

Review

Hereditary Cancer Genomics: Current Practice, Translational Challenges, and Future Priorities

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Abstract

The expanding scope and sensitivity of genomic testing are reshaping hereditary cancer assessment, shifting attention from the detection of genomic variants alone to the more complex task of determining their significance, biological origin, and relevance to clinical management. This review examines the analytical, biological, and clinical factors that shape this process and integrates them within a five-stage translational framework encompassing variant detection, variant interpretation, biological attribution, clinical validity, and utility. Reliable interpretation requires appropriate test and gene selection, recognition of assay limitations and technically challenging variant classes, and evidence-based variant classification. Computational prediction can support variant interpretation when appropriately validated. Polygenic risk models may further refine risk estimation, although their clinical implementation remains limited by validation, calibration, and population-specific performance. Ancestry-related limitations in genomic resources remain relevant to both variant interpretation and risk estimation. Particular attention is given to biological attribution, illustrated by liquid biopsy, where clonal hematopoiesis can confound the origin and clinical interpretation of plasma variants. Genomic findings must ultimately be considered together with phenotype, family history, gene–disease validity, and disease-specific evidence when informing risk assessment, surveillance, prevention, counseling, or treatment. As hereditary cancer testing continues to expand, its value will depend less on the number of findings generated than on how reliably those findings can be translated into meaningful benefit for patients and families.

Keywords: hereditary cancer genomics; next-generation sequencing; variant classification; artificial intelligence; clonal hematopoiesis; liquid biopsy; clinical actionability



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1. Introduction

1.1. Molecular Basis and Clinical Phenotypes

Hereditary cancer predisposition is characterized by an increased susceptibility to malignancy resulting from pathogenic or likely pathogenic (P/LP) germline variants. Clinical features that increase suspicion for hereditary predisposition include unusually early age at cancer diagnosis, multiple primary malignancies, characteristic tumor combinations, and familial clustering of related cancers [1]. Most established hereditary cancer syndromes follow autosomal-dominant inheritance, although autosomal-recessive and, more rarely, X-linked inheritance patterns also occur. Susceptibility genes participate in diverse biological pathways involved in DNA repair, cell-cycle control, apoptosis, growth signaling, and maintenance of genomic integrity [2].

However, because penetrance and expressivity vary, the absence of these classical features does not exclude an underlying hereditary cancer predisposition [3]. Consequently, fully evaluating this predisposition requires navigating a complex landscape defined by variable patterns of penetrance, non-canonical epigenetic mechanisms, and systemic ancestry biases in genomic databases.

1.2. Penetrance Spectrum and Cumulative Cancer Risks

Inherited cancer risk is best understood as a continuum rather than as a set of fixed categories. High-, moderate-, and lower-penetrance categories are clinically useful for risk stratification, but the thresholds separating them do not represent discrete biological boundaries and may vary between professional guidelines. Penetrance itself is influenced by age, sex, the specific gene and variant, family history, ancestry, reproductive and environmental factors, and study design [4]. Estimates derived from clinically ascertained, phenotype-first cohorts may differ from those obtained through population-based, genotype-first approaches [5].

Pathogenic variants in *BRCA1* confer a striking cumulative lifetime breast cancer risk of up to 72% alongside an ovarian cancer risk of 44%. Meanwhile, *BRCA2* carrier status yields an estimated 69% lifetime breast cancer risk and a 17% ovarian cancer risk. Furthermore, recent epidemiological cohort updates have reclassified *PALB2* as a high-penetrance locus, given that its germline variants carry a cumulative lifetime breast cancer risk of 40–53% by age 80, closely mirroring the clinical severity and management mandates of *BRCA2* [6,7]. Particularly high cancer risks characterize *TP53*-associated Li-Fraumeni syndrome and *CDH1*-associated hereditary diffuse gastric cancer [8,9]. *CHEK2* and *ATM* generally confer more moderate and variable risks. However, gene-level classification alone may obscure clinically relevant variant-specific differences in risk. For example, *CHEK2* p.(Ile157Thr) is associated with lower risk than typical truncating *CHEK2* variants [10], whereas *ATM* c.7271T>G, p.(Val2424Gly) is associated with substantially higher breast cancer risk than most pathogenic *ATM* variants [11]. These examples illustrate that pathogenicity and penetrance are related but distinct concepts: identification of a P/LP variant does not, by itself, define the magnitude of cancer risk for an individual carrier.

Polygenic risk scores (PRS), which combine the effects of multiple common genetic variants, may further refine risk estimates by capturing a component of genetic susceptibility beyond that attributable to pathogenic variants in established cancer predisposition genes. In breast cancer, multifactorial models such as CanRisk integrate PRS with pathogenic variants, family history, and other established risk factors to provide individualized risk estimates [12]. However, clinical application requires appropriate validation and calibration, particularly because many PRS have been developed predominantly in populations of European ancestry and their predictive performance may not transfer reliably across populations [13]. More broadly, the role of PRS in routine clinical care continues to evolve,

with ongoing questions regarding appropriate indications, implementation, and evidence of clinical utility [14].

Evidence from external validation studies illustrates both the value and the limits of these models. In the population-based Swedish KARMA (KARolinska Mammography Project for Risk Prediction of Breast Cancer) cohort, CanRisk/BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm) version 6 was evaluated in 66,415 women of European ancestry, including 816 incident breast cancers; the full model incorporating family history, questionnaire-based risk factors, mammographic density, a 313-variant PRS and pathogenic-variant status, achieved an area under the ROC curve (AUC) of 0.70 (95% CI, 0.66–0.73), with an expected-to-observed (E/O) ratio of 0.88 (95% CI, 0.75–1.04) and a calibration slope of 0.97 (95% CI, 0.95–0.99) [15]. In the independent Generations Study nested case–control sample of 1337 women of European ancestry (619 incident cases), adding PRS and lifestyle/reproductive factors produced only a modest AUC increase in women younger than 50 years (69.1% to 69.7%); calibration was generally good for BOADICEA version 5, whereas the PRS-extended Tyrer–Cuzick model showed evidence of overestimation in the highest risk decile [16]. Among 2979 European-ancestry *BRCA1/2* pathogenic-variant carriers in the prospective TRANsIBCCS cohort, the CanRisk/BOADICEA version 6 multifactorial model was well calibrated (E/O 1.07, 95% CI 0.92–1.24) and showed an AUC of 0.79 (95% CI 0.76–0.82), although the analysis included 209 incident cases and does not establish that model-guided decisions improve long-term outcomes [17]. These studies support risk stratification in the populations studied, but do not establish equivalent calibration in other ancestry groups or justify transferring thresholds without local evaluation. Moreover, improvements in discrimination and calibration do not, by themselves, demonstrate that incorporating PRS into clinical decision-making improves patient outcomes.

1.3. Lynch Syndrome: Genetic Heterogeneity and the EPCAM Epigenetic Mechanism

Lynch syndrome is a hereditary cancer predisposition syndrome caused predominantly by constitutional P/LP variants in the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6*, or *PMS2*. Loss of MMR function promotes microsatellite instability and accumulation of somatic variants [18].

EPCAM deletions represent a mechanistically distinct cause of Lynch syndrome. Constitutional deletions involving the 3′ end of *EPCAM*, particularly those disrupting the transcription termination and polyadenylation signals, can result in transcriptional read-through and subsequent hypermethylation and epigenetic silencing of the adjacent *MSH2* promoter in *EPCAM*-expressing tissues [19]. The resulting phenotype can resemble *MSH2*-associated Lynch syndrome. Accordingly, diagnostic testing for Lynch syndrome should include analysis capable of detecting clinically relevant 3′ *EPCAM* deletions rather than relying on *EPCAM* sequence analysis alone [20].

Constitutional epimutations represent an additional mechanism of hereditary cancer predisposition that may not be detected by conventional sequence analysis. In particular, constitutional *MLH1* promoter methylation can cause Lynch syndrome through epigenetic silencing of *MLH1*, although the underlying mechanisms and patterns of inheritance may differ between primary and secondary epimutations [21,22]. These mechanisms illustrate a broader analytical principle in hereditary cancer testing: the importance of selecting assays capable of detecting the relevant molecular alteration rather than relying exclusively on sequence-based testing.

1.4. Genomic Inequities and Ancestry Bias in Reference Databases

The reliability of hereditary cancer variant interpretation is affected by the unequal representation of global populations in genomic resources. gnomAD is primarily a population-frequency resource, whereas ClinVar aggregates clinical assertions and should not be treated as a population reference database. Nevertheless, ancestry-related disparities in both population-frequency data and clinically submitted variant evidence can limit the evidence available for variant interpretation [23,24]. Underrepresentation can contribute to uncertainty, including a higher burden of variants of uncertain significance (VUS), and may lead to overestimation of variant rarity or inappropriate application of population-frequency criteria, particularly in underrepresented populations [25]. Consistent with this disparity, higher VUS rates have been reported among individuals of African ancestry than among those of European ancestry, with subsequent reclassification patterns also differing across ancestry groups [26]. Population-specific resources can help address these limitations by providing more representative allele-frequency data. For example, CanVaS documents germline variation in Greek individuals with a personal and/or family history of cancer providing population-specific allele frequencies together with clinical and familial information [27]. As a clinically ascertained resource, however, CanVaS should not be interpreted as providing allele frequencies representative of the general Greek population. However, population frequency is only one component of variant classification and conclusions should integrate all relevant American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) evidence categories [28].

Against this background, this review examines the challenges that arise as hereditary cancer genomics moves from variant detection to clinical application. Rather than considering analytical performance, variant interpretation, biological origin, and clinical relevance in isolation, we examine how these issues interact across the translational pathway. Particular attention is given to biological attribution, the assignment of a detected variant to its likely biological source, using liquid biopsy and clonal hematopoiesis as an illustrative example. These considerations are integrated into a five-stage translational framework encompassing variant detection, variant interpretation, biological attribution, clinical validity and utility, providing a structured perspective on where uncertainty can arise between generating a genomic finding and using it to inform clinical management.

1.5. Literature Selection

In this narrative review, relevant literature was identified primarily through PubMed/MEDLINE, with the search updated through September 2026 and no predefined restriction on publication year. Clinical practice guidelines were also identified through the official websites of relevant professional organizations, including NCCN, ESMO, ACMG, and ASCO. Search terms, used individually and in combination, included hereditary cancer, hereditary cancer syndromes, cancer predisposition genes, next-generation sequencing, bioinformatic analysis, germline, variant interpretation, ACMG/AMP classification, artificial intelligence, polygenic risk scores, clonal hematopoiesis, mosaicism, liquid biopsy, and clinical actionability.

Priority was given to relevant clinical practice guidelines, consensus statements, methodological studies, and peer-reviewed publications, including earlier landmark studies where appropriate. Publications were selected according to their relevance to the topics covered, methodological quality, including study design and appropriateness of validation and clinical implications. The future priorities discussed in this review were identified by considering recurring limitations in variant detection and interpretation, uncertainties in biological attribution, gaps in population-specific validation, and the distinction between

predictive performance and demonstrated clinical utility. No formal systematic review protocol or quantitative evidence synthesis was applied.

2. Contemporary Genetic Testing Modalities

Selection of a germline genetic testing strategy for hereditary cancer predisposition should balance clinical indication, expected diagnostic yield, analytical performance, detectable variant classes, incidental findings, turnaround time, cost, and available interpretation resources. No single sequencing modality detects all clinically relevant variant classes equally well. Testing should therefore be selected according to the clinical question and the validated capabilities of the laboratory rather than sequencing breadth alone [29]. Optimizing diagnostic yield requires balancing these primary sequencing platforms with phenotype-driven gene selection boundaries and incorporating specialized complementary assays capable of catching variant classes that escape standard next-generation sequencing architectures.

2.1. Multigene Targeted NGS Panels, Whole-Exome Sequencing, and Whole-Genome Sequencing

Next-generation sequencing (NGS)-based multigene panel testing has largely replaced sequential single-gene testing for many hereditary cancer indications and is now widely used in clinical practice [29]. Nevertheless, targeted single-gene or variant-specific testing remains appropriate in selected circumstances, such as when a characteristic clinical phenotype strongly suggests a specific gene-associated syndrome, when a known familial pathogenic variant is being investigated, or when a tumor-detected variant warrants germline follow-up to establish its constitutional origin [30]. Targeted multigene panels provide high read depth across selected clinically relevant genes and are effective for detecting single-nucleotide variants and small insertions/deletions; copy-number analysis can also be incorporated when specifically validated. Importantly, analytical performance depends not only on the genes included in a panel but also on assay design, target coverage, sequencing chemistry, bioinformatic analysis, and validation for the relevant variant classes. Their focused design may reduce incidental findings, but their restricted genomic scope limits detection outside the regions and genes included in the assay. Conversely, broader panels may increase interpretive complexity when they include genes with less well-established disease associations, penetrance, or clinical management recommendations [29,31,32]. Whole-exome sequencing (WES) interrogates most protein-coding regions and extends analysis beyond predefined gene panels, which may be useful when the phenotype does not clearly indicate a specific hereditary cancer syndrome or when a wider genomic evaluation is warranted. Whole-genome sequencing (WGS) provides relatively uniform genome-wide coverage and extends interrogation beyond coding regions, potentially improving the detection of structural variants, deep-intronic variants, and variants affecting regulatory elements. However, the clinical interpretation of non-coding variation remains challenging, and variant detection depends on the sequencing platform, bioinformatic pipeline, and extent of analytical validation [33]. Thus, the choice between a targeted panel, WES, and WGS should be guided by the clinical indication, the expected genetic architecture, the required breadth of analysis, and the validated performance of the assay [34].

2.2. Clinical Considerations in Test and Gene Selection

The scope of germline genetic testing should be driven by the clinical phenotype and family history, the validity of gene–disease associations, and the anticipated clinical relevance of results. When several susceptibility genes are plausible, multigene panel testing is generally appropriate; however, broader testing does not automatically translate into greater clinical utility [35]. This is particularly relevant for genes with limited or

disputed evidence for cancer predisposition, whose inclusion may increase interpretive uncertainty without providing a corresponding increase in clinically meaningful diagnostic yield [36]. Prior testing should also be considered, particularly when earlier assays had restricted gene content, analytical scope, or validation for specific variant classes.

2.3. Complementary Methods Beyond Routine Short-Read NGS

Structural and copy-number variants, splice-altering variants, and variants in technically difficult genomic regions may require complementary methods for reliable detection or characterization. Depending on the variant class, complementary approaches may include copy-number assays, RNA analysis for suspected splice effects, and long-read sequencing for complex or technically challenging genomic regions [29,37,38]. Methylation-specific assays may be required when a constitutional epigenetic mechanism is suspected, as illustrated by *MLH1* epimutations in Lynch syndrome [39]. The choice of a complementary method should therefore be driven by the suspected molecular mechanism and by variant classes that fall outside the validated scope of the primary assay. Laboratories should clearly define each assay's validated analytical scope and limitations.

3. Bioinformatic Analysis & Variant Curation

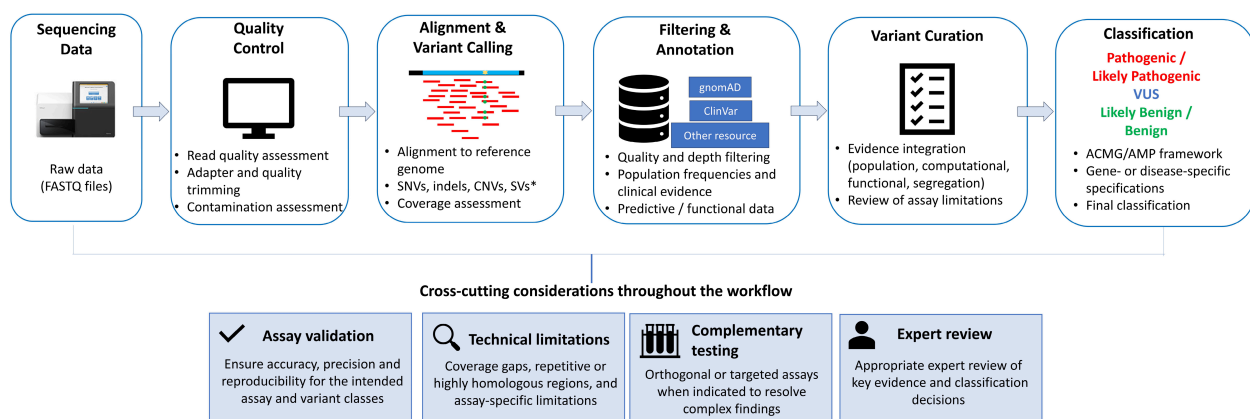
A critical step in clinical genomics is transforming sequencing data into a clinically meaningful interpretation. Analytical artifacts, incomplete coverage, allele dropout, mapping ambiguity, and bioinformatic errors can compromise variant detection and subsequent interpretation. A robust pipeline therefore requires validated quality-control procedures, transparent filtering criteria, orthogonal confirmation where indicated, and appropriate expert review. Managing these bioinformatic complexities requires an integrated approach involving validated analytical pipelines, evidence-based variant classification, and appropriately evaluated computational tools.

3.1. Architecture of the Analytical Pipeline

Converting raw sequencing data into a clinical report generally involves quality assessment, alignment to an appropriate, version-specified reference genome, variant calling, quality filtering, annotation, variant curation, and classification [40]. Raw sequencing reads in FASTQ format undergo quality assessment using tools such as FastQC. Adapter trimming and quality filtering, when required, are performed using separate dedicated tools. Following alignment to the reference genome, single-nucleotide variants (SNVs) and small insertions and deletions (indels) can be identified using variant callers such as GATK HaplotypeCaller or DeepVariant [41]. Copy Number Variants (CNVs) may be detected using dedicated tools such as ExomeDepth [42] or CNVkit [43], depending on the sequencing assay and its validated analytical performance. The specific tools and algorithms may vary between laboratories, but the bioinformatics pipeline should be validated for the intended assay and variant classes [44,45]. Annotation commonly incorporates population-frequency resources such as gnomAD and clinical databases such as ClinVar. Variant classification follows ACMG/AMP standards and guidelines and, where available, gene- or disease-specific specifications of these criteria [46]. Pipeline performance should be evaluated using appropriate reference materials and benchmark datasets, with particular attention to genomic regions that remain technically challenging for short-read analysis. The high sequence homology between *PMS2* and its pseudogene *PMS2CL*, particularly in the 3' region of *PMS2*, illustrates this limitation and may require specialized assay design or complementary methods for reliable variant detection. Depending on the variant class, complementary approaches may include gene-specific long-range PCR for locus-specific sequence analysis or appropriately designed multiplex ligation-dependent

probe amplification (MLPA) assays for copy-number assessment [47]. This example underscores that sequencing depth alone does not ensure analytical reliability when read mapping or locus specificity is compromised [38,40]. Alignment and variant-calling performance also depend on the algorithms used and the characteristics of the sequencing assay. BWA-MEM is a widely used short-read aligner, whereas DRAGEN provides an integrated, hardware-accelerated secondary analysis platform. Comparative studies have evaluated alternative alignment and variant-calling workflows, including BWA-MEM2 and DRAGEN-GATK, in germline whole-exome sequencing using reference samples, reporting improved SNV/indel detection performance and reduced analysis time for the evaluated BWA-MEM2/DRAGEN-GATK workflow compared with the standard GATK workflow under the study conditions [48]. Other benchmarking studies have demonstrated variability in analytical sensitivity and false-positive rates across targeted sequencing workflows [49]. These findings emphasize that performance depends on the assay, variant class, reference datasets, and analytical workflow, and that the complete pipeline must be validated for its intended clinical application.

A simplified overview of the clinical bioinformatics workflow, from sequencing data to variant classification, is shown in Figure 1.



*SNVs: single-nucleotide variants; indels: insertions/deletions; CNVs: copy-number variants; SVs: structural variants

Figure 1. Clinical bioinformatics workflow from sequencing data to variant classification. The workflow integrates quality control, variant detection and annotation, evidence-based curation, and classification, while assay validation, technical limitations, complementary testing, and expert review remain relevant throughout the analytical process. In the classification step, red indicates pathogenic or likely pathogenic variants, blue indicates variants of uncertain significance (VUS), and green indicates likely benign or benign variants. The remaining colors are used for visual organization of the workflow.

3.2. Variant Classification and Evidence Integration

The evidentiary weight of individual criteria inherently depends on the quality, validity, and context of the supporting data, and subsequent refinements of the framework provide more specific guidance for evaluating computational, functional, segregation, and phenotypic evidence [50–52]. Gene-specific specifications are particularly relevant in hereditary cancer genetics because the applicability and strength of each criterion may depend on the gene, disease mechanism, and available clinical or functional data. Their practical impact is illustrated by the ClinGen ENIGMA *BRCA1/BRCA2* Variant Curation Expert Panel pilot, where the application of gene-specific specifications successfully resolved 11 out of 13 variants previously flagged as VUS or marked by conflicting assertions [53].

Computational predictions can contribute to ACMG/AMP classification when appropriately calibrated, with their evidentiary weight determined by validated tool-specific

thresholds rather than by the prediction score alone. Calibration studies have shown that selected predictors can reach moderate or strong evidence levels for subsets of variants [50,54]. The same principle applies to functional evidence: its strength depends on assay validation, biological relevance, and concordance with the established disease mechanism rather than on the presence of an experimental result alone [51].

Reproducibility of variant classification depends on transparent documentation of the evidence evaluated. Relevant databases, computational tools and software versions, reference transcripts, the genome build, and analytical limitations should therefore be documented. Expert review by appropriately trained professionals is particularly important when evidence is discordant, gene-specific specifications are unavailable, or computational evidence has not been calibrated for the relevant variant or gene–disease context.

4. Artificial Intelligence in Genomic Medicine

AI and machine-learning approaches may reduce the burden of manual variant prioritization, particularly for variants lacking sufficient clinical or functional evidence. However, their clinical utility depends on the specific task for which a model was developed and the context in which it has been validated.

AI-based prediction tools address different consequences of genomic variation and should therefore be selected according to the variant mechanism under investigation. For missense variation, established ensemble methods like REVEL [55] combine multiple conservation scores to evaluate pathogenicity, whereas deep-learning models such as AlphaMissense, which builds on AlphaFold-derived representations and evolutionary information, estimate the pathogenicity of missense variants [56]. Importantly, AlphaMissense does not directly predict the structural or functional consequences of an individual amino acid substitution; rather, its predictive performance may vary between genes, highlighting the importance of gene-specific validation in clinical variant interpretation [57]. In hereditary cancer genetics, the integration of gene-level constraint metrics (e.g., pLI, LOEUF, and syn_z) from population databases like gnomAD [23] requires careful calibration; because adult-onset predisposition genes like *BRCA1* and *BRCA2* primarily impact post-reproductive lifespan, they frequently display lower evolutionary constraint than early-onset disease loci, potentially confounding general AI scoring systems. In contrast, SpliceAI is designed to predict splice-altering variants, including those located beyond canonical splice-site regions [58], offering a critical alternative for variants in cancer susceptibility genes that may otherwise be misannotated as simple missense or synonymous alterations. Consequently, moving these specialized computational algorithms from theoretical frameworks into active clinical pipelines requires a balanced evaluation of their real-world utility and technical boundaries.

Applications and Limitations of AI-Based Prediction

AI-based models can prioritize variants as candidates for functional investigation, although appropriately calibrated computational predictions may also contribute directly to variant classification when applied at validated thresholds within the relevant ACMG/AMP or gene-specific classification framework [44,48].

Representative validation studies also require careful interpretation. REVEL was developed as an ensemble for rare missense variants and evaluated against two test sets that excluded variants used for training REVEL itself or its component predictors: 935 disease variants with 123,935 putatively neutral exome variants, and 1953 pathogenic/likely pathogenic versus 2406 benign/likely benign ClinVar variants. It outperformed comparator predictors by AUC in these datasets, but its performance depends on the composition and curation of the benchmark sets and does not directly establish clinical accuracy for

every hereditary-cancer gene [49]. AlphaMissense was calibrated against ClinVar variants to assign categories at a target precision of 90%; this is not equivalent to 90% sensitivity, nor does it mean that every gene or clinical context achieves that precision. Independent assessments have found heterogeneous performance across genes, reinforcing the need for gene- and disease-context-specific evaluation [50,51].

SpliceAI was evaluated using experimentally supported splice-altering variants and has demonstrated the ability to predict splice-altering effects, including those associated with variants outside canonical splice sites, but a predicted splice effect is not by itself proof of a disease-relevant transcript consequence or pathogenicity in a particular gene [52]. An additional concern is dependence between predictors and validation data. REVEL itself combines multiple correlated features, including conservation and scores from other tools; therefore, treating REVEL, its component predictors, and related conservation metrics as separate ACMG/AMP evidence items can double-count substantially overlapping information. More generally, predictors may share training variants, databases, labels, or underlying features, and nominally separate evaluations may not be fully independent of model development. The ClinGen calibration framework therefore supports applying computational evidence at appropriately calibrated strengths within the relevant classification framework, while avoiding the treatment of correlated predictor outputs as independent evidence [44,48]. Evaluation should prioritize external datasets not used in training or threshold selection, transparent handling of overlapping variants, and performance stratified by gene, variant class, ancestry, and mechanism.

Finally, predictive performance should be distinguished from clinical utility. The WISDOM randomized clinical trial compared risk-based with annual breast screening in 28,372 women aged 40–74 years in the United States; risk-based screening was non-inferior for the rate of stage IIB or higher cancers after a median of 5.1 years, but did not significantly reduce biopsy rates despite fewer mammograms [59]. This supports the feasibility and non-inferiority for the evaluated advanced-stage cancer endpoint of a multifactorial risk-guided screening strategy in the trial setting, but does not isolate the independent contribution of PRS from other genetic and clinical inputs, and generalizability is limited by the predominantly non-Hispanic White study population and imperfect adherence. Thus, evidence that a model improves discrimination or changes risk categories should not be equated with evidence that the PRS component itself improves patient outcomes.

However, AI-based tools have important limitations. Predictive performance may vary across variant classes and models trained or evaluated using unrepresentative genomic datasets may inherit biases that affect their performance in underrepresented populations. These limitations should be considered when computational evidence is incorporated into clinical variant interpretation. Accordingly, AI-based predictions should support variant prioritization and interpretation within established classification frameworks rather than substitute for validated clinical, functional, population, or segregation evidence. In practice, evidence of clinical utility requires showing that use of the prediction changes decisions appropriately and improves patient-relevant outcomes or safely reallocates care, rather than merely improving AUC, calibration, or reclassification.

5. Liquid Biopsy and the Challenge of Clonal Hematopoiesis

Liquid biopsy provides a particularly relevant example of the challenges involved in biological attribution of genomic findings. It enables minimally invasive analysis of circulating nucleic acids, including circulating tumor DNA (ctDNA) for tumor genotyping, treatment monitoring, and molecular residual disease assessment [60]. A major interpretive challenge is that plasma cell-free DNA also contains DNA released from hematopoietic cells.

Somatic variants arising from clonal hematopoiesis may therefore be detected alongside tumor-derived alterations in plasma, complicating determination of variant origin. Thus, detection of a variant in plasma alone does not establish its tumor origin. Resolving this diagnostic ambiguity requires a detailed understanding of how aging bone marrow introduces genomic noise, the implementation of sequencing of matched blood-cell DNA to help determine the origin of detected variants, and an evaluation of emerging multimodal fragmentomic modalities.

5.1. Clonal Hematopoiesis as a Confounder of Plasma DNA Interpretation

Clonal hematopoiesis (CH) refers to the expansion of hematopoietic clones carrying acquired somatic mutations and becomes increasingly common with age. Prior chemotherapy may also exert selective pressure favoring the expansion of pre-existing hematopoietic clones, particularly those carrying mutations in DNA damage response genes such as *TP53* and *PPM1D* [61]. Clonal hematopoiesis of indeterminate potential (CHIP) represents a defined subset of CH in the absence of an overt hematologic neoplasm or cytopenia attributable to a clonal disorder. Frequently affected genes include *DNMT3A*, *TET2*, and *ASXL1* [62]. Indeed, for hereditary cancer assessment, clonal expansion under the selective pressure of prior genotoxic therapies frequently targets key DNA damage response (DDR) pathway genes, most notably *PPM1D* and *TP53* [61]. The presence of these somatic hematopoietic mutations directly confounds germline interpretation, as they can masquerade as constitutional alterations associated with syndromes like Li–Fraumeni [8,63]. Consequently, CH-associated variants can enter the circulating DNA compartment and confound liquid-biopsy interpretation.

Misattribution of hematopoietic variants to the tumor can affect both plasma-based tumor profiling and molecular residual disease assessment, where low ctDNA levels make accurate attribution of variant origin particularly important. This issue is also relevant to hereditary cancer assessment because a plasma variant may be germline, tumor-derived somatic, or CH-derived. In a large retrospective cohort of 16,812 patients with advanced cancer, paired plasma and white blood cell sequencing identified at least one CH variant among reportable clinical genes in 42.3% of patients. Among detected variants in selected hereditary cancer genes, the proportion attributed to CH was 39.0% for *BRCA2*, 37.9% for *CHEK2*, 27.4% for *BRCA1*, 20.1% for *ATM*, and 18.5% for *TP53* [64]. These gene-specific percentages represent the proportion of detected variants attributed to CH in this advanced-cancer cohort, rather than the prevalence of CH among carriers of each gene. They should not be extrapolated to unaffected individuals undergoing hereditary cancer assessment. These findings illustrate that overlap between CH-associated and cancer-predisposition genes can complicate interpretation when plasma sequencing is considered in isolation.

5.2. Matched Hematopoietic Sequencing for Variant-Origin Assessment

Matched sequencing of plasma cfDNA and a hematopoietic reference sample, such as buffy-coat leukocyte DNA, can help distinguish hematopoietic variants from tumor-derived alterations, thereby narrowing the possible origin of a plasma variant [65]. However, it does not always resolve variant origin. Detection or absence of a variant in buffy coat depends on sequencing depth and the analytical sensitivity of the assay [66]. A variant detected in both compartments may be consistent with a hematopoietic or germline origin. Conversely, a plasma-restricted variant may be compatible with a tumor-derived origin, although this interpretation depends on adequate sensitivity of the matched hematopoietic analysis (Figure 2). It is important to note that the reported CH frequencies come from patients with advanced cancer and must not be generalized to unaffected individuals undergoing hereditary cancer assessment. In cancer patients, CH estimates are significantly influenced

by age, treatment exposure (e.g., cytotoxic chemotherapy), disease status, inflammation, and metabolic syndromes, as well as the underlying analytical methodologies and specific demographic denominators utilized across different cohorts.

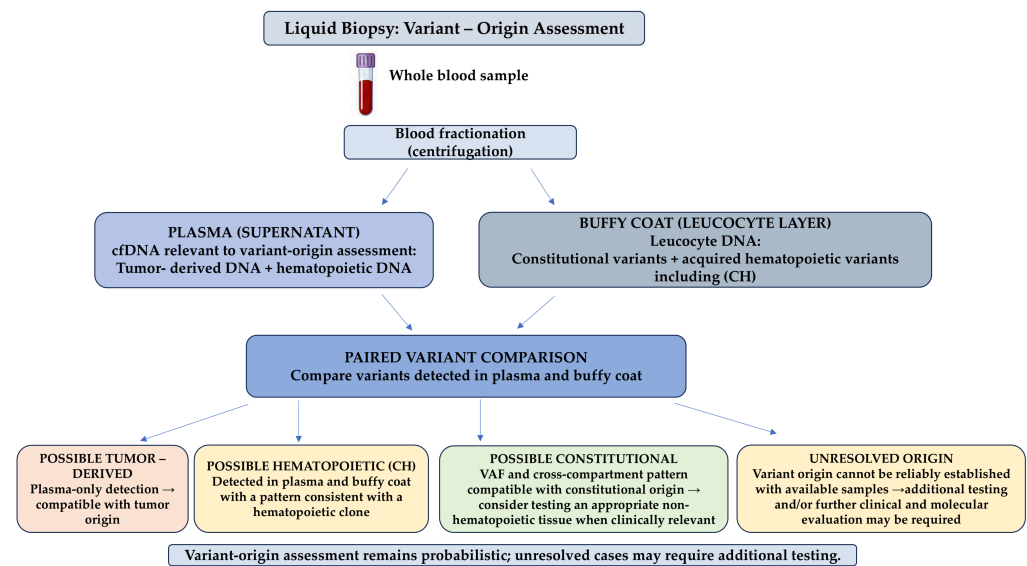


Figure 2. Variant-origin assessment in liquid biopsy using paired plasma and hematopoietic analysis. Plasma cfDNA frequently contains a mixture of tumor-derived and hematopoietic DNA, whereas matched leukocyte DNA provides a hematopoietic reference that can help distinguish likely tumor-derived alterations, hematopoietic mutations, and constitutional germline variants. Because this assessment remains inherently probabilistic, secondary testing of non-hematopoietic tissues may be required when data are equivocal, particularly to help distinguish high-fraction clonal hematopoiesis from constitutional mosaicism or germline variation. Cases in which variant origin cannot be reliably established are indicated as having unresolved origin.

Particular caution is required for genes such as *TP53* which are implicated in both clonal hematopoiesis and hereditary cancer predisposition. Constitutional *TP53* variants may arise de novo, including through postzygotic mosaicism [67]. A VAF approaching 50% may be compatible with a heterozygous germline variant. However, VAF alone cannot establish germline status, and low-level *TP53* variants detected in blood may reflect constitutional mosaicism, CH, circulating tumor DNA, or technical artifacts [8,63]. When the distinction is clinically consequential, testing of an appropriate non-hematopoietic tissue, such as cultured skin fibroblasts or plucked hair follicles using validated methods, together with expert integration of the molecular findings with the patient’s phenotype and family history, should be considered [67,68]. Buccal DNA may itself contain hematopoietic cells, and therefore may not always represent an independent germline source; sample selection should consequently follow laboratory-validated procedures.

When serial samples are available, changes in VAF may provide additional evidence for variant attribution. If the biological origin of a clinically relevant variant remains unresolved, repeat testing of a newly collected blood sample may be considered, depending on the clinical context. Tumor-derived VAF may decrease or increase with changes in tumor burden and treatment response, whereas CH clones may remain relatively stable or change over time, including under treatment-related selective pressure [61]. However, changes in VAF over time should be interpreted in the context of treatment and tumor burden rather than using fixed thresholds across cancers or assays [69]. Such changes alone cannot establish or exclude constitutional mosaicism.

5.3. Emerging Applications and Complementary Approaches

Beyond its established applications in patients with cancer, liquid biopsy is also being investigated for surveillance in hereditary cancer syndromes. In Li–Fraumeni syndrome, a multimodal cfDNA approach integrating targeted sequencing, shallow whole-genome sequencing, and methylation analysis provided proof-of-principle for cancer detection, including cancer-associated signals detected before diagnosis by conventional surveillance in some carriers [70]. These findings illustrate the potential value of combining different circulating signals rather than relying on individual sequence variants, although clinical utility in hereditary cancer surveillance remains to be established. Emerging methylation-based and fragmentomic approaches may provide complementary information on tissue of origin or the likely origin of circulating DNA [71]. These signals may support inference that circulating DNA is tumor-derived, but they address a different question from establishing the biological origin of an individual sequence variant. Their role in routine source attribution remains under evaluation and they should not be considered substitutes for matched hematopoietic analysis when variant-level attribution is required.

6. Clinical Actionability and Guideline-Based Management

The clinical value of genomic testing depends on whether test results can be translated into evidence-based surveillance, prevention, counseling, or treatment. Clinical actionability is determined not only by variant classification but also by the gene–disease association, the origin and clinical significance of the finding, the patient’s clinical context, and the availability of an evidence-based intervention. Translating these variables into actionable workflows requires a comprehensive assessment across established tumor-specific syndromes, an understanding of the balance between preventive surgery and active surveillance, and the rigorous implementation of family-wide cascade screening protocols.

6.1. Hereditary Breast and Ovarian Cancer (HBOC)

In hereditary breast and ovarian cancer (HBOC), pathogenic variants in *BRCA1*, *BRCA2*, *PALB2*, and other established susceptibility genes may influence surveillance, risk-reducing surgery, and treatment selection, although the nature and strength of these recommendations vary according to the gene and, where relevant, variant-specific evidence [72]. Current clinical guidelines recommend gene-specific breast surveillance and consideration of risk-reducing interventions for carriers of pathogenic or likely pathogenic variants in established cancer predisposition genes. For *BRCA1* and *BRCA2* carriers, decisions regarding risk-reducing mastectomy and salpingo-oophorectomy should consider gene-specific cancer risks, age, family history, family planning considerations, and individual preferences [73,74]. Multifactorial models such as CanRisk can integrate pathogenic variants in cancer predisposition genes, polygenic risk scores, family history, and other risk factors to support individualized risk assessment and inform surveillance and prevention decisions [12,75]. Therapeutic implications are tumor- and indication-specific. PARP inhibitors have established clinical indications for tumors harboring *BRCA1/2* alterations, whereas therapeutic evidence for alterations in other homologous recombination repair genes is more context-dependent and should not be extrapolated across genes or tumor types. Treatment selection should therefore reflect the specific biomarker, tumor type, and approved clinical indication [76,77].

6.2. Lynch Syndrome and Familial Adenomatous Polyposis

Many contemporary pathways for Lynch syndrome incorporate tumor testing for mismatch-repair deficiency (dMMR) by immunohistochemistry (IHC) or microsatellite instability (MSI), particularly in colorectal and endometrial cancer. A dMMR/MSI-high

tumor phenotype does not by itself establish a diagnosis of Lynch syndrome and should prompt evaluation for sporadic mechanisms, including *MLH1* promoter hypermethylation where relevant, and constitutional causes [78]. Confirmed Lynch syndrome is associated with intensive colonoscopic surveillance, with the recommended starting age and interval determined by the affected gene and current guidelines. For example, surveillance is generally initiated earlier in *MLH1* and *MSH2* carriers than in *MSH6* and *PMS2* carriers [79]. Risk-reducing gynecological surgery may also be considered in women with Lynch syndrome, with decisions individualized according to the affected gene, age, family history, and completion of childbearing [80]. In advanced colorectal cancer, MSI-high/dMMR status may support immune-checkpoint inhibitor therapy in defined disease settings, with treatment decisions guided by the tumor biomarker and current tumor-specific recommendations rather than by germline Lynch syndrome status alone [81]. Other hereditary colorectal cancer syndromes, including familial adenomatous polyposis (FAP), require distinct surveillance and prevention strategies. Classic FAP, caused by pathogenic germline variants in *APC*, is associated with a markedly increased lifetime risk of colorectal cancer in the absence of appropriate surveillance and intervention, whereas attenuated FAP generally presents with fewer adenomas, later disease onset, and a lower colorectal cancer risk. Colonoscopic surveillance typically begins in childhood or early adolescence for classic FAP and later for attenuated FAP. The timing and type of surgery should be individualized according to the polyp burden, clinical phenotype, feasibility of endoscopic management, and patient-specific considerations [80,82].

6.3. Li–Fraumeni Syndrome and Multimodal Surveillance

Because germline *TP53* pathogenic variants are associated with a broad spectrum of childhood and early-onset malignancies, including soft-tissue sarcomas, osteosarcoma, adrenocortical carcinoma, central nervous system tumors, and very early-onset breast cancer, surveillance strategies emphasize early cancer detection while avoiding unnecessary exposure to ionizing radiation. The modified Chompret criteria include breast cancer diagnosed before the age of 31 years as a criterion for *TP53* testing, irrespective of family history [8]. The Toronto protocol provided an important framework for multimodal surveillance incorporating whole-body MRI, brain MRI, breast MRI in women, abdominal imaging, and clinical assessment [83]. The intensity and exact components of surveillance should follow contemporary Li–Fraumeni guidelines with age-specific implementation and consideration of individual clinical circumstances [84]. Risk-reducing mastectomy should be discussed with female carriers, while therapeutic decisions should consider the increased risk of subsequent primary malignancies associated with DNA-damaging therapies. Whenever possible, radiotherapy and genotoxic chemotherapy should be avoided to reduce the potential risk of subsequent primary malignancies. However, these treatments may still be necessary for effective cancer management, and decisions should be made through individualized multidisciplinary assessment [8,85].

6.4. *CDH1*-Associated Hereditary Diffuse Gastric Cancer: Balancing Risk and Intervention

CDH1-associated hereditary diffuse gastric cancer illustrates particularly clearly how clinical actionability depends on balancing cancer risk against the consequences of preventive intervention. In hereditary diffuse gastric cancer associated with pathogenic *CDH1* variants, risk-reducing total gastrectomy is an important preventive option, while endoscopic surveillance in expert centers may be considered as an alternative for selected individuals who defer or decline gastrectomy. However, endoscopic surveillance has limitations in detecting and assessing early gastric lesions and cannot be assumed to provide the same degree of risk reduction as gastrectomy [80].

Management decisions should consider family history, the clinical context in which the *CDH1* variant was identified, the potential consequences of surgery, and individual preferences [9]. Women carrying pathogenic *CDH1* variants also have an increased risk of lobular breast cancer. Current guidelines recommend breast surveillance with MRI and mammography, with the timing and modalities individualized according to current recommendations and family history, while risk-reducing mastectomy may be discussed based on individual risk and preferences [9,74].

6.5. Cascade Testing and Reinterpretation of Germline Genetic Findings

Identification of a pathogenic or likely pathogenic germline variant in an affected or asymptomatic individual may have implications for at-risk relatives and support cascade testing, with appropriate genetic counseling and informed consent. Cascade testing enables at-risk relatives who carry the familial variant to access appropriate surveillance and risk-reducing interventions, while a negative result for the known familial variant may help avoid unnecessary syndrome-specific surveillance [86]. Although testing for the known familial variant is generally appropriate, broader testing may be warranted when the personal or family history is not fully explained by the familial variant [87]. Because responsibilities surrounding recontact and reinterpretation vary across healthcare systems, professional guidance, and local legal frameworks, laboratories and healthcare providers should define how significant reclassifications will be reviewed, communicated and clinically implemented [88,89].

6.6. Clinical Interpretation of Negative and Uncertain Results

Negative and uncertain results require careful clinical interpretation. A true negative result for a known familial pathogenic variant should be distinguished from an uninformative negative result in a family without an established molecular diagnosis; the latter should be interpreted in the context of the patient's phenotype and family history and does not definitively exclude inherited susceptibility. Likewise, a variant of uncertain significance should not be considered an actionable positive finding or used to guide clinical management [28]. Management should remain guided by the patient's personal and family history and applicable disease-specific recommendations [72].

7. Translational Challenges, Implementation Gaps, and Future Priorities

The central challenge in genomic medicine has shifted from the mere generation of genomic data to its reliable translation into clinical decisions across diverse populations, platforms, and healthcare settings. Key translational hurdles include the transportability and calibration of AI-based predictors alongside the accurate attribution of plasma variants when tumor-derived and hematopoietic signals coexist. Crucially, increasing analytical sensitivity may elevate variant detection rates without necessarily improving clinical discrimination, particularly when the biological significance or origin of a detected signal remains unresolved.

These complex challenges can be systematically organized across five interdependent translational stages: variant detection, variant interpretation, biological attribution, clinical validity, and clinical utility (Table 1). Beyond these analytical and interpretive bottlenecks, substantial implementation gaps persist. For emerging technologies, the critical question is no longer simply whether they can uncover additional findings, but whether these results improve patient diagnosis, clinical management, or outcomes beyond established standard-of-care workflow. Consequently, future frameworks in genomic medicine must rigorously distinguish raw analytical capability from demonstrated clinical utility. Widespread clinical adoption should depend on robust, prospective, or real-world evidence of utility, alongside

analytical validation, reproducibility across diverse ancestries, and sustainable, equitable implementation. Moving forward, key priorities must include: optimizing the detection of technically challenging variant classes through validated complementary methods [27,37]; expanding ancestry-diverse genomic resources to properly calibrate risk-prediction tools across populations [13]; establishing definitive approaches to differentiate constitutional, hematopoietic, and tumor-derived variation [61,63,65–67,69]; and developing sustainable models for variant reinterpretation. Finally, real-world implementation will ultimately hinge on addressing structural barriers such as payer reimbursement, laboratory resources, and regulatory compliance, which frequently restrict patient access even after analytical performance has been fully established [90].

Table 1. Author-proposed five-stage conceptual framework for translating genomic findings into clinical application in hereditary cancer genomics.

Translational Stage	Central Question	Representative Bottlenecks
Variant detection	Can the relevant genetic alteration be detected reliably?	Coverage gaps, difficult genomic regions, structural variants, assay-specific limitations
Variant interpretation	What is the clinical significance of the detected variant?	Classification uncertainty, limited functional evidence, computational prediction
Biological attribution	What is the origin of the detected signal?	Constitutional versus acquired variation, mosaicism, CH/CHIP versus tumor-derived DNA
Clinical validity	What does the finding mean for cancer risk, disease, or phenotype?	Gene–disease validity, penetrance, variant-specific effects, ancestry, phenotype integration
Clinical utility	Does the finding provide meaningful benefit for patient or family management?	Evidence-based interventions, gene-specific recommendations, treatment indications, demonstrated patient benefit

Applying the Translational Framework to Clinical Decision-Making

The five-stage framework can be illustrated with two clinical scenarios that demonstrate how variant detection, interpretation, biological attribution, clinical validity, and utility inform patient management.

Example 1: A *BRCA1* Variant of Uncertain Significance.

A patient with a personal and family history suggestive of hereditary breast and ovarian cancer undergoes multigene panel testing, revealing a *BRCA1* variant of uncertain significance (VUS).

Stage 1—Variant detection: Analytical validity is assessed through sequencing coverage, read quality, mapping quality, and, where indicated, confirmatory testing. The assessment should also encompass copy-number variant detection, particularly for *BRCA1* and *BRCA2*, using a validated NGS-based CNV analysis pipeline or complementary methods such as MLPA, as appropriate. CNV analysis should extend to other clinically relevant genes covered by the panel where analytical performance has been adequately validated.

Stage 2—Variant interpretation: The variant is evaluated using ACMG/AMP criteria and applicable *BRCA1*-specific specifications, integrating population, clinical, segrega-

tion, computational, functional, and RNA evidence where relevant. If evidence remains insufficient or conflicting, the variant retains its VUS classification.

Stage 3—Biological attribution: The specimen type and clinical context are reviewed to assess whether the finding is constitutional or potentially mosaic or acquired.

Stage 4—Clinical validity: Interpretation considers the established *BRCA1*–disease association alongside the patient’s phenotype and family history, and relevant histopathological findings documented in the medical record. A VUS does not establish a molecular diagnosis of hereditary breast and ovarian cancer.

Stage 5—Clinical utility: A *BRCA1* VUS should not independently guide clinical management, including risk assessment, surveillance, risk-reducing interventions, or treatment selection. Clinical decisions should instead rely on established clinical indications, personal and family history, and other validated molecular findings. Periodic variant reassessment and informative segregation studies may contribute to future reclassification but should not be confused with predictive testing for clinical management.

Example 2: A Low-VAF TP53 Variant in Blood.

A low-variant-allele-frequency (VAF) *TP53* variant is identified in peripheral blood during hereditary cancer assessment. Possible explanations include constitutional variation, postzygotic mosaicism, clonal hematopoiesis (CH), circulating tumor DNA, or technical artifact.

Stage 1—Variant detection: Read quality, sequencing depth, strand balance, mapping quality, and the assay’s limit of detection are reviewed; confirmation with a suitably sensitive method may be required. Conventional germline variant-calling pipelines may filter out low-VAF variants; therefore, variant-calling thresholds and assay-specific detection limits should also be considered.

Stage 2—Variant interpretation: The variant is classified using relevant clinical, population, computational, functional, and gene-specific evidence. Importantly, pathogenicity classification does not establish constitutional origin.

Stage 3—Biological attribution: Patient age, prior exposure to chemotherapy or radiotherapy, and hematological findings should be considered when evaluating the possibility of clonal hematopoiesis. Testing DNA from non-hematopoietic tissues, such as cultured skin fibroblasts or plucked hair follicles, may help distinguish constitutional mosaicism from CH. Hair follicles offer a less invasive sampling option, provided that the analytical method has been validated for this specimen type [68]. Blood-contaminated specimens, including saliva and buccal samples, require cautious interpretation. When biological attribution remains unresolved, repeat testing of a newly collected blood sample may provide additional evidence from changes in VAF over time, although analysis of an appropriate non-hematopoietic tissue may still be required.

Stage 4—Clinical validity: The variant’s classification, biological origin, VAF, phenotype, and family history are considered together. A confirmed constitutional pathogenic *TP53* variant may support Li–Fraumeni syndrome, whereas a blood-limited variant should not independently establish this diagnosis. Constitutional mosaicism requires individualized risk assessment. The absence of a suggestive personal or family history does not exclude constitutional mosaicism.

Stage 5—Clinical utility: Syndrome-specific management and irreversible interventions should not be based solely on an unresolved low-VAF blood finding. Confirmed constitutional pathogenic or likely pathogenic variants warrant genetic counseling and management according to current recommendations, with consideration of family testing. Findings attributable to CH should be evaluated in the appropriate clinical and hematologic context and should not be used to justify predictive cascade testing for hereditary *TP53*-related cancer risk. In cases of constitutional *TP53* mosaicism, genetic counseling

should address the possibility of transmission to offspring, who may carry the variant in a constitutional heterozygous state.

This example highlights biological attribution as a distinct translational step: even a pathogenic variant may have different clinical implications depending on whether it is constitutional, mosaic, hematopoietic, or tumor-derived.

8. Conclusions

NGS has transformed hereditary cancer genomics from predominantly sequential testing toward broader, increasingly integrated genomic assessment. As testing expands, however, the principal challenge is not only detecting genomic variation but determining which findings are analytically reliable, biologically relevant, and clinically meaningful. Meeting this challenge requires linking reliable variant detection and interpretation with accurate biological attribution, clinical validity, and demonstrated clinical utility.

The next phase of hereditary cancer genomics should be defined not by how much genomic information can be generated, but by how reliably that information can be translated into meaningful benefit for patients and families. Each additional finding should therefore be evaluated for its capacity to improve diagnosis, risk assessment, prevention, or treatment.

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Abbreviations

The following abbreviations are used in this manuscript:

ACMG	American College of Medical Genetics and Genomics
AMP	Association for Molecular Pathology
AI	Artificial Intelligence
CH	Clonal Hematopoiesis
CHIP	Clonal Hematopoiesis of Indeterminate Potential
ctDNA	Circulating Tumor DNA
dMMR	Mismatch Repair Deficiency
HBOC	Hereditary Breast and Ovarian Cancer
MLPA	Multiplex Ligation-dependent Probe Amplification
MMR	Mismatch Repair
NGS	Next-Generation Sequencing
P/LP	Pathogenic or Likely Pathogenic
VAF	Variant Allele Frequency
VUS	Variant of Uncertain Significance
WES	Whole-Exome Sequencing
WGS	Whole-Genome Sequencing

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