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Proposed framework for triage of putative germline variants detected via tumour genomic testing in UK oncology practice

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ABSTRACT

In the UK, most patients receive publicly funded medical care through the National Health Service (NHS), which funds tumour and/or germline testing for eligible patients with cancer to inform clinical management.

Testing on tumour-derived DNA may identify putative heritable variants, with implications for the proband and their wider family, but is not a reliable substitute for germline genetic testing when hereditary cancer predisposition is suspected.

The likelihood that a variant identified through tumour testing is of germline origin depends on multiple clinical and technical factors. Certain genotypes significantly influence a patient's cancer risk, and intervention in those carriers may facilitate cancer prevention or early detection, while other genotypes are associated with lower cancer risk, and associated intervention in such cases have limited clinical utility.

We convened a national meeting of clinical cancer genetics and scientific leads to rationalise germline follow-up testing of variants identified through tumour-based testing. After contrasting potential approaches, implementation of an NHS-contextualised 'intermediate conservative' approach was agreed and refined by the authors, with the final pathway recirculated to the UK clinical and scientific community for consensus agreement and publication.

We outline relevant patient, genetic and technical considerations informing likely origin of variants, a review of current relevant guidance and NHS laboratory practices and a workflow for laboratory and clinical teams to triage tumour-detected variants requiring onward germline follow-up. This approach aims to direct limited resources towards identifying germline variants associated with the greatest potential clinical impact, with a view to supporting more efficient and equitable delivery of genomic medicine in oncology.

WHAT IS ALREADY KNOW ON THIS TOPIC

⇒ Tumour-based testing may lead to inadvertent identification of putative heritable genetic variants
⇒ The estimated likelihood of germline origin of variants picked up through tumour testing is influenced by:
⇒ factors related to the variant (gene in which it has been identified, variant allele frequency, population frequency, type of variant)
⇒ patient-specific features (cancer type, age at diagnosis, ethnicity, personal and family history)
⇒ Technical considerations (proportion of DNA from nucleated cancer cells compared relative to that from normal cells, type and extent of testing)
⇒ Some, but not all, variants of putative germline origin will require confirmation and onward management.
⇒ Tumour-testing should not be considered a substitute for formal germline genetic testing where an underlying hereditary cause is suspected.

WHAT THIS STUDY ADDS

⇒ We propose a workflow by which laboratory and clinical teams can triage variants ascertained through tumour testing, such that resources for germline follow-up can be focussed to those associated with greatest clinical impact.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Germline follow-up testing should be reserved for variants associated with highest clinical utility, i.e. those associated with highest clinically actionable cancer risk and/or those likely causal for the patient phenotype. We hope this work will help rationalise testing in the face of increasing demands on overstretched clinical and laboratory services.

BACKGROUND

Traditionally, frameworks for evaluation of genetic tests focused on factors related to the analytical and clinical validity, ethical and medicolegal considerations and clinical utility.[1] The National Health Service (NHS) in the UK is a publicly funded system. To ensure appropriate and fair allocation of resources, decisions are underpinned by ethical principles related to justice, equity, beneficence and non-maleficence.[2] Services should be prioritised for individuals in greatest clinical need, and to whom the service will provide the greatest benefit, giving due consideration for cost implications of testing and associated infrastructure required to interpret and manage results.[3] Increasing demands for limited resources have prompted efforts in rationalising and standardising indications for testing ('who to test') and genes included on relevant panels ('what to test'), based on the clinical utility of the test and likelihood of identification of carriers, balanced

against the workload associated with analysis, interpretation and reporting of results.[4]

NHS-funded standard-of-care testing is typically phenotype driven, but the extent of test targets and eligibility criteria is expanding, such that additional and incidental findings are more likely. Furthermore, NHS laboratories and clinical genetics services frequently receive referrals related to patients undergoing broader pan-cancer gene testing on tumour and/or germline DNA as part of research studies or clinical trials, through direct-to-consumer testing or in the private sector.

WHO TO TEST?

In England and Scotland, indications for NHS-funded germline genetic testing are outlined in the NHS England (NHSE) Genomic Test Directory and Scottish Genomic Test Directory, respectively, with testing practices in Northern Ireland and Wales broadly aligned depending on local capacity. Germline genetic testing is generally recommended where the a priori probability of identifying a constitutional variant in a relevant gene is at least 10%.[5] However, testing may be indicated at much lower predicted diagnostic yield where the clinical utility of a result is high. For example, where the result has implications for treatment eligibility, or where a variant predisposes to cancers, where the risk may be reduced through surgery, or where effective surveillance for early detection exists. Eligibility for testing may also be extended more widely where identification of presymptomatic carriers enables risk-reducing interventions that may be cost-effective or even cost-saving for the NHS.[6 7] The clinical utility of a test is limited where risks are low, uncertain, where options for risk management are limited, or where survival advantage has not been proven.[8]

WHAT TO TEST/REPORT?

In the UK, diagnostic germline genetic testing has traditionally been phenotype driven and restricted to those genes for which strong, consistent data for gene–disease association exist. Curation of gene panels is undertaken via PanelApp (https://panelapp.genomicsengland.co.uk/), a crowdsourced platform developed by Genomics England with the aim of standardising panels of genes analysed for patients with specific phenotypes.[9] The penetrance and phenotype—and therefore benefit of risk-reducing interventions—associated with constitutional variants in such genes may be variant specific. Based on current knowledge, classifying individual variants as 'high', 'moderate' or 'low' risk is difficult. Instead, the genes in which variants occur are classified according to these categories, recognising that not all variants in these genes will be associated with the purported risk category, necessitating different management of variants associated with 'standard' compared with 'reduced' penetrance within a high-risk gene.[10] Complicating matters, penetrance may be further modified by coexisting genetic and other modifiers, as well as family history, such that the context in which a variant is identified must also be considered in order to provide accurate advice to patients and their at-risk relatives.[11] In the UK, it is generally accepted that testing for and reporting of variants should be restricted to those with an associated OR in excess of 2 (lower CI in excess of 1.5)[12 13]—prompting reporting guidance for genes where variant-specific risks straddle this threshold.[14]

INCIDENTAL FINDINGS

Although testing in the UK has, for the most part, been phenotype driven, using small panels, broader testing using whole exome or genome sequencing has become increasingly available, in the context of cancer and in rare disease settings. Identification of incidental findings (including recessive traits or clinically relevant variants not known to be associated with the patient phenotype) is consequently becoming an increasing issue. Some programmes or research studies may purposefully report 'secondary' findings not immediately related to the patient phenotype if the findings are deemed clinically important, in which case they are considered 'additional' rather than 'incidental' findings.[15] The British Society for Genetic Medicine (BSGM) has developed guidelines for the management of incidental findings from germline genetic tests undertaken as part of rare disease investigations.[15]

When considering cancer, there is widespread availability of pan-cancer predisposition panels that can be accessed in the private sector, or direct-to-consumer, and variants not immediately related to the patient/family phenotype are routinely reported as additional findings. Furthermore, analysis of tumour-derived DNA from tissue or circulating tumour DNA (ctDNA) (through NHS standard of care testing or through privately or self-funded commercial testing) may identify variants of putative germline origin, that may be 'on-tumour' (associated with the cancer in which it has been associated), 'off-tumour' (not known to be associated with the cancer in question). Efforts are ongoing to align the classification of variants detected in somatic and germline contexts, but some tensions remain given the different purposes of testing, and subtleties in distinction between pathogenicity and oncogenicity.[16] While the aim of constitutional (germline) genetic testing is to detect heritable variants associated with clinically meaningful cancer risk, the intention of somatic testing is to identify relevant variants in tumour-derived DNA to inform targeted therapeutic strategies or to aid diagnosis. Variants of putative germline origin may be reported when detected via testing of tumour-derived DNA that would not be reported if identified through standard diagnostic germline testing—for example, missense variants in *ATM* or *CHEK2*.[17 18]

Recessive traits

Identification of a recessive trait may not have immediate implications for the proband, unless there is a reasonable suspicion that the variant is contributory and another variant in trans has not been identified. Arguments for identification of recessive traits centre around implications for reproductive decision-making, particularly if the likelihood of having a child affected by a recessive disorder is high, for example, if they are in a consanguineous relationship, or if their partner is from a population where associated carrier frequency is high.[19] NHS-funded testing of partners of carriers of recessive traits in a particular gene is offered in cases of consanguinity or if the spouse/partner is from a population where carrier frequency is at least one in seventy—a threshold set to balance the burden of work associated with cascade and carrier testing against the likelihood of conception of a child with a clinically relevant autosomal recessive phenotype, while similar strategies have been recommended in other jurisdictions.[20 21] The UK Cancer Genetics Group (UKCGG) has developed specific reporting and testing guidance for variants in *MUTYH*, given disproportionate workload compared with clinical utility, given that the associated phenotype is adult in onset, and carrier frequency relatively high in certain populations, and considering relative genetic heterogeneity of polyposis and bowel cancer.[4]

Where a monoallelic variant is unlikely to be contributory to the patient phenotype, the utility of follow-up testing depends on the risk of having a child affected by an associated recessive phenotype. This depends on the patient's age and reproductive

status, and the likelihood that their partner is also a carrier of a variant in the same gene. If the carrier frequency in the general population is low (rarer than one in seventy), cascade testing of relatives/partner testing is not recommended as per the current NHS genomic test directory. Therefore, reporting of recessive traits unlikely to be contributory to the patient phenotype *is not* routinely recommended.[15] When a single variant in a gene associated with a recessive condition has been identified in a patient with features potentially compatible with the disorder, the decision regarding reporting depends on the likelihood that the variant may be contributory as well as the genetic heterogeneity of the patient phenotype.[15]

Dominant traits unrelated to patient phenotype

BSGM recommends that resources in NHS genomic laboratories should be focused on identifying and reporting of variants directly related to the indication for which the test was requested. Secondary findings in cancer predisposition genes are therefore not *actively* sought if unrelated to a rare disease indication. However, on occasion, a variant associated with dominant cancer predisposition may be identified as an incidental finding in the context of investigations undertaken for a rare disease. In this situation, BSGM guidance recommends that reporting is restricted to *pathogenic* variants only and that decisions to report should be guided by the age of onset of an associated phenotype, associated penetrance, as well as actionability (possible intervention) and clinical context of the individual in whom it has been identified.[15]

In the context of 'unselected' testing—where an incidental variant has been identified in a patient without a clear manifesting phenotype, a more cautious approach is required in declaring a variant as clinically actionable, for which reason, BSGM does not advocate reporting of variants for which evidence is insufficient to classify as pathogenic. The BSGM guidance applies to incidental constitutional variants detected in the context of rare disease and, as written, is not directly intended to be applicable to incidental findings of putative germline origin detected through somatic testing in patients with cancer.

ANALYSIS OF TUMOUR-DERIVED DNA

Genetic variation is a fundamental driver of oncogenesis, with relevant pathogenic variants occurring in either somatic or germline contexts. Comprehensive analysis requires testing of DNA derived from a sample enriched for neoplastic cells, and of DNA from a paired representative constitutional DNA sample. Where paired testing of neoplastic and non-neoplastic samples is not possible, unpicking the origin of an identified variant may be difficult. Inadvertent identification of germline genetic variants is not infrequent, reported in 3–18%, depending on the number and type of test targets,[22–25] with detection likely to increase as tumour-based testing of genes associated with cancer susceptibility expands and with increasing application of broader, pan-cancer panels.

The germline conversion rate (GCR) refers to the proportion of variants detected in tumour-derived DNA that are likely pathogenic/pathogenic and of germline origin relative to the total number of tumour-detected variants in a particular gene. Factors that should be considered in predicting likelihood of constitutional origin of a variant picked up through testing of tumour-derived DNA include the gene in which the variant has been identified, proportion of neoplastic relative to non-neoplastic cells, variant allele frequency (VAF) of the variant in question as well as of any other coexisting variants, coexisting genomic aberrations, type of variant, frequency of variant in germline and somatic context, and the patient's personal and family phenotype. Distinguishing between relevant somatic drivers and (possibly incidental) germline variants may have immediate implications for patient treatment, as well as for estimating the risk of future cancers and informing cancer risk management, for them and their family members.

Variant allele frequency

VAF refers to the proportion of sequencing reads containing a specific genetic variant at a given locus relative to the total reads covering that position. Accurate interpretation of VAF relative to the technical and clinical context is helpful in distinguishing between variants of likely germline origin and those more likely to be somatic.

The expected VAF of germline variants in constitutional DNA is typically approximately 50% (heterozygous) or 100% (homozygous/hemizygous), apart from exceptional cases of constitutional mosaicism. However, in tumour-derived DNA, the VAF of a significant proportion of germline variants deviates from these typical numbers depending on the gene in question and coexisting copy number events or other mechanisms in cis or in trans that may lead to spuriously low or spuriously high measures, respectively, as well as issues related to bioinformatic pipelines and other technical considerations.[26 27]

Because of potential loss of the variant allele in tumour cells, and because of technical limitations of testing (particularly of fragmented DNA/DNA from Formalin-Fixed Paraffin-Embedded (FFPE)-preserved tumours), tumour testing is *not* a substitute for germline testing.[28–30] Formal constitutional (germline) genetic testing should be offered where clinically indicated and possible in an eligible individual, even if testing of the relevant genes using tumour-derived DNA is uninformative.

Somatic variants typically exhibit variable VAFs due to tumour heterogeneity and inherent genomic instability, clonal evolution and coexisting genomic aberrations. Furthermore, it is important to remember the influence of technical considerations such as sequencing artefacts, particularly at shallow depths or in regions of genomic complexity.[31] VAF is also influenced by the proportion of DNA derived from neoplastic compared with non-neoplastic cells across different samples. Nuclear genomic DNA can be obtained from any nucleated cell, including cancer cells, supporting stromal cells, tumour-associated normal cells and tumour-infiltrating lymphocytes. Accurate assessment of neoplastic cell content (NCC) is important to ensure a representative DNA sample. NCC may be influenced by the physical area of the tumour relative to non-cancerous tissue, cellularity of the sample and the size of the cancer cells relative to normal cells (bearing in mind that the amount of DNA per nucleated cell is unchanged). Manual assessment of NCC has been shown to be variable between pathologists,[32] further complicating the interpretation of VAFs.

The probability of a variant detected in tumour-derived DNA being of germline origin is very low if the VAF is less than approximately 30–35%.[33 34] The likelihood of variants detected in tumour-derived DNA being of germline origin if VAF is greater than 35% depends on the gene in which the variant is identified. Some genes are so commonly somatically altered either pan-tumour (eg, *TP53*, *RB1*) or in related tumour phenotypes (eg, *APC* in colorectal cancer, *VHL* in renal cell carcinoma) that the GCR is low even at high VAF, and conversely, somatic variation in other genes is rare such that a variant picked up in such a gene is much more likely to be of constitutional origin.[33]

Relevance of patient and family phenotype

Constitutional variants in certain genes are associated with a syndromic phenotype, such that the likelihood of constitutional origin in a variant in such a gene in an otherwise ostensibly unaffected individual is very low, although a cautious approach is recommended for syndromic conditions associated with variable expressivity and mild phenotypes (eg, neurofibromatosis type 1).[35] GCR is also impacted by ascertainment, and whether or not the gene in which the variant is identified is associated with predisposition to the cancer tested ('on-tumour') or not ('off-tumour').[33] Clinical clues may help point to the origin of a variant, as well as the tumour type in question. Other relevant factors include age at diagnosis and personal/family cancer history. Ethnicity is also relevant, given that patients from certain populations are more likely to carry certain constitutional variants than others.

Founder/recurrent variants

Certain populations are enriched for certain variants—so-called *founder variants*, arising from a common ancestor many generations previously.[36] Variants may become enriched because of population bottlenecks or isolation—for example, small island populations with little inward migration, or in populations where genetic admixture is limited because endogamy is routine.[37 38] Examples include *BRCA1*/*BRCA2* founder variants in individuals of Ashkenazi Jewish heritage (*BRCA1* c.68_69del p.(Glu23ValfsTer17)or c.5266dup p.(Gln1756ProfsTer74), *BRCA2* c.5946del p.(Ser1982ArgfsTer22)).[39] Where a known founder variant is detected as part of analysis of tumour-derived DNA, there should be a high index of suspicion of germline origin, particularly if the patient has a relevant heritage, irrespective of the VAF or tumour type.[28] It should be noted that certain types of variants (including large genomic rearrangements) may be more challenging to detect during analysis of tumour-derived DNA, particularly if DNA is highly fragmented, such that certain recurrent variants reported in different populations (deletion of exon 16 in *MLH1* in Finnish patients,[40] or deletions of exons 1–23 in *BRCA1* in patients from the West of Ireland[41]) may not be very obvious—formal genetic testing on a representative sample of constitutional DNA is recommended for eligible patients even if no variants are detected on tumour-derived DNA, although the likelihood of identifying a variant is lower if not from a population where large genomic rearrangements are uncommon.

Targeted germline follow-up testing

Given that variants in most cancer susceptibility genes are associated with incomplete penetrance, variable expressivity and non-syndromic cancer predisposition, the only definitive way to determine the origin of variants identified in tumour is to undertake follow-up testing in constitutional DNA. In some jurisdictions, paired tumour-normal testing happens as a matter of routine practice, but in most circumstances in NHS services, testing of tumour and constitutional samples is undertaken asynchronously and very rarely contemporaneously. In a publicly funded health system, germline follow-up of all variants in cancer susceptibility genes picked up through tumour testing is unrealistic, given limited capacity and resources. Follow-up targeted germline testing of variants picked up during tumour testing should therefore be rationalised, not only guided by the GCR but also giving due consideration to the clinical utility of confirming (or not) germline origin of a particular variant.

Table 1 Genes categorised by actionability as per the European Society for Medical Oncology Precision Medicine Working Group[33]

	Age at which germline follow-up of variants detected through tumour testing should be considered	
CSG actionability	**All ages**	**Age<30 years**
Most	*BRCA1, BRCA2, PALB2, MLH1, MSH2, MSH6, RET**	
High	*BRIP1, RAD51C, RAD51D, PMS2, VHL†, TSC2, SDHB, SDHC, SDHAF2, SDHD, TMEM127, MUTYH‡*	*APC, PTEN, RB1, TP53†**
Standard	*ATM, BARD1, CHEK2, NF1, FLCN, FH, POLE, POLD1, SDHA, SMAD4, DICER1, SMARCB1, SUFU, PTCH1, BAP1*	*CDKN2A, SMARCA4*

*see Gene-specific and genotype-specific considerations section below.
†Certain tumours excluded because of high frequency of somatic events (*TP53*—brain, *VHL*—kidney).
‡Biallelic events only.
CSG, cancer susceptibility gene; MA, most actionable; SA, standard actionability.

RECOMMENDATIONS OF THE EUROPEAN SOCIETY FOR MEDICAL ONCOLOGY (ESMO) PRECISION MEDICINE WORKING GROUP (PMWG)

The ESMO PMWG has provided data and recommendations to inform somatic to germline testing pathways.[33] Their analysis of 49 264 paired tumour-normal samples indicated that GCR of pathogenic variants detected in tumour tissue is high (defined as >10%), for certain genes (such as *BRCA1*, *BRCA2* and *PALB2*), when filtering based on a VAF of 30% (for Single Nucleotide Variants (SNVs)) or 20% (for indels) is applied. Comparatively, for certain other genes, GCR is very low, even after strict filtering based on VAF has been applied, because somatic variation in those genes is a common and early event.

The group has categorised 40 genes in which filtered tumour variants demonstrate high GCR into three groups in terms of 'actionability' (most, high and standard), based on associated penetrance, efficacy of risk-reducing interventions and 'off-tumour' GCR (table 1) and have suggested four strategies for follow-up germline testing of variants identified in these genes in tumour-derived DNA (table 2) that could be applied depending on locoregional capacity and resources, ranging from permissive—germline follow-up for filtered variants in all 40 genes in all tumour types—to conservative, with germline follow-up only in 'associated' tumour types ('on-tumour') for all genes. Additional caveats are suggested for those genes in which somatic variation

Table 2 Strategies for germline follow-up of variants detected through tumour testing as per the European Society for Medical Oncology guidance[33]

Approach	
Permissive	Germline follow-up for all 40 genes in all tumour types.
Intermediate permissive	Germline follow-up for all 23 MA-CSGs/HA-CSGs in all tumour types, but germline follow-up only in 'associated' tumour types for 17 SA CSGs.
Intermediate conservative	Germline follow-up in all tumour types for the 7 MA-CSGs but germline follow-up only in 'associated' tumour types for the other 33 HA-CSGs/SA-CSGs.
Conservative	Germline follow-up only in 'associated' tumour types for all 40 genes.

CSGs, cancer susceptibility genes; HA, highly actionable; MA, most actionable; SA, standard actionability.

is particularly common in certain tumour types (eg, *TP53* variants in brain tumours, *VHL* variants in kidney cancers), and germline follow-up is not required in such instances, although patients meeting eligibility criteria for diagnostic germline testing should still be offered the same.[20] For other genes, follow-up germline testing is only recommended when the patient is younger than 30 years of age.

ctDNA specific considerations

The increasing availability of the 'liquid biopsy' for the purpose of analysis of ctDNA provides additional challenges. This testing is now routinely available in the NHS for patients with advanced lung or breast cancers.[42] Application of pan-cancer, tumour-agnostic panels for analysis of ctDNA means that incidental findings are more likely. Although data regarding GCR of variants ascertained through ctDNA testing is lacking, the prior probability of a variant being of germline origin is higher, particularly if tumour fraction (TF) (proportion of tumour-derived cfDNA) is low—in which case DNA being analysed is primarily constitutional. It is important that due consideration be given to factors such as patient age (influencing likelihood of coexisting age-related clonal haematopoiesis) and tumour burden/fraction, as well as other biological and clinical considerations as outlined here above.

Gene-specific and genotype-specific considerations

RET

New data from population studies and broad diagnostic testing for other indications have demonstrated that the penetrance associated with (likely) pathogenic constitutional variants in *RET* may not be as high as previously estimated.[43–45] Ascertainment of such variants in the context of a personal and/or family history of phenotypic features of multiple endocrine neoplasia type 2 would traditionally lead to an irreversible intervention (prophylactic thyroidectomy) at very young ages, with potential associated morbidity, in presymptomatic carriers.[46 47] However, it is unclear if the risks associated with such intervention in carriers without a personal/family history of a relevant phenotype outweigh the relatively low medullary thyroid cancer risk in carriers of most incidental *RET* variants, with a handful of notable high-risk exceptions (box 1).

Box 1 Highest risk *RET* genotypes

High risk genotypes—NM_020975.6(RET):

c.2753T>C (p.Met918Thr)
codon 634 variants
⇒ c.1900T>C (p.Cys634Arg)
⇒ c.1900T>G (p.Cys634Gly)
⇒ c.1901G>C, (p.Cys643Ser)
⇒ c.1901G>A, p.Cys634Tyr
⇒ c.1902C>G (p.Cys634Trp)

c.2647_2648delinsTT (p.Ala883Phe)
Double mutations[71–76]:
Exon 14/Exon14: c.2410G>A (p.Val804Met)/c.2417A>G (p.Tyr806Cys) (V804M/Y806C)
Exon 14/Exon 14: c.2410G>A (p.Val804Met)/c.2413G>A (p.Glu805Lys) (V804M/E805K)
Exon 14/Exon 15: c.2410G>A (p.Val804Met)/c.2711C>G (p.Ser904Cys) (V804M/ S904C),
Exon 13/Exon 14: c.2410G>A (p.Val804Met)/c.2342A>G (p.Gln781Arg) (V804M/Q781R)
Exon 13/Exon 16: c.2372A>T (p.Tyr791Phe)/c.2753T>C (p.Met918Thr) (Y791F/M918T)
Exon 16/Exon16: c.2753T>C (p.Met918Thr)/c.2765C>A (p.Ser922Tyr) (M918T/S922Y)

TP53

TP53 is the most frequently somatically mutated gene, with variation evident across a host of cancer types. Somatic *TP53* variants are associated with reduced survival rates, increased resistance to chemoradiation and increased risk of disease relapse and may be associated with aggressive transformation in certain cancers.[48 49] Most pathogenic variants are missense variants, but benign missense variation is also common in *TP53,* complicating variant interpretation. Deleterious variants are commonly identified in the DNA binding domain, and a significant proportion of deleterious somatic variants occurs in hotspots at codons 133, 175, 213, 220, 245, 248, 273, 282 and 337.[50] The deleterious impact of *TP53* variants may be apparent through loss of function, including of transcriptional regulation of genes mediating growth suppression, apoptosis and DNA repair, or conversely, through gain of function and upregulation of proliferation, drug resistance and metastatic potential.[51–53] Variant *TP53* alleles may also exert a dominant negative effect on the wild-type allele.[53]

Constitutional (likely) pathogenic variants are, in general, rare, with a frequency of 1 in 3555 to 1 in 5476, except for a relatively common founder variant in South Brazil.[50 54] The phenotype associated with constitutional variants in *TP53* was initially described as Li Fraumeni syndrome, which was described as a clinical entity some fifty years ago.[55] Early data suggested a very high lifetime cancer risk, particularly of the breast, brain and adrenal glands, as well as sarcoma. Early penetrance estimates were subject to ascertainment bias, and it is becoming increasingly apparent that cancer risks are dependent on variant type and clinical context, including the patient's personal and family history, and other potential coexisting modifiers of disease.[56 57] Increasing availability of germline *TP53* testing, in addition to improved survival of affected carriers, has provided data indicating a broader phenotype than that suggested by classic or Chompret diagnostic criteria for Li Fraumeni syndrome, such that 'heritable *TP53*-related cancer (hTP53rc) syndromes' has been proposed as a more inclusive descriptive term for phenotypes associated with constitutional *TP53* variation.[57] Providing bespoke patient-specific or variant-specific risk estimates is, at present, unfeasible, given the rarity of individual variants. Furthermore, the relatively high contribution of de novo variation and mosaicism means that family history is not always informative, and the patient's own phenotype may be atypical.[58 59] *TP53* is also one of the genes most commonly implicated in clonal haematopoiesis of indeterminate potential—a common phenomenon in older patients such that it is necessary to clarify the origin of a variant identified in blood-derived DNA where the phenotype of the patient in question is inconsistent with constitutional variation in *TP53*.[60–62]

Because of the wide phenotypic spectrum in carriers of constitutional variants in *TP53*, cancer risk management is challenging. For certain *TP53*-associated cancers, evidence for surveillance/risk-reducing interventions is limited, although annual whole-body, breast and brain MRI scans may facilitate early cancer detection.[63 64] Guidelines for surveillance in patients

with confirmed constitutional (likely) pathogenic variants in *TP53* have been defined by ERN Genturis (European Reference Networks Genetic Tumour Risk Syndromes) and UKCGG, with clinical examination, biochemical tests and imaging (abdominal ultrasound, brain and whole body MRI) starting from birth, with additional interventions later in life.[57 65] Mastectomy is preferred to breast conservation for female carriers who develop breast cancer, to minimise potential risk of radiation-induced primary cancers, and risk-reducing breast surgery may be offered to presymptomatic female carriers.[66] A diagnosis of hTP53rc syndrome in a family is devastating, and the diagnosis itself, as well as the burden associated with lifelong and intense surveillance, is associated with a significant psychosocial morbidity.[67]

TP53-specific variant interpretation guidance has been developed.[68] Robust application of this guidance to all variants ascertained in the somatic context may not be realistic, given that some relevant points related to family history may be missing at the time of reporting and given the high frequency of somatic variants in this gene across different cancer types. However, because of the significant implications of a potential diagnosis of heritable *TP53*-related cancer syndromes, this guidance should be applied for all variants of putative germline origin, to inform when germline follow-up testing is indicated.

The GCR of *TP53* variants is low in certain tumour types (eg, brain tumours) and in patients older than 30 years, such that germline follow-up of variants detected in brain tumours or in any tumour in patients older than this age is likely not required, although consideration for formal diagnostic *TP53* germline testing should be given to patient fulfilling relevant eligibility criteria.[20 33]

Moderate-risk susceptibility genes

Gene panels used for germline genetic testing are increasing in size as well as availability, with more frequent inclusion of genes in which variants are associated with more modest risks. The clinical utility of identifying moderate-risk susceptibility alleles is less clear than the identification of high-risk variants, particularly if the associated disease is not typically associated with high mortality.[8] The clinical utility of identifying low-risk variants is questionable and unlikely to justify the significant workload associated with interpretation, reporting or onward clinical activity, such that current UK best practice guidance recommends that variants associated with odds of disease less than twofold are not reported.[12 17 18] This is particularly relevant for non-truncating variants in *ATM, CHEK2, RAD51C, RAD51D, BRIP1* and *BARD1* (with some notable exceptions), given that the risks associated with such variants are lower than those associated with truncating variants.[69] Gene-specific reporting should be considered for genes associated with multiple allelic disorders or specific mechanisms of disease (eg, *APC*, *RET, POLE, POLD1*).[14] It is generally recommended that germline follow-up of variants ascertained through tumour-based testing is only offered if those same variants would be reported if identified through formal diagnostic germline genetic testing.

UKCGG/CANCER VARIANT INTERPRETATION GROUP (CANVIG) PROPOSAL FOR GERMLINE FOLLOW-UP OF VARIANTS PICKED UP THROUGH TUMOUR TESTING

Consensus meeting and working group activity

The UKCGG convened a meeting of clinical cancer genetics and scientific leads (January 2025) with geographical representation across all seven Genomic Laboratory Hubs, Genomic Medicine Service Alliances and Regional Genetics Services in England, with additional representation from clinical and scientific colleagues in Scotland, Wales and Northern Ireland (Devolved Nations). The NHSE Genomic Test Directory indication for confirmatory germline testing of tumour-derived findings was discussed. This discussion was also informed by communications related to NHSE plans for extension of tumour testing to include *MLH1, MSH2, MSH6, BRCA1, BRCA2* and *PALB2*, with purposeful reporting in all tumour types of likely pathogenic/pathogenic variants identified at VAF consistent with putative germline origin as part of their clinical trials recruitment strategy. The meeting was attended by 39 clinical and scientific leads.

A proposal for implementation of an 'intermediate conservative' approach to germline follow-up testing of variants identified through tumour-based testing was agreed. This was selected as the most appropriate option for the NHS context, considering cost and resource implications related to testing and downward result management, clinical utility and potential patient benefit, as well as alignment with the clinical trials strategy. This approach was preferred to the permissive or intermediate-permissive strategies, as it was felt to align more closely with current indications for diagnostic germline testing of the genes in question. The conservative strategy was deemed overly restrictive given expanding application of broader testing in a host of cancer type, with associated likelihood of 'off-tumour' high-impact findings. Discussions regarding VAF thresholds were informed by scientific leads and comments regarding local practices. Consensus was reached that germline follow-up testing would be recommended for variants meeting other criteria at a VAF greater than 40% and considered for those relevant variants at VAF 30–40%. The consensus was that variants at VAF lower than 30% would be unlikely to warrant germline follow-up, unless the variant in question was a known founder/recurrent variant, or if the variant was identified in an individual who would in any case fulfil criteria for diagnostic germline genetic testing. NHS-specific modifications were suggested, including major modification for the follow-up of variants in *RET*. Following this provisional consensus approval, the framework was discussed and refined by a small working group (the authors). The final framework was drafted and circulated for comment to all relevant cancer leads, and members of the UKCGG Council and CanVIG-UK Steering and Advisory Group, before wider dissemination of the finalised framework to all ordinary members of UKCGG and CanVIG for final consensus and agreement for publication.

In what circumstances should germline follow-up of variants detected through tumour/ctDNA testing be offered?

Following this meeting, we propose that germline follow-up testing of variants detected through tumour testing, using a modified *intermediate conservative* strategy, should be considered in certain circumstances (figure 1). Follow-up germline testing should be considered for variants in certain genes when:

- A variant is classified as likely pathogenic *or* pathogenic based on relevant CanVIG-UK (gene-specific where available) variant interpretation guidelines.[70]
- Tumour testing has been undertaken through a valid diagnostic laboratory (ie, not research).
- The variant is present at a VAF of at least approximately 30–40%, with due consideration to NCC/TF, testing technology and relevant biological and genomic context *or* the identified variant is a known recurrent/founder variant.

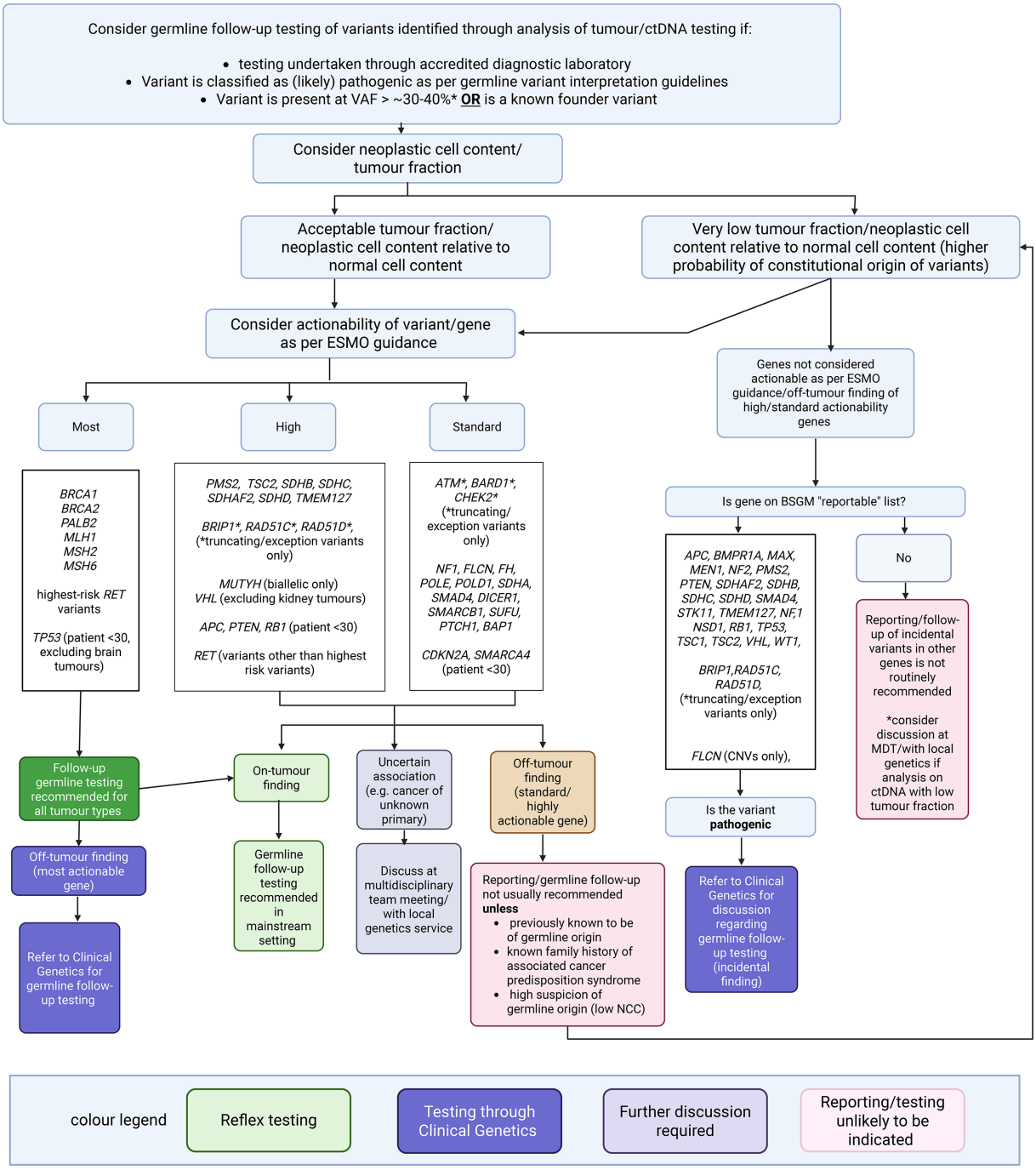

Figure 1 Proposed workflow when considering reporting/germline follow-up testing of variants of putative germline origin detected through tumour/circulating tumour DNA (ctDNA) testing. BSGM, British Society for Genetic Medicine; ESMO, European Society for Medical Oncology; MDT, Multidisciplinary Team; NCC, neoplastic cell content.

Modified 'most actionable genes' list—all tumour types

BRCA1, BRCA2, PALB2, MLH1, MSH2, MSH6

We propose that germline follow-up of variants detected through tumour testing should be offered for all variants meeting criteria above in *six* of the most actionable genes (*BRCA1*, *BRCA2*, *PALB2*, *MLH1*, *MSH2*, *MSH6*).

RET

While we do not recommend automatic germline follow-up of *all* variants detected in *RET*, particularly if identified outside of a relevant phenotype, given the uncertainties regarding associated penetrance highlighted by recent population data, we do recommend that follow-up germline testing should be offered for the most highly penetrant variants (Box 1) in all tumour types and meeting the criteria above.[43]

TP53

We propose that germline follow-up of *TP53* variants detected through tumour testing be offered where the patient is aged younger than 30 years, for all tumours except brain tumours and meeting the criteria above.

If a *TP53* variant is identified in the tumour of a patient aged older than 30 years, germline follow-up is recommended where the patient's personal/family history is consistent with

hTP53rc syndrome such that they fulfil eligibility for diagnostic germline *TP53* testing.

High/standard actionability genes—some tumours, some variants

For other high and standard actionability genes, we propose germline follow-up only when variants are ascertained in an 'on tumour' context and fulfil other criteria as outlined here above. Variants identified in the context of cancer of unknown primary will need a multidisciplinary decision on whether germline follow-up is appropriate given the lack of evidence for/against an association in this scenario.

Germline follow-up is only recommended for variants for which testing/reporting is routinely undertaken as part of diagnostic germline testing.[14] Germline follow-up of *RET* variants not considered 'highest risk' identified through tumour testing should be considered for patients with a relevant phenotype, but the majority of such patients will be eligible for formal germline diagnostic *RET* testing in any case.[20]

'Other' variants highly likely to be of germline origin

Particular consideration should be given to incidental variants of putative germline origin detected through ctDNA analysis when NCC is low, where constitutional origin is almost certain and where reporting following the proposed somatic to germline pathway would not be recommended routinely. In such circumstances, follow-up testing may be considered if the variant meets criteria for reporting and onward clinical action as per BSGM incidental findings guidance.[15] It should be noted that this guidance is intended for use when *germline* testing identifies pathogenic variants in cancer susceptibility genes in a patient with a rare disease phenotype, so it cannot be directly applied to variants of likely but unconfirmed germline origin detected in a somatic context. In the specific scenario where 'ctDNA testing' identifies a variant that is almost certainly of germline origin, we propose that follow-up be restricted to pathogenic variants in the same list of genes in which germline variants are considered reportable in the rare disease context (online supplemental table).

When variants in non-standard/most/high actionability genes are incidentally identified, reporting and onward follow-up should be restricted to *pathogenic variants* only, in line with BSGM recommendations.[15]

When, and by whom, should the patient be offered germline confirmatory testing?

The majority of diagnostic germline genetic testing is now available in the mainstream setting, that is, through routine oncology, surgery or other secondary care services. We propose that germline follow-up testing for variants identified in an 'on-tumour' setting be offered in the mainstream setting, to facilitate timely confirmation and facilitate therapeutic decision-making, informed by the result.

Confirmatory germline testing for 'off-tumour' findings, or for incidentally ascertained variants of putative germline origin in genes other than those standard/most/high actionability genes, should be supported by a clinical genetics service according to local policies and infrastructure. This will enable the nuances associated with penetrance estimates in an unselected individual to be discussed and bespoke advice to be provided as required.

For those patients where clinical suspicion of a putative germline variant persists but variants reported through tumour testing are reported at VAF lower than the proposed ~30% threshold, we recommend that the treating clinician discuss the case with their local clinical genetics service, or at a local genomic tumour advisory board.

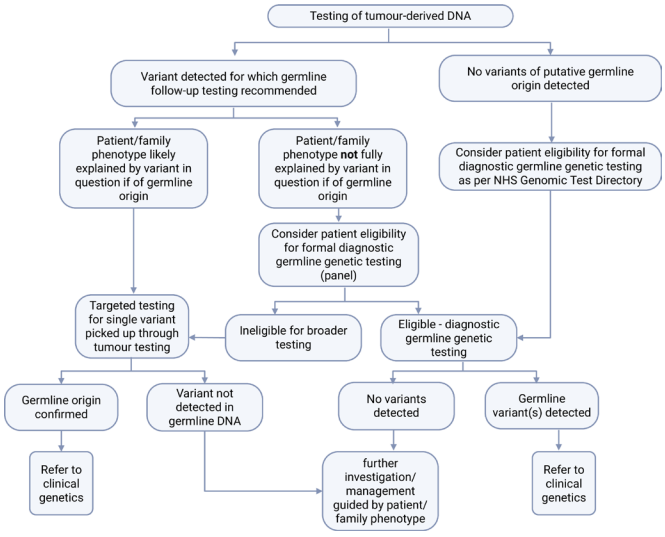

Figure 2 Workflow—when to consider further diagnostic germline genetic testing. NHS, National Health Service.

Diagnostic germline panel testing after somatic testing

As previously highlighted, tumour-based testing should not be considered a substitute for germline genetic testing.

Where germline follow-up for a variant of likely germline origin is indicated, the extent of testing may range from targeted testing of the specific variant to full panel testing, depending on the personal and family history of the patient (figure 2). If the patient's personal and family history is likely to be explained by the variant picked up through tumour testing, extended testing beyond targeted follow-up testing for the variant in question is unlikely to yield any additional variants. However, if a high suspicion exists that the personal/family phenotype is not fully explained by the variant of likely germline origin picked up through tumour testing, full panel testing may be considered as a first-line test. In this case, the laboratory team undertaking germline testing should be provided with details of the variant picked up through somatic testing, to inform reporting regarding the presence/absence of the same in constitutional DNA.

If *no* variants are identified during tumour testing, or if a variant detected as part of tumour testing is confirmed somatic in origin, the patient may be eligible for further, formal diagnostic germline genetic testing of a gene/panel of genes, depending on their personal and family cancer history.

Worked example

A 23-year-old female patient was diagnosed with spindle cell sarcoma at age 21. She had a strong family history of cancer, including multiple paternal relatives with colorectal, endometrial and urinary tract cancers. Her father was known to carry a pathogenic variant in *MSH2*. In addition, her paternal aunt had a brain tumour at age 19, and one of her paternal cousins had a known diagnosis of ataxia–telangiectasia.

Her oncology team arranged testing on DNA from her tumour. Analysis was extended beyond standard-of-care testing at the request of the treating team.

A number of variants were identified at high VAFs including NM_000051.4(ATM):c.875C>T (p.Pro292Leu) (VAF 85.3%),

NM_000251.3(MSH2): c.150_191delinsCC p.(Leu51ProfsTer20) (VAF 80.8%), NM_000321.3(RB1):c.958C>T (p.Arg320Ter) (70.8%) and NM_000546.6(TP53):c.455del (p.Pro152fs) (76.8%).

This case highlights a number of key points. The *MSH2* variant in question is the same as the known familial variant, indicating inadvertent identification of a known familial germline variant, which should have been considered in advance of arranging analysis including this gene. Confirmatory *MSH2* testing using DNA from whole blood was arranged.

Finding a number of variants at approximately the same VAF is not an unusual finding in cancer, and typically indicates a clonal event. However, in this instance, distinguishing between variants of somatic and variants of germline origin is impossible in the absence of a paired normal sample.

The known family history of ataxia–telangiectasia increases suspicion that the identified *ATM* variant is of germline origin. However, confirmatory germline testing was not offered for the *ATM* variant identified in her sample, given that this is not a variant for which reporting would be recommended if detected through germline genetic testing based on UKCGG/CanVIG reporting guidance, and the carrier frequency of *ATM* variants in the general population does not meet the threshold at which carrier testing for recessive traits is recommended.

Although the patient is younger than 30 years, follow-up germline genetic testing for the *RB1* variant was not recommended given that the subtype of sarcoma in question is not typically RB1 associated, and considering the lack of a personal or family history of retinoblastoma.

In this case, formal diagnostic *TP53* testing was offered, given her own history of sarcoma and her paternal aunt's history of a brain tumour.

Non-SNV variation

Tumour testing may lead to the identification of copy number variation and/or other structural variation involving relevant cancer susceptibility genes. As well as considering the factors here above, the size of the variant is relevant—very large genomic rearrangements (such as whole gene deletions) are more likely to represent somatic events occurring as a consequence of inherent genomic instability. Often, very large rearrangements of constitutional origin (ie, those detectable by microarray) involving loss or gain of multiple genes are associated with a syndromic phenotype, such that ascertainment of similar rearrangements in the somatic context would not necessarily lead to concern regarding a heritable event if the patient had no other discernible phenotype. CNVs also require specialist interpretation, especially duplications where orientation may not be immediately obvious, or those at extreme 5′ or 3′ ends of the gene.

Results of tests from non-NHS laboratories

Where variants of likely or confirmed germline origin have been ascertained by testing via non-NHS laboratories, results should be discussed within the context of a multidisciplinary team meeting to determine actionability and onward follow-up. It is important to consider whether the laboratory undertaking the testing has relevant accreditation necessary for germline testing and reporting, and if the variant has been interpreted in line with best practice variant interpretation guidelines. It is also important to consider if the reported variant is considered reportable/actionable through standard-of-care NHS testing—that is, is it a gene for which diagnostic testing would be offered and is the variant one for which reporting is routine.

SUMMARY

In summary, we propose implementation of a modified 'intermediate-conservative' approach to germline follow-up of variants of putative germline origin in cancer susceptibility genes ascertained through tumour testing, with certain gene-specific considerations (figure 1).

Germline follow-up testing should be considered for variants classified as likely pathogenic or pathogenic, if present at a VAF ~30–40% or higher (depending on clinical/biological/technical context), or if the variant is a known recurrent/founder germline variant, depending on the clinical context. Germline follow-up testing is recommended for variants fulfilling these criteria in genes in the most actionable category in all tumour types; but for variants fulfilling these criteria that are identified in high and standard actionable categories, testing is recommended only when ascertained in an associated tumour.

There may be instances where germline follow-up of variants reported at VAF lower than the proposed thresholds may be considered (particularly in the context of ctDNA with low TF, or where other reported variants are detected at much lower VAF), if clinical suspicion persists. In such cases, discussion at the local genomic tumour advisory board may be helpful.

Germline follow-up testing of variants ascertained in a relevant associated cancer should be offered in the mainstream context, while onward action related to 'off-tumour' findings and decisions regarding germline follow-up of variants of putative germline origin in genes other than standard/high or most actionable genes should be undertaken by a relevant professional within a clinical genetics service.

Formal diagnostic germline testing using a relevant multigene panel may still be indicated in eligible patients where tumour-based testing does not identify a putative heritable variant, or where identified variants are confirmed somatic in origin.

All germline genetic testing should be undertaken with appropriate consent in line with best practice. Where variants are confirmed to be germline in origin, carrier patients should be referred to clinical genetics for contextualised risk assessment and for onward management of the patient and family.

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REFERENCES

1 Sanderson S, Zimmern R, Kroese M, *et al*. How can the evaluation of genetic tests be enhanced? Lessons learned from the ACCE framework and evaluating genetic tests in the United Kingdom. *Genet Med* 2005;7:495–500.
2 NHS England. How commissioning is changing. 2025. Available: https://www.england.nhs.uk/commissioning/how-commissioning-is-changing
3 Ellard S, Morgan S, Wynn SL, *et al*. Rare disease genomic testing in the UK and Ireland: promoting timely and equitable access. *J Med Genet* 2024;61:1103–12.
4 McVeigh TP, Lalloo F, Monahan KJ, *et al*. Carrier testing for partners of *MUTYH* variant carriers: UK Cancer Genetics Group recommendations. *J Med Genet* 2024;61:813–6.
5 NICE. National institute for health and care excellence familial breast cancer: classification, care and managing breast cancer and related risks in people with a family history of breast cancer. Clinical guideline [Cg164]; 2013. Available: https://www.nice.org.uk/guidance/cg164
6 Park J, Karnati H, Rustgi SD, *et al*. Impact of population screening for Lynch syndrome insights from the All of Us data. *Nat Commun* 2025;16:523.
7 Sun L, Wei X, Fierheller CT, *et al*. Economic Evaluation of Population-Based BRCA1 and BRCA2 Testing in Canada. *JAMA Netw Open* 2024;7:e2432725.
8 Turnbull C, Achatz MI, Balmaña J. Breast cancer multigene germline panel testing in mainstream oncology based on clinical-public health utility (cancer mortality benefit): esmo precision oncology working group recommendations. *Ann Oncol* 2025;36:853–65.
9 Martin AR, Williams E, Foulger RE, *et al*. PanelApp crowdsources expert knowledge to establish consensus diagnostic gene panels. *Nat Genet* 2019;51:1560–5.
10 Garrett A, Allen S, Durkie M, *et al*. Classification of variants of reduced penetrance in high-penetrance cancer susceptibility genes: Framework for genetics clinicians and clinical scientists by CanVIG-UK (Cancer Variant Interpretation Group-UK). *Genet Med* 2025;27:101305.
11 Paterson AD, Seok SC, Vieland VJ. The effect of ascertainment on penetrance estimates for rare variants: Implications for establishing pathogenicity and for genetic counselling. *PLoS One* 2023;18:e0290336.
12 McVeigh TP, Lalloo F, Frayling IM, *et al*. Challenges in developing and implementing international best practice guidance for intermediate-risk variants in cancer susceptibility genes: *APC* c.3920T>A p.(Ile1307Lys) as an exemplar. *J Med Genet* 2024;61:810–2.
13 Spurdle AB, Greville-Heygate S, Antoniou AC, *et al*. Towards controlled terminology for reporting germline cancer susceptibility variants: an ENIGMA report. *J Med Genet* 2019;56:347–57.
14 UKCGG. UK cancer genetics group - exceptional variants/gene-specific variant reporting. 2024. Available: https://www.ukcgg.org/information-education/exceptional-variantsgene-specific-variant-reporting
15 BSGM. Managing incidental findings: guidance for rare and inherited disease diagnostic genomic testing. British Society for Genetic Medicine; 2024. Available: https://bsgm.org.uk/healthcare-professionals/guidance-on-incidental-findings/
16 Mehta N, He R, Viswanatha DS. Correspondence on "Standards for the classification of pathogenicity of somatic variants in cancer (oncogenicity): Joint recommendations of Clinical Genome Resource (ClinGen), Cancer Genomics Consortium (CGC), and Variant Interpretation for Cancer Consortium (VICC)" by Horak et al. *Genet Med* 2022;24:1986–8.
17 UKCGG. UKCGG/cstag statement on reporting practice for variants in ATM v.1.0. 2024. Available: https://www.ukcgg.org/information-education/exceptional-variantsgene-specific-variant-reporting
18 UKCGG. UKCGG statement on reporting practice for missense variants in CHEK2. 2024. Available: https://www.ukcgg.org/information-education/exceptional-variantsgene-specific-variant-reporting
19 Kristoffersson U, Johansson-Soller M. Pregnancy Planning and Genetic Testing: Exploring Advantages, and Challenges. *Genes (Basel)* 2024;15:1205.
20 NHSE. National genomic test directory testing criteria for rare and inherited disease version 7.1. 2025. Available: https://www.england.nhs.uk/publication/national-genomic-test-directories
21 Gregg AR, Aarabi M, Klugman S, *et al*. Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2021;23:1793–806.
22 Lebedeva A, Shaykhutdinova Y, Seriak D, *et al*. Incidental germline findings during molecular profiling of tumor tissues for precision oncology: molecular survey and methodological obstacles. *J Transl Med* 2022;20:29.
23 Catenacci DVT, Amico AL, Nielsen SM, *et al*. Tumor genome analysis includes germline genome: are we ready for surprises? *Int J Cancer* 2015;136:1559–67.
24 Mandelker D, Zhang L, Kemel Y, *et al*. Mutation Detection in Patients With Advanced Cancer by Universal Sequencing of Cancer-Related Genes in Tumor and Normal DNA vs Guideline-Based Germline Testing. *JAMA* 2017;318:825–35.
25 Tung N, Dougherty KC, Gatof ES, *et al*. Potential pathogenic germline variant reporting from tumor comprehensive genomic profiling complements classic approaches to germline testing. *NPJ Precis Oncol* 2023;7:76.
26 Hayashi H, Kunimasa K, Tanishima S, *et al*. Germline BRCA2 variant with low variant allele frequency detected in tumor-only comprehensive genomic profiling. *Cancer Sci* 2024;115:682–6.
27 Montgomery ND, Selitsky SR, Patel NM, *et al*. Identification of Germline Variants in Tumor Genomic Sequencing Analysis. *J Mol Diagn* 2018;20:123–5.
28 Li MM, Chao E, Esplin ED, *et al*. Points to consider for reporting of germline variation in patients undergoing tumor testing: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2020;22:1142–8.
29 Lincoln SE, Nussbaum RL, Kurian AW, *et al*. Yield and Utility of Germline Testing Following Tumor Sequencing in Patients With Cancer. *JAMA Netw Open* 2020;3:e2019452.
30 Stout LA, Hunter C, Schroeder C, *et al*. Clinically significant germline pathogenic variants are missed by tumor genomic sequencing. *NPJ Genom Med* 2023;8:30.
31 Kraft IL, Godley LA. Identifying potential germline variants from sequencing hematopoietic malignancies. *Hematology Am Soc Hematol Educ Program* 2020;2020:219–27.
32 Smits AJJ, Kummer JA, de Bruin PC, *et al*. The estimation of tumor cell percentage for molecular testing by pathologists is not accurate. *Mod Pathol* 2014;27:168–74.
33 Kuzbari Z, Bandlamudi C, Loveday C, *et al*. Germline-focused analysis of tumour-detected variants in 49,264 cancer patients: ESMO Precision Medicine Working Group recommendations. *Ann Oncol* 2023;34:215–27.
34 Morgan RD, Burghel GJ, Flaum N, *et al*. Predicting the likelihood of a *BRCA1/2* pathogenic variant being somatic by testing only tumour DNA in non-mucinous high-grade epithelial ovarian cancer. *J Clin Pathol* 2023;76:684–9.
35 Lee T-SJ, Chopra M, Kim RH, *et al*. Incidence and prevalence of neurofibromatosis type 1 and 2: a systematic review and meta-analysis. *Orphanet J Rare Dis* 2023;18:292.
36 Aretz S, Tricarico R, Papi L, *et al*. MUTYH-associated polyposis (MAP): evidence for the origin of the common European mutations p.Tyr179Cys and p.Gly396Asp by founder events. *Eur J Hum Genet* 2014;22:923–9.
37 Lerat J, Bonnet C, Cartault F, *et al*. High prevalence of congenital deafness on Reunion Island is due to a founder variant of LHFPL5. *Clin Genet* 2019;95:177–81.
38 Quinn S, Walsh N, Streata I, *et al*. Catalogue of inherited autosomal recessive disorders found amongst the Roma population of Europe. *Eur J Med Genet* 2025;73:104989.
39 Schubert EL, Mefford HC, Dann JL, *et al*. BRCA1 and BRCA2 mutations in Ashkenazi Jewish families with breast and ovarian cancer. *Genet Test* 1997;1:41–6.
40 Sipilä LJ, Aavikko M, Ravantti J, *et al*. Detection of a major Lynch Syndrome-causing MLH1 founder variant in a large-scale genotyped cohort. *Fam Cancer* 2024;23:647–52.
41 McVeigh TP, Cody N, Carroll C, *et al*. Recurrent large genomic rearrangements in BRCA1 and BRCA2 in an Irish case series. *Cancer Genet* 2017;214–215:1–8.
42 NHSE. National genomic test directory for cancer. V11. 2025. Available: https://www.england.nhs.uk/publication/national-genomic-test-directories
43 West C, Ruth K, Owens M. Penetrance of MEN2A-causing RET mutations in clinically unselected population is very low – important implications for clinical practice. Presented at Society for Endocrinology BES 2023. Endocrine Abstracts; 2023.
44 West CE, Mirshahi UL, Ruth KS. Mutation spectrum and associated risks of medullary thyroid cancer and all-cause mortality in incidentally identified men2a-causing ret variants. *medRxiv* [Preprint] 2024.
45 Loveday C, Josephs K, Chubb D, *et al*. p.Val804Met, the Most Frequent Pathogenic Mutation in RET, Confers a Very Low Lifetime Risk of Medullary Thyroid Cancer. *J Clin Endocrinol Metab* 2018;103:4275–82.
46 Vouchara A, Chorti A, Tzikos G, *et al*. Thyroidectomy in children and adolescents: a systematic review. *Gland Surg* 2024;13:697–712.

47 de Jong M, Nounou H, Rozalén García V, *et al*. Children are at a high risk of hypocalcaemia and hypoparathyroidism after total thyroidectomy. *J Pediatr Surg* 2020;55:1260–4.
48 Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol* 2010;2:a001008.
49 Wang H, Guo M, Wei H, *et al*. Targeting p53 pathways: mechanisms, structures and advances in therapy. *Sig Transduct Target Ther* 2023;8:92.
50 Garritano S, Gemignani F, Palmero EI, *et al*. Detailed haplotype analysis at the TP53 locus in p.R337H mutation carriers in the population of Southern Brazil: evidence for a founder effect. *Hum Mutat* 2010;31:143–50.
51 Kato S, Han S-Y, Liu W, *et al*. Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proc Natl Acad Sci U S A* 2003;100:8424–9.
52 Kotler E, Shani O, Goldfeld G, *et al*. A Systematic p53 Mutation Library Links Differential Functional Impact to Cancer Mutation Pattern and Evolutionary Conservation. *Mol Cell* 2018;71:178–90.
53 Giacomelli AO, Yang X, Lintner RE, *et al*. Mutational processes shape the landscape of TP53 mutations in human cancer. *Nat Genet* 2018;50:1381–7.
54 de Andrade KC, Frone MN, Wegman-Ostrosky T, *et al*. Variable population prevalence estimates of germline TP53 variants: A gnomAD-based analysis. *Hum Mutat* 2019;40:97–105.
55 Li FP, Fraumeni JF Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann Intern Med* 1969;71:747–52.
56 Bougeard G, Renaux-Petel M, Flaman J-M, *et al*. Revisiting Li-Fraumeni Syndrome From TP53 Mutation Carriers. *J Clin Oncol* 2015;33:2345–52.
57 Frebourg T, Bajalica Lagercrantz S, Oliveira C, *et al*. Guidelines for the Li-Fraumeni and heritable TP53-related cancer syndromes. *Eur J Hum Genet* 2020;28:1379–86.
58 Ward A, Farengo-Clark D, McKenna DB, *et al*. Clinical management of TP53 mosaic variants found on germline genetic testing. *Cancer Genet* 2024;284–285:43–7.
59 Gonzalez KD, Buzin CH, Noltner KA, *et al*. High frequency of de novo mutations in Li-Fraumeni syndrome. *J Med Genet* 2009;46:689–93.
60 Evans DG, Woodward ER. New surveillance guidelines for Li-Fraumeni and hereditary TP53 related cancer syndrome: implications for germline TP53 testing in breast cancer. *Fam Cancer* 2021;20:1–7.
61 Berry DK, Gillis N, Padron E, *et al*. Interpretation of ambiguous TP53 test results: Mosaicism, clonal hematopoiesis, and variants of uncertain significance. *J Genet Couns* 2024;33:916–26.
62 Weitzel JN, Chao EC, Nehoray B, *et al*. Somatic TP53 variants frequently confound germ-line testing results. *Genet Med* 2018;20:809–16.
63 Saya S, Killick E, Thomas S, *et al*. Baseline results from the UK SIGNIFY study: a whole-body MRI screening study in TP53 mutation carriers and matched controls. *Fam Cancer* 2017;16:433–40.
64 Achatz MI, Villani A, Bertuch AA, *et al*. Update on Cancer Screening Recommendations for Individuals with Li-Fraumeni Syndrome. *Clin Cancer Res* 2025;31:1831–40.
65 Hanson H, Brady AF, Crawford G, *et al*. UKCGG Consensus Group guidelines for the management of patients with constitutional *TP53* pathogenic variants. *J Med Genet* 2021;58:135–9.
66 Schon K, Tischkowitz M. Clinical implications of germline mutations in breast cancer: TP53. *Breast Cancer Res Treat* 2018;167:417–23.
67 McBride KA, Ballinger ML, Schlub TE, *et al*. Psychosocial morbidity in TP53 mutation carriers: is whole-body cancer screening beneficial? *Fam Cancer* 2017;16:423–32.
68 Fortuno C, Lee K, Olivier M, *et al*. Specifications of the ACMG/AMP variant interpretation guidelines for germline TP53 variants. *Hum Mutat* 2021;42:223–36.
69 Dorling L, Carvalho S, Allen J, *et al*. Breast Cancer Risk Genes - Association Analysis in More than 113,000 Women. *N Engl J Med* 2021;384:428–39.
70 Garrett a., Allen S, Loong L, *et al*. CanVIG-uk consensus specification for cancer susceptibility genes (csgs) of ACGS best practice guidelines for variant classification v3.0. 2024. Available: https://www.cangene-canvaruk.org/canvig-uk-guidance
71 Kihara M, Miyauchi A, Yoshida H, *et al*. Tandem Germline RET Mutations in a Family Pathogenetic for Multiple Endocrine Neoplasia 2B, Confirmed by a Natural Experiment. *Eur Thyroid J* 2014;3:272–7.
72 Cranston AN, Carniti C, Oakhill K, *et al*. RET is constitutively activated by novel tandem mutations that alter the active site resulting in multiple endocrine neoplasia type 2B. *Cancer Res* 2006;66:10179–87.
73 Hedayati M, Heydarzadeh S. RET proto-oncogene mutations: impact on diagnosis, treatment and prognosis of mtc. In: Kostoglou-Athanassiou I, ed. *Thyroid Cancer - The road From genes to successful treatment*. Rijeka: IntechOpen, 2023.
74 Sheikholeslami S, Zarif Yeganeh M, Hoghooghi Rad L, *et al*. Haplotype Frequency of G691S/S904S in the RET Proto-Onco-gene in Patients with Medullary Thyroid Carcinoma. *Iran J Public Health* 2014;43:235–40.
75 Nakao K-T, Usui T, Ikeda M, *et al*. Novel tandem germline RET proto-oncogene mutations in a patient with multiple endocrine neoplasia type 2B: report of a case and a literature review of tandem RET mutations with in silico analysis. *Head Neck* 2013;35:E363–8.
76 Valente FOF, Dias da Silva MR, Camacho CP, *et al*. Comprehensive analysis of RET gene should be performed in patients with multiple endocrine neoplasia type 2 (MEN 2) syndrome and no apparent genotype-phenotype correlation: an appraisal of p.Y791F and p.C634Y RET mutations in five unrelated Brazilian families. *J Endocrinol Invest* 2013;36:975–81.