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p53 major hotspot variants are associated with poorer prognostic features in hereditary cancer patients

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Abstract

TP53 pathogenic germline variation is associated with the multi-cancer predisposition Li–Fraumeni syndrome (LFS). Next-generation sequencing and multigene panel testing are highlighting variability in the clinical presentation of patients with *TP53* positive results. We aimed to investigate if the p53 variants considered as major hotspots at both germline and somatic levels (p.Arg175His, p.Gly245Asp, p.Gly245Ser, p.Arg248Gln, p.Arg248Trp, p.Arg273Cys, p.Arg273His, and p.Arg282Trp) were associated with poorer prognostic features compared to other pathogenic missense variants in the DNA-binding domain. To do so, we assessed clinical features from 1025 carriers of germline *TP53* pathogenic variants (749 probands and 276 relatives) from three independent datasets (IARC *TP53* Database, Ambry Single Gene Testing, and Ambry Multigene Panel Testing). We observed that, compared to carriers of non-hotspot germline variants, individuals that carried a hotspot germline variant were more likely to present with a Classic LFS phenotype, earlier age of first breast cancer onset, and shorter time to diagnosis to any cancer. Further studies with larger datasets addressing differences in cancer phenotypes by genotype are thus needed to replicate our findings and consider variant effect and position, towards future personalized clinical management of pathogenic variant carriers.

Keywords *TP53*, Hotspot, Germline, Li–Fraumeni syndrome, Genotype–phenotype.

Introduction

The p53 protein is encoded by the *TP53* gene, and acts as a tumor suppressor by controlling a range of cellular responses such as apoptosis, cell-cycle arrest, senescence, or modulation of autophagy [1]. Pathogenic germline variants in the *TP53* gene underlie the predisposition to multiple cancer types as part of Li–Fraumeni syndrome (LFS). Clinical criteria to prioritize testing of individuals for *TP53* disease-causing variation have relaxed over time, as it has become apparent that not all *TP53* carrier individuals present with a strong family history of LFS cancers. The current National Comprehensive Cancer Network (NCCN) guidelines [2] recommend *TP53* testing for patients meeting Classic LFS or Chompret 2015 criteria, as well as for individuals from families with a known *TP53* pathogenic variant (Table 1). However, increasing application of next-generation sequencing, and in particular multigene panel testing, has identified a substantial proportion of *TP53* carriers meeting neither of these criteria (reviewed in [3]).

The IARC *TP53* Database [4] represents a unique collection of *TP53* variants published in the literature. This database has been very useful to investigate the possibility that patient presentation might differ depending on variant position, or the mechanism by which a variant affects protein function. For

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Table 1 NCCN criteria version 2.2017 for LFS testing [3].

Criteria	Description
Classic LFS	Proband diagnosed with sarcoma before 45 years of age, and a first-degree relative with cancer before 45 years of age, and another first- or second-degree relative with any cancer diagnosed under 45 years of age or with sarcoma at any age
Chompret 2015	Proband with sarcoma, brain tumor, breast cancer, or adrenocortical carcinoma before age 46 years, and at least one first- or second-degree relative with cancer (other than breast cancer if the proband has breast cancer) under the age of 56 years or a relative with multiple primaries at any age. A proband with multiple primary tumors, two of which are sarcoma, brain tumor, breast cancer, and/or adrenocortical carcinoma, with the initial cancer occurring before the age of 46 years, regardless of the family history. A proband with adrenocortical carcinoma or choroid plexus tumor at any age of onset, regardless of the family history. A proband with breast cancer before 31 years
Individual from a family with a known *TP53* pathogenic variant	

example, it has been reported an association between p53 missense variants in the DNA-Binding domain (DBD) and early sarcoma [5] or earlier age of breast cancer [6]. Another study [7] reported a positive association of presentation with an LFS cancer, and the age of first cancer of any type, with reduced transactivation capability (<25% versus ≥25% activity of wild-type allele, as defined by that study), but not with dominant negative effect (DNE). In contrast, a subsequent study of 322 *TP53* carriers addressed by comprehensive cancer centers or university hospitals [8] reported an earlier age at cancer onset for patients carrying missense variants with DNE in comparison to all types of loss of function variants and genomic rearrangements.

Most variants in the *TP53* gene are missense substitutions, causing single amino acid changes [9]. There are six amino acid residues in the p53 protein historically considered as hotspot locations due to mutational frequency noticeably much higher than any other residue [10–12]; of which five are impacted at both the germline and somatic levels (Fig. 1). These residues are involved in DNA contact (positions 248, 273), or in maintaining the structural integrity of the DNA-binding surface (175, 245, 282). The specific p53 major hotspot variants with the highest frequency are: p.Arg175His, p.Gly245Asp, p.Gly245Ser, p.Arg248Gln, p.Arg248Trp, p.Arg273Cys, p.Arg273His, and p.Arg282Trp. The p.Arg249Ser variant (and several other variants at residue 249) is a common somatic variant due to the effect of specific carcinogens [9]. Further, the p.Arg337His missense variant is extremely common as a germline variant in cancer affected individuals from the Brazilian population due to a founder effect [13]. All of the p53 major hotspot variants are reported to have DNE in the IARC *TP53* Database [4], as confirmed by a recent systematic functional study [14].

The clinical associations of the p53 major hotspot variants have been widely studied, mainly in the context of somatic alterations in tumors from the general population. For example, in a study of 2916 patients with multiple malignancies, it was observed that patients carrying somatic variants affecting residues 248 and 282 were associated with shorter survival after diagnosis in comparison to patients carrying nonsense variants in the *TP53* gene [15]. However, there are no reports for similar comprehensive studies focused on p53 hotspot variants in hereditary cancer patients.

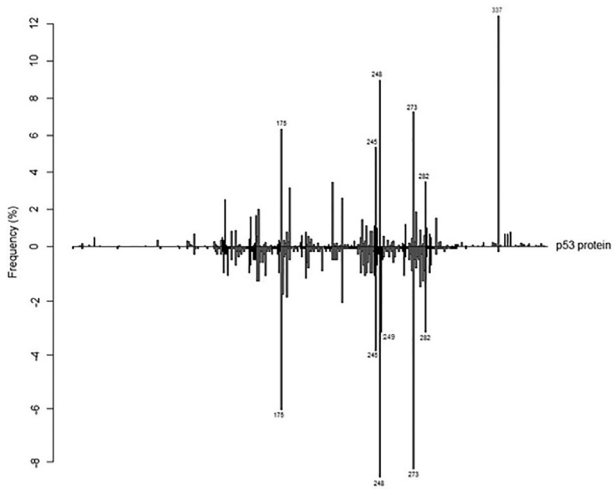

Fig. 1 Residues frequently affected by germline (positive Y axis) or somatic (negative Y axis) missense variation along the p53 protein. Data extracted from the IARC *TP53* Database (R19, August 2018) [4]. For some residues, there are several hotspot variant substitutions at that position. The following substitutions are observed as hotspot variants at both germline and somatic level: p.Arg175His, p.Gly245Asp, p.Gly245Ser, p.Arg248Gln, p.Arg248Trp, p.Arg273Cys, p.Arg273His, and p.Arg282Trp. Residue 249 has higher frequency at the somatic level only (primarily p.Arg249Ser) due to carcinogen effects [9]. Residue 337 has an extremely high frequency in the germline dataset only, ascribed to the founder variant (p.Arg337His) in the Brazilian population [13].

Three possibilities were recently summarized in a review to explain why the hotspot variants in the *TP53* gene occur in most human cancer tissues [16]. Paraphrasing, these proposals were that: (i) the structure of the p53 protein generated by hotspot mutants is highly altered; (ii) environmental mutagens are acting in selection of hotspot mutants; and/or (iii) hotspot mutants are located at CpG codons which have high mutation rates. Another possibility suggested by the same study [16] is that hotspot variants produce proteins with a gain-of-function ability, that gives a positive selection to cells during the process of tumorigenesis. The existence of somatic

Table 2 Total number of patients identified from the three main datasets for p53 major hotspot variants analyses with pathogenic missense variants in the DBD*.

Dataset	Classic LFS n (n DNE)	Chompret 2015 n (n DNE)	Non-LFS n (n DNE)	Patients with breast cancer and information on age of diagnosis n (n DNE)
IARC *TP53* Database	97 (78 DNE)	57 (39 DNE)	296 (196 DNE)	129 (112 DNE)
Ambry SGT	5 (5 DNE)	45 (32 DNE)	26 (14 DNE)	39 (29 DNE)
Ambry MGT	1 (1 DNE)	45 (31 DNE)	177 (123 DNE)	146 (101 DNE)

*Numbers in brackets represent patients carrying only variants with a reported DNE, included in restricted analyses. Note that DNE status was not available for all variants.

hotspot variants is not unique to *TP53* and often occurs in other key cancer genes. Another well-known somatic example is the p.Val600Glu variant in the *BRAF* gene [17]. Detection of hotspot variants is usually associated with a negative functional impact [18], and hotspots have been proposed to be useful as therapeutic targets [19].

We aimed to investigate if inheritance of p53 major hotspot variants (p.Arg175His, p.Gly245Asp, p.Gly245Ser, p.Arg248Gln, p.Arg248Trp, p.Arg273Cys, p.Arg273His, and p.Arg282Trp) was associated with variation in clinical presentation. We assessed if hotspot and non-hotspot carrier groups differed in the proportion of individuals meeting different clinical LFS criteria, age of first breast cancer diagnosis, and time to diagnosis of any cancer. To avoid differences that may be caused by variant effect (e.g. missense versus truncating) or variant location in specific domains, we intentionally restricted this analysis to assumed pathogenic missense variants in the DBD. We also conducted secondary analyses including only DBD-region variants with a reported DNE, in order to identify differences in clinical presentation of hotspot variants regardless of DNE.

Methods

Included datasets and patient selection

Analyses included germline data from confirmed probands in the IARC *TP53* germline Database (R19, August 2018) [4], as well as *TP53* results from index patients undergoing genetic testing at Ambry genetics through *TP53*-single gene testing (Ambry SGT) or multigene panel testing (Ambry MGT) (Table 2). Patients with variants considered to be mosaic (i.e. not of germline origin), as determined using previously published approaches [20], were excluded from the Ambry datasets. Annotation of proven or suspected mosaic carriers was not possible for the IARC *TP53* dataset, and it is possible that some apparent germline variant carriers in this dataset may actually represent mosaic carriers, including individuals with mosaicism due to clonal hematopoiesis of indeterminate potential (CHIP) [20]. However, this is not expected to be a substantial issue since somatic mosaicism is reported to be particularly increased for older individuals [20,21], and probands in the IARC *TP53* Database had average age of diagnosis of 25.3 years (±18.3), and average age of testing of 28.6 (± 16.2).

Only variants already classified as pathogenic or likely pathogenic by Ambry Genetics were included for analysis of the Ambry dataset. This classification was based on the ACMG/AMP guidelines for germline variant classification [22]. For variants in the IARC *TP53* Database, it is important to note that this database does not provide (or aim to provide) curated clinical-grade classifications for *TP53* variants, and presence of a variant in this database is not evidence to assume that it is pathogenic. However, one should expect that the germline variants in this database are enriched for pathogenic variants, since they were largely identified in individuals ascertained for clinical testing because of personal and family history suggestive of LFS. In a conservative approach to remove (likely) benign variants as well as variants of uncertain significance (VUS) from the IARC *TP53* Dataset, we excluded missense variants meeting at least one of two criteria, according to the information present in the latest version of the database (R19): either (i) functional/supertrans transactivation class (mean transactivation activity >75%), or (ii) predicted non-deleterious by Align-GVGD and BayesDel [23]. Importantly, no variants in the final dataset drawn from the IARC *TP53* Database were classified as (likely) benign by Ambry Genetics. There were a total of 197 unique non-hotspot variants across all datasets. All the variants included in this study are detailed in Supplementary Table 1, with their corresponding functional and computational data.

In order to compare the proportion of hotspot variant carriers between groups with contrasting phenotypes, we selected patients from the three datasets meeting: (i) Classic LFS criteria; (ii) Chompret 2015 criteria as an intermediate phenotype; and (iii) none of the NCCN criteria for LFS testing as per Table 1 (termed “non-LFS”). When a patient met both classic LFS and Chompret 2015 criteria, they were considered only in the classic LFS group. The average age of first breast cancer diagnosis was compared between hotspot variant carriers and non-hotspot DBD region variant carriers, in the three datasets by selecting all breast cancer-affected *TP53* carriers with available information, independent of family history. Finally, the analyses were repeated restricted to carriers of DNE variants. Note that we used DNE data as reported in the IARC *TP53* Database and not as recently reported in a systematic functional study [14], to be in agreement with the analysis of Bougeard et al. [8]. The number of patients from each group in each dataset is shown in Table 1.

The relationship between hotspot germline variation and age at first diagnosis of any cancer type (all, and secondary analysis excluding breast cancer) was assessed in a cancer-free survival analysis (i.e. time to first cancer diagnosis), using information from the IARC *TP53* Database. After excluding probands (to avoid overlap with the breast cancer analysis dataset), the analysis included 276 relatives carrying pathogenic missense variants in the DBD (germline

confirmed): 107 hotspot carriers (all DNE) versus 169 non-hotspot carriers (92 individuals with known DNE variants).

Statistical analysis

When combining datasets, pooled proportions were calculated using the metaprop random effects meta-analysis function in RStudio (meta package version 4.8–1). Due to the small size of some datasets (below 10), the Freeman–Tukey double arcsine transformed proportion was calculated. The Kruskal–Wallis test was used to find significant differences in the pooled and individual proportions of hotspot variants identified in Classic LFS versus Chompret vs non-LFS patients. After applying log transformations to normalize data, the Student *t*-test was used to find significant differences in average age of first breast cancer onset between patients carrying hotspot variants and patients carrying non-hotspot variants within the same dataset. Ages from all datasets were combined and weighted using the metacont function in RStudio (meta package version 4.8–1). Kaplan-Meier survival analysis was used to compare time to diagnosis of any cancer in family members from the IARC *TP53* Database, for individuals carrying hotspot variants versus individuals carrying non-hotspot variants (survival package version 2.39–4). All plots and analyses were created using RStudio version 1.1.456 (https://www.rstudio.com/).

Results

Classic LFS patients showed a higher proportion of p53 major hotspot variants than non-LFS patients

When combining all datasets, the proportion of hotspot variants was significantly different between all three groups, and specifically higher in Classic LFS (52%) versus non-LFS patients (32%) ($p = 0.001$) (Fig. 2). Interestingly, the proportion of p53 major hotspots in patients meeting Chompret 2015 criteria was found to be at an intermediate value between that of Classic LFS versus non-LFS patients for each dataset (43% combined). We performed a secondary analysis including only carriers of DNE variants using data from the same three datasets, and the proportion of major hotspot variants was still significantly greater ($p = 0.027$) in Classic LFS versus non-LFS patients.

p53 major hotspot variant carriers demonstrated lower average age of first breast cancer onset and shorter time to diagnosis of any cancer

Irrespective of carriage of a germline hotspot variant, the age at first breast cancer diagnosis was younger in patients from the IARC *TP53* Database compared to patients undergoing SGT and especially MGT, as might be expected given the differences in ascertainment for historical clinical testing versus current clinical diagnostic sequencing (Table 3). However, within each dataset, the average age of first breast cancer diagnosis was lower for hotspot variant carriers in comparison to non-hotspot variant carriers. This difference in age at diagnosis reached significance for individuals from IARC *TP53* Database ($p = 0.002$). When combined, no significant heterogeneity was found between the three datasets ($p = 0.624$), and the average age of first breast cancer diagnosis was significantly lower in hotspot variant carriers than in non-hotspot variant carriers ($p < 0.001$) (Table 3).

In the analysis restricted to only DNE variants in the non-hotspot carriers group, the average age of first breast cancer diagnosis was still significantly lower for the hotspot carriers compared to non-hotspot DNE carriers, namely: 36.4y ($p < 0.001$) for the IARC *TP53* Database, 37.5y ($p = 0.176$) for Ambry SGT, and 45.4y ($p = 0.103$) for Ambry MGT, with p for combined analysis < 0.001).

When comparing time to diagnosis of any cancer using germline data from 276 family members with multiple tumors in the IARC *TP53* database (Fig. 3), this was significantly shorter in individuals carrying hotspot variants than for other individuals carrying non-hotspot variants ($p < 0.001$). This remained significant when the analysis was restricted to only variants with DNE in 199 family members ($p < 0.001$). When excluding breast cancers in relatives (217 relatives), time to diagnosis was also significantly shorter for hotspot variant carriers ($p = 0.031$), but did not remain significant with only DNE variants (154 family members) ($p = 0.204$).

Discussion

In the p53 protein, a number of major missense changes, termed major hotspot variants [10–12], have been extensively studied in a variety of studies, mainly at the somatic level. As tumor DNA sequencing evolves, new recurrent missense variants in other residues are being identified [24,25], suggesting that the spectrum of residues identified as hotspots may increase (for example, the p53-Y220 residue). In this study, we focused on the eight p53 major hotspot variants that are also common at the germline level (p.Arg175His, p.Gly245Asp, p.Gly245Ser, p.Arg248Gln, p.Arg248Trp, p.Arg273Cys, p.Arg273His, and p.Arg282Trp), and compared clinical features of individuals with hotspot variants to those of individuals with other pathogenic missense variants in the DBD, also taking into account reported DNE of the missense variants.

Our analysis of three datasets provides evidence that the p53 major hotspot variants, when present as a germline alteration, are associated with more severe clinical presentation, namely stronger family history of LFS cancers (Classic LFS). Further, our pooled analysis of three datasets demonstrated an earlier age of breast cancer diagnosis for hotspot carriers versus non-hotspot carriers, and we also observed shorter time to first diagnosis of any cancer in relatives of probands in the IARC *TP53* Database. This is in agreement with Kotler et al. [12], who also reported earlier age at diagnosis of all cancers for six of the eight major germline hotspot variants in comparison to all other missense, frameshift, and nonsense *TP53* variants in the DBD. Our observations persisted when the non-hotspot group was restricted to include only carriers of variants with a reported DNE in the IARC *TP53* Database. This is notable since DNE variants have previously been reported to be associated with an earlier age of cancer diagnosis in comparison to all types of loss of function

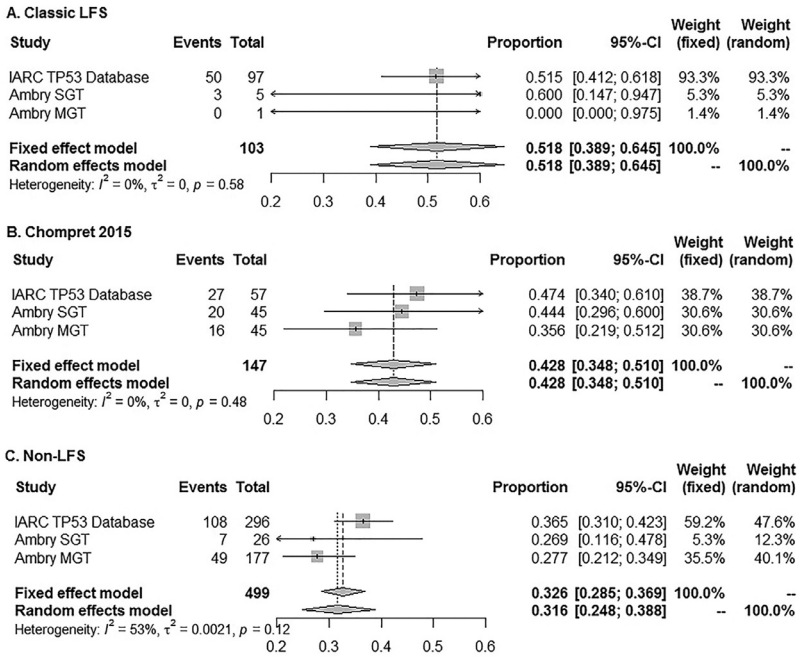

Fig. 2 Proportion of p53 major hotspot variant carriers relative to all DBD pathogenic missense variant carriers in (A) Classic LFS patients, (B) Chompret 2015 patients, and (C) non-LFS patients. Overall, the pooled proportion of hotspot versus non-hotspot DBD variant carriers was significantly different between the three groups ($p=0.001$). Comparisons for individual datasets reached significance for the IARC *TP53* database ($p=0.019$), but not the Ambry single gene testing (SGT) dataset ($p=0.221$) or Ambry multigene testing (MGT) dataset ($p=0.476$).

Table 3 Average age of first breast cancer diagnosis in p53 major hotspot variant carriers versus non-hotspot variant carriers across three different datasets.

Dataset (n)	Average age for hotspot carriers (± standard deviation)	Average age for non-hotspot carriers (± standard deviation)	p-value
IARC *TP53* Database (129)	29.3y (± 7.4)	34.3y (± 10.2)	0.002
Ambry SGT (39)	33.5y (± 7.1)	40.1y (± 12.7)	0.062
Ambry MGT (146)	41.5y (± 12.2)	44.6y (± 11.7)	0.127
Difference in age at onset for hotspot vs. non-hotspot carriers, all 3 datasets combined*			<0.001

*Refer to methods for details of analysis approach.

variants and genomic rearrangements [8], suggesting that p53 major hotspot variants (all DNE) could be driving these observations.

Petitjean et al. [26] reported lower levels of transactivation activity to be associated with earlier age of cancer diagnosis. We note that, on average, the p53 major hotspot variants have significantly lower transactivation activity (1.57%) than the other variants considered in our study (13.3%) ($p<0.001$), which could partly explain the observed differences in the phenotypes. However, Giacomelli et al. recently reported that the *TP53* variants with the highest frequency, such as hotspots, did not confer the greatest impact in terms of loss of function and DNE. In addition, this study suggested that hotspots must therefore exist due to an inherent mutability of their encoding DNA sequence e.g. presence of methylated CpG dinucleotides. We note that, out of 11/38 (29%) probands with a *de novo* variant in the IARC *TP53* carry a hotspot variant, as opposed to 36/191 (19%) of probands without a *de novo* variant. This may be interpreted to support the hypothesis of inherent mutability of these sequence positions.

Our study did not specifically assess the hypothesis of Baugh et al. that these hotspot variants produce proteins with GOF ability, giving positive selection to cancer cells. All eight major hotspot variants exhibit evidence for GOF from at least one of a range of assays in the IARC *TP53* Database; five were tested for and demonstrated growth advantage, including p.Arg249Ser. Of the non-hotspot variants in the DBD included in this study, eight exhibit growth advantage, while five do not. Data arising from comprehensive assessment of GOF ability for *TP53* variants would thus be necessary to better address the question as to whether GOF abilities of hotspot variants contribute to the association with worse phenotypic features observed in our study. That is, evidence to date suggests that inherent mutability, and likely other factors,

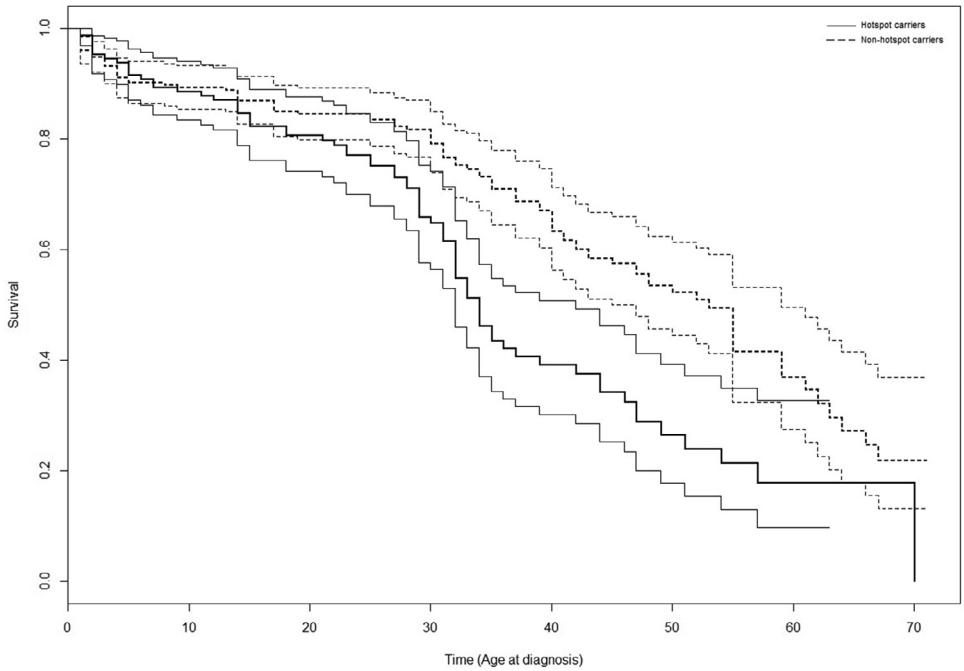

Fig. 3 Time to diagnosis of any cancer in p53 major hotspot variant carriers (solid lines) versus non-hotspot variant carriers (dashed lines) using germline data from 276 family members in the IARC TP53 Database (data with 95% CI, as represented by thinner lines) ($p<0.001$).

will explain why some pathogenic variants focus on specific residues.

This analysis focused on p53 major germline hotspot variants, with other DBD-region missense variants as reference group. Results from other previous studies [6,27] suggest that phenotypic presentation may vary by variant effect (truncating versus not) or location (missense DBD vs. not DBD). Another more recent study [28] also found loss-of-function variants to be associated with characteristic LFS cancer histories. Further studies with larger datasets addressing differences in LFS phenotypes by genotype are thus needed to replicate our findings supporting more severe clinical phenotype for germline carriers of major hotspot missense variants, and those considering variant effect and position. Along with the known reduced penetrance alleles, these data are further evidence of the potential to personalize clinical management of pathogenic variant carriers beyond just applying the best estimates of the average risks for *TP53* pathogenic variants.

Conflict of interest

Tina Pesaran, Jill Dolinsky, Amal Yussuf, and Kelly McGoldrick are paid employees of Ambry Genetics. All other authors have declared no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.05.002.

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