Current review of TP53 pathogenic germline variants in breast cancer patients outside Li-Fraumeni syndrome

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ABSTRACT

Pathogenic germline variants in *TP53* predispose carriers to the multi-cancer Li-Fraumeni syndrome (LFS). Widespread multigene panel testing is identifying *TP53* pathogenic variants in breast cancer patients outside the strict clinical criteria recommended for LFS testing. We aimed to assess frequency and clinical implications of *TP53* pathogenic variants in breast cancer cohorts ascertained outside LFS. Classification of *TP53* germline variants reported in 59 breast cancer studies, and publicly available population control sets was reviewed and identified evidence for misclassification of variants. *TP53* pathogenic variant frequency was determined for: breast cancer studies grouped by ascertainment characteristics; breast cancer cohorts undergoing panel testing; and population controls. Early age of breast cancer onset, regardless of family history or *BRCA1/BRCA2* previous testing, had the highest pick-up rate for *TP53* carriers. Patients at risk of hereditary breast cancer unselected for features of LFS carried *TP53* pathogenic variants at a frequency comparable to that of other non-*BRCA1/2* breast cancer predisposing genes, and ~3-fold more than reported in population controls. These results have implications for the implementation of *TP53* testing in broader clinical settings, and suggest urgent need to investigate cancer risks associated with *TP53* pathogenic variants in individuals outside the LFS spectrum.

Keywords

Breast cancer, *TP53*, genetic testing, multi-gene panels, germline

Introduction

Li-Fraumeni syndrome (LFS; MIM# 151623) is a genetic disorder characterized by the occurrence of multiple malignancies, mainly early onset breast cancers, soft tissue sarcomas, brain tumors and adrenal gland carcinomas (F. P. Li & Fraumeni, 1969). Its underlying cause is pathogenic germline variation in the *TP53* gene (MIM# 191170), a key tumor suppressor gene. With the advent of lower cost next-generation sequencing (NGS) and introduction of multigene panel testing, the identification rate of *TP53* variants in breast cancer patients outside the context of LFS is increasing, but is still not clear how frequent *TP53* variants are in such cohorts. It has been reported that *TP53* pathogenic variants account for <1% of hereditary breast cancer cases (Martin et al., 2003), and between 5%-8% of early-onset breast cancer cases with no family history (McCuaig et al., 2012).

Recommendations for *TP53* testing were previously restricted to patients meeting conventional LFS criteria. In 2015, the Chompret criteria 2009 (Tinat et al., 2009) were updated to include women with breast cancer diagnosed before age 31 years, due to the findings that up to 6% of such patients not fulfilling such criteria could carry *TP53* pathogenic variants (Bougeard et al., 2015). Supp. Table S1 shows the recommendations for LFS testing according to the NCCN guidelines version 2.2017 (Daly et al., 2017).

Several published studies have reported the frequency of *TP53* pathogenic variants in different cohorts of breast cancer patients unselected for LFS. Similarly, other publications described frequency of *TP53* carriers as detected by multi-gene panel testing of breast (and other) cancer patients without obvious LFS features. We undertook a literature review to: (i) calculate the frequency of *TP53* carriers in ***TP53*-targeted studies** for breast cancer patients independent of LFS criteria - according to age of onset, family history, previous *BRCA1* (MIM# 113705) and *BRCA2* (MIM# 600185) testing, or HER2+ tumor status - in order to identify factors that may optimize detection rate of variants in breast cancer patients, and (ii)

estimate the frequency of *TP53* carriers identified in **multigene panel studies of breast cancer patients,** documenting differences in detection rate of variants according to ancestry and/or previous *BRCA1/2* testing. We also consider the implications of these findings for setting clinical criteria for *TP53* testing, and the clinical utility of including *TP53* in multigene cancer panels.

Methods

This research has been approved by the Human Research Ethics Committee of the QIMR Berghofer Medical Research Institute. All variants are described on *TP53* genomic reference sequence NC_000017.10, transcript variant NM_000546.5, and on SwissProt protein sequence #P04637 when relevant.

Study and cohort selection

Study selection included: (i) whole studies or patient subsets within studies that consisted of breast cancer patients not selected by LFS-criteria, sequenced for germline DNA variants across the complete *TP53* coding region (in some instances together with *BRCA1/2*); or (ii) studies where *TP53* germline DNA sequencing was performed as a component of multigene panels for patients referred for hereditary breast cancer testing.

The search strategy and an overview of the selection process of breast cancer cohort sequencing studies published until 2017 are shown in Supp. Figure S1. Specifically, studies were excluded if they: did not report *TP53* results; were overlapping with other larger studies conducted by the same centre/group; or that patients were selected on the basis of LFS suspicion or a known pathogenic variant in the family. Details of studies included, with information on study design, are shown in Supp. Tables S2 and S3. Ascertainment criteria and other features were extracted for each study, and similar cohorts of patients were

combined as appropriate in order to calculate a pooled proportion of *TP53* carriers for each set of clinical features. For the *TP53*-targeted studies, patients who were identified as meeting conventional LFS criteria (Classic LFS, LFL, or Chompret 2009, Supp. Figure S1) after being found to be *TP53*-positive on gene testing were noted and excluded from the final analysis, in order to provide a cleaned dataset to specifically estimate the prevalence of *TP53* pathogenic variants in breast cancer patients outside LFS. For studies with cohorts of patients with similar clinical features but different ancestral backgrounds, results were separated by ancestry.

Re-evaluation of variant pathogenicity

There were notable differences in classification methods used by studies considered for this review. A number of studies followed the ACMG/AMP guidelines for variant classification (Richards et al., 2015), but the majority of studies used their own classification method based mainly on bioinformatic predictions, existing functional data, control databases, or classifications reported on the ClinVar portal (https://www.ncbi.nlm.nih.gov/clinvar/).

Individual-level variant information was reported by all but three *TP53*-targeted (total 22) and seven multigene panel studies (total 37) that met inclusion criteria for this review. Where individual level information was provided, we re-evaluated the classification of the reported variants. To do this, we excluded variants originally reported in the published studies as (likely) pathogenic if they were consistently reported in ClinVar as "(Likely) Benign". In addition, variants found in ClinVar with conflicting classifications between "(Likely) Benign"/"Uncertain" were excluded if additional computational (Fortuno et al., 2018) and functional (Kato et al., 2003) evidence supported this exclusion. We also included in the meta-analyses variants reported as uncertain by the original publications if they were consistently reported in ClinVar as "(Likely) Pathogenic", or in ClinVar with conflicting

classifications between "(Likely) Pathogenic"/"Uncertain" and if additional computational and functional evidence supported this inclusion.

Overall, after re-evaluation, six variants originally reported as (likely) pathogenic in the original publications were excluded, and three variants originally reported as uncertain were included. These variants are detailed in Supp. Tables S2 and S3, along with additional evidence supporting the exclusion/inclusion of these variants.

Where information was not provided to permit individual variant review, we assessed confidence in the original assertions by considering the testing provider (large commercial companies with publicly provided classification criteria were considered to be more likely reliable compared to small research studies), and year of testing (with older studies, especially those pre-ACMG guidelines, considered less reliable). The three *TP53*-targeted studies with no individual variant information were assessed as having possible low (sample sizes 136 and 14) or moderate (sample size 50) confidence in assertions. For the seven multigene panel studies that did not give variant-level information, the two largest studies arose from clinical diagnostic laboratories considered to have high confidence assertions and another two used ACMG/AMP guidelines (denoted moderate confidence assertions). The remaining three studies, which were considered to have moderate confidence assertions, identified a total of three *TP53* carriers and provided little weight in the subsequent analyses.

A more strict approach to variant re-evaluation was used for estimation of breast cancer risk in multigene panel-tested cohorts, In this approach, we only included variants if they were reported in ClinVar as "(Likely) Pathogenic" or with conflicting classifications between "(Likely) Pathogenic"/"Uncertain" (if same additional computational and functional evidence supported this exclusion). For the case samples set, we included only those studies that explicitly stated that patients had a personal history of breast cancer. We used data from a

recent study that estimated the prevalence of *TP53* pathogenic variants using "population controls" drawn from ExAC (excluding TCGA), FLOSSIES (Fabulous Ladies Over Seventy), and whole exome sequencing (WES) (de Andrade et al., 2017). After strict variant re-evaluation as denoted above, 70 individuals (carrying 14 unique variants) were re-assigned as non-carriers for the control dataset, but no variants were excluded from relevant case studies. Evidence for variant inclusion or exclusion is detailed in Supp. Tables S3 and S4.

Statistics

All estimations of pooled and individual *TP53* proportions were calculated using the metaprop random effects meta-analysis function in RStudio version 1.1.456 (meta package version 4.8-1). Based on the scarcity of events (e.g. many studies with none or small numbers of *TP53* carriers), the Freeman-Tukey double arcsine transformed proportion was calculated. The byvar command in the metaprop function was used to determine whether the differences between subgroups were significant. The risk of breast cancer associated with a *TP53* pathogenic variant detected in multigene panel studies was estimated by comparing the frequency of pathogenic variants in breast cancer patients of European ancestry, to that observed for individuals of European ancestry from healthy control cohorts (de Andrade et al., 2017).

Results

TP53-targeted studies: frequency of *TP53* carriers in non-conventional LFS breast cancer cohorts

A total of 22 studies included in this review specifically sequenced the entire *TP53* coding region in cohorts of breast cancer patients selected on the basis of a range of different characteristics, including family history, age of onset, *BRCA1/2* pathogenic variant status, breast tumor pathology and personal history of other cancers. Sample size for the individual

studies ranged from 5 to 333, with only 4 studies sequencing more than 100 individuals. If not specified, ancestry was assumed to be European. Details of individual studies, including selection criteria, are shown in Supp. Table S2.

Four studies that selected patients on the basis of family history of breast and/or ovarian cancer alone reported an absence of *TP53* carriers (Balz, Prisack, Bier, & Bojar, 2002; Lehman et al., 2000; Martin et al., 2003; Prosser et al., 1991). When patients with a positive family history were also pre-screened and shown to be negative for *BRCA1/2* pathogenic variants (BRCAx), this frequency increased to 1% (Arcand et al., 2015; Arcand et al., 2008; Walsh et al., 2006). Unsurprisingly, no carriers were identified in a single study of 171 patients unselected for both family history and age of onset (Lehman et al., 2000).

On the other hand, when patients with no previous *BRCA1/2* testing were selected according to age of onset, and either presence or absence of family history, the weighted proportion of pathogenic variants ranged from 0% to 6%. In women diagnosed in their 30s with positive family history, the frequency of *TP53* carriers was 2.4% (Mouchawar et al., 2010). In women diagnosed before 30 years with no reported family history of cancer, the combined frequency from two studies was 2.0% (Lalloo et al., 2006)(Gonzalez, Noltner, et al., 2009). For women unselected by family history, with a personal diagnosis of breast cancer up to 31 years of age, the proportion of *TP53* carriers ranged between 3.8% (Mouchawar et al., 2010) and 6.0% (Bougeard et al., 2015), and where breast cancer was diagnosed before the age of 50 the proportion was reported as 4.0% (O'Shea et al., 2017).

Other studies have examined the role of *TP53* in BRCAx women with early onset breast cancer and differences in family history selection criteria, and found variable results. In women with positive family history, the highest *TP53* carrier frequency was estimated at 0.8% in Chinese women diagnosed before 35 years (Cao et al., 2010), while in only 43

women diagnosed before 30 years, Ginsburg et al., 2009 found no *TP53* carriers. A single study found one pathogenic variant out of 67 Pakistani BRCAx women (1.5%) with negative family history diagnosed before 30 years (Rashid et al., 2012). When it comes to BRCAx early onset breast cancer patients unselected for family history, *TP53* carrier frequencies were estimated at 7.7% for women diagnosed before age 30 (McCuaig et al., 2012), or between 2.3%-4.2% for women of different ancestries diagnosed before 36 years (Ang, Lim, Yong, & Lee, 2009; Carraro et al., 2013; Lee et al., 2012; Tinat et al., 2009). Ginsburg et al., 2009, however, found no carriers out of only 52 BRCAx women of varying ancestries, all diagnosed with breast cancer before 30 years.

Amplification of HER2 has been reported as more common in women who carry a *TP53* pathogenic variant compared to the population as a whole (Melhem-Bertrandt et al., 2012). In studies of patients with HER2+ breast tumors in the absence of LFS criteria, the *TP53* frequency was higher, reported as 7.0% for women diagnosed before 31 years (Eccles et al., 2016), but only 0.5% when women were diagnosed before age 50 (Rath et al., 2013).

Finally, a single small study found three *TP53* carriers out of 8 BRCAx patients diagnosed with breast cancer before 36 years, when there was a previous diagnosis of another LFS-linked tumor - but no family history suggestive of LFS; a frequency of 37.5% (Tinat et al., 2009).

A summary of these results according to the recruitment criteria for the cohort is shown in Table 1.

Key points

- **The frequency of *TP53* carriers reported in breast cancer cases with no significant personal history of LFS cancers ranged from 0% to 7.7%, with the highest frequencies observed in BRCAx patients with breast cancer <30 years (7.7%), and in HER2+ BRCAx patients <31 years (7%).**
- **In general, the frequency of *TP53* carriers was lower in cases selected on the basis of family history of breast and other cancers only (and not other selection criteria such as early age of onset, HER2+).**
- **When selecting patients based on early age of onset but not family history, reported *TP53* carrier rates outside LFS have been between 3.8%-7.7% when aged <30/31 years, overlapping with 4.0% frequency reported for women aged <50 years.**

***TP53* carrier frequency in multigene panel studies of breast cancer patients**

TP53 is routinely included in commercial/research-based multigene panels for breast cancer predisposition, and this form of testing is likely to be far more common than specific *TP53*-targeted testing, except where patients clearly meet LFS-based criteria. Most panel studies select patients on the basis of suspicion of hereditary cancer, therefore these patients are diverse in terms of age of onset, family history, presence of male breast cancer, and other clinical features. We compared differences in *TP53* carrier frequency among different ancestries or by BRCA status, and selected mainly patients that were referred due to personal or family history of breast cancer, when the information was available. Details of individual studies, including selection criteria, are shown in Supp. Table S3.

Differences according to ancestry

While most of the published multigene panel studies tested patients of (mostly) European ancestry, a limited number of studies have analyzed other populations. We therefore compared the frequency of *TP53* carriers among cohorts of patients with different ancestries and without previous *BRCA1/2* testing (BRCA unknown) for Europeans (Bunnell et al., 2017; Buys et al., 2017; Castera et al., 2014; Couch et al., 2015; Couch et al., 2017; Doherty, Bonadies, & Matloff, 2015; Eliade et al., 2017; Kapoor et al., 2015; Kraus et al., 2017; Moran et al., 2017; Pinto et al., 2016; Rummel, Lovejoy, Shriver, & Ellsworth, 2017; Shirts et al., 2016; Schroeder et al., 2015; Susswein et al., 2016; Tedaldi et al., 2017; Tung et al., 2015; Tung et al., 2016) Asians (Kwong et al., 2016; Lin et al., 2016; Ng et al., 2016; Rajkumar, Meenakumari, Mani, Sridevi, & Sundersingh, 2015; Yang et al., 2015; Wong et al., 2016), and Middle Easterns (Jalkh et al., 2017; Lolas Hamameh et al., 2017).

The frequency of *TP53* carriers was 0.1% for the European cohort, 1.6% for both the Asian and Middle Eastern cohorts (Figure 1). In the study of Zick et al., 2017, the variant c.541C>T (p.Arg181Cys) was detected in 2/5 Arab patients (40%), and then again in 5/42 (11.9%) of Arab unrelated breast cancer patients, leading to the suggestion that Arab patients with early age of breast cancer, multiple malignancies or suggestive family history should be tested for this recurrent variant. This small study was removed from the meta-analysis as the variant identified appears to be a potential founder in that population, with the potential to exaggerate the prevalence in comparison to other studies. A single panel study with patients of African American ancestry reported a frequency of 0.35% (Churpek et al., 2015). The higher frequency in Asian and Middle Eastern patients was significant in comparison to studies of Europeans (p-values = 0.0023 and <0.0001, respectively). One study of 85 patients from Colombia (Latino) failed to identify any *TP53* carrier (Cock-Rada, Ossa, Garcia, & Gomez, 2017).

This observed higher frequency of *TP53* carriers in patients of non-European ancestry may reflect that multigene panel testing is less common outside USA or Europe, and patients may only be considered for testing after they have developed multiple tumors (Wong et al., 2016). In addition, some of the studies in patients of European ancestry had selection criteria likely to have selected against *TP53*-positive status. For example, Couch *et al.* (Couch et al., 2015) only selected women with triple negative breast cancer (TNBC), which has no known correlation with *TP53* and 11.20% were positive for *BRCA1/2*.

Differences according to BRCA status

Other multigene panel studies included only BRCAx patients (confirmed *BRCA1/2*-negative). We compared the frequency of *TP53* carriers reported in these studies with the frequency in previous studies of patients with unknown *BRCA1/2* status. This analysis included only multigene panel studies selecting patients with European ancestry for *BRCA1/2* unknown (same cohort as previous section) and BRCAx patients (Maxwell et al., 2015; Minion et al., 2015; Desmond et al., 2015; Aloraifi et al., 2015; Susswein et al., 2016; Thompson et al., 2016; J. Li et al., 2016; Slavin et al., 2017; Crawford et al., 2017).

As observed earlier (Figure 2A), the frequency of *TP53* carriers for the *BRCA1/2* unknown cohort was 0.1%, not significantly lower than for the BRCAx cohort which was 0.3% (P=0.0505) (Supp. Figure S2). This result highlights that the overall frequency of *TP53* carriers is very low, and there is considerable imprecision in estimates for patients screened by non-LFS selection criteria.

Comparison with carrier frequency of other breast cancer genes

In the total of 37 multigene panel studies reviewed, the frequency of *TP53* carriers identified in multigene panel studies ranged from 0% to 4.4% (Supp. Table S5). For patients not previously screened for *BRCA1/2*, the carrier frequency was highest for *BRCA1/2* combined

genes in all studies (range 2.2% to 22.3%). Notably, for some studies (Bunnell et al., 2017; Jalkh et al., 2017; Lin et al., 2016; Lolas Hamameh et al., 2017; Maxwell et al., 2015; Rummel et al., 2017; Yang et al., 2015) the frequency of *TP53* carriers was higher than that observed for any of other breast cancer predisposing genes included in multigene panels (*ATM, CHEK2, PALB2*), although these pathogenic frequencies were not curated. In all these studies, early age of onset (before 40 or 35) was at least one of the selection criteria for the patients tested.

Identification of patients not meeting current NCCN criteria for LFS testing

The use of multigene panel studies allows for the analysis of *TP53* germline variants in cancer patients without obvious LFS characteristics, who would not meet LFS testing criteria based on the NCCN criteria version 2.2017 (Supp. Table S1). However, in some instances, patients identified as positive for *TP53* have subsequently been found to meet these criteria ("LFS NCCN 2017 criteria") after further clinical review. We documented the proportion of patients who met LFS NCCN 2017 criteria based on the information reported, across all panel studies (Table 2). Overall, the proportion of *TP53* carriers identified in multigene panels who did not meet LFS NCCN 2017 criteria was similar to that of patients who did meet criteria. This highlights the advantage of multigene panel studies to identify *TP53* carriers that would be missed if only specific testing guidelines were followed.

Estimation of breast cancer risk based on multigene panel studies

The multigene panel studies reviewed ascertained cases independently of the NCCN/LFS criteria. Comparison of the frequency of *TP53* high-risk pathogenic variants observed in these studies to that in population-based "control" samples provides an opportunity to examine the effect of ascertainment on the estimate of breast cancer risk for patients outside of the classic LFS clinical scenario. In the past, the population frequency of *TP53* pathogenic

variants has been estimated to be one in 5,000 (0.0002) (Lalloo et al., 2006). In our meta-analysis of 84,806 European ancestry patients specifically denoted as having a personal history of breast cancer (and unselected for previous *BRCA1/2* testing), 141 cases were identified to carry an assumed pathogenic *TP53* variant (frequency 0.0017). More recently, a study reported the prevalence of (likely) pathogenic variants in "population" control individuals drawn from three datasets (de Andrade et al., 2017); in non-Finnish European/European American/European populations, 93 of 35,492 individuals were reported to carry a (likely) pathogenic variant (combined frequency 0.0026). Including only variants with enough evidence for pathogenicity as per ClinVar, the estimated population frequency of a (likely) pathogenic *TP53* variant would drop to 0.0006 (23/35,492 individuals) (see methods, Supp. Table S4). Based on this revised population frequency, the estimated breast cancer risk associated with a *TP53* pathogenic variant would be OR 2.57 (95% CI 1.65, 3.99; P=<.0001), respectively.

Key points

- **The overall weighted frequency of *TP53* carriers was 0.1% for European ancestry patients undergoing breast cancer multigene panel testing.**
- **The frequency of *TP53* carriers was higher in patients of non-European ancestry.**
- **The frequency of *TP53* carriers was comparable to that of other non-*BRCA1/2* breast cancer predisposing genes.**
- **Approximately half of the *TP53* carriers identified by multigene panel testing did not fulfil LFS NCCN 2017 criteria.**
- **Differences in ascertainment are likely to impact estimates of breast cancer risk due to *TP53* pathogenic variants.**

Discussion

This review aimed to estimate the frequency of patients with *TP53* pathogenic variants among different groups of breast cancer patients unselected for features of LFS.

The frequency in early onset breast cancer patients (diagnosed before age 31) has been reported to be as high as 6%, leading to the 2009 update of the Chompret criteria (Bougeard et al., 2015), and inclusion of early onset breast cancer (before 31 years) in the most recent version of the NCCN guidelines for *TP53* testing. We note that *TP53* pathogenic rate was sometimes but not always "increased" in studies of patients diagnosed <31 years, but also that *TP53* pathogenic variant frequency was comparable in some *TP53*-targeted studies selecting BRCAx patients diagnosed <36 years (from 2.3% in a Brazilian sample to 4.2% in Asian samples), or when European ancestry patients without a significant family history were diagnosed with breast cancer before 50 years (4%) (Table 1). This suggests that it may be beneficial to consider expanding the age testing criteria for *TP53* to before 36 years, although larger studies would be helpful to provide more robust frequency estimates. Although one previous study has proposed that there is no support for *TP53* testing for women with early onset breast cancer in the absence of family history (Ginsburg et al., 2009), the results of this review indicate the converse, namely that the detection rate is higher when patients are selected according to early age of onset, *irrespective* of reported family history. The detection rate may be especially increased in women with HER2+ breast tumors, although this is based only on one study. Interestingly, there is published evidence that patients with the reduced penetrance Brazilian founder pathogenic variant c.1010G>A (p.Arg337His) are less likely to develop HER2+ breast tumors than other *TP53* carriers (Fitarelli-Kiehl et al., 2015), inferring that HER2-positive tumor status may be less predictive for p.Arg337His and potentially other reduced penetrance variants.

In 37 multigene panel studies reviewed, the overall frequency of *TP53* carriers identified ranged from 0% to 4.4%, and was variable between patient groups according to ancestry or previous BRCA testing (Supp. Table S5). This frequency was highest in reports from Asia and the Middle East, although this may reflect differences in patient referral patterns (Wong et al., 2016). Despite high uptake of panel testing in the USA (Robson, 2014), debate about the clinical utility of all genes included in cancer panels, and the high detection rate of variants of unknown significance (van Marcke, De Leener, Berliere, Vikkula, & Duhoux, 2016) has delayed routine use of multigene panel testing in clinics worldwide. It is common to offer targeted gene tests to individuals meeting criteria for specific syndromes, and there are arguments in support of multigene panel testing for individuals not meeting specific syndromic phenotypes (Robson, 2014). Multigene panel testing of patients suspected to have a genetic predisposition to breast cancer has proved useful for detecting potentially actionable pathogenic variants in genes other than *BRCA1/2* (Bunnell et al., 2017; Desmond et al., 2015; Kurian et al., 2014; Susswein et al., 2016). The inclusion of *TP53* in multigene testing panels as a proven susceptibility gene for breast and other cancers is not generally disputed. On the other hand, while results from a cost-effectiveness analysis provided evidence for inclusion of *BRCA1, BRCA2, PALB2* and *CHEK2* in multigene panel testing, this same study advised caution about the inclusion of other genes including *TP53* given the rarity of variants and their association with syndromes with clear features (Lerner-Ellis, Khalouei, Sopik, & Narod, 2015). However, as we have shown, for some multigene panel studies (Bunnell et al., 2017; Jalkh et al., 2017; Lin et al., 2016; Lolas Hamameh et al., 2017; Maxwell et al., 2015; Rummel et al., 2017; Yang et al., 2015) where early age of onset (before 35 or 40) was at least one of the selection criteria, the frequency of *TP53* carriers identified was comparable to other breast-cancer predisposing genes, such as *PALB2*, *ATM* or *CHEK2* (Suppl. Table S5) even after our reevaluation of *TP53* variant classification only, making the comparison

potentially a conservative one. Importantly, we have also shown that about half of *TP53* carriers detected using multigene panels testing would not meet the existing NCCN clinical criteria for LFS testing (Table 2).

There has been extensive discussion of the benefits and concerns raised by the identification of individuals and families carrying pathogenic *TP53* variants. Due to the wide range of the cancer predisposition involved, determining the optimal risk management has been complex and evidence based guidelines have only recently begun to be established (Ballinger et al., 2017). There are also important implications for treatment and the role of radiotherapy (Evans, Birch, Ramsden, Sharif, & Baser, 2006), and for family planning and the use of assisted reproductive technologies for carriers of a child-bearing age (Verlinsky et al., 2001). Together, these reasons argue for the utility of including *TP53* in multigene panels despite the low frequency reported for pathogenic variants.

In contrast, the literature also provides clear illustration of the issues that complicate genetic testing for *TP53* in clinical practice. As well as the continuing uncertainty around many elements of risk management, genetic counselling is impacted by a number of emerging issues that are particularly prominent in relation to *TP53*. These include the frequency of *de novo* variants (Gonzalez, Buzin, et al., 2009; Renaux-Petel et al., 2018) and recent data suggesting an exceptionally high rate of somatic mosaicism and aberrant clonal expansion of *TP53* variation (Coffee et al., 2017; Weitzel et al., 2017). This has led to the recommendation that patients with a (likely) pathogenic variant in *TP53*, particularly presenting outside the context of a typical LFS family history, should be followed-up to confirm if the variant is truly germline (Coffee et al., 2017; Weitzel et al., 2017). If further data continues to bear out the high levels of somatic variants described so far, it is likely to necessitate a broad re-evaluation of the literature summarised here.

Even taking these issues into consideration, the low overall rate of (likely) pathogenic variants observed in breast cancer cohorts, along with the observation of a non-trivial frequency in healthy control cohort, raises questions about the extent to which the strong diagnostic criteria used for LFS have influenced the current understanding of the natural history associated with *TP53* pathogenic variants. After re-evaluation of variant pathogenicity, the estimate of breast cancer risk for carriers based on the reports of multigene panel studies was estimated to be OR 2.57 (95% CI 1.65, 3.99) which is essentially incompatible with previous estimates derived from LFS families or from sarcoma cohorts (Mitchell et al., 2013). Understanding the specific risk implications for a *TP53* variant carrier in these different clinical contexts is an urgent challenge as clinical practice moves quickly towards broader genetic testing with less specific clinical indications.

In summary, this review and meta-analysis of *TP53* carrier frequency in patients with breast cancer outside LFS found an overall low pathogenic variant detection rate, which was highest when selecting patients according to an early age of onset, regardless of other criteria, . Multigene panel testing of breast cancer patients unselected for features of LFS was found to detect *TP53* pathogenic variant carriers at frequencies comparable to that of other non-*BRCA1/2* breast cancer predisposing genes. Future studies need to address the penetrance and cancer risks associated with *TP53* pathogenic variation in patients outside LFS spectrum, with particular consideration of the possible role of mosaicism or somatically acquired variation.

Contributors

All authors conceived the study. CF performed the literature review and conducted the statistical analysis. CF and ABS produced the initial draft of the manuscript, and all authors contributed to completion of the final version.

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Conflict of Interest

None declared.

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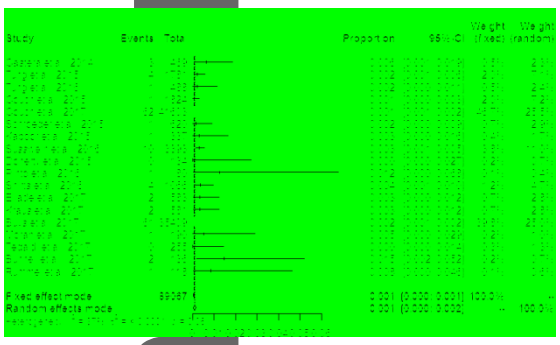
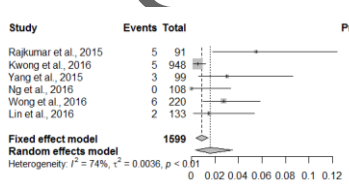
Legends

Figure 1. Meta-analyses of *TP53* carrier frequency (Events) in multigene panels for BRCA unknown breast cancer patients according to ancestry: European (A), Asian (B), and Middle Eastern (C)





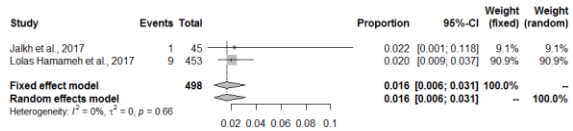


Table 1. Summary of frequencies of *TP53* carriers outside LFS estimated for each cohort of breast cancer patients

Selection criteria for cohort	Weighted frequency of *TP53* carriers outside LFS with 95% CI	Total sample size*	Reference
+FH	0 (0; 0.004)	238	Balz et al., 2002; Lehman et al., 2000; Martin et al., 2003; Prosser et al., 1991
BRCAx, +FH	0.010 (0; 0.0045)	110	Arcand et al., 2015; Arcand et al., 2008; Walsh et al., 2006
-FH	0 (0; 0.021)	171	Lehman et al., 2000
+FH, early onset (30s)	0.024 (0.001; 0.126)	42	Mouchawar et al., 2010
+FH, early onset (before 30)	0 (0; 0.11)	32	Lalloo et al., 2006
-FH, early onset (before 30)	0.020 (0; 0.09)	77	Gonzalez, Noltner, et al., 2009; Lalloo et al., 2006
Early onset (before 30/31)	0.060: 0.038	NA**; 52	Bougeard et al., 2015; Mouchawar et al., 2010
Early onset (before 50)	0.040 (0.014; 0.094)	50	O'Shea et al., 2017
BRCAx, +FH (before 30)	0 (0; 0.082)	43	Ginsburg et al., 2009
BRCAx, +FH (before 35)	0.008 (0.001; 0.03) (Asian)	240	Cao et al., 2010
BRCAx, -FH (before 30)	0.015 (0; 0.08) (Middle Eastern)	67	Rashid et al., 2012
BRCAx (before 30)	0 (0; 0.068) (Mixed ancestries); 0.077 (0.002; 0.36)	52; 13	Ginsburg et al., 2009; McCuaig et al., 2012
BRCAx (before 36)	0.042 (0.016; 0.091) (Asian); 0.031 (0.009; 0.078); 0.023 (0.0001; 0.123) (Brazilian)	113 (Asian); 128; 43 (Brazilian)	Ang et al., 2009; Carraro et al., 2013; Lee et al., 2012; Tinat et al., 2009
HER2+ (before 31)	0.070 (0.023; 0.157)	71	Eccles et al., 2016
HER2+ (before 50)	0.005 (0; 0.026)	213	Rath et al., 2013
BRCAx, with other LFS tumors (before 36)	0.375 (0.085; 0.755)	8	Tinat et al., 2009

FH = Family history; BRCAx = negative for *BRCA1/2* pathogenic variants
*Details of individual studies included can be seen in Supp. Table S2.
**NA = Not available, rates only reported in this study.

Table 2. Report of LFS and non-LFS cases (NCCN 2017 criteria) for patients identified to carry a *TP53* pathogenic variant in multigene panel studies reviewed

LFS cases (%)	Non-LFS cases (%)	Reference
1 (33.33)	2 (66.67)	Castera et al., 2014
2 (50)	2 (50)	Maxwell et al., 2015
1 (25)	3 (75)	Rajkumar et al., 2015
12 (66.67)	6 (33.33)	Susswein et al., 2016
2 (66.67)	1 (33.33)	Yang et al., 2015
0 (0)	1 (100)	Pinto et al., 2016
0 (0)	5 (100)	Thompson et al., 2016
4 (80)	1 (20)	J. Li et al., 2016
4 (66.67)	2 (33.33)	Wong et al., 2016
1 (25)	3 (75)	Shirts et al., 2016
0 (0)	2 (100)	Eliade et al., 2017
1 (50)	1 (50)	Kraus et al., 2017
1 (50)	1 (50)	Zick et al., 2017
Total: 29 (49.15%)	30 (50.85%)	

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