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**Differences in patient ascertainment affect the use of gene-specified
ACMG/AMP phenotype-related variant classification criteria: evidence for *TP53***

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ABSTRACT

The ACMG/AMP guidelines for variant classification are widely used for clinical interpretation of gene test results. These guidelines may be specified to genes/syndromes of interest in order to improve their utility in the clinical setting. As part of these specifications, phenotype-related criteria can be detailed and weighted

This is the author manuscript accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/humu.23972](https://doi.org/10.1002/humu.23972).

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depending on the personal history of disease for a given variant carrier. We investigated how ascertainment can affect the significance and/or weight of patient phenotype as predictor of germline variant pathogenicity, using the Li-Fraumeni Syndrome gene *TP53* as an example. Likelihood ratios in favor of variant pathogenicity were determined for report of personal history of several *TP53*-related cancers, using data from 2,656 probands undergoing single-gene testing (SGT) and 15,483 undergoing multigene panel testing (MGPT). Overall, *TP53*-associated cancers were more predictive of pathogenicity, and demonstrated greater evidence weight, in the MGPT versus SGT dataset. This observation is almost certainly explained by differences in proband ascertainment for the two streams of testing, and these findings have implications for germline variant classification using ACMG/AMP guidelines.

Keywords: ACMG, ascertainment, VCEP, TP53, variant classification

INTRODUCTION

Genetic testing for hereditary cancer allows carriers of pathogenic variants to undergo recommended surveillance and risk-reduction strategies. However, these benefits rely on accurate interpretation of test results, but interpretation is not straightforward, and a large number of genetic tests identify variants of unknown significance. The American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines are commonly used to provide standard germline variant classifications (Richards et al., 2015). These guidelines were originally designed to be used for any Mendelian disease gene, but in an effort to improve and standardize classification strategies, they have also been further specified to individual genes of interest by ClinGen Expert Panel groups, including *PTEN*

(Mester et al., 2018), *CDHI* (Lee et al., 2018), *PAH* (Zastrow et al., 2018), hearing loss genes (Oza et al., 2018), RASopathy genes (Gelb et al., 2018), *MYH7* (Kelly et al., 2018), and *TP53* (https://clinicalgenome.org/site/assets/files/3876/clingen_tp53_acmg_specifications_v1.pdf, manuscript in preparation). This involves determining which forms of data are most informative for a specific gene and the corresponding phenotype, and may include considering disease penetrance and prevalence to establish frequency cut-offs, assessing the relevance of functional assays, and in some cases modifying the weight assigned to an ACMG/AMP code depending its relevance to the gene-disease relationship in question.

Of particular interest when specifying the ACMG/AMP guidelines to genes of interest are the criteria that refer to the patient's personal or family history of a disease, the nature of which is clearly completely different for different genes. Examples of these criteria (with their evidence codes noted) are:

- PS2 De novo (both maternity and paternity confirmed) in a *patient with the disease* and no family history.
- PS4 The prevalence of the variant in *affected individuals* is significantly increased compared with the prevalence in controls.
- PM6 Assumed de novo in a *patient with the disease*, but without confirmation of paternity and maternity.
- PP1 Cosegregation with disease in *multiple affected family members* in a gene definitively known to cause the disease.
- PP4 *Patient's phenotype* or *family history* is highly specific for a disease with a single genetic etiology.

- BS4 Lack of segregation in *affected members* of a family.

The specification of these guidelines for cancer predisposition syndromes present an additional difficulty compared to some other Mendelian diseases, since most well-established cancer syndromes include a spectrum of cancer types. For cancer syndromes, the extent to which an individual or a family is *affected* by a condition can vary greatly; the original strength level of the corresponding criteria can potentially be upgraded or downgraded to take this into account. As an example, a criterion could be designated at a higher strength level for a patient presenting with a severe personal or family history of cancers considered to be highly distinctive for pathogenic variants in the gene concerned, such as early age at onset or multiple diagnoses of specific cancer types in the proband and numerous relatives. It is possible to derive information about how strongly phenotypic features predict the pathogenicity of a variant directly from large cohorts that have undergone genetic testing based on a range of phenotypic indications. Representative datasets of this sort can be used to calibrate the strength level of phenotype-based evidence for practical application in the diagnostic setting. To apply this approach in the context of hereditary cancer, it is important to keep in mind that some “signature” cancer types occur relatively commonly in the general population, and the corresponding frequency with which they are reported in individuals undergoing testing will be influenced by any clinical criteria for testing applied by either clinicians, laboratories, or funders i.e. the effect of ascertainment.

We investigated this issue, using *TP53* as an example. Pathogenic germline variants in *TP53* cause the multi-cancer-predisposition Li-Fraumeni syndrome (LFS), with core malignancies recognized as early onset breast cancer, sarcomas, adrenocortical carcinomas, and brain tumors. The current National Comprehensive Cancer Network

(NCCN) guidelines recommend *TP53* testing for individuals meeting the classic LFS criteria (Li et al., 1988) or the less strict Chompret 2015 criteria (Bougeard et al., 2015), as well as individuals from a family with a known pathogenic variant (Daly et al., 2017) (Table 1). In the past, *TP53* gene testing was generally only offered as a single gene test (SGT), and only to patients identified to have LFS features. However, diagnostic gene sequence tests are now commonly carried out in the context of multigene panels; most commercial multi-gene panel tests (MGPT) offered for diagnosis of hereditary cancer include *TP53*, and therefore this gene is increasingly being tested in individuals with a wide range of presenting phenotypes. Indeed, a recent study (Rana et al., 2018), found that phenotypes listed in the LFS testing criteria were much less frequent in *TP53* carriers identified by MGPT versus those found by SGT. We hypothesized that differences in patient ascertainment (SGT versus MGPT) may affect the predictive capacity of patient phenotype for use in germline *TP53* variant classification using adapted ACMG/AMP guidelines. To investigate this, we used data from a major diagnostic company (Ambry Genetics) to determine statistically the significance and/or weight of patient phenotype as a predictor of germline variant pathogenicity, comparing findings for SGT and MGPT.

METHODS

We used data from a total of 2,656 probands undergoing SGT between April, 2010 and December, 2014 and 15,483 undergoing MGPT between March, 2012 and April, 2017 at Ambry Genetics (results for relatives or probands undergoing single-site testing were not included in the analysis). From these datasets, carriers were defined as probands carrying a *TP53* (likely) pathogenic variant, excluding patients confirmed to be mosaic or with a (likely) pathogenic variant in another high-moderate risk

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cancer gene (*MRE11A*, *BRCA1*, *BRCA2*, *BRIP1*, *APC*, *ATM*, *PMS2*, *MLH1*, *MSH2*, *PALB2*, *MSH6*). Non-carriers were defined as probands who tested negative for all the genes in the corresponding test series. Individuals were categorized by cancer diagnosis age-group for LFS-associated cancers (breast cancer, sarcoma, adrenocortical carcinoma, brain tumor, leukemia, and lung cancer); characteristics of *TP53* carriers and non-carriers are shown in Table 2. The age-groups denoted were selected based on the traditional recommendations for *TP53* genetic testing (Daly et al., 2017).

We then assessed the personal history of LFS cancers as a predictor of *TP53* variant pathogenicity by logistic regression analysis (using SPSS, Version 22), comparing results between SGT and MGPT. The phenotypes detailed in Table 2 were used as independent predictor variables in the model, while the outcome was the carrier status of probands. For each cancer type, individuals unaffected by that cancer were designated as the reference group in the logistic regression. This analysis is based on the assumption that the personal history of cancer is different between carriers and non-carriers, and that the pathogenic variant prevalence depends on factors such as cancer diagnosis and age group (Easton et al., 2007). For each phenotype statistically significantly associated with pathogenic variant carrier status, the odds ratio estimates (OR) for the phenotypic feature and for the constant term from the model were obtained and the associated likelihood ratio (LR) of pathogenicity was calculated as: $LR = OR(\text{phenotype}) * OR(\text{constant}) * (1-P)/P$, where P is the proportion of *TP53* pathogenic variant carriers in the dataset used (SGT, MGPT).

Last, the LRs were translated to the corresponding ACMG/AMP strength level, following proposed conversions of a recent Bayesian re-analysis of these guidelines

(Tavtigian et al., 2018). ACMG/AMP criteria applicability and strength level for the SGT and MGT datasets were compared.

RESULTS AND DISCUSSION

This study aimed to investigate if – in the context of gene-specifications of variant classification guidelines - differences in ascertainment could impact the ACMG/AMP strength level assigned to a given phenotype.

The results (Table 3) demonstrate that the significance and weight of a patient's phenotype as a predictor of pathogenicity differs markedly for patients ascertained for SGT versus MGPT. For the MGPT dataset, nine of the 13 phenotype-age categories were significantly predictive of *TP53* pathogenic variant carrier status, and eight translated into LRs that could be considered appropriate for conversion into ACMG/AMP strength levels following the code strength calculations recently published (Tavtigian et al., 2018). Only six of these nine phenotype-age categories were also significantly predictive for the SGT dataset: sarcoma (any age), adrenocortical carcinoma ($\leq 45y$), brain tumor ($\leq 45y$), and leukemia ($\leq 45y$), and only two translated into LRs for ACMG/AMP (sarcoma $\leq 45y$ and adrenocortical carcinoma $\leq 45y$). Further, the corresponding strength level based on the LR was lower in SGT than MGPT for all significant phenotype-age categories. Two phenotypes could be assigned the ACMG/AMP strength level “Strong” in the MGPT dataset (adrenocortical carcinoma, $\leq 45y$ and $>45y$), but none in the SGT dataset.

Overall, the phenotypes analyzed were more strongly associated with *TP53* pathogenic variant carrier status in patients undergoing MGPT compared to SGT. This observation is almost certainly explained by differences in proband ascertainment for the two streams of testing. It might be expected that virtually all

probands who undergo SGT would meet the NCCN criteria for *TP53* testing, thus making the corresponding phenotypes less informative as a predictor of *TP53* pathogenicity compared to the situation where carriers are ascertained in the context of MGPT. For example, 2.18% of probands tested through SGT had adrenocortical carcinoma, which is one of the NCCN criteria for *TP53* testing, as opposed to only 0.03% probands tested through MGPT. The proportion of individuals with other LFS-associated cancers is similarly much higher in SGT than in MGPT. Further evidence for differences in ascertainment between the two streams of testing is provided by the observation that overall 5.5% of SGT subjects (143/2656) carried pathogenic variants versus 2.2% of MGPT subjects (348/15483).

It should be noted that for this a proof-of concept study we chose to determine the strength of prediction of LFS cancers traditionally used to select individuals for *TP53* testing (Daly et al., 2017) - when observed in a *proband* tested by Ambry Genetics. We emphasize several points to clarify the utility of the specific LR of pathogenicity presented here in the context of future *TP53* variant classification. Strictly speaking, LR (and associated evidence code strength levels) derived for a given dataset should only be applied for classification of variants of uncertain significance (VUS) detected in that same dataset; in this instance they may be applied as relevant for VUS detected by Ambry Genetics SGT or MGPT. That is, additional analysis would be required to determine if the evidence strengths are generally applicable to other testing settings for which VUS classification is to be undertaken. Further, the analyses here were focused on personal history of cancer, and did not capture family history of cancer as another important predictor of variant pathogenicity. More complex models that capture the combination of patient personal cancer presentation (including age at cancer onset, multiple cancer diagnoses in an individual, and possibly tumor pathology features), as well as reported family history, are more appropriate for deriving and applying predictors for use in variant classification within a specific dataset. We note that the specific predictive clinical features used in our analysis differ from those recently described as part of a separate exercise carried out in the context of the ClinGen *TP53* Variant Curation Expert Panel (VCEP). This VCEP used a combination of expert opinion and published data to define clinical criteria and evidence strengths to be used for variant classification within *TP53*-adapted ACMG/AMP guidelines (https://clinicalgenome.org/site/assets/files/3876/clingen_tp53_acmg_specifications_v1.pdf, manuscript in preparation).

While we used *TP53* as an example to show the importance of patient ascertainment in assessing predictive capacity of clinical features, this issue translates to any gene where clinical criteria may have been used to select individuals for gene testing. We thus present these findings to exemplify the need to calibrate evidence strength level for clinical predictors of variant pathogenicity for any gene. For practical application, we recommend that VCEPs (and individual testing laboratories) are aware of this issue, and carry out a similar analysis with their genes of interest when deriving and specifying phenotype-related criteria, both when developing new guidelines or when revising existing ones. If significant differences are found between different sources of test results, a given criterion might need to have different strength levels depending on the specific approach to testing that is used. Alternatively, the most conservative strength level may be suggested to simplify use more broadly across multiple sources.

We would also recommend that such analyses be revisited over time to detect changes in predictive capacity of patient presentation, for example due to: changes in clinical recommendations for SGT; absorption of SGT test requests into the MGPT stream; relaxation of criteria and/or health insurance support for MGPT; expansion of gene content and thus patient phenotypes recommended for multigene panels that include a gene of interest.

In conclusion, these findings encourage the need to consider ascertainment when developing and applying gene-specific guidelines for variant classification, in particular the criteria in which the phenotype or family history of a variant carrier is used as evidence of variant pathogenicity.

ACKNOWLEDGEMENTS

C.F is supported by a University of Queensland (UQ) International Scholarship from the UQ School of Medicine. A.B.S is supported by an NHMRC Senior Research Fellowship (ID1061779).

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.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Tables

Table 1. NCCN criteria version 2.2017 for TP53 testing (Daly et al., 2017)

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	<p>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.</p> <p>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.</p> <p>A proband with adrenocortical carcinoma or choroid plexus tumor at any age of onset, regardless of the family history.</p> <p>A proband with breast cancer before 31 years</p>
Individual from a family with a known TP53 pathogenic variant	

Table 2. Characteristics of probands included in the single-gene testing (SGT) and multi-gene panel testing (MGPT) analysis datasets

Ascertainment	SGT		MGPT	
Phenotype	N carriers (total 143)	N non-carriers (total 2513)	N carriers (total 348)	N non-carriers (total 15135)
No breast cancer	66	866	126	5911
Breast cancer $\leq 30y$	35	589	40	499
Breast cancer 31-40y	29	496	58	1935
Breast cancer $\geq 41y$	13	562	125	6786
No sarcoma	88	2090	322	15003
Sarcoma $\leq 45y$	48	306	19	64
Sarcoma $> 45y$	7	117	8	68
No adrenocortical carcinoma	134	2464	346	15133
Adrenocortical carcinoma $\leq 45y$	9	26	2	1
Adrenocortical carcinoma $> 45y$	0	23	1	1
No brain tumor	130	2399	339	15069
Brain tumor $\leq 45y$	12	82	9	34
Brain tumor $> 45y$	1	32	1	32
No leukemia	137	2439	345	15071
Leukemia $\leq 45y$	6	59	4	26

Leukemia >45y	0	15	0	38
No lung cancer	141	2458	343	15053
Lung cancer ≤45y	2	17	3	10
Lung cancer >45y	0	40	4	72
None of the cancers	16	405	101	5762

Table 3. Likelihood ratio of pathogenicity (LR) for *TP53* variants based on personal history of LFS cancers for single-gene testing (SGT) and multi-gene panel testing (MGPT) datasets*

Dataset	SGT (N = 2,656)				MGPT (N = 15,483)			
Phenotype	P-value	OR (95% CI)	LR	Corresponding ACMG/AMP strength level	P-value	OR (95% CI)	LR	Corresponding ACMG/AMP strength level
Breast cancer ≤30y	0.01	1.95 (1.15, 3.29)	0.92	None	0.00	3.88 (2.66, 5.66)	3.10	Supporting
Breast cancer 31-40y	0.05	1.67 (0.99, 2.83)	NA	NA	0.00	1.53 (1.11, 2.11)	1.22	None
Breast cancer ≥41y	0.09	0.58 (0.30, 1.09)	NA	NA	0.69	0.95 (0.73, 1.22)	NA	NA
Sarcoma ≤45y	0.00	4.97 (3.18, 7.76)	2.35	Supporting	0.00	10.46 (5.97, 18.33)	8.37	Moderate
Sarcoma >45y	0.03	2.49 (1.11, 5.75)	1.17	None	0.00	6.26 (2.97, 13.23)	5.01	Moderate
Adrenocortical carcinoma ≤45y	0.00	10.60 (4.56, 24.68)	4.99	Moderate	0.00	76.32 (6.30, 923.83)	61.06	Strong

Adrenocortical carcinoma >45y	1.00	0.00 (NA)	NA	NA	0.00	55.50 (3.45, 892.98)	44.40	Strong
Brain tumour ≤45y	0.00	3.40 (1.70, 6.83)	1.60	None	0.00	8.27 (3.60, 19.00)	6.62	Moderate
Brain tumour >45y	0.85	1.22 (0.16, 9.24)	NA	NA	0.67	1.54 (0.21, 11.55)	NA	NA
Leukemia ≤45y	0.047	2.49 (1.01, 6.10)	1.17	None	0.00	6.44 (2.15, 19.23)	5.15	Moderate
Leukemia >45y	1.00	0.00 (NA)	NA	NA	1.00	0.00 (NA)	NA	NA
Lung cancer ≤45y	0.47	1.76 (0.38, 8.23)	NA	NA	0.02	6.71 (1.31, 34.29)	5.37	Moderate
Lung cancer >45y	1.00	0.00 (NA)	NA	NA	0.055	2.71 (0.98, 7.56)	NA	NA

*LRs of pathogenicity converted to ACMG/AMP strength level as per Tavtigian et al., 2018. Strength levels based on LR_s considered statistically significant are shown in **bold**. Levels with higher strength as designated from the MGPT analysis compared to the SGT analysis are shown as darker red-shaded cells. NA = Non-applicable, where OR_s were not significant. **Note that we are not proposing to use these phenotypes as evidence for pathogenicity using ACMG/AMP guidelines, but to emphasize the difference in their strength level depending on the dataset (see discussion for further explanation).**

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Date:

2020-01-16

Citation:

Fortuno, C., Pesaran, T., Dolinsky, J., Yussuf, A., McGoldrick, K., Goldgar, D., James, P. A. & Spurdle, A. B. (2020). Differences in patient ascertainment affect the use of gene-specified ACMG/AMP phenotype-related variant classification criteria: Evidence for TP53. HUMAN MUTATION, 41 (3), pp.537-542. <https://doi.org/10.1002/humu.23972>.

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