants with certainty, an issue that our model aims to begin to address.

Future larger studies with a greater number of variants will be essential to validate and confirm our findings. We acknowledge the substantial phenotypic variability within LFS, which adds to the complexity of interpreting clinical implications for reduced penetrance variants. This heterogeneity highlights the need for caution before drawing clinical conclusions from current data. However, the occurrence of early-onset cancers among *TP53* reduced penetrance variant carriers suggests that, in the future, variant-level differences may inform more tailored risk assessment and management strategies. Additional larger studies will also play a key role in quantifying age-specific risks for reduced penetrance variants highlighted in this study, aiming to improve the clinical management of carriers and enhance the accuracy of cancer risk prediction for affected individuals and their relatives.

Data and code availability

All datasets used for analyses are publicly available in the literature or databases, as described. Variant-level clinical data from Ambry Genetics is available on request. The predictions generated during this study are available as supplemental information.

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Table 5. ORs associated with core LFS cancers among *TP53* pathogenic and reduced penetrance variants against 256,868 controls

	Pathogenic group ($n = 505$)[a]				Reduced penetrance group ($n = 203$)[a]			
Phenotype (1st cancer)	*p* value	OR	Lower 95% CI	Upper 95% CI	*p* value	OR	Lower 95% CI	Upper 95% CI
Breast cancer <31 y	<0.001	16.07	12.08	21.38	0.01	3.05	1.25	7.45
Breast cancer 31–35 y	<0.001	3.57	2.36	5.42	0.95	0.97	0.31	3.03
Breast cancer 36–60 y	0.41	0.90	0.70	1.16	0.89	1.03	0.72	1.47
Brain tumor ≤45 y	<0.001	14.40	6.36	32.60	0.10	5.19	0.72	37.27
Brain tumor 46–60 y	No data				No data			
Sarcoma ≤45 y	<0.001	27.85	16.92	45.85	<0.01	7.09	1.75	28.73
Sarcoma 46–60 y	<0.001	20.34	8.29	49.92	No data			
Adrenocortical carcinoma ≤60 y	0.01	12.29	1.69	89.22	No data			

CI, confidence interval; OR, odds ratio (enrichment of the phenotype within these case-case cohorts, and *not* cancer risk relative to baseline population risk).
[a]Controls include probands with no pathogenic/likely pathogenic or uncertain variants identified via multigene panel testing.

Declaration of interests

M.E.R., T.P., and K.M. are paid employees of Ambry Genetics.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2025.100484.

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