# Cancer Variant Interpretation Group UK (CanVIG-UK): An exemplar national specialist multi-disciplinary genomics forum

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### Clinical utility of Cancer Susceptibility Genes

Germline Cancer Genetics, analysis for constitutional variation relating to cancer susceptibility, constitutes approximately one quarter of activity in NHS Molecular Diagnostic Laboratories in England1. Following identification of a Pathogenic Variant (PV) in a Cancer Susceptibility Genes (CSG), mitigation of the impact of future cancers may be possible via (i) surgical risk reduction (e.g. mastectomy, gastrectomy, oophorectomy, colectomy), (ii) chemoprevention (iii) intensive screening to enable early detection (iv) lifestyle modification2. Family members negative for the familial CSG-PV can be spared anxiety and unnecessary screening. On account of the late-onset, variably penetrant, autosomal dominant inheritance that characterises most CSGs, it is typical that multiple PV-positive family members are identified via cascade screening and may be geographically distributed across disparate genomics services.

Erroneous interpretation of CSG variant pathogenicity can therefore (i) result in discordant management within families, (ii) have serious clinical consequences for individuals and (iii) at population level result in misdirection of resources 3-5. For all these reasons, robust, rapid, accurate variant analysis and interpretation of disease risk is critical to effective delivery of germline cancer genetics and advancing outcomes for patients.

### The evolving landscape of variant interpretation in germline cancer genetics

CSG analysis became available in the late 1990s via family cancer clinics, within a few years of identification of the relevant genes2. If the cancer phenotype ascribed to the gene matched that found in the proband/family under study, typically a rare variant would be taken to be pathogenic and causative with little additional evidence6. Subsequently, large-scale population sequencing studies revealed the degree of innocuous variation present in the human genome (and indeed in disease-associated genes) and “down-grading” was required for many erroneously-labelled pathogenic variants7. An era of caution followed, with much greater recourse to labelling of variants as VUS/VOUS (“variants of uncertain significance”). However, due to the lack of systems for sharing emerging evidence, families have often sat for years in limbo with their “VUS”, even when data had long become available by which their variant could be up- or down-graded.

Sharing of clinical variant data was somewhat improved with the advent of Locus Specific Databases (LSDs), such as Breast Cancer Information Core (BIC) and Leiden Open Variant Databases (LOVDs)8-11. However, the curation of clinical and molecular data was often suboptimal, with (i) erroneous nomenclature, (ii) duplication of entries and (iii) classifications that were highly subjective and frequently contradictory12.

Utilising data from ~70,000 genetic tests for hereditary breast ovarian cancer (HBOC) performed by Myriad Genetics¸ in 2007 Easton et al published a landmark multifactorial analysis by which using clinical, pedigree and allelic data ‘odds of causality’ were mathematically generated for 1433 variants13. In 2008, the first formal 5 point variant interpretation system for CSGs was published by IARC, which included numeric thresholds for probability of pathogenicity14. Expert cancer susceptibility consortia such as ENIGMA and InSIGHT further evolved these multifactorial variant classification systems to incorporate tumour phenotype and in-silico predictions15-17. However, ENIGMA/InSIGHT approaches required statistical genetic-epidemiologic analyses of large curated research data series, and were not reproducible by an individual diagnostic laboratory seeking to classify in a clinical timescale a newly-identified variant.

In 2015, the American College of Medical Geneticists published a framework prescribing how multiple disparate evidence sources could be integrated into a classification by a diagnostic laboratory for a newly identified genomic variant18. Acknowledged upfront by the authors as preliminary; the ACMG framework has subsequently been further evolved under the auspices of ClinGen including (i) application to particular genes and/or diseases (including TP53, CDH1 and PTEN) (ii) specification of particular criteria (eg functional assays)19 (iii) the underpinning Bayesian model20-23 .

### Coordinated national UK approaches in Variant Interpretation

In 2016, with endorsement from NHS England and Health Education England, it was agreed by the UK Association of Clinical Genomic Scientists (UK-ACGS) to adopt formally the ACMG framework for variant interpretation18,24. The UK-ACGS establishing a central national group to agree UK specification of the ACMG framework, members of which would then disseminate practice and training locally24. To better address disease-specific clinical use-cases, UK-ACGS recommended focused variant classification specification and training for rare paediatric disease, germline cancer genetics, cardiac disease and hypercholesterolemia. In response to this recommendation, CanVIG-UK (Cancer Variant Interpretation Group UK) was initiated in 2017.

### CanVIG-UK: Cancer Variant Interpretation Group UK

The purpose of CanVIG-UK (Cancer Variant Interpretation Group UK) is to advance outcomes for patients by improving the accuracy and consistency of interpretation of variants in Cancer Susceptibility genes across the UK clinical and diagnostic laboratory communities (hereafter termed the UK clinical-laboratory community).

We shall do this by advancing six objectives (see Box 1)

**Creation of a UK national multi-disciplinary professional network and forum for variant interpretation in Germline Cancer Genetics**

CanVIG-UK currently comprises >100 members including clinical and laboratory representation from each of the 25 Molecular Diagnostic Laboratories and Clinical Genetics Services of the UK and ROI. This group comprises roughly equal proportions of clinical scientists and clinical geneticists, with two thirds work exclusively or predominantly in cancer genetics (see Figure 1). There are two mechanisms for accessing the expertise of the CanVIG network.

* Firstly, there is a monthly WebEx meeting to which topical issues and problematic variants/cases can be submitted. The ‘variant(s)-of-the-month’ are circulated one week in advance of the monthly meeting. CanVIG-UK members then (i) ascertain whether additional cases and/or laboratory data exist locally (ii) undertake local, independent classification of the variant. The relevant clinical and laboratory data are presented by the nominating laboratory, followed by input of any additional information by the broader CanVIG-UK group and discussion regarding strength of evidence for each of the ACMG criteria. A consensus CanVIG classification is generated following this discussion via a post-discussion on-line poll. A date-stamped detailed CanVIG variant summary sheet is generated (Appendix 3): this is made available online to CanVIG-UK members via CanVar-UK and also submitted to ClinVar (see below).
* Secondly, there is regular email activity within the group, whereby queries can be addressed for more immediate response and/or debate within the CanVIG-UK community.

**Education of the UK Clinical-Laboratory Community**

In addition to providing a consensus classification, the discussions provide an important educational role in variant interpretation and ACMG variant classification (see Figure 2). The CanVIG-UK group is also involved in education of the broader genetics and oncology communities in variant interpretation for cancer susceptibility genes.

**Detailed specification for Germline Cancer Genetics of the UK-ACGS Best Practice Guidelines for Variant Classification**

The UK-ACGS has generated and updates annually a detailed specification of the ACMG variant interpretation framework to be applied across the NHS UK clinical-laboratory community (ACGS Best Practice Guidelines for Variant Classification 2019)24. Like the original ACMG framework, the ACGS specification was developed primarily for rare Mendelian paediatric disease. In germline cancer genetics, de novo and biallelic paradigms are less common: instead we are typically reliant on frequency in case series and functional assays. Thus, an important remit for CanVIG-UK has been to develop consistent with the ACGS specification, detailed specification for CSG variants relating in particular to these criteria (PS4, PS3/BS3) and.

CanVIG-UK task-and-finish subgroups were established to develop each theme (e.g. splicing, functional, case-control) involving literature review, consultation with national experts and evaluative testing against positives and true negatives. We include version 1.0 of the CanVIG-UK specification of the ACGS Best Practice Guidelines for Variant Classification, pertaining to criteria PS3, PS4, PM1, PP2, PM2, PM3, PM5, PP3, PP4, PP5, BS2, BS3, BP2, BP4, BP5 and BP6, including for each criterion explanatory notes detailing rationale/methodology (see Appendix 1). It is anticipated that there will be ongoing iteration by CanVIG-UK of these criteria as new evidence and international recommendations emerge and the UK-ACGS specification evolves.

**Ratification of additional guidance in Germline Cancer Genetics relevant to the UK Clinical-Laboratory community**

Historically, first presentation to the family cancer clinic was of an unaffected individual, concerned by a significant family cancer history. Increasingly, genetic analysis is now performed as part of routine work-up at cancer diagnosis, either through analysis of a germline sample or through therapeutically-motivated molecular analysis of the tumour. In both contexts (i) focused testing of one or two genes has often been superseded by broad ‘cancer panels’ containing dozens or hundreds of genes (ii) patients may be unselected for family history (iii) analysis and reporting in a tight time frame is typically required. A number of challenging issues have emerged, including:

1. Categorisation and management of reduced penetrance variants in high penetrance genes
2. Variant interpretation and clinical management for moderate penetrance genes
3. Adaptation of variant interpretation and risk for different contexts of clinical ascertainment
4. Inference of germline findings from tumour-only sequencing

Whilst germane across genomics, consideration of these issues has become pressing earliest within germline cancer genetics. Benefitting from its regular forum, multidisciplinary membership and affiliation to UK-ACGS and the UK Cancer Genetics Group (UK-CGG), the CanVIG–UK group is also evolving national UK approaches on such issues (see Appendix 2: CanVIG-UK definitions and reporting recommendations for managing a variant of reduced penetrance in a high penetrance gene).

**Development of an online platform to facilitate information-sharing and variant interpretation within the UK Clinical-Laboratory community**

In germline cancer genetics, enrichment in cases (especially “strong families”) is one of the most valuable clinical observations indicating variant pathogenicity. However, to date we have struggled to quantify such observations on account of (a) failure to aggregate data distributed across laboratories (ii) lack of a robust denominator.

In a collaborative venture between Public Health England and the CanVIG-UK national network of molecular diagnostic laboratories, historic and prospective data from molecular testing of cancer susceptibility genes have been submitted via a pseudonymisation portal to the NCRAS Section 251 compliant environment data environment of Public Health England25. National variant totals (numerator and denominator) are released back to the clinical-laboratory community via the CanVar-UK datasystem (<http://www.canvaruk.org/>).

CanVar-UK also provides multiple annotations for 1,008,643 variants from 95 cancer susceptibility genes, including selected gene-specific variant level annotations such as Locus Specific Databases case counts, functional assays, splicing assays and multifactorial analyses. Accessible only to registered CanVIG-UK clinical-laboratory users is a community area for sharing of local classifications, comments/notes, upload of documents and results from local laboratory assays (e.g. RNA analyses of potential splicing variants), all non-identifiable.

**UK contribution to international variant interpretation endeavours**

CanVIG-UK facilitates is a valuable conduit between the UK clinical-laboratory germline cancer genetics community and relevant international variant interpretation endeavours. Firstly, CanVIG-UK provides a means of dissemination to the broader UK clinical-laboratory communities of emerging projects and outputs from international endeavours such as ENIGMA, INSIGHT, and ClinGen expert groups. Secondly, CanVIG-UK provides a regular and broad clinical-laboratory forum upon which principles/issues/models emerging from these international groups can be readily and rapidly tested. Thirdly, CanVIG-UK data are submitted to international projects, for example use by ENIGMA of the PHE UK laboratory data on BRCA1/BRCA2. Fourthly, CanVIG-UK consensus classifications and underpinning details are shared internationally via ClinVar, making CanVIG-UK the first UK organisation to submit clinical-laboratory variant classifications to ClinVar.

**Sustainability**

Maintaining a national level network, regular meetings and development of the underpinning datasystem (CanVar-UK) requires sustained support: the activities of CanVIG-UK are currently supported by a Cancer Research UK Catalyst Award (CanGene-CanVar xxx).

**Conclusion**

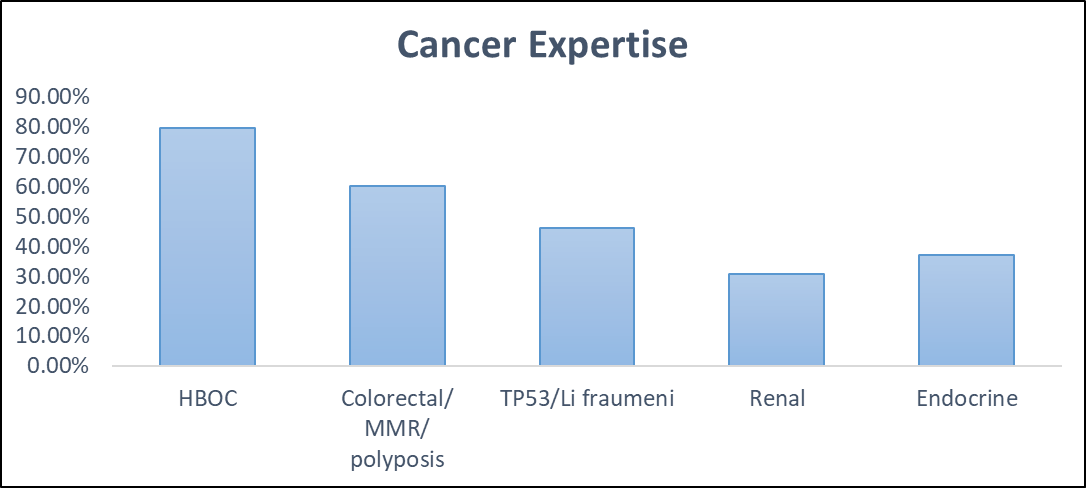
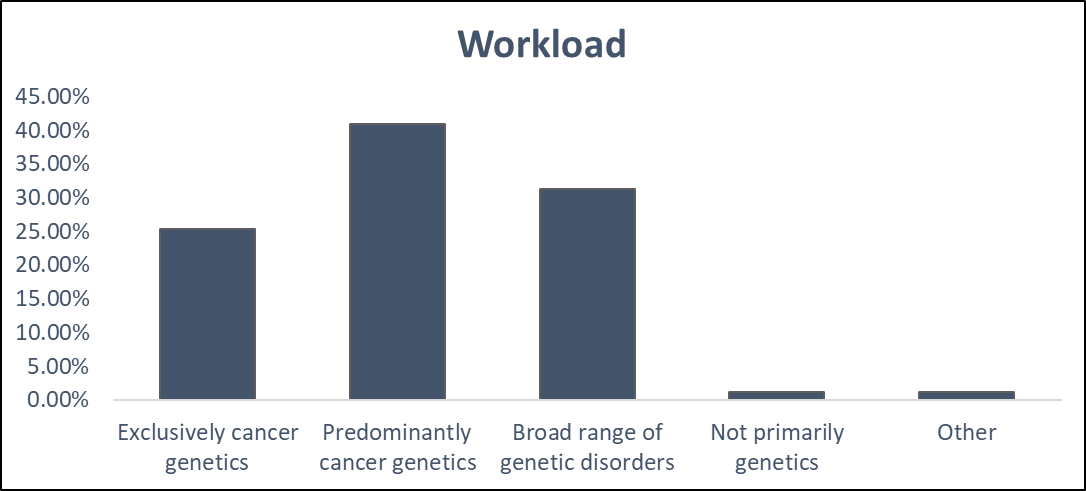
CanVIG-UK is a multidisciplinary group compriing >100 diagnostics laboratory scientists and clinicians expert in germline cancer genetics with representation from the 25 molecular diagnostic laboratories of the UK and ROI. Through CanVIG-UK, the UK clinical-laboratory germline cancer genetics community have evolved (i) an e-forum for realtime consultation on problematic variants (ii) a monthly WebEx multidisciplinary meeting and ‘variant surgery’ for detailed review of challenging cases and group education (iii) infrastructure for secure submission and centralisation of molecular data through Public Health England (iv) an online datasystem (CanVar-UK) for sharing of variant level-level data either publicly and within a secure community region (v) fruitful interactions with international CSG variant interpretation endeavours. In summary, we propose CanVIG-UK as an exemplar of a disease-specific national multidisciplinary genomics network, the like of which are essential for effective specialist collaborative case-review, information-sharing and education in this era of rapid emergence of genomic knowledge.

**Box 1: The CanVIG Mission Statement**

The purpose of CanVIG-UK (Cancer Variant Interpretation Group UK) is to advance outcomes for patients by improving the accuracy and consistency of interpretation of variants in Cancer Susceptibility genes across the UK clinical-laboratory community. We have six specific objectives:

1. **Creation of a UK national multi-disciplinary professional network and forum for Germline Cancer Genetics**
2. **Education of the UK Clinical -Laboratory Community**
3. **Detailed specification for Germline Cancer Genetics of the UK-ACGS Best Practice Guidelines for Variant Classification**
4. **Ratification of additional guidance in Germline Cancer Genetics relevant to the UK Clinical-Laboratory community**
5. **Development of an online platform to facilitate information-sharing and variant interpretation within the UK Clinical-Laboratory germline cancer genetics community**
6. **UK contribution to international variant interpretation endeavours**

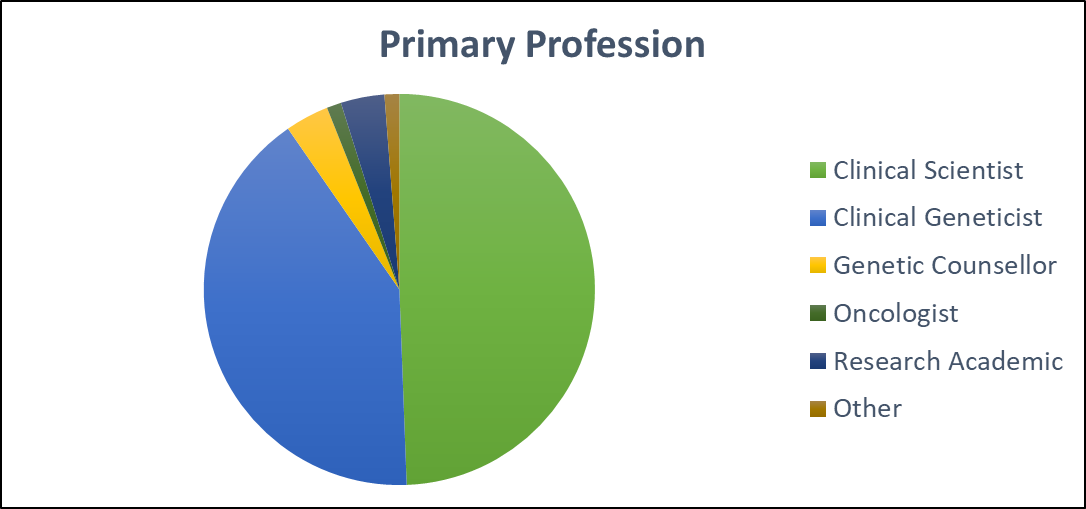
**Figure 1 (Below): Overview of the characteristics of the membership of CanVIG-UK.** Data from survey of CanVIG members (survey return rate 83/103 [80%], survey performed on 29/10/2019), asking about (a) primary profession (b) division of work (c) areas of expertise within germline cancer genetics



a

b

c



**Figure 2: Responses from members regarding perceived utility of different CanVIG activities.** Participants were asked to rate the utility of aspects of CanVIG for their local CSG variant interpretation practice (5 very useful to 0-not useful at all). Data from survey of CanVIG members (survey return rate 83/103 [80%], survey performed on 29/10/2019).

### Appendix 1: Specification for cancer susceptibility genes of 2019 ACGS Best Practice Guidelines for Variant Classification

For the following categories, there is no additional specification for CSGs beyond that provided in ACGS Best Practice Guidelines for Variant Classification 201924

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| **PVS1** | null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where LOF is a known mechanism of disease. |
| **PS1** | Same amino acid change as a previously established pathogenic variant regardless of nucleotide change. |
| **PS2** | De novo (both maternity and paternity confirmed) in a patient with the disease and no family history. |
| **PM4** | Protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants. |
| **PP1** | Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease. |
| **BS4** | Non-segregation with disease |
| **BP3** | In-frame deletions/insertions in a repetitive region without a known function |
| **BP7** | A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved |

For the following categories, CanVIG-UK has developed detailed specification to supplement with that provided in ACGS Best Practice Guidelines for Variant Classification 2019.

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| **PS3**  VSTR  STR  \_MOD  \_SUP |
| Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product |
| ***For assays of protein function***   |  |  |  |  | | --- | --- | --- | --- | |  | Discrimination | Controls | Reproducibility | | **Strong** | relative protein activity assay or functional impact  <25% compared to level for wildtype | ≥10 ‘true positive’  ≥10 ‘true-negative’ | ≥2 laboratories OR results demonstrably reproducible from a single laboratory | | **Mod** | ≥5 ‘true positive’  ≥5 ‘true-negative’ | | **Sup** | ≥2 ‘true positive’  ≥2 ‘true-negative’ | single laboratory |   **Explanatory** **Notes:**   * For use by CanVIG-UK, a published assay requires review by two independent CanVIG-UK registered clinical laboratory scientists. * This criterion is for variant-specific analyses. Where functional data provides support at the gene rather than variant level (e.g. biochemical analysis) this typically should be incorporated within the phenotypic specificity criterion PP4. |
| ***For assays of splicing function***   |  |  | | --- | --- | | **Vstrong** | **2 orthogonal assays:** exhibiting abnormal transcripts; no evidence of leakiness 4,8 | | **Strong** | **1 assay:** exhibiting abnormal transcripts; no evidence of leakiness8 | | **Mod** | **≥1** **assay:** exhibiting abnormal transcripts; evidence of some leakiness8 | | **Sup** | **≥1** **assay:** exhibiting abnormal/alternative transcripts which have been reported as present in normal controls (implying naturally occurring isoforms)12 | | **Do not apply** | **≥1** **assay:** exhibiting abnormal/alternative transcripts with evidence of extreme leakiness8 |   **Explanatory Notes:**   1. Experimental data may include quantitative assays (e.g. realtime-PCR, Sanger assay with formal quantitation of peak height +/-tape-station, minigene, NGS RNAseq) and semi/non-quantitative assays (e.g. visual evaluation of relative peaks height of Sanger, gel-based, analysis for splice variant-containing allele indicating loss via nonsense mediated decay (ie SNV in trans with the putative splicing variant appears homozygous on RNA sequencing despite being heterozygous on DNA sequencing indicating the loss of expression of the transcript containing the putative splicing variant)). 2. The assays must be performed in a diagnostically ISO accredited laboratory or recognized research laboratory with which direct consultation can be undertaken. **If an alternative source of evidence (e.g. publication) downgrade by one level of evidence.**  All assays should evidence appropriate validations and controls3 3. Laboratory methodology should be appropriately validated: primers must have been tested in ≥5 independent normal control reactions, not necessarily run at the same time (i.e. primers could be validated using 5 normal controls across several runs or runs as a batch on a single run). 4. Orthogonal assays include (a) PCR-based assays using different primers (b) ≥2 different platforms e.g. RT-PCR and minigene 5. To attain very strong/strong, the criteria by which the disease mechanism is interpreted as loss of function should be met (as per PVS1 recommendations26) 6. The exon in question must be present in the biologically relevant transcript 7. Splicing impact must fulfil one of the criteria below, **otherwise downgrade by one level of evidence**    1. out of frame + predicted to undergo NMD+ removal of >10% of the protein    2. in-frame but removal of a critical hotspot (as listed in PM1)    3. in-frame but removal of >10% of the protein 8. In the absence of specific data for a given gene/exon, the following thresholds of ‘leakiness’ should be applied:  * **No evidence of leakiness**: ratio for allele of >80:20 (abnormal: normal) ==overall ratio of > 40:60 (abnormal: normal) * **Evidence of some leakiness:** ratio for allele of >20:80 (abnormal: normal) ==overall ratio of > 10:90 (abnormal: normal) * **Evidence of extreme leakiness:** ratio for allele of <20:80 (abnormal: normal) ==overall ratio of < 10:90 (abnormal: normal). Typically abnormal transcript will be visible on gel but present only at extremely low level or not visible by Sanger sequencing.  1. For ±1&2, PVS1 criteria should be used instead of PS3. 2. When PS3 is applied for splicing, PP3 (in silico evidence), PM4 (in-frame aberration), PVS1 (truncating) can not be applied. 3. Although PP3 cannot be applied alongside PS3, the assay results for variants at the intron-exon boundaries should nevertheless be supported by in silico predictions (MaxEntScan ≥15% difference **OR** SSFL ≥5% difference) **Otherwise downgrade by one level of evidence.** Exceptions where in silico concordance not required: (i) U12 splice sites, (ii) TCCTTAAC at the 3’ end, (iii) variants outside of intron-exon boundaries (namely 5’:Last 3 bases of exon plus 8 bases on intron, 3’:12 bases of intron plus 2 bases of exon) 4. As per naturally occurring (ie non-pathogenic) splice variants,( for example those delineated by ENIGMA at <https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf>)27 and reference transcriptome resources (for example GTEX, <https://gtexportal.org/home/>)28 |
| **Additional comments:**   * There is variation gene by gene, exon by exon in the lower limit of % normal transcript (‘leakiness’) at which normal protein function is maintained. * Furthermore, the accuracy of different assays in correctly quantifying ratios of different transcripts will vary and is often poorly quantified. * As improved data on the precision of different assays emerges, these standards will likely be amended. |

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| **PS4**  VSTR  STR  \_MOD  \_SUP |
| The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls |
| **Explanatory** **Notes:**   * The Pexact is generated from Fishers exact 2-way case control comparison. Non-duplicated case data and control data from an equivalent ethic group, both robustly genotyped, are required. * For Western European case data, comparison to the gnomAD NFE population is recommended (ie 64,603 individuals for gnomAD v2.1). * A more stringent p-value should be applied commensurate with uncertainty regarding duplicates. * The Odds Ratio (OR) from this case control comparison (ad/bc) should be consistent with the effect size anticipated for that gene type.   + For a ‘high penetrance’ gene or variant, OR >5 for unselected cancer series or OR>10 for enriched familial cases.   + For an ‘intermediate penetrance’ gene or reduced penetrance variant in high penetrance gene, OR >2 for unselected cancer series or OR>4 for enriched familial cases. * If the control frequency is 0, to generate an OR, the Haldane-Anscombe correction may be required (add 0.5 to cells a,b,c,d). * For non-coding variants, restriction to the WGS partition of gnoMAD is required. |
| **Additional comments:**   * The p-value of association is used to reflect the relative likelihood that observed differences in frequency arose due to association versus chance. For ‘strong’ evidence, p<0.05 equates to the stipulation that CI95 around the OR do not include 1.0. * As the p-value does not reflect effect size, attainment of minimum effect size (OR) is also stipulated. * A larger minimum effect size (OR) should be observed when an ‘enriched’ series is examined (compared to unselected disease). * Where paired numerator-denominator frequencies are unavailable, a case-counting approach is required, which takes into account the specificity of phenotype observed in the proband +/- family. This approach does not take into account the denominator or the reference case series. For TP53 and PTEN, case-counting guidance has been issued via the respective ClinGen expert groups20,22. For MMR, a case-counting approach is under development by CanVIG-UK. |

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| **PM1, PP2**  \_MOD  \_SUP |
| **PM1: Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation**  **PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease.** |
| Use PM1 for missense variants arising in a CSG domain well characterised as a “hotspot” for pathogenic missense variants  Use at **Mod** when there is no benign variation present in the hotspot region/domain.  Use at **Sup** when there is some benign variation present in the hotspot region/domain.  Use PP2 at **Sup** where there is overall constraint for missense variation at the level of the region/exon/gene (Z≥3.09)  **Explanatory Notes:**   1. The majority of CSGs act by loss of function. Hence, for many of these genes, the majority of established pathogenic mutations are truncating (early linkage analyses, agnostic to mechanism, support this). Examples: *BRCA1, BRCA2, PALB2, PAD51C, RAD51C, RAD51D, MLH1, MSH2, MSH6, PMS2.*   However, in these genes, there are typically specific domains in which missense variation at key residues can cause loss of function; some benign variation typically also occura in these regions. PM2\_sup can be used for these specific residues/domains **e.g** residues listed in by ENIGMA within **BRCA1** BRCT and RING domains, **BRCA2:** DNA binding domain (<https://enigmaconsortium.org/wp-content/uploads/2018/10/ENIGMA_Rules_2017-06-29-v2.5.1.pdf>) 27   1. PM1 and PP2 can be used together |

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| **PM2**  \_MOD  \_SUP |
| Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium |
| Use at **Mod:** 0 observation in large control series >50,000 individuals  Use at **Sup** : 1 observation in large control series >50,000 individuals  **Explanatory Notes:**   * Ensure that sequencing coverage is sufficient. * Be cautious in using this criterion for small insertions/deletions, as calling of these variant types in NGS/exome/genome data can vary widely according to sequencing approaches/analytical methodologies. * If a control series has been used for PS4 (case control analysis), the same dataset can not be re-used for PM2. * If the GNOMAD NFE has been used for PS4, for PM2 the remainder of the GNOMAD populations may be used (ie 76,853 individuals for gnomAD v2.1) |

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| **PM3**  STR  \_MOD  \_SUP |
| For recessive disorders, detected in trans with a pathogenic variant |
| Use where variant found in trans with a pathogenic variant and the patient-level clinical features match those anticipated for the gene in question  Use at **Strong** where variant found in ≥2 unrelated cases, and the features are distinctive for that gene  Use at **Mod** where variant found in 1 case, and the features are distinctive for that gene  Use at **Sup** where variant found in 1 case, and the features are distinctive for a set of genes  **Explanatory Notes:**   * Comprehensive analysis should be undertaken for the gene to exclude an alternative second pathogenic mutation (e.g.incl MLPA) in that gene * Comprehensive analysis should be undertaken for all other genes for which the phenotypic features overlpa. * Requires testing of parents (or offspring) to confirm phase * Can use for homozygous variants but downgrade by one evidence level (as per ClinGen SVI points-based system)29 * Use PP4 for cellular/molecular phenotypes. |

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| **PM5**  \_MOD  \_SUP |
| Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before |
| Use at **Sup** where   * Single known P/LP variant at that codon   Use at **Mod:** where   * ≥2 known P/LP variants at that codon and the variant under evaluation is more deleterious than ≥2 P/LP variants at the codon using Revel or Align GVGD   **Explanatory Notes:**   * This requirement than the original ACMG stipulation, consistent with recommendations from the TP53 and CDH1 expert groups, and reflects data generated by CanVIG-UK for BRCA1/BRCA2 (manuscript in preparation). |

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| **PP3**  \_SUP |
| Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact). |
| * Protein impact: 3/3 tools (one tool may be borderline below threshold) using a ***predefined*** strategy of (i) a set of three tools (ii) a single tool   + SIFT (deleterious), Polyphen HumVar ≥(probably damaging) plus:     - Align GVGD (C45, C65), (for BRCA1, BRCA2)     - MAPP (bad) (for MMR genes)     - CADD ( >15) (for any other CSG)   + Or use Revel (>0.7) as a single score * Splicing impact:   + Intron-exon boundary: MaxEnt >15% difference **AND** SSFL >5% difference30   + Deep intronic: predicted creation of a novel splice site of any strength, absent in the normal sequence. |

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| **PP4**  STR  \_MOD  \_SUP |
| Patient’s phenotype or family history is highly specific for a disease with a single genetic aetiology |
| |  |  |  |  | | --- | --- | --- | --- | | Level | Points | A cellular/molecular phenotype that is: | Example | | **Sup** | 1 | Highly predictive for germline aberration of one of a small set of genes | Aberration on mitomycin-induced chromosomal breakage (for genes related to Fanconi Anaemia) | | **Sup** | 1 | Moderately predictive for germline aberration of the specific gene | LOH on at chromosomal locus for tumour-suppressor gene | | Loss on immunohistochemistry (for mismatch repair deficiency)  MSH6 loss 🠆 MSH6  PMS2 loss 🠆 PMS2 | | - | 0.5 | Moderately predictive for germline aberration of one of a small set of genes | MSI (for mismatch repair deficiency) | | **Mod** | 2 | Highly predictive for germline aberration of the specific gene | Depletion of BRCA2 in lymphocytes **and** aberration on mitomycin-induced chromosomal breakage (for BRCA2-related Fanconi Anaemia) | | Loss on immunohistochemistry (for mismatch repair deficiency)  MSH and MSH6 loss 🠆MSH2 | | Loss of MLH1/PMS2 on immunohistochemistry **and** normal MLH1 promoter methylation (for MLH1-related mismatch repair deficiency) |   **Explanatory Notes:**   * For CSGs, PP4 is applied for a cellular/molecular phenotype * For CSGs the high level clinical phenotype is often too non-specific (eg breast and/or ovarian cancer). For a number of pleiomorphic rare tumour and/or syndromic presentation of cancer susceptibility, the specificity of high level clinical phenotype has been captured within PS4 within the case counting (eg ClinGen criteria for CDH1, PTEN, TP5320-22). For other pleiomorphic rare tumour and/or syndromic presentations (eg MEN1, HLRCC) these specifications are awaited. * Comprehensive analysis of the gene and related genes should have been undertaken to exclude an alternative pathogenic mutation (incl MLPA) * Individuals/tumours included must have been demonstrated to carry the germline mutation * Evidence can be summed across multiple cases:   + Total points: Suporting:1; Moderate: 2; Strong: 4   + Only one individual per family can contribute   + Up to two ***independent*** tumour phenotype assays can be included per case (e.g. MSI AND LOH). Strongly correlated tumour phenotypes cannot both be included, e.g. MSI and IHC from the same case. |

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| **PP5**  \_SUP |
| Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation |
| **Explanatory Notes:**   * Any classification of LP/P after 2016 from   + ≥2 accredited North American diagnostic laboratory OR   + a single North American diagnostic laboratory where the utilised evidence is clearly listed   + ClinGen Expert Group, eg INSIGHT, ENIGMA. * When a single laboratory has classified as LP/P with provision of insufficient detail, it is advised that the individual laboratory is contacted to procure directly the evidence used for classification. |
| **Additional comments:**   * This is an **exceptional** application, as per UK-ACGS specification, as for commonly tested cancer susceptibility genes classifications by large laboratories may have be derived from their substantial series of case data not otherwise publicly available |

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| **BA1/BS1** |
| Allele frequency is “too high” for disorder ExAC or gnomAD |
| Use **BA1** as **Stand\_Alone** when allele frequency in a heterogeneous outbred population is >1%.  Use **BS1** as **Strong** when allele frequency in a heterogeneous outbred population is > value specified for specific gene by respective expert group.  **Explanatory Notes:**  BA1 should not be applied where the variant has already been well demonstrated as a pathogenic founder mutation (eg CHEK2 1100delC). The reduction of threshold of BA1 from 5% to 1% for Cancer Susceptibility Genes is predicated on existence of sufficiently high volumes of sequencing data to preclude existence of common undescribed founder mutations (>1% MAF). |

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| **BS2** |
| Observation in controls inconsistent with disease penetrance. Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age |
| Where variant is reportedin homozygous state in unaffected individual  Use at **Sup** where no further genotyping or clinical/cellular phenotyping is possible  Use at **Strong** where   * laboratory analysis has been repeated using an orthogonal approach (eg different primers) to confirm homozygosity for allele AND * patient is of age at which biallelic mutations would be anticipated to be penetrant for a distinctive phenotype AND * patient has been actively examined to exclude relevant phenotype AND/OR had analysis of cellular phenotype   OR   * the homozygote is observed in a specified control population in addition to a heterozygote frequency meeting BS1   **Explanatory Notes:**   * Use BP2 for compound heterozygotes |

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| **BS3**  \_SUP  \_STR |
| **Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing** |
| ***For assays of protein function***   |  |  |  |  | | --- | --- | --- | --- | |  | Discrimination | Controls | Reproducibility | | **Strong** | relative protein activity assay or functional impact  >25% compared to level for wildtype | ≥10 ‘true positive’  ≥10 ‘true-negative’ | ≥2 laboratories OR  Results demonstrated as reproducible in single laboratory | | **Sup** | ≥2 ‘true positive’  ≥2 ‘true-negative’ | single laboratory |   . |
| ***For assays of splicing function***   |  |  |  | | --- | --- | --- | | **Strong** | **1 assay:** with no evidence of abnormal transcripts (% normal transcript>90%) | ISO accredited laboratory or recognized research laboratory with which direct consultation can be undertaken. | | **Sup** | **1 assay:** with no evidence of abnormal transcripts (% normal transcript>90%) | **alternative source of evidence (e.g. publication)** |   **Explanatory Notes:**   * BS3 should only be applied for an assay of protein function whereby the assay has been validated for variants in the relevant domain to ensure that the mechanism of pathogenicity captured by the assay in question is relevant to that variant. BS3 should not be applied for an assay of protein function when in silico tools predict effect on splicing and/or for the first or last three bases of the exon * A splicing assay can only be used for BS3 for intronic variants and those in the first or last three bases of the exon. * Evidence of amplification of both alleles is required (i.e. sequencing should demonstrate the SNV in question or another nearby SNV, on the background of the wildtype sequence). This is necessary to exclude generation of a ‘normal’ RNA result when the splicing aberration has not been detected by the assay used (e.g. due to intron retention, size too large for the PCR to amplify) * When BS3 is applied for splicing OR protein function, BP4 (in silico evidence), can**not** be applied. * For specification of acceptable assays and QC standards, see PS3. |

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| **BP1**  \_SUP |
| Missense variant in a gene for which primarily truncating variants are known to cause disease |
| Use at **Sup** for genes/gene regions in which >95% of reported pathogenic mutations are truncating  **Explanatory note:**   * Can be used for BRCA1 outside of BRCT and RING domains * Can be used for BRCA2 outside of DNA-binding domain * Can be used for MLH1 xxxxxxxxxxxxxxxxxxx |

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| **BP2**  \_STR  \_SUP |
| Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern |
| Where variant is reportedin conjunction with a pathogenic variant in unaffected individual   * Use at **Sup** where no further clinical/cellular phenotyping is possible   Use at **Strong** where   * alleles have been confirmed as in trans (for heterozygous variants in trans) AND * patient is of age at which biallelic mutations would be anticipated to be penetrant for an distinctive phenotype AND * patient has been actively examined to exclude relevant phenotype AND/OR had analysis of cellular phenotype |
| **Explanatory note:**   * BS2 should be used when the variant is homozygous |

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| **BP4**  \_SUP |
| **Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.** |
| * Splicing impact:   + Intron-exon boundary: MaxEnt <15% difference AND SSFL<5% difference (Houdayer) AND   + No evidence of deep intronic: predicted creation of a novel splice site, absent in the normal sequence, of any strength.   **AND**   * protein impact: 3/3 tools (one tool may be borderline above threshold) using a ***predefined*** set of tools (recommendations below) or single tool   + SIFT (tolerate), Polyphen HumVar (benign) plus:     - Align GVGD (C0, C15), (for BRCA1, BRCA2)     - MAPP (good) (for MMR genes)     - CADD ( <10) (for any other CSG)   + Or Revel (<0.4) as a single score |

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| **BP5**  \_SUP |
| Variant found in a case with an alternate molecular basis for disease |
| This should not be applied in paradigms of non-syndromic autosomal dominant incompletely penetrance cancer susceptibility eg HBOC  **Explanatory note:**   * Co-occurrence of ≥2 pathogenic variants in different cancer susceptibility genes is widely reported. Typically the phenotype exhibited is indistinguishable from that of a single pathogenic mutation |
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| **BP6**  \_SUP |
| Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation |
| **Explanatory Notes:**   * Any classification of LB/B after 2016 from   + ≥2 accredited North American diagnostic laboratory OR   + a single North American diagnostic laboratory where the utilised evidence is clearly listed   + ClinGen Expert Group, eg INSIGHT, ENIGMA. * When a single laboratory has classified as LB/B with provision of insufficient detail, it is advised that the individual laboratory is contacted to procure directly the evidence used for classification. |
| **Additional comments:**  This is an **exceptional** application, as per UK-ACGS specification, as for commonly tested cancer susceptibility genes, classifications by large laboratories may have be derived from their substantial series of case data not otherwise publicly available |

### Appendix 2: Variants of reduced penetrance in high penetrance cancer susceptibility genes

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| * Variant interpretation and classification should be undertaken using the ACMG framework (with ACGS and CanVIG-UK specifications) * If any of the below criteria are met, the variant should be assigned the relevant ACMG class but with addendum of “reduced penetrance”. * The report should reference and recommend the nationally ratified clinical management recommendations for that gene for variants of reduced penetrance. * Clinical management recommendations for variants of reduced penetrance for each gene should established by disease-specific experts. |
| **Criterion 1: Down-modification of classic biallelic phenotype**  Abnormal physical AND cellular phenotype associated with biallelic mutations is present but notably milder.  ***Example:*** BRCA2-related Fanconi anaemia:   * Cancer is not penetrant by 5 years **AND** * Congenital abnormalities and physical features are mild **AND** * Incomplete functional abrogation of chromosomal breakage following mitomycin C exposure **OR** BRCA2-specific assays show only modest depletion of BRCA2 in quantity and/or function |
| **Criterion 2: Well calibrated assay gives intermediate effect**   * Highly predictive and well-calibrated published functional assay demonstrate intermediate effect, ie significant impairment of protein function but not at level demonstrated for truncating mutations in gene (e.g. Guidugli et al for BRCA231,32, Findlay et al 2019 for BRCA133) |
| **Criterion 3: Segregation analysis gives lower estimate of penetrance**   * Formal genetic epidemiologic analyses demonstrate variant to be associated with disease but of penetrance statistically significantly reduced compared to established estimates eg: BRCA1 c.5096G>A p.Arg1699Gln34,35 |

### Appendix 3: Example of CanVIG-UK classification summary report

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| **Variant classification by Can-VIG UK (Cancer Variant Interpretation UK)**  Can-VIG UK is a working group convened on behalf of the UK ACGS (Association of Clinical Genetic Scientists), which includes registered clinical scientists and clinical geneticists representing the following UK Regional molecular diagnostic laboratories: Aberdeen, Belfast, Birmingham, Bristol, Cambridge, Cardiff, Dublin, Exeter, Glasgow, GOSH, Guy's, Leeds, Liverpool (Cheshire & Merseyside), Oxford, Manchester, Newcastle, Nottingham, Sheffield , SW Thames (St George's), Wessex (Salisbury/Southampton), University Hospitals of Leicester. | | | | | |
| **Submitter** | Dr Clare Turnbull MD PhD FRCP FRCPath | | | **Date** | 28/11/18 |
| **Gene** | BRCA1 | **Transcript** | NM\_007294.3  ENST00000357654  LRG\_292t1 | **Variant** | c.53T>C (p.Met18Thr) |
| **Population data** | The variant was observed in 4 independent UK families undergoing clinical diagnostic testing, the denominator of which dataset of clinical testing was 16,600. Case control comparison against ethnically matched population data (4/16,600 in familial cases against 0/63,369 GNOMAD NFE controls pexact= 0.0019  There are additional reports of this variant in ClinVar (5), BIC (3) and BRCA1 LOVD (11), UMD(7), DMuDB(7)  The variant is absent in the remainder of the GNOMAD populations (75,263 individuals). | | | | |
| **Prediction (based on variant type/location), IN silico tools** | **AlignGVGD class:** C45  **SIFT prediction:** deleterious  **MAPP prediction:** bad  **Polyphen2 HumVar prediction:** benign  **CADD scaled score [0-99]:** 16.18 | | | | |
| **Functional data** | Findlay *et al.* 2018: Non functional via saturation editing analysis using on haploid *BRCA1* construct. | | | | |
| **Segregation data** |  | | | | |
| **De novo data** |  | | | | |
| **Allelic data (biallelic observations)** |  | | | | |
| **Other classifications** | Ambry LP 2018, Gene Dx LP 2014, Counsyl LP 2018 | | | | |
| **Other** |  | | | | |

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| **PATHOGENIC**  **Criteria** | **Weight (supporting, moderate, strong, very strong)** | **BENIGN**  **Criteria** | **Weight (supporting, strong)** |
| PVS1(null) |  | BS1/BA1 (controls) |  |
| PS4 (case control) | Very strong | BP4 (in silico) |  |
| PM2 (absent control) | Mod | BP1 (only trunc) |  |
| PP3 (in silico) |  | BP7 (synonymous) |  |
| PM5 (same residue) |  | BP3 (in frame, no func) |  |
| PM1 (hot spot) |  | BS3 (functional assay) |  |
| PP1 (Segregation) |  | BS4 (non segregation) |  |
| PS3 (functional assay) | Strong | BP2 (biallelic) |  |
| PM3 (biallelic) |  | BP6 (other databases) |  |
| PP5 (other databases) | Sup | Alternative cause (BP5) |  |
| Specific phenotype (PP4) |  |  |  |
| De novo (PM6, PS2) |  |  |  |
| **Total** | 1 very strong, 1 strong, 1 mod, 1 sup | **Total** |  |
| **Classification** | 5-Pathogenic | | |

**Synopsis for ClinVar**

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| ***Data included in classification:***  The variant was observed in 4 independent UK families undergoing clinical diagnostic testing, the denominator of which dataset of clinical testing was 16,600. Case control comparison against ethnically matched population data (4/16,600 in familial cases against 0/63,369 GNOMAD NFE controls pexact= **0.0019** (PS4\_VS).  The variant is absent in the remainder of the GNOMAD populations (75,263 individuals) (PM2).  Non-functional in SGE haploid BRCA1-assay (Findlay *et al.* 2018), an assay well validated against ENIGMA/ClinVar (PS3).  Classified as Likely Pathogenic by Ambry 2018, Gene Dx 2014, Counsyl 2018 (PP5).  ***Data not included in classification:***  Predicted deleterious on SIFT, Align GVGD; Polyphen HumVar prediction benign .  There are additional reports of this variant in ClinVar (5), BIC (3) and BRCA1 LOVD (11), UMD(7), DMuDB(7). |

**Supplementary Note: CanVIG-UK membership**

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| **Name** | **Organisation** |
| Stephen Abbs | Cambridge University Hospitals NHS Foundation Trust |
| Patrick Tarpey | Cambridge University Hospitals NHS Foundation Trust |
| Jonathan Bruty | Cambridge University Hospitals NHS Foundation Trust |
| James Drummond | Cambridge University Hospitals NHS Foundation Trust |
| James Whitworth | Cambridge University Hospitals NHS Foundation Trust |
| Anne Ramsay Bowden | Cambridge University Hospitals NHS Foundation Trust |
| Marc Tischowitz | Cambridge University Hospitals NHS Foundation Trust |
| Eamonn Maher | Cambridge University Hospitals NHS Foundation Trust |
| Shirley Heggarty | Belfast Health And Social Care Trust |
| Sean Hegarty | Belfast Health And Social Care Trust |
| Rosalind Martin | Belfast Health And Social Care Trust |
| Peter Logan | Belfast Health And Social Care Trust |
| Claire Byrne | Belfast Health And Social Care Trust |
| Yvonne Wallis | Birmingham Women's And Children's NHS Foundation Trust |
| Samantha Butler | Birmingham Women's And Children's NHS Foundation Trust |
| Rachel Hart | Birmingham Women's And Children's NHS Foundation Trust |
| Lowri Hughes | Birmingham Women's And Children's NHS Foundation Trust |
| Kim Reay | Birmingham Women's And Children's NHS Foundation Trust |
| Kai-Ren Ong | Birmingham Women's And Children's NHS Foundation Trust |
| Joanne Mason | Birmingham Women's And Children's NHS Foundation Trust |
| Ian Tomlinson | Birmingham Women's And Children's NHS Foundation Trust |
| Ian Frayling | Cardiff & Vale University Health Board |
| Sheila Palmer-Smith | Cardiff & Vale University Health Board |
| Julian Sampson | Cardiff & Vale University Health Board |
| Alex Murray | Cardiff & Vale University Health Board |
| Munaza Ahmed | Great Ormond Street Hospital For Children NHS Foundation Trust |
| Louise Kiely | Great Ormond Street Hospital For Children NHS Foundation Trust |
| Louise Busby | Great Ormond Street Hospital For Children NHS Foundation Trust |
| Claire Brooks | Great Ormond Street Hospital For Children NHS Foundation Trust |
| Alison Taylor-Beadling | Great Ormond Street Hospital For Children NHS Foundation Trust |
| Ajith Kumar | Great Ormond Street Hospital For Children NHS Foundation Trust |
| Vishakha Tripathi | Guy's And St Thomas' NHS Foundation Trust |
| Mina Ryten | Guy's And St Thomas' NHS Foundation Trust |
| Louise Izatt | Guy's And St Thomas' NHS Foundation Trust |
| Anjana Kulkarni | Guy's And St Thomas' NHS Foundation Trust |
| Adam Shaw | Guy's And St Thomas' NHS Foundation Trust |
| Joanna Campbell | Guy's And St Thomas' NHS Foundation Trust |
| Huw Thomas | St. Mark's Hospital, Northwick Park Hospital, Harrow |
| Daniel Chubb | Institute Of Cancer Research |
| Mary Alikian | Institute Of Cancer Research |
| Cankut Cubuk | Institute Of Cancer Research |
| Rachel Robinson | Leeds Teaching Hospitals NHS Trust |
| Brendan Mullaney | Leeds Teaching Hospitals NHS Trust |
| Karen-Lynn Greenhalgh | Liverpool Women’s NHS Foundation Trust |
| Virginia Clowes | London North West University Healthcare NHS Trust |
| Angela Brady | London North West University Healthcare NHS Trust |
| George Burghel | Manchester University NHS Foundation Trust |
| Emma Woodward | Manchester University NHS Foundation Trust |
| Ronnie Wright | Manchester University NHS Foundation Trust |
| Gareth Evans | Manchester University NHS Foundation Trust |
| Fiona Lalloo | Manchester University NHS Foundation Trust |
| Andrew Wallace | Manchester University NHS Foundation Trust |
| John Burn | Newcastle Upon Tyne Hospitals NHS Foundation Trust |
| James Tellez | Newcastle Upon Tyne Hospitals NHS Foundation Trust |
| Sarah Mackenzie | Newcastle Upon Tyne Hospitals NHS Foundation Trust |
| Helen Powell | Newcastle Upon Tyne Hospitals NHS Foundation Trust |
| Stephen Tennant | NHS Grampian, Aberdeen |
| Joanna Tolmie | NHS Grampian, Aberdeen |
| Dawn O'Sullivan | NHS Grampian, Aberdeen |
| Rosemarie Davidson | NHS Greater Glasgow & Clyde |
| Jonathan Grant | NHS Greater Glasgow & Clyde |
| Daniel Stobo | NHS Greater Glasgow & Clyde |
| Aisha Ansari | NHS Greater Glasgow & Clyde |
| Jennie Murray | NHS Lothian, Edinburgh |
| David Moore | NHS Lothian, Edinburgh |
| Rachael Tredwell | Nottingham University Hospital NHS Trust |
| Joanne Field | Nottingham University Hospital NHS Trust |
| Kirsty Bradshaw | Nottingham University Hospital NHS Trust |
| Rachel Harrison | Nottingham University Hospital NHS Trust |
| Logan Walker | University of Otago, Christchurch, New Zealand |
| Trudi Mcdevitt | Our Lady's Children's Hospital, Crumlin, Dublin |
| Marie Duff | Our Lady's Children's Hospital, Crumlin, Dublin |
| Catherine Clabby | Our Lady's Children's Hospital, Crumlin, Dublin |
| Treena Cranston | Oxford University Hospitals NHS Foundation Trust |
| Tina Bedenham | Oxford University Hospitals NHS Foundation Trust |
| Evgenia Petrides | Oxford University Hospitals NHS Foundation Trust |
| Lara Hawkes | Oxford University Hospitals NHS Foundation Trust |
| Fiona McRonald | Public Health England |
| Sian Ellard | Royal Devon And Exeter NHS Foundation Trust |
| Ruth Cleaver | Royal Devon And Exeter NHS Foundation Trust |
| Carole Brewer | Royal Devon And Exeter NHS Foundation Trust |
| Emma Baple | Royal Devon And Exeter NHS Foundation Trust |
| Nick Woodwaer | Royal Free London NHS Foundation Trust |
| Stacey Daniels | Salisbury NHS Foundation Trust |
| Alison Callaway | Salisbury NHS Foundation Trust |
| Khalid Tobal | Sheffield Children's NHS Foundation Trust |
| Shadi Albaba | Sheffield Children's NHS Foundation Trust |
| Sarah DELL | Sheffield Children's NHS Foundation Trust |
| Rodney Nyanhete | Sheffield Children's NHS Foundation Trust |
| Richard Kirk | Sheffield Children's NHS Foundation Trust |
| Mark Watson | Sheffield Children's NHS Foundation Trust |
| Miranda Durkie | Sheffield Children's NHS Foundation Trust |
| Katie Snape | Sheffield Children's NHS Foundation Trust |
| Jackie Cook | Sheffield Children's NHS Foundation Trust |
| Hazel Clouston | Sheffield Children's NHS Foundation Trust |
| Anne-Cecile Hogg | Sheffield Children's NHS Foundation Trust |
| Sabrina Talukdar | St George's University Hospitals NHS Foundation Trust |
| Lorraine Hawkes | St George's University Hospitals NHS Foundation Trust |
| Laura Cobbold | St George's University Hospitals NHS Foundation Trust |
| Kate Tatton-Brown | St George's University Hospitals NHS Foundation Trust |
| Helen Hanson | St George's University Hospitals NHS Foundation Trust |
| Charlene Crosby | St George's University Hospitals NHS Foundation Trust |
| Ayaovi Hadonou | St George's University Hospitals NHS Foundation Trust |
| Zoe Kemp | The Royal Marsden NHS Foundation Trust |
| Terri Mcveigh | The Royal Marsden NHS Foundation Trust |
| Clare Turnbull | The Royal Marsden NHS Foundation Trust |
| Alice Garrett | The Royal Marsden NHS Foundation Trust |
| Cathal O'Brien | Trinity College Dublin, The University Of Dublin, Ireland |
| Laura Yarram | University Hospitals Bristol NHS Foundation Trust |
| Kenneth Smith | University Hospitals Bristol NHS Foundation Trust |
| Helen Williamson | University Hospitals Bristol NHS Foundation Trust |
| Alan Donaldson | University Hospitals Bristol NHS Foundation Trust |
| Julian Barwell | University Hospitals Of Leicester NHS Trust |
| Matilda Bradford | University Hospitals Plymouth NHS Trust |
| Lucy Side | University Hospital Southampton NHS Foundation Trust |
| Diana Eccles | University Hospital Southampton NHS Foundation Trust |
| Diana Baralle | University Hospital Southampton NHS Foundation Trust |
| Anneke Lucassen | University Hospital Southampton NHS Foundation Trust |

#### References

1. Norbury, G. Association of Clinical Genomic Scientists Pan-UK Census of Molecular Testing Activity 2015-16. (2017).

2. Turnbull, C., Sud, A. & Houlston, R.S. Cancer genetics, precision prevention and a call to action. *Nat Genet* **50**, 1212-1218 (2018).

3. Tandy-Connor, S. *et al.* False-positive results released by direct-to-consumer genetic tests highlight the importance of clinical confirmation testing for appropriate patient care. *Genet Med* (2018).

4. Kilbride, M.K., Domchek, S.M. & Bradbury, A.R. Ethical Implications of Direct-to-Consumer Hereditary Cancer Tests. *JAMA Oncol* **4**, 1327-1328 (2018).

5. <https://www.theguardian.com/science/2019/jul/21/senior-doctors-call-for-crackdown-on-home-genetic-testing-kits>. in *The Guardian* (2019).

6. Eccles, D.M. *et al.* BRCA1 and BRCA2 genetic testing-pitfalls and recommendations for managing variants of uncertain clinical significance. *Ann Oncol* **26**, 2057-65 (2015).

7. Lek, M. *et al.* Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**, 285-91 (2016).

8. Pinard, A. *et al.* WES/WGS Reporting of Mutations from Cardiovascular "Actionable" Genes in Clinical Practice: A Key Role for UMD Knowledgebases in the Era of Big Databases. *Hum Mutat* **37**, 1308-1317 (2016).

9. Pinard, A. *et al.* Actionable Genes, Core Databases, and Locus-Specific Databases. *Hum Mutat* **37**, 1299-1307 (2016).

10. Szabo, C., Masiello, A., Ryan, J.F. & Brody, L.C. The breast cancer information core: database design, structure, and scope. *Hum Mutat* **16**, 123-31 (2000).

11. Fokkema, I.F. *et al.* LOVD v.2.0: the next generation in gene variant databases. *Hum Mutat* **32**, 557-63 (2011).

12. Greenblatt, M.S. *et al.* Locus-specific databases and recommendations to strengthen their contribution to the classification of variants in cancer susceptibility genes. *Hum Mutat* **29**, 1273-81 (2008).

13. Easton, D.F. *et al.* A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. *Am J Hum Genet* **81**, 873-83 (2007).

14. Plon, S.E. *et al.* Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* **29**, 1282-91 (2008).

15. Spurdle, A.B. *et al.* ENIGMA--evidence-based network for the interpretation of germline mutant alleles: an international initiative to evaluate risk and clinical significance associated with sequence variation in BRCA1 and BRCA2 genes. *Hum Mutat* **33**, 2-7 (2012).

16. Parsons, M.T. *et al.* Large scale multifactorial likelihood quantitative analysis of BRCA1 and BRCA2 variants: An ENIGMA resource to support clinical variant classification. *Hum Mutat* **40**, 1557-1578 (2019).

17. Thompson, B.A. *et al.* Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the InSiGHT locus-specific database. *Nat Genet* **46**, 107-115 (2014).

18. Richards, S. *et al.* 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**, 405-24 (2015).

19. Brnich, S.E. *et al.* Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *bioRxiv* (2019).

20. Savage, S.A. TP53 Rule Specifications for the ACMG/AMP Variant Curation Guidelines. Vol. 2019 (ClinGen, clinicalgenome.org, 2019).

21. ClinGen CDH1 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2. (2019).

22. Mester, J.L. *et al.* Gene-specific criteria for PTEN variant curation: Recommendations from the ClinGen PTEN Expert Panel. *Hum Mutat* **39**, 1581-1592 (2018).

23. Tavtigian, S.V. *et al.* Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework. *Genet Med* **20**, 1054-1060 (2018).

24. Ellard, S. *et al.* ACGS Best Practice Guidelines for Variant Classification 2019. (Association for Clinical Genetics Science (ACGS), 2019).

25. Cox, J.H.

26. Abou Tayoun, A.N. *et al.* Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. *Hum Mutat* **39**, 1517-1524 (2018).

27. Spurdle, A. ENIGMA BRCA1/2 Gene Variant Classification Criteria. (2017).

28. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* **45**, 580-5 (2013).

29. ClinGen. SVI Recommendation for in trans Criterion (PM3) - Version 1.0. (2019).

30. Houdayer, C. *et al.* Guidelines for splicing analysis in molecular diagnosis derived from a set of 327 combined in silico/in vitro studies on BRCA1 and BRCA2 variants. *Hum Mutat* **33**, 1228-38 (2012).

31. Guidugli, L. *et al.* Functional assays for analysis of variants of uncertain significance in BRCA2. *Hum Mutat* **35**, 151-64 (2014).

32. Guidugli, L. *et al.* Assessment of the Clinical Relevance of BRCA2 Missense Variants by Functional and Computational Approaches. *Am J Hum Genet* **102**, 233-248 (2018).

33. Findlay, G.M. *et al.* Accurate functional classification of thousands of <em>BRCA1</em> variants with saturation genome editing. *bioRxiv* (2018).

34. Spurdle, A.B. *et al.* BRCA1 R1699Q variant displaying ambiguous functional abrogation confers intermediate breast and ovarian cancer risk. *J Med Genet* **49**, 525-32 (2012).

35. Moghadasi, S. *et al.* The BRCA1 c. 5096G>A p.Arg1699Gln (R1699Q) intermediate risk variant: breast and ovarian cancer risk estimation and recommendations for clinical management from the ENIGMA consortium. *J Med Genet* **55**, 15-20 (2018).