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LETTER TO THE EDITOR

WILEY Human Mutation HGVS HUMAN GENOME VARIATION SOCIETY

Concern regarding classification of germline *TP53* variants as likely pathogenic

To the Editor,

We read with some concern the recent article in *Human Mutation* on the frequency of potentially actionable germline variants in the *TP53* gene (de Andrade et al., 2019). A prevalence estimate, including *TP53* variants widely accepted as pathogenic/likely pathogenic (LP), was reported as one carrier in 3,555-5,476 individuals. However, an additional estimate of >1 in 500 was also proffered in the abstract, generated from the inclusion of several variants labelled as "likely pathogenic" using an arguably spurious classification system devised by and unique to the author, albeit that their classification as "likely pathogenic" for three common variants (p.N235S, p.V31I, and p.R290H) were juxtaposed with the term "controvertible."

We acknowledge this approach is distinct from and less stringent than that would be appropriate for clinical use. However, it is of great concern that readers interpreting *TP53* variants, often found on panel testing in individuals with no personal history of cancer, may use the author's classification and counsel as having Li Fraumeni syndrome individuals carrying these de Andrade et al. (2019) so-called "likely pathogenic" variants.

We have used four sources of data to review the pathogenicity of variants present in gnomAD at a frequency of $n \geq 6$, and assessed by de Andrade et al. (2019) using 2,3, or 4 classifications as being LP/P (Table 1). First the author's own table on likely frequency in the Cancer Genome Atlas (TCGA) to develop a "case control" assessment, second to use the proportions of the variants as a percentage of all gnomAD variants compared with the proportions of the variant in the IARC *TP53* database. Germline variants reported in IARC are largely the result of testing of families with Li Fraumeni criteria and of sentinel Li Fraumeni tumours at young ages. As such, this provides evidence of the specific pattern of tumors linked to germline *TP53* pathogenic variants and is potentially stronger evidence than a simple case control analysis of all cancer. If all the variants reported by de Andrade as LP/P had the same effect the ratio would be close to 1.0 for all variants. If a common variant in Gnomad was only rarely associated with a typical Li Fraumeni pattern, perhaps as a chance finding, then the ratio would drop well below 1.0 providing evidence against causality. For the TCGA data, ratios of TCGA to gnomAD of less than 2.5-fold were considered against pathogenicity (in terms of high risk) and in IARC a ratio of below 0.5 was considered against. In reverse, ratios of case control from TCGA above 2-fold and above 1.5-fold in the IARC ratios were considered supportive. Third, for core binding domain missense variants a recent functional classification has been made available (Kotler et al., 2018). We also obtained computational analyses of the pathogenic likelihood using the PolyPhen-2, SIFT, and Align GVGD algorithms. In addition, on the basis of the data outputs available, we also classified the variants using the recent ACMG guidelines. Examples of variants with gnomAD frequencies $< n = 6$ were also included to act as controls for our analyses.

The results of these analyses indicate that for the four variants, (c.848G>A; p.R283H, c.374C>T; p.T125M, c.655C>T; p.P219S, c.784G>A; p.G262S), that were classified as pathogenic by de Andrade et al. (2019), there is no case control data to support this conclusion with c.784G>A; p.G262S having three entries in gnomAD and 0 in IARC, insufficient to prove pathogenicity (see Table 1). In total, 15 of the LP and these four labeled pathogenic variants would be reclassified below LP on the basis of the Kotler data.

For those 10 variants classified by de Andrade et al. (2019) as likely pathogenic and having a frequency in gnomAD $n \geq 6$, we show: (a) for eight of these variants the case control data is against pathogenicity (b) there is no support for pathogenicity for the 7/8 variants for which functional data are available and (c) the formal ACMG classification is either benign, likely benign or VUS. For c.847C>T; p.R283C, the data outputs are conflicting but its gnomAD frequency ($n = 24$) and lack of representation in IARC would suggest that even if there is pathogenicity (which is not supported by the functional studies) at most this can only be of low-moderate penetrance. Similarly, c.523C>T; p.R175C gives conflicting results and, therefore, the ACMG classification as a VUS is appropriate.

Of interest of the three "controvertible" variants, there is extremely strong evidence against pathogenicity for p.N235S and p.R290H with all four assessments being in favor of a benign classification. For p.N235S the frequency of one in 2,347 for non-TCGA gnomAD alone would count strongly against pathogenicity.

There are nonetheless a small group of LP variants that had support for pathogenicity with c.527G>T p.C176F being supported by Kotler and c.1,010G>A p.R337H (both TCGA proportion/gnomAD non TCGA proportion and IARC proportion/gnomAD only proportion > 10), c.970G>C p.D324H (TCGA proportion/gnomAD non TCGA proportion = 9.12) from TCGA and IARC ratios. These three only contribute three individuals to the overall total of variants where there is good support for pathogenicity. As such, the conservative estimate for the frequency of *TP53* LP and pathogenic variants in non-TCGA gnomAD drops to only one in 6,258

TABLE 1 Analysis of likely pathogenicity of 18 of the 48 variants from de Andrade et al (2019) using (a) case control comparisons with the TCGA, IARC, and gnomAD datasets, (b) functional analyses of variants within the DNA binding domain from Kotler et al. (2018) (c) computational analyses of likely pathogenicity (iv) previous descriptions of variant pathogenicity from ClinVar and (d) likely variant classification based on the ACMG guidelines. The 18 variants are those present in gnomAD at a frequency ≥ 6 and classified as P/LP using 2, 3, or 4 criteria by de Andrade et al (2019). Case control comparison ratios (see footnotes for explanation) of TCGA/gnomAD < 2.5 and IARC/gnomAD < 0.5 are considered against likely pathogenicity whilst ratios of TCGA/gnomAD > 2 and IARC/gnomAD > 1.5 are considered supportive. For the functional data, a relative fitness score (RFS) > −1 is indicative of compromised antiproliferative function, whereas a RFS ≤ −1 indicates retained antiproliferative function. Reliability measures and details of scoring for the computational analyses are show in Table S1. Variants reported are based on the canonical transcript NM_000546.4 and protein NP_000537.3. Nucleotide numbering starts with the A of the ATG translation initiation site as nucleotide 1.

				TCGA proportion/ gnomAD nonTCGA proportion†		IARC proportion/ gnomAD only proportion§		Kotler (2018)		Computational*					
Variant	gnomAD* (n)	IARC* (n)	TCGA* (n)	Ratio	Indication pathogenicity	Ratio	Indication pathogenicity	RFS score	Indication pathogenicity	PolyPhen-2	SIFT	Align GVGD	ClinVar*	ACMG (our evaluation)	
P, de Andrade et al (2019)															
c.659A>G; p.Y220C	2	23	1	18.23	strong support	10.35	strong support	0.09	support	probably damaging	deleterious	C65	P(6)	PS3, PS4, PM5, PP3	P
c.844C>T; p.R282W	1	38	1	>10	strong support	>10	strong support	0.56	support	probably damaging	deleterious	C65	P(12), LP (1), LB(1)	PS3, PS4, PM5, PP3	P
c.742C>T; p.R248W	1	67	1	>10	strong support	>10	strong support	−0.04	support	probably damaging	deleterious	C65	P(7)	PS3, PS4, PM5, PP3	P
c.473G>A; p.R158H	1	23	1	>10	strong support	>10	strong support	−0.60	support	probably damaging	deleterious	C25	P(4), LP(4)	PS3, PS4, PM5, PP3	P
c.374C>T; p.T125M	1	1	0	0	insufficient	0.45	insufficient	−2.16	against	probably damaging	deleterious	C65	LP(5), VUS(1)	BS3, PP3	VUS
c.848G>A; p.R283H	10	1	1	2.03	against	0.05	strongly against	−1.79	against	possibly damaging	deleterious	C0	LP(5), VUS(2)	BS3	VUS
c.655C>T; p.P219S	1	2	0	0	insufficient	0.90	equivocal	−1.35	against	probably damaging	deleterious	C65	LP(2), VUS(1)	BS3, PP3	VUS
c.784G>A; p.G262S	3	1	0	0	insufficient	0.15	against	−1.44	against	probably damaging	deleterious	C55	VUS(5)	BS3, PP3	VUS
LP, de Andrade (2019)															
c.91G>A; p.V31I	57	3	4	1.38	against	0.03	strongly against	outside DBD	-	benign	tolerated	C0	LP(1), LB (3), B(1), VUS (3)	BP4, BS1	LB
c.847C>T; R283C	24	9	8	9.12	strong support	0.25	against	−1.50	against	benign	deleterious	C55	LP(1), VUS(9)	BS3, BS1	B

(Continues)

TABLE 1 (Continued)

Variant	gnomAD* (n)	IARC* (n)	TCGA* (n)	TCGA proportion/ gnomAD nonTCGA proportion†		IARC proportion/ gnomAD only proportion§		Kotler (2018)		Computational*			ClinVar*	ACMG (our evaluation)	
				Ratio	Indication pathogenicity	Ratio	Indication pathogenicity	RFS score	Indication pathogenicity	PolyPhen-2	SIFT	Align GVGD			
c.566C>T; p.A189V	6	4	0	0	insufficient	0.30	against	−2.11	against	possibly damaging	deleterious	C65	P(1), VUS(4)	BS3, PP3	VUS
c.704A>G; p.N235S	58	20	2	0.65	strongly against	0.16	against	−2.67	against	benign	tolerated	C0	LB(5), VUS(3)	BS3, BP4, BS1	B
c.869G>A; p.R290H	42	10	1	0.44	strongly against	0.11	against	−1.54	against	benign	tolerated	C0	VUS(8)	BS3, BP4, BS1	B
c.1096T>G; p.S366A	14	2	1	1.40	against	0.07	strongly against	outside DBD	-	benign	tolerated	C0	LB(3), VUS(1)	BP4	VUS
c.329G>A; p.R110H	13	1	1	1.52	against	0.04	strongly against	−1.79	against	benign	tolerated	C0	VUS(5)	BP4, BS3	LB
c.509C>T; p.T170M	12	1	0	0	against	0.04	strongly against	−2.75	against	benign	deleterious	C15	VUS(5)	BS3	VUS
c.997C>T; p.R333C	6	2	0	0	insufficient	0.15	against	outside DBD	-	damaging	deleterious	C55	VUS(4)	PP3	VUS
c.523C>T; p.R175C	6	1	2	9.12	strong support	0.11	against	−2.84	against	probably damaging	deleterious	C65	VUS(3)	BS3, PP3	VUS

*gnomAD entries (from Table 2, de Andrade et al 2019).

∫ IARC entries (from Table 2, de Andrade et al 2019).

¶TCGA entries (from Table 2, de Andrade et al 2019).

#Accessed 8th November 2018 using http://canvar.ml/db/ and comprising http://genetics.bwh.harvard.edu/pph2/ (PolyPhen 2), http://sift.bii.a-star.edu.sg/ (SIFT), http://agvgd.hci.utah.edu/agvgd_input.php (AlignGVGD).

+Accessed 8th November 2018 https://www.ncbi.nlm.nih.gov/clinvar/.

Numbers of germline entries for each variant to ClinVar are given in parentheses.

TCGA proportion / gnomAD nonTCGA proportion†, refers to:

A/B ÷ C/D, where

A = number entries for that variant in TCGA

B = 7,208 (number of TCGA samples in gnomAD, from de Andrade et al 2019)

C = gnomAD entries for that variant – TCGA entries for that variant

D = 131, 424 (gnomAD non TCGA data set from de Andrade et al 2019)

IARC proportion / gnomAD only proportion§, refers to:

A/B ÷ C/D, where

A = number of entries for that variant in IARC

B = 631 (from Table 2, total IARC entries from 2,3 & 4 classification criteria from de Andrade et al 2019)

C = gnomAD entries for that variant – TCGA entries for that variant

D = 284 (from Table 2, gnomAD – TCGA entries for total of 2,3 & 4 classification criteria from de Andrade et al 2019)

Note. B: benign, DBD: DNA binding domain, gnomAD: Genome Aggregation Database, IARC: International Agency for Research on Cancer, LP: likely pathogenic, P: pathogenic; LB: likely benign; TCGA: The Cancer Genome Atlas; VUS: variant of uncertain significance.

individuals, very similar to our original estimate of one in 5,000 on the basis of a population series of very young breast cancers (Lalloo et al., 2003).

Whilst we understand the authors' desire to document the frequencies of potentially actionable variants in *TP53*, it is of utmost importance that readers do NOT take their classification as support for actionability for cancer surveillance or to offer presymptomatic testing for variants that would certainly not be classified as LP or P using the recent American College of Medical Genetics guidelines (Richards et al., 2015), which have also just been adopted in the United Kingdom (Ellard et al., 2018) as the standard for variant interpretation in the clinic.

PUBLISHER'S NOTE

A rebuttal is published online and in the same journal issue entitled "Response to: Concern regarding classification of germline TP53 variants as likely pathogenic" by de Andrade et al.: DOI: 10.1002/humu.23749.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interests.

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