New surveillance guidelines for Li-Fraumeni and hereditary *TP53* related cancer syndrome: implications for germline *TP53* testing in breast cancer

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Abbreviations

ACC	Adrenocortical carcinoma
ACMG	American College of Medical Genetics and Genomics
AMP	Association for Molecular Pathology
CNS	Central nervous system
ERN	European reference network
GBCA	Gadolinium based contrast agents
HCP	Health care professional
h <i>TP53</i> rc	Heritable <i>TP53</i> -related cancer
LFS	Li-Fraumeni syndrome
STS	Soft-tissue sarcoma
WBMRI	Whole

Introduction

Heterozygous pathogenic germline variants were identified in *TP53* in 1990 as a cause of Li-Fraumeni syndrome (LFS) [1]. LFS was originally labelled SBLA syndrome to reflect the predominance of Sarcoma, Breast/Brain, Leukaemia/Lung cancer and Adrenal carcinoma in the original families [2, 3]. LFS is typically characterized by familial aggregations of very early-onset malignancies covering many tumour sites. These include the characteristic core LFS tumours: soft-tissue sarcomas (STS), osteosarcomas (OS), adrenocortical carcinomas (ACC), central nervous system

(CNS) tumours and very early-onset female breast cancers, typically occurring ≤ 30 years. Germline testing for variants in *TP53* has been most frequently employed in individuals with core LFS malignancies who fulfil clinical criteria such as classical LFS or “Chompret criteria” (Table 1a/b) [4, 5]. Nevertheless testing for individual tumours without a family history has been carried out in childhood malignancy [6–8] or among adult females with extremely early-onset breast cancers with germline *TP53* mutations being identified in apparently isolated cases [5, 9]. Such findings have led to the idea being posited that a heritable *TP53*-related cancer (h*TP53*rc) syndrome would be a better term than LFS [10, 11]. Very recently two Europe based guidelines for surveillance in h*TP53*rc syndrome that include intensive surveillance with annual whole body MRI (WBMRI), brain MRI, breast MRI in women from age 20 years and blood testing (for ACC hormones) have been published [12, 13], to add to an American guideline [14]. The guidelines are based around the so-called ‘Toronto’ protocol that has been associated with a reported survival advantage [15]. The introduction of these intensive surveillance programmes, together with the increased availability of cancer predisposition gene testing, means it is particularly timely to understand both accurate variant interpretation and the clinical features where a genuinely pathogenic germline *TP53* mutation is likely to be present.

Likelihood of identifying a germline *TP53* variant in breast cancer patients

A review suggested that between 3.8–7.7% of women with breast carcinoma aged ≤ 30 harboured pathogenic *TP53* variants [16]. However, lower rates have since been reported at 2.2% in 370 Dutch women [17]. Nevertheless these data

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Table 1 Clinical diagnostic criteria for Li Fraumeni syndrome and *TP53* testing

Criteria	Description	Reference
(A) Classic	Proband diagnosed with a sarcoma before age 45 AND; One first-degree relative with any malignant tumour diagnosed before age 45 AND; Another first- or second-degree relative diagnosed with any malignant tumour before age 45 or a sarcoma at any age	[4]
(B) Chompret 2009, modified 2015	Proband with a typical LFS tumor diagnosed before age 46 AND; At least one first- or second-degree relative diagnosed with a typical LFS tumor (except breast cancer if the proband is/was affected by breast cancer) before age 56 or with multiple primary tumours OR; Proband with multiple primary tumours (other than multiple breast tumors)—including at least two from LFS tumor spectrum - with the first diagnosed before age 46 OR; Proband diagnosed with ADR or CPC , or RMS of embryonal anaplastic subtype* at any age irrespective of family history; OR Female proband with breast cancer < 31*	[8]

CNS central-nervous system tumors, ADR adrenocortical carcinoma, CPC choroid-plexus carcinoma, RMS rhabdomyosarcoma

*Added in modified Chompret criteria[8]

have shown that a family history of cancer is not a pre-requisite when considering genetic testing of *TP53*. As early reports showed that the likelihood of germline *TP53* reduced sharply after 30 years [18] and that germline *TP53* only very rarely caused familial breast cancer unexplained by *BRCA1* or *BRCA2* [19], testing outside this extremely early onset group was rarely undertaken until recently unless the woman with breast cancer's personal or family history fulfilled at least Chompret/modified Chompret criteria [5]. Nonetheless, very low rates of germline *TP53* mutation detected in breast cancer > 30 years has recently been confirmed from the BRIDGES study of 60,466 cases and 53,461 controls [20]. In this study 4/346 had truncating *TP53* variants < 30 years compared to 2/53,461 controls (OR = 309) compared to only 3/60,120 (OR = 1.33) > 30 years (Easton D-personal communication).

The development of multi-gene cancer panels has resulted in much more widespread testing of *TP53*, as it is included in many commercial gene panels offered for germline cancer gene testing in breast cancer, the most frequent indication for commercial panel testing. For instance Ambry genetics provide an online tool [21] that can be interrogated. From this 79,368 women with breast cancer have been tested and the prevalence of pathogenic *TP53* variants was 0.3% (<https://www.ambrygen.com/providers/resources/prevalence-tool>, accessed 29th August 2020). Aged ≤ 30 years this was 2.5% in 2033 cases compared to 0.18% in 42197 > 46 years. We do not know, however, whether there was pre-testing of those with LFS criteria to reduce the rates in those ≤ 30 or whether some at > 46 had elements of Chompret criteria. Testing in Manchester has found of 381 female breast cancers ≤ 30, *TP53* was found in 22 (5.80%), whereas of 921 women > 46 only 2 (0.22%) had *TP53* with one having clear

LFS criteria reducing those > 46 to only 0.11% without LFS criteria ($P < 0.0001$). The frequency in women aged 31–46 was still only 0.33% ($n = 27623$) using the Ambry tool.

Breast cancer gene panel testing and the need for a refined approach

The inclusion of cancer predisposition genes with a wide and variably penetrant phenotype when disrupted, on breast cancer gene panels, is a recognised challenge. Thus many commercial gene panels for breast cancer hereditary predisposition include, in addition to *TP53*, the *CDH1* tumour suppressor gene. Whilst germline *CDH1* mutations have been described in 0.34% of lobular breast cancer cases [22], they are more commonly associated with diffuse gastric cancer, a diagnosis with a dismal prognosis and prophylactic gastrectomy being the main preventable measure. In view of the uncertainties around true penetrance in breast cancer and the real potential for psychological harm, *CDH1* is not included in NHS England hereditary breast cancer panel testing, as was determined by the UK Cancer Genetics Group [23].

Similarly, is it therefore worthwhile including *TP53* in gene panels for women at older ages? The potential benefits of the new surveillance protocols in early detection of future cancers and decision making to potentially avoid second cancers from radiotherapy [12] are clear, as well as the importance of reproductive decision making and offering testing to other potentially at risk family members. However, there are some potential serious harms that can be caused to women who do not have a definite pathogenic variant and potential psychological harm to the tiny proportion that do. Therefore a personalised and refined approach to diagnostic

TP53 testing is needed, so that where a variant is detected, it is interpreted accurately and appropriate management put in place as required, with minimal distress.

Interpreting *TP53* variants

There are two main concerns when interpreting variants discovered during germline *TP53* testing in people with cancer

1. Is the variant detected the cause of the underlying cancer?
2. What is the allele frequency of the variant and is it confined to lymphocyte DNA?

Pathogenicity of variants

TP53 is not common for dominantly inherited cancer predisposing genes in that the majority of pathogenic variants are missense [5]. Therefore, complex analyses are required, unless there is already definitive evidence that the variant is pathogenic. Most pathogenic *TP53* missense variants reside in the important DNA core binding domain equating to exons 4–8 where the original cases were found [1]. Most pathogenic missense variants act in a dominant negative fashion with protein products forming tetramers with wild type p53 [5]. Information that can help classify variants can be obtained from sources such as the IARC database (<https://p53.iarc.fr/>) and for general population frequency estimates from the Genome Aggregation Database (gnomAD; <https://gnomad.broadinstitute.org/>). It is critically important that missense variants should be classified by the widely used ACMG/AMP guidelines [24]. A subcommittee of which, has developed further guidance specific to *TP53* [25]. The stringent clinical criteria are in place so that (together with strict functional criteria) missense variants from mass gene panel testing don't get over-called. These include:

1. Phenotype information (scores are given for fulfilling Classical LFS or the Chompret criteria, but not for single tumours outwith the criteria);
2. Functional analyses of variants using different in vitro assays performed either in yeast or cultured cells [26–29].

Importantly at least two different functional tests that predict pathogenicity are required to score in that category [25]. The new ACMG/AMP criteria for germline *TP53* variants classification are deliberately stringent to avoid mis-classification [25]. The importance of this has been demonstrated by the potential for 'over-classification' of *TP53* variants resulting in as high as a frequency as 1 in 500 in gnomAD [30]. If a more stringent approach is used, this provides a

more likely 1 in 5000 frequency [30–32]. The less parsimonious approaches to classification have resulted in colorectal cancer risk being almost certainly overestimated in LFS [12]; one report mis-classified five of the six germline reported variants as pathogenic [12, 33]. Given that over age 46 potentially only 0.1–0.2% of women with breast cancer will have a pathogenic variant, there is an almost similar chance that some laboratories will over classify variants that are unlikely to be associated with high or even moderate cancer risk [30, 31].

TP53 allele frequency

Most molecular reports on germline testing from commercial laboratories did not include the allele frequency of the variant until recently (many still do not!). Providing these has become straightforward since moving from Sanger to Next Generation sequencing (NGS) where identification of low DNA allele frequencies has been substantially enhanced. This has been shown to be very valuable in identifying underlying mosaic variants in monogenic conditions such as NF2 [34]. However, there is a particular pitfall in *TP53* testing as well as for a number of other genes such as *PPM1D* and several oncogenes. A frequent cause of low level allele frequencies detected is clonal haematopoiesis of indeterminate potential (CHIP) [35–39]. CHIP has recently become apparent in lymphocyte DNA panel testing by NGS. Older smokers or those who have had chemotherapy or radiotherapy treatments are particularly likely to manifest this. CHIP was first reported in patients over 70 years of age, but can be detected from 30 years of age. The frequency of CHIP increases with age, smoking and chemotherapy or radiotherapy treatments [35–39]. Even with an allele frequency consistent with a germline variant of 50%, clonal haematopoiesis is still possible and should be considered where a *TP53* variant is found in a context not usually associated with germline *TP53* [36, 39]. The concern is that probably > 50% of *TP53* variants found on panel testing are due to CHIP and therefore for those aged over 46 this is likely to be the predominant reason for identifying an apparently pathogenic *TP53* variant at all. The presence of true mosaic *TP53* alterations should be considered in patients with sporadic cancers strongly suggestive of a pathogenic *TP53* variant, such as paediatric ACC, choroid plexus carcinoma, and breast cancer ≤ 30 years, and patients with multiple *TP53* core primary tumours [12]. However, the detection of a *TP53* variants at significantly < 50% allele frequency on lymphocyte DNA NGS should prompt confirmation of the variant in the tumour and preferably another tissue without lymphocytes such as hair follicle or skin biopsy with fibroblast culture [12, 39]. Where the variant is absent from the tumour (best tested on section without clear infiltration with lymphocytes/macrophages) and other tissues, clonal haematopoiesis is

by far the most likely cause (Fig. 1). Even in those with an apparent 50% allele frequency who do not meet LFS criteria, other tissues should be tested because, as many as 6/16 (37.5%) tested in one study who were apparently heterozygote had no evidence of the variant in fibroblast culture [37].

Breast cancer risks and overall penetrance

That there is a very high risk of breast cancer aged ≤ 30 years is clear from the high detection rates of *TP53* pathogenic variants in women with breast cancer at this age. The BRIDGES study showed a 300 fold relative risk potentially equivalent to a 30% risk by the 31st birthday using a 0.1% risk by age 30 for the population in the UK [40]. Even using a 2% detection frequency from the lowest detection rate studies, this would equate to a 100-fold relative risk and a 10% cumulative risk [17]; the 5% rate [5, 9] would therefore equate to a 25% risk similar to that estimated from cohorts [41]. What is also clear is that this relative risk drops off fairly dramatically after 30 years of age with detection rates in commercial testing of 0.71% for *TP53* in 4344 samples aged 31–36, and 0.24% in over 20,000 samples aged 37–46 (<https://www.ambrygen.com/providers/resources/prevalence-tool>, accessed 28/08/2020, [21]). Whilst, this might still represent relative risks of 10-fold for that latter period this

is now less than would be expected for *BRCA1*. No reliable cumulative risks are available from large-scale prospective studies and cohort studies that include retrospectively ascertained cases probably exaggerate the lifetime penetrance estimated at around 90% in one study [41]. After age 45 it is not impossible that risks are little different to population risks; in Manchester we have seen no prospective breast cancers in women over 34 years of age with 6/9 occurring aged ≤ 30 . Indeed in 15 female *TP53* carriers with 83 years of follow up post pathogenic variant report aged > 45 years no breast cancers have been observed.

There is clearly also a very high rate of contralateral breast cancer approaching 4–7% per annum which was significantly higher than for *BRCA1* or *BRCA2* in those diagnosed aged < 35 [42]. This remained true in prospective analysis and should justify a discussion of risk reducing contralateral mastectomy as long as survival chances are good from the first primary.

The overall cumulative all cancer incidence of germline pathogenic variants in *TP53* from familial cases has been estimated at 73–100% by age 70, with risks close to 100% in women [41, 43–45]. It is highly likely nonetheless that penetrance is variable. This may be explained in some part by the dominant-negative effects of most pathogenic missense variants. These *TP53* dominant-negative missense variants are usually found in families with childhood malignancies and are

Fig. 1 Flow-chart to aid in distinguishing mosaicism from CHIP and subsequent variant interpretation

Table 2 Recommendations for *TP53* testing in women with breast cancer [11]

(A) When testing is definitely recommended	(B) When testing could be considered	(C) When NOT to test <i>TP53</i>
Women with invasive breast cancer or DCIS ≤ 30	Women with breast cancer ≤ 46 not fulfilling Chompret criteria or other criteria in (A)	Breast cancer aged > 46 with no fulfilment of Chompret criteria and no previous malignancy or DCIS
Women with breast cancer ≤ 46 fulfilling Chompret criteria		

generally highly penetrant [5, 46]. In contrast, typical loss of function variants (frameshift or nonsense variants, splicing variants, large genomic rearrangements, and non-dominant-negative missense variants), are predominantly identified in families with mainly adult onset cancers and are likely to have a lower overall cancer penetrance [5, 12, 44]. A particularly important example of such a low penetrance, but still pathogenic variant, is the non-dominant-negative missense p.Arg337His variant, present in 0.3% of the Southern Brazilian population [45–49]. Nevertheless this variant is definitely associated with breast cancer risk [48, 49], but its overall penetrance for breast cancer is still to be determined.

Risks from standard breast cancer therapies in *TP53* carriers

There is a very high likelihood of subsequent primary tumours in patients with pathogenic *TP53* variants [5, 44]. This appears to occur in $> 40\%$ of *TP53* carriers despite often poor survival from the first primary [5, 44]. Radiotherapy particularly, but also some types of chemotherapy, appear to increase this metachronous primary cancer risk with a particular risk within the radiation field [5, 12, 50]. Studies of chemotherapy/radiotherapy effects on lymphocytes with wild-type or mutant *TP53* and on *TP53* mouse models appears to provide additional evidence for this risk [51]. Therefore, for women with a significant likelihood of a germline *TP53* related breast cancer, testing for pathogenic variants should ideally be performed prior to commencing treatment [12]. If a pathogenic *TP53* variant is found particularly in young women with good survival chances mastectomy should be undertaken rather than breast conserving surgery and radiotherapy as per recent guidance [12]. In these young women with high contralateral risk the option of bilateral mastectomy should also be discussed [12, 42].

Effects of germline *TP53* pathogenic variant on psychological distress

There is not a great deal published on the impact of *TP53* testing on psychological factors. However, there are overall higher levels of distress in families with *TP53*

pathogenic variants even in those testing negative [52]. More research is needed in this area particularly on the impact of an unexpected *TP53* test result from panel testing as in our experience identifying a *TP53* variant is often associated with high levels of anxiety.

Breast cancer testing criteria for *TP53*

Women with breast cancer > 46 years of age have a very low likelihood of a germline pathogenic variant in the absence of Chompret criteria [12]. As described above, this is probably only around 0.1%. These women are probably more likely to have a CHIP diagnosis which will require fibroblast culture and/or obtaining tissue block material. They are also at least as likely to have a variant of uncertain significance that might be considered as ‘hot’ [27]. This does not even take into account the many variants that would still be considered class 3 [53]. The recent guidance recommends that breast cancer patients > 46 years at diagnosis should NOT be tested for *TP53* [12]. Given the above this seems a sensible approach as the dis-benefits of testing and potential huge anxieties around whether there is variant misclassification, CHIP or the waiting game around a ‘hot’ class 3 that probably will never be classified as high risk are considerably higher than a meaningful result. There are clear recommendations for testing aged ≤ 30 and with Chompret criteria [12], but this leaves many women with breast cancers aged 31–46 with no clear guidance where testing might be considered (Table 2) albeit still with a low likelihood of detection ($\sim 0.33\%$). National testing panels for breast cancer in the UK now only include *BRCA1*, *BRCA2* and *PALB2* and given the above it would seem appropriate to have breast panels for older women with breast cancer that also exclude *TP53*. Website: The complete ERN guidelines can be uploaded from the ERN website: <https://www.genturis.eu> [12].

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Compliance with ethical standards

Conflict of interest DGE has received travel grants from AstraZeneca.

References

- Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH et al (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 250:1233–1238
- Li FP, Fraumeni JF Jr (1969) Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann Intern Med* 71:747–752
- Lynch HT, Guirgis HA (1979) Childhood cancer and the SBLA syndrome. *Med Hypotheses* 5:15–22
- Li FP, Fraumeni JF, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA, Miller RW (1988) A cancer family syndrome in twenty-four kindreds. *Cancer Res* 48:5358–5362
- Bougeard G, Renaux-Petel M, Flaman JM, Charbonnier C, Femy P, Belotti M, Gauthier-Villars M, Stoppa-Lyonnet D, Consolino E, Brugieres L, Caron O, Benusiglio PR, Bressac-de Paillerets B, Bonadona V, Bonaiti-Pellie C, Tinat J, Baert-Desurmont S, Frebourg T (2015) Revisiting li-fraumeni syndrome from TP53 mutation carriers. *J Clin Oncol* 33(21):2345–2352. <https://doi.org/10.1200/JCO.2014.59.5728>
- Magnusson S, Gisselsson D, Wiebe T, Kristoffersson U, Borg Å, Olsson H (2012) Prevalence of germline TP53 mutations and history of Li-Fraumeni syndrome in families with childhood adrenocortical tumors, choroid plexus tumors, and rhabdomyosarcoma: a population-based survey. *Pediatr Blood Cancer* 59:846–853
- Hettmer S, Archer NM, Somers GR, Novokmet A, Wagers AJ, Diller L et al (2014) Anaplastic rhabdomyosarcoma in TP53 germline mutation carriers. *Cancer* 120:1068–1075
- Tabori U, Shlien A, Baskin B, Levitt S, Ray P, Alon N et al (2010) TP53 alterations determine clinical subgroups and survival of patients with choroid plexus tumors. *J Clin Oncol* 28:1995–2001
- Evans DG, Moran A, Hartley R, Dawson J, Bulman B, Knox F, Howell A, Lalloo F (2010) Long-term outcomes of breast cancer in women aged 30 years or younger, based on family history, pathology and BRCA1/BRCA2/TP53 status. *Br J Cancer* 102:1091–1098
- Gonzalez KD, Noltner KA, Buzin CH, Gu D, Wen-Fong CY, Nguyen VQ et al (2009) Beyond li fraumeni syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol* 27:1250–1256
- Ruijs MW, Verhoef S, Rookus MA, Pruntel R, van der Hout AH, Hogervorst FB et al (2010) TP53 germline mutation testing in 180 families suspected of Li-Fraumeni syndrome: mutation detection rate and relative frequency of cancers in different familial phenotypes. *J Med Genet* 47:421–428
- Frebourg T, Bajalica Lagercrantz S, Oliveira C, Magenheimer R, Evans DG, European Reference Network GENTURIS (2020) Guidelines for the Li-Fraumeni and heritable TP53-related cancer syndromes. *Eur J Hum Genet*. <https://doi.org/10.1038/s41431-020-0638-4>
- Hanson H, Brady AF, Crawford G, Eeles RA, Gibson S, Jorgensen M, Izatt L, Sohaib A, Tischkowitz M, Evans DG, Consensus Group Members (2020) UKCGG Consensus Group guidelines for the management of patients with constitutional TP53 pathogenic variants. *J Med Genet*. <https://doi.org/10.1136/jmedgenet-2020-106876>
- Kratz CP, Achatz MI, Brugières L, Frebourg T, Garber JE, Greer MC et al (2018) Cancer screening recommendations for individuals with li-fraumeni syndrome. *Clin Cancer Res* 23:e38–e45
- Villani A, Shore A, Wasserman JD, Stephens D, Kim RH, Druker H et al (2016) Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study. *Lancet Oncol* 17:1295–1305
- Fortuno C, James PA, Spurdle AB (2018) Current review of TP53 pathogenic germline variants in breast cancer patients outside Li-Fraumeni syndrome. *Hum Mutat* 39:1764–1773
- Bakhuizen JJ, Hogervorst FB, Velthuis ME, Ruijs MW, van Engelen K, van Os TA, Gille JJ, Collée M, van den Ouweland AM, van Asperen CJ, Kets CM, Mensenkamp AR, Leter EM, Blok MJ, de Jong MM, Ausems MG (2019) TP53 germline mutation testing in early-onset breast cancer: findings from a nationwide cohort. *Fam Cancer* 18:273–280
- Sidransky D, Tokino T, Helzlsouer K, Zehnbauser B, Rausch G, Shelton B, Prestigiacomo L, Vogelstein B, Davidson N (1992) Inherited p53 gene mutations in breast cancer. *Cancer Res* 52:2984–2986
- Zelada-Hedman M, Børresen-Dale AL, Claro A, Chen J, Skoog L, Lindblom A (1997) Screening for TP53 mutations in patients and tumours from 109 Swedish breast cancer families. *Br J Cancer* 75:1201–1204
- Dorling L, Carvalho S, Allen J, González-Neira A, Luccarini C, Wahlström C, Pooley KA, Parsons MT, Fortuno CL (2020) Breast cancer risk genes: association analysis of rare coding variants in 34 genes in 60,466 cases and 53,461 controls. *N Engl J Med*. In press
- Hart SN, Polley EC, Yussuf A, Yadav S, Goldgar DE, Hu C, LaDuca H, Smith LP, Fujimoto J, Li S, Couch FJ, Dolinsky JS (2020) Mutation prevalence tables for hereditary cancer derived from multigene panel testing. *Hum Mutat* 41:e1–e6. <https://doi.org/10.1002/humu.24053>
- Petridis C, Arora I, Shah V, Moss CL, Mera A, Clifford A, Gillett C, Pinder SE, Tomlinson I, Roylance R, Simpson MA, Sawyer EJ (2019) Frequency of pathogenic germline variants in CDH1, BRCA2, CHEK2, PALB2, BRCA1, and TP53 in sporadic lobular breast cancer. *Cancer Epidemiol Biomarkers Prev* 28:1162–1168
- Taylor A, Brady AF, Frayling IM, Hanson H, Tischkowitz M, Turnbull C, Side L, UK Cancer Genetics Group (UKCGG) (2018) Consensus for genes to be included on cancer panel tests offered by UK genetics services: guidelines of the UK Cancer Genetics Group. *J Med Genet* 55:372–377
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL (2015) ACMG Laboratory Quality Assurance Committee (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 17:405–424
- Fortuno C, Lee K, Olivier M, Pesaran T, Mai PL, de Andrade KC et al (2020) Specifications of the ACMG/AMP variant interpretation guidelines for germline TP53 variants. *Hum Mutat* in press
- Kato S, Han SY, Liu W, Otsuka K, Shibata H, Kanamaru R, Ishioka C (2003) Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc Natl Acad Sci USA* 100:8424–8429
- Zerdoumi Y, Lanos R, Raad S, Flaman JM, Bougeard G, Frebourg T, Tournier I (2017) Germline TP53 mutations result into a constitutive defect of p53 DNA binding and transcriptional response to DNA damage. *Hum Mol Genet* 26:2591–2602
- Giacomelli AO, Yang X, Lintner RE, McFarland JM, Duby M, Kim J et al (2018) Mutational processes shape the landscape of TP53 mutations in human cancer. *Nat Genet* 50:1381–1387
- Kotler E, Shani O, Goldfeld G, Lotan-Pompan M, Tarcic O, Gershoni A et al (2018) A systematic p53 mutation library links differential functional impact to cancer mutation pattern and evolutionary conservation. *Mol Cell* 71:178–90
- de Andrade KC, Frone MN, Wegman-Ostrosky T, Khincha PP, Kim J, Amadou A et al (2019) Variable population prevalence

- estimates of germline TP53 variants: a gnomAD-based analysis. *Hum Mutat* 40:97–105
31. Evans DG, Turnbull C, Woodward ER (2019) Concern regarding classification of germline TP53 variants as likely pathogenic. *Hum Mutat* 40:828–831
 32. Laloo F, Varley J, Ellis D, Moran A, O'Dair L, Pharoah P, Evans D, Onset Breast Cancer Study Group (2003) Prediction of pathogenic mutations in patients with early-onset breast cancer by family history. *Lancet* 361:1101–1102
 33. Yurgelun MB, Masciari S, Joshi VA, Mercado RC, Lindor NM et al (2015) Germline TP53 mutations in patients with early-onset colorectal cancer in the colon cancer family registry. *JAMA Oncol* 1:214–221
 34. Evans DG, Hartley CL, Smith PT, King AT, Bowers NL, Tobin S, Wallace AJ, Perry M, Anup R, Lloyd SKW, Rutherford SA, Hammerbeck-Ward C, Pathmanaban ON, Stapleton E, Freeman SR, Kellett M, Halliday D, Parry A, Gair JJ, Axon P, Laitt R, Thomas O, Afridi SK, Obholzer R, English Specialist NF research group, Duff C, Stivaros SM, Vassallo G, Harkness EF, Smith MJ (2020) Incidence of mosaicism in 1055 de novo NF2 cases: much higher than previous estimates with high utility of next-generation sequencing. *Genet Med* 22:53–59
 35. Batalini F, Peacock EG, Stobie L, Robertson A, Garber J, Weitzel JN, Tung NM (2019) Li-Fraumeni syndrome: not a straightforward diagnosis anymore—the interpretation of pathogenic variants of low allele frequency and the differences between germline PVs, mosaicism, and clonal hematopoiesis. *Breast Cancer Res* 21:107
 36. Weber-Lassalle K, Harter P, Hauke J, Ernst C, Kommos S, Marmé F et al (2018) Diagnosis of li-fraumeni syndrome: differentiating TP53 germline mutations from clonal hematopoiesis: results of the observational AGO-TR1 trial. *Hum Mutat* 39:2040–2046
 37. Weitzel JN, Chao EC, Nehoray B, Van Tongeren LR, LaDuca H, Blazer KR et al (2018) Somatic TP53 variants frequently confound germ-line testing results. *Genet Med* 20:809–816
 38. Chen S, Liu Y (2019) p53 involvement in clonal hematopoiesis of indeterminate potential. *Curr Opin Hematol* 4:235–240
 39. Mester JL, Jackson SA, Postula K, Stettner A, Solomon S, Bissonnette J, Murphy PD, Klein RT, Hruska KS (2020) Apparently heterozygous TP53 pathogenic variants may be blood limited in patients undergoing hereditary cancer panel testing. *J Mol Diagn* 22:396–404
 40. <https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/incidence-invasive#heading-One> Accessed 28 Aug 2020
 41. Shin SJ, Dodd-Eaton EB, Peng G, Bojadzieva J, Chen J, Amos CI et al (2020) Penetrance of different cancer types in families with Li-Fraumeni syndrome: a validation study using multi-center cohorts. *Cancer Res* 80:354–360
 42. Hyder Z, Harkness EF, Woodward ER, Bowers NL, Pereira M, Wallace AJ, Howell SJ, Howell A, Laloo F, Newman WG, Smith MJ, Evans DG (2020) Risk of contralateral breast cancer in women with and without pathogenic variants in BRCA1, BRCA2, and tp53 genes in women with very early-onset (<36 Years) breast cancer. *Cancers (Basel)* 12:378
 43. Chompret A, Brugières L, Ronsin M, Gardes M, Dessarps-Freichey F, Abel A et al (2000) P53 germline mutations in childhood cancers and cancer risk for carrier individuals. *Br J Cancer* 82:1932–1937
 44. Mai PL, Best AF, Peters JA, DeCastro RM, Khincha PP, Loud JT et al (2016) Risks of first and subsequent cancers among TP53 mutation carriers in the National Cancer Institute Li-Fraumeni syndrome cohort. *Cancer* 122:3673–3681
 45. Amadou A, Waddington Achatz MI, Hainaut P (2018) Revisiting tumor patterns and penetrance in germline TP53 mutation carriers: temporal phases of Li-Fraumeni syndrome. *Curr Opin Oncol* 30:23–29
 46. Figueiredo BC, Sandrini R, Zambetti GP, Pereira RM, Cheng C, Liu W et al (2006) Penetrance of adrenocortical tumours associated with the germline TP53 R337H mutation. *J Med Genet* 43:91–96
 47. Achatz MI, Olivier M, Le Calvez F, Martel-Planche G, Lopes A, Rossi BM et al (2007) The TP53 mutation, R337H, is associated with Li-Fraumeni and Li-Fraumeni-like syndromes in Brazilian families. *Cancer Lett* 245:96–102
 48. Palmero EI, Schüler-Faccini L, Caleffi M, Achatz MI, Olivier M, Martel-Planche G et al (2008) Detection of R337H, a germline TP53 mutation predisposing to multiple cancers, in asymptomatic women participating in a breast cancer screening program in Southern Brazil. *Cancer Lett* 261:21–25
 49. Andrade KC, Santiago KM, Fortes FP, Mambelli LI, Nóbrega AF, Achatz MI (2016) Early-onset breast cancer patients in the South and Southeast of Brazil should be tested for the TP53 p. R337H mutation. *Genet Mol Biol* 39:199–202
 50. Kasper E, Angot E, Colasse E, Nicol L, Sabourin JC, Adriauch S et al (2018) Contribution of genotoxic anticancer treatments to the development of multiple primary tumours in the context of germline TP53 mutations. *Eur J Cancer* 101:254–262
 51. Evans DG, Birch JM, Ramsden RT, Sharif S, Baser ME (2006) Malignant transformation and new primary tumours after therapeutic radiation for benign disease: substantial risks in certain tumour prone syndromes. *J Med Genet* 43:289–294
 52. Lammens CR, Aaronson NK, Wagner A, Sijmons RH, Ausems MG, Vriends AH, Ruijs MW, van Os TA, Spruijt L, Gómez García EB, Kluijdt I, Nagtegaal T, Verhoef S, Bleiker EM (2010) Genetic testing in Li-Fraumeni syndrome: uptake and psychosocial consequences. *J Clin Oncol* 28(18):3008–3014
 53. Plon SE, Eccles DM, Easton D, Foulkes WD, Genuardi M, Greenblatt MS, Hogervorst FB, Hoogerbrugge N, Spurdle AB, Tavtigian SV, IARC Unclassified Genetic Variants Working Group (2008) Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* 29:1282–1291

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