

Swiss Genomics Association consensus guideline for evidence-based genomic variant interpretation in Mendelian disease

Quant Group¹, Dylan Lawless ^{*2}, and the joint SGA committee³

¹The quantitative omic epidemiology group.

³The Swiss Genomics Association committee.

²Department of Intensive Care and Neonatology, University Children's Hospital Zurich,
University of Zurich, Zurich, Switzerland.

November 23, 2025

DRAFT – NOT FINAL

This document is a **live public working draft** and does not represent a completed or officially endorsed release. All statements, analyses, and opinions are those of the authors and have not yet undergone joint committee review or external peer review. Content is provided for transparency and collaborative development and may be revised substantially prior to publication.

Abstract

Genomic medicine programmes across the world are advancing rapidly, especially with AI, but variant interpretation remains implemented differently across national, institutional and commercial efforts, with no shared agreement on how supporting evidence should be structured, recorded or exchanged. As a result, equivalent data are repeatedly recomputed, manually curated or privately remodelled, while shared learning, verification and reuse remain limited. Key evidence including inheritance, provenance, population context, functional data and conflicting observations is inconsistently captured, reducing transparency and interoperability and slowing collective progress.

We define a harmonised, tool-agnostic specification that establishes a shared data architecture and scientific approach for variant interpretation in Mendelian disease. The guideline sets minimum requirements for representing, linking and auditing evidence, including sequence and sample provenance, variant normalisation, segregation logic, phenotype alignment, evidence grading, conflict handling and versioned synthesis statements. It emphasises structured, reviewable reasoning rather than fixed classification labels and is designed to complement, not replace, existing standards, tools and workflows.

By formalising a standardised approach to quantifying interpretation, this framework enables distributed groups to solve genomics problems in alignment rather than in parallel isolation. It reduces duplication, supports independent validation, improves automation and ensures that advances in one sector directly strengthen others. The model establishes a shared, exchangeable foundation for interpretation that remains transparent, interoperable and evolvable, strengthening cooperation between national initiatives, healthcare, research and industry. ¹

¹* Addresses for correspondence: Dylan.Lawless@uzh.ch.

Availability: This data is integrated in <https://iei-genetics.github.io>.

Contents

1	Introduction	4
2	Purpose and scope of the guideline	5
3	Architectural context and national motivation	5
4	Design principles for evidence-based variant interpretation	6
5	Data provenance and sample quality requirements	6
6	Evidence rule framework	7
6.1	Structure of evidence rules	7
6.2	Versioning and audit trace	8
6.3	Optional reference implementation (QV framework)	8
6.4	Implemented rule set	12
7	Evidence flags	12
7.1	Evidence domains	13
7.2	Final flag set	13
7.3	Interpretation of flag combinations	14
8	Structured representation and interoperability	15
8.1	Alignment with GA4GH Variant Representation and Variant Annotation models	15
8.2	GA4GH Variant Annotation model	16
8.3	Exchange formats and machine readable outputs	17
9	Probabilistic interpretation in Pillar 3	17
9.1	Relationship to Pillar 1 and Pillar 2 inputs	17
9.2	Evidence aggregation logic	17
10	Clinical review checkpoints	17
10.1	Required manual assessments	17
10.2	Boundary of automated reasoning	17
11	Reporting and synthesis statements	17
12	Auditability, versioning, and reproducibility	17

13 Limitations and scope	17
14 Future extensions	17
15 Conclusion	17
16 Supplemental	20
17 Appendix	20
17.1 Example rule files	20
17.2 Example YAML or JSON evidence objects	20
17.3 Worked examples of flagged variants	20
18 Evidence flags	20
18.1 Evidence domains	20

1 Introduction

Genome sequencing is widely used in clinical and research settings, yet the evidence used to justify variant interpretations is recorded and applied inconsistently. Many systems treat algorithmic scores or proprietary rankings as conclusions rather than inputs, and key evidence such as provenance, variant identity, inheritance fit, population context, phenotype alignment, and contradictory signals is often incomplete or incomparable across institutions. This limits reproducibility, complicates verification, and restricts the safe use of automated or AI supported interpretation.

The Swiss Genomics Association is developing a national evidence architecture that supports consistent interpretation across healthcare, research, and industry. The objective is not to replace existing pipelines or tools, but to define the minimal evidential contract they depend on. This contract specifies how evidence is structured, how uncertainty is recorded, and how falsification tests are applied to every proposed variant. It provides a transparent, computable basis for reasoning that can be traced, audited, and exchanged across systems.

By establishing shared expectations for variant identity, provenance, and evidence evaluation, the guideline enables interoperability, supports automation, and ensures that genomic data remain clinically meaningful and reusable across future analytical

frameworks.

2 Purpose and scope of the guideline

This guideline defines the national minimum requirements for evidence based interpretation of variants in Mendelian disease. It sets out the computable rules and evidence flags that form the uniform evidence state for each variant. These rules apply falsification tests to all upstream outputs and record the presence, absence, or contradiction of supporting evidence.

The guideline focuses on how evidence is represented, computed, and interpreted. It does not prescribe sequencing workflows, vendors, prioritisation tools, or clinical reporting formats. Any institution may use its own pipelines, provided it supplies the minimal identifiers and evidence required for interoperability and reproducibility across the national system.

3 Architectural context and national motivation

A national genomics system must balance durability with adaptability. Clinical records, identifiers, and audit trails require structures that remain stable over decades, while biological knowledge, phenotype models, and interpretive resources evolve continuously. The guideline is designed for this mixed environment. It defines the stable elements needed for long term interoperability, including variant identity, provenance fields, and the structure of evidence states, while allowing evidence rules, knowledge sources, and computational logic to be updated without invalidating past interpretations. This separation ensures that new annotations, ontologies, or analytical frameworks can be incorporated as they mature, and that historical decisions remain traceable to the evidence that supported them at the time. The approach reflects the broader national strategy to build genomic infrastructure that is open, verifiable, and compatible with both public and private systems.

4 Design principles for evidence-based variant interpretation

The guideline is grounded in principles that support clarity, reproducibility, and transparent reasoning. Each evaluative step is defined as computable logic rather than as narrative instruction, ensuring that results can be reproduced exactly across institutions. Uncertainty is recorded explicitly so that missing or partial evidence is visible rather than inferred. Provenance links each flag to the rule, resource, and input that produced it, allowing complete reconstruction of decisions. The framework prioritises consistency across time and across analytic environments, enabling automation while maintaining clinical traceability. By treating variant interpretation as a set of falsification tests applied to each candidate, the system provides a stable evidential foundation that can be used reliably in clinical care, research, and industry without prescribing any particular workflow or toolchain.

5 Data provenance and sample quality requirements

Reliable interpretation depends on clear documentation of how each variant was generated. The system therefore requires a minimal set of provenance fields that describe the sample, the sequencing method, the reference build, and the primary variant calling pipeline. These identifiers do not constrain laboratories to specific technologies but ensure that downstream evaluation can be traced to defined inputs. Provenance is recorded as structured fields rather than free text so that evidence rules can reference them directly and so that analyses remain comparable across time and institutions.

Sample quality must also be sufficient to support interpretation. Basic indicators such as coverage, call completeness, and genotype quality are required for the core falsification tests to operate correctly. Where quality or provenance information is missing, the system records explicit flags rather than inferring validity. This approach ensures that downstream conclusions reflect the trustworthiness of the inputs and that gaps in evidence remain visible to clinical reviewers and automated systems alike.

6 Evidence rule framework

The evidence model in this guideline is based on reverse reasoning. For every variant proposed by an upstream tool, the system evaluates each evidence domain for signals that contradict, weaken, or fail to support the hypothesis that the variant explains disease. Each evaluation is implemented as a computable rule that inspects defined inputs and emits one or more evidence flags. Together, these rules produce a uniform evidence state for every variant that can be interpreted, exchanged, and audited in a consistent way.

Rules do not attempt to reproduce upstream prioritisation logic or clinical judgement. They operate on normalised variant identifiers, provenance fields, and standard annotation outputs, and they test whether key expectations are met. All outcomes, including missing or indeterminate results, are recorded explicitly as machine readable flags rather than left implicit in narrative reports or tool specific scores.

6.1 Structure of evidence rules

Each evidence rule is defined as a small, independent logical unit. In implementation, rules are encoded in a structured format such as YAML or JSON. They specify the required inputs, the conditions under which the rule can be applied, and the flags that are set when those conditions hold.

Inputs include normalised variant identifiers, population frequencies, inheritance information, functional annotations, phenotype mappings, and any necessary provenance or quality indicators. The rule then evaluates a clearly defined predicate, for example whether the allele frequency is incompatible with a rare Mendelian disorder given a reference dataset, or whether the observed segregation pattern contradicts the expected mode of inheritance. The result is one or more discrete flags that describe the evidence state, including counter evidence, uncertainty, or missing data.

Rules are designed to be composable and pipeline independent. Any laboratory or company can compute the same evidence state provided it supplies the required inputs and executes the rules as specified. This separation of logic from implementation allows the national standard to evolve without dictating local software choices.

6.2 Versioning and audit trace

To ensure reproducibility, every rule is associated with a versioned schema and a stable identifier. Rules are grouped into rule sets, which are released as numbered versions with defined validity periods. Each rule set can be referenced by a unique name and checksum so that any evidence state can be linked back to the exact logic that produced it.

When a rule is updated, for example to incorporate a new reference dataset or corrected threshold, the change is recorded as a new version rather than a silent modification. Historical rule sets remain available so that past interpretations can be reconstructed and compared with current behaviour. Audit trails therefore include both the flags assigned to each variant and the rule set version that generated them.

This versioning model applies equally to national recommendations and to local extensions. Institutions may define additional rules for internal use, provided these are clearly namespaced and versioned. The national minimum contract is defined by the core rule set; everything else is additive and does not alter the interpretation of those core flags.

6.3 Optional reference implementation (QV framework)

The guideline is accompanied by an optional reference implementation based on Qualifying Variant (QV) protocols (4). A QV protocol encodes the complete evidence rule set in a single, machine readable file. It specifies the sequencing method, reference build, analysis tools, coverage and quality thresholds, genomic regions or panels, and the evidence rules applied to each variant. This configuration separates interpretive logic from software execution and allows identical workflows to be reproduced across sites.

QV files are expressed in YAML, with a simple key value structure that can be parsed by any modern programming environment. Each file includes metadata, version identifiers, and a checksum so that downstream outputs can be linked unambiguously to the protocol used. The same file can be used to drive both clinical and research analyses, ensuring that evidence rules are applied consistently and that any changes are transparent.

An excerpt from a QV interpretation prompt is shown in Listing 1. In practice, full QV sets are constructed using a public builder tool and released with the

corresponding rule set versions. Laboratories and companies are free to implement the national rules directly or to adopt the QV framework as a ready to use reference.

Listing 1: Excerpt from the QV interpretation prompt used with the QV Builder app. Line wrapping is shown for display only. For actual use, refer to the original source file or the corresponding official QV set release.

```
draft style of prompts used to make the variant interpretation flagging
  ↳ rules before converting to YAML/JSON:

...

meta qv_set_id="qv_rare_singlecase_interpretation_v1_20251105"
meta version="1.0.0"
meta title="Rare variant single case interpretation"
meta created="2025-11-05"
meta authors=DylanLawless

# GnomAD flags
criteria gnomad_flags logic=or desc="Variant has no gnomAD flags or
  ↳ will be reviewed in report generation"
criteria gnomad_flags field=gnomad_flag operator==" value=NA
criteria gnomad_flags field=gnomad_flag operator="in" value="AC0,AS-
  ↳ VQSR,InbreedingCoeff,RF,Not in exomes,Not in genomes,No data,
  ↳ Discrepant Frequencies,CHIP,Monoallelic,Only heterozygous,MNV,LCR
  ↳ ,LC pLoF,pLoF Flag,NC Transcript,SEGDUPE,Common low heteroplasmy"
note "gnomad: gnomAD flags are retained for review; presence of any
  ↳ flag requires explicit mention or justification in final causal
  ↳ report. For information see https://gnomad.broadinstitute.org/
  ↳ help"

# ACMG flags
criteria acmg_criteria logic=or desc="Variant has no benign ACMG
  ↳ evidence or will be reviewed in report generation"
criteria acmg_criteria field=ACMG_criteria operator="not_contains"
  ↳ value="B"
criteria acmg_criteria field=ACMG_criteria operator="contains" value="
  ↳ BA1,BS1,BS2,BS3,BS4,BP1,BP2,BP3,BP4,BP5,BP6,BP7,BP8"
note "ACMG: ACMG benign evidence codes are retained for review;
  ↳ variants carrying these codes must be justified in the final
```

```

    ↪ causal interpretation report"
note "ACMG: For details on ACMG criteria see Richards et al. 2015, Li
    ↪ et al. 2017, Riggs et al. 2020, Tavtigian et al. 2020"
note "ACMG: Variants containing benign evidence codes (BA, BS, BP) are
    ↪ not excluded but must be explicitly reviewed in the final report"

criteria ppie_reporting logic=or desc="Record presence and fulfilment
    ↪ of patient or public representation (PPIE) requirements"
criteria ppie_reporting field=ppie_status operator="in" value="
    ↪ fulfilled,not_applicable,declined,pending"
note "PPIE: For each case, record whether patient or public
    ↪ representation requirements were met according to context (
    ↪ clinical, research, or commercial)."
```

note "PPIE: Minimum record includes: opportunity for expert

```

    ↪ consultation, whether discussion occurred, and whether non-
    ↪ actionable findings were addressed."
```

note "PPIE: In commercial contexts, the report itself may satisfy this

```

    ↪ requirement if it conveys the relevant information clearly and
    ↪ accessibly without requiring direct consultation."
```

note "PPIE: In research or cohort studies, record whether participants

```

    ↪ were informed about data use and whether individual feedback was
    ↪ planned."
```

Inheritance pattern

```

criteria gt_valid field="proband_genotype" operator="in" value="HET,HOM,
    ↪ HEMI"

criteria comp_het group_by="sample,SYMBOL" count=">=2" desc="Two
    ↪ distinct variants in one gene"

criteria not_hom_parent field="proband_genotype" operator="==" value="HOM"
criteria not_hom_parent field="mother_genotype" operator="!=" value="HOM"
criteria not_hom_parent field="father_genotype" operator="!=" value="HOM"

criteria not_hemi_parent field="proband_genotype" operator="==" value="HEMI
    ↪ "
```

```

criteria not_hemi_parent field="father_genotype" operator="!=" value="HEMI"

criteria not_same_parent field="PARENT_GENOTYPE_MATCH" operator="=="
    ↪ value="false"

criteria phase_ok field="PHASE" operator="in" value="TRANS,UNKNOWN"

criteria allow_dn field="DE_NOVO" operator="==" value="true" logic="or"

note "inheritance priority ordering applied downstream: compound_het >
    ↪ biallelic_hom > de_novo > possible_compound_het >
    ↪ single_het_parent > proband_genotype"
note "compound_het includes inherited, inherited+de novo, and dual de
    ↪ novo in trans (phasing required downstream)"
note "missing parent genotypes allow de_novo and possible_compound_het,
    ↪ and may still support compound_het depending on counts"
note "genotype mapping applied upstream: 00=REF, 01=HET, 10=HET, 11=HOM
    ↪ "

# RNA transcript support from controls

criteria rna_in_controls field="RNA_TPM_controls_median" operator=">"
    ↪ value=0.1 desc="Transcript detected in controls"
criteria rna_in_any_control field="RNA_TPM_controls_max" operator=">"
    ↪ value=0.0 desc="Transcript seen in at least one control"
criteria rna_patient_quantifiable field="RNA_TPM_patient" operator=">"
    ↪ value=0 desc="Patient transcript quantified"

criteria rna_pass logic="and" desc="RNA quant suitable for
    ↪ interpretation"
criteria rna_pass field="RNA_TPM_controls_max" operator=">" value=0
criteria rna_pass field="RNA_TPM_patient" operator=">" value=0

criteria rna_fail logic="and" desc="Transcript not seen in any control,
    ↪ quantification unreliable"
criteria rna_fail field="RNA_TPM_controls_max" operator="==" value=0

```

```
note "RNA filter: transcript must be observed in at least one control
    ↳ sample to consider patient quantification reliable"
note "RNA FAIL indicates no expression in all controls, patient value
    ↳ should not be interpreted quantitatively"
....
```

6.4 Implemented rule set

The implemented rule set defines the current national minimum contract for computable evidence. Each rule is encoded in a stable YAML structure and operates independently of any specific sequencing or annotation pipeline. The rules cover population frequency, inheritance and segregation, functional annotation, genotype validity, and quality related provenance checks. Together they produce the complete evidence state represented by the flag set defined in this guideline.

Qualifying Variant (QV) sets provide a practical reference implementation of these rules. A QV file specifies the rule versions, required inputs, and the conditions under which each flag is generated. The format is simple and machine readable, enabling consistent execution across institutions and software environments. QV sets can be assembled using the public builder at https://switzerlandomics.ch/pages/qv_builder/, which validates rule definitions, ensures correct structure, and allows users to preview the resulting YAML. This promotes transparent, reproducible configuration and supports FAIR principles by separating interpretive logic from local pipelines.

The implemented rule set is updated through versioned public releases. Older versions remain available for audit, comparison, and reconstruction of historical interpretation states. This ensures that interpretations can always be traced to the exact rules applied at the time and that changes to the national standard remain transparent and reviewable.

7 Evidence flags

Evidence flags represent the computable outcome of all evidence rules defined in this guideline. Each flag records whether a specific domain provides support, uncertainty, or counter evidence for a variant's role in disease. The flags form a uniform, machine

readable evidence state that can be compared and audited across institutions and over time.

7.1 Evidence domains

Each evidence domain tests whether the observable data are compatible with the hypothesis that a variant explains disease. These domains include population frequency, inheritance and segregation, molecular function, protein level consequence, genotype validity, and other external evidence sources. The system applies the same principle to all domains: if the available data provide a reason to question the hypothesis, a flag is set and recorded explicitly.

The appendix provides detailed descriptions of each domain, including the logic applied, examples of counter evidence, and extended illustrations for reference. The main text presents the domains only at conceptual level to maintain clarity.

7.2 Final flag set

The following list defines the current authoritative set of national evidence flags. These flags are produced directly by the rule framework and represent the complete evidence state for each variant. Future versions of the rule set will be released publicly and are intended to remain back compatible so that older interpretations can be reproduced without modification. All technical details, examples, and rule definitions are provided in the supplemental appendix.

Box 1: Flags

```
flag_gt_valid
flag_moi_parent_gt_missing_mother
flag_moi_parent_gt_missing_father
flag_moi_parent_gt_missing_any
flag_moi_parent_gt_hom_mother
flag_moi_parent_gt_hom_father
flag_moi_parent_gt_hom_any
flag_moi_parent_conflict_AD
flag_moi_parent_conflict_AR
flag_moi_parent_conflict_XR
flag_moi_parent_conflict_any
flag_popfreq_common
flag_popfreq_rare
flag_popfreq_ultrarare
flag_missing_popfreq
flag_uniprot_hits_any_feature
flag_uniprot_hits_domain_like
flag_uniprot_hits_structural_like
flag_uniprot_hits_ptm_like
flag_uniprot_hits_binding_like
flag_uniprot_hits_variant_like
flag_uniprot_is_lof
flag_uniprot_predicted_nmd
flag_uniprot_truncates_feature
```

7.3 Interpretation of flag combinations

Individual flags describe the behaviour of specific evidence domains, but interpretation depends on their combined pattern. A variant may carry flags indicating missing evidence, technical uncertainty, biological inconsistency, or direct contradiction of an expected disease mechanism. The aggregation of these signals defines the overall evidential state used in Pillar 3 probabilistic reasoning.

Combinations are not reduced to a single classification. Instead, they form a transparent summary of all falsification attempts across domains. This preserves

nuance, allows systematic reanalysis as new knowledge or rule versions emerge, and ensures that clinical interpretation remains grounded in explicit evidence rather than implicit assumptions.

8 Structured representation and interoperability

Interoperability is essential for a national evidence framework that must operate across laboratories, hospitals, research groups, and commercial systems. The guideline therefore relies on established, internationally recognised data standards rather than bespoke formats. These standards ensure that variant identity, phenotypic descriptions, and evidence statements can be exchanged without loss of meaning and that automated systems can interpret and recompute evidence consistently across environments.

8.1 Alignment with GA4GH Variant Representation and Variant Annotation models

This framework aligns with the **ga4gh!** (**ga4gh!**) standards for representing genomic variation and associated evidence. The **vrs!** (**vrs!**) defines computable, globally unique representations of sequence variation, enabling unambiguous exchange independent of file format or provider. The **va!** (**va!**) model specifies how evidence, conditions, and interpretive assertions can be encoded as structured objects with clear provenance. Together, these standards provide the semantic backbone for portable variant identity and portable evidence statements.

The guideline builds on this foundation by requiring that key identifiers and evidence outputs remain compatible with **vrs!** objects and **va!** evidence structures. This ensures that the national flag based system can interoperate with global infrastructures and that variant level assertions can be integrated into downstream applications, registries, or decision support systems.

This alignment is complemented by established community standards. variant call format (VCF) and **bam!** (**bam!**)/**cram!** (**cram!**) remain the primary formats for variant calls and alignments, while structured phenotype and disease descriptors use the **hpo!** (**hpo!**) and **ordo!** (**ordo!**). The *Phenopacket* schema provides a GA4GH backed model for transmitting harmonised case level data, and the *Beacon* protocol

supports federated discovery across distributed datasets. These standards allow institutions to participate in national and international initiatives without modifying their core workflows. They also provide a consistent substrate for automated interpretation tools such as Exomiser, LIRICAL, and related phenotype driven frameworks.

8.2 GA4GH Variant Annotation model

The **va!** model provides a structured approach for representing variant level assertions together with the evidence that supports or contradicts them. Traditional interpretation frameworks rely on human written summaries or categorical labels, which are difficult to recompute and cannot easily integrate quantitative or external data sources. In contrast, **va!** represents each assertion as a machine readable object linking the variant, the condition, the evidence lines, and their provenance.

This structure supports quantitative reasoning, where multiple evidence sources such as cohort frequencies, functional assays including MAVE, RNA or protein data, or model organism results can be encoded using controlled vocabularies and weighted consistently. It allows variant interpretations to evolve transparently as new evidence emerges, without losing the historical context required for audit or reproducibility.

By ensuring compatibility with **va!**, the national framework enables each flag and evidence state to be incorporated into a wider ecosystem of automated reasoning tools and variant knowledge bases. The structured flag set can be referenced directly in **va!** evidence collections, and the falsification logic defined in this guideline can be embedded as computable rules beneath future probabilistic or decision support models.

The **vrs!**, developed within the **gks!** (**gks!**) work stream, complements this by defining the core objects for representing alleles, haplotypes, and variations in a consistent and implementation independent manner. Together, **vrs!** and **va!** provide the global foundation for the structured, interoperable, and evolvable representation of variant evidence that this guideline depends on.

8.3 Exchange formats and machine readable outputs

The evidence framework is designed so that every output can be exchanged and reused without custom transformation. All rule definitions are distributed in **YAML** so

that logic remains readable, versioned, and portable across programming environments. Variant level evidence states are produced as structured **JSON** objects that mirror the identifiers and semantics defined in **vrs!** and the **va!** specification. This ensures that flags, provenance fields, and supporting metadata can be consumed directly by clinical systems, research pipelines, registries, or future decision support tools.

The same representation applies across institutions. Each variant receives a stable identifier, a complete flag set, and a minimal provenance record encoded as machine readable fields rather than free text. Historical interpretations can be reconstructed by replaying the rule version associated with each output. This approach avoids fragmentation, supports long term compatibility, and allows independent implementations to reach identical conclusions from the same inputs.

9 Probabilistic interpretation in Pillar 3

9.1 Relationship to Pillar 1 and Pillar 2 inputs

9.2 Evidence aggregation logic

10 Clinical review checkpoints

10.1 Required manual assessments

10.2 Boundary of automated reasoning

11 Reporting and synthesis statements

12 Auditability, versioning, and reproducibility

13 Limitations and scope

14 Future extensions

15 Conclusion

Acknowledgements

Contributions

DL designed the analyses and wrote the manuscript.

Competing interest

The authors declare no competing interest.

Ethics statement

This study only used data which was previously published and publicly available, as cited in the manuscript.

Data availability

The data used in this manuscript is derived from open sources which are cited in methods. The data generated is available from ...

Funding

References

- [1] Michael H. Duyzend, Pilar Cacheiro, Julius O. B. Jacobsen, Jessica Giordano, Harrison Brand, Ronald J. Wapner, Michael E. Talkowski, Peter N. Robinson, and Damian Smedley. Improving prenatal diagnosis through standards and aggregation. *Prenatal Diagnosis*, 44(4):454–464, April 2024. ISSN 0197-3851, 1097-0223. doi: 10.1002/pd.6522.
- [2] Reece K. Hart, Ivo F. A. C. Fokkema, Marina DiStefano, Ros Hastings, Jeroen F. J. Laros, Rachel Taylor, Alex H. Wagner, and Johan T. Den Dunnen. HGVS Nomenclature 2024: Improvements to community engagement, usability, and computability. *Genome Medicine*, 16(1):149, December 2024. ISSN 1756-994X. doi: 10.1186/s13073-024-01421-5.
- [3] Johan T. Den Dunnen, Raymond Dalglish, Donna R. Maglott, Reece K. Hart, Marc S. Greenblatt, Jean McGowan-Jordan, Anne-Francoise Roux, Timothy Smith, Stylianos E. Antonarakis, Peter E.M. Taschner, and on behalf of the Human Genome Variation Society (HGVS), the Human Variome Project (HVP), and the Human Genome Organisation (HUGO). HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Human Mutation*, 37(6):564–569, June 2016. ISSN 10597794. doi: 10.1002/humu.22981.
- [4] Dylan Lawless, Ali Saadat, Mariam Ait Oumelloul, Simon Boutry, Veronika Stadler, Sabine Österle, Jan Armida, David Haerry, D. Sean Froese, Luregn J.

Schlapbach, and Jacques Fellay. Application of qualifying variants for genomic analysis. May 2025. doi: 10.1101/2025.05.09.25324975.

- [5] Ksenia Lavrichenko, Emilie Sofie Engdal, Rasmus L. Marvig, Anders Jemt, Jone Marius Vignes, Henrikki Almusa, Kristine Bilgrav Saether, Eiríkur Briem, Eva Caceres, Edda María Elvarsdóttir, Magnús Halldór Gíslason, Maria K. Haanpää, Viktor Henmyr, Ronja Hotakainen, Eevi Kaasinen, Roan Kanninga, Sofia Khan, Mary Gertrude Lie-Nielsen, Majbritt Busk Madsen, Niklas Mähler, Khurram Maqbool, Ramprasad Neethiraj, Karl Nyrén, Minna Paavola, Peter Pruisscher, Ying Sheng, Ashish Kumar Singh, Aashish Srivastava, Thomas K. Stautland, Daniel T. Andreasen, Esmee Ten Berk De Boer, Søren Vang, Valtteri Wirta, and Frederik Otzen Bagger. Recommendations for bioinformatics in clinical practice. *Genome Medicine*, 17(1):124, October 2025. ISSN 1756-994X. doi: 10.1186/s13073-025-01543-4.

Acronyms

AD Autosomal Dominant	23
AR Autosomal Recessive	23
GRCh38 Genome Reference Consortium Human Build 38	21
HGVS Human Genome Variation Society	20
HVNC HGVS Variant Nomenclature Committee	20
HUGO Human Genome Organisation	20
MOI Mode of Inheritance	23
VCF variant call format	15
WGS Whole Genome Sequencing	22
XL X-Linked	23

16 Supplemental

Supplemental data are presented under the same headings that correspond to their relevant main text sections.

17 Appendix

17.1 Example rule files

17.2 Example YAML or JSON evidence objects

17.3 Worked examples of flagged variants

18 Evidence flags

18.1 Evidence domains

18.1.1 Counter-evidence and conflict handling

18.1.2 Normalised variant representation and nomenclature

Accurate variant description depends on consistent use of internationally recognised nomenclature systems. The Human Genome Variation Society (HGVS) nomenclature provides the authoritative standard for describing sequence variants at the DNA, RNA, and protein levels. It ensures that each variant is expressed unambiguously and reproducibly across clinical reports, publications, and databases.

The HGVS Nomenclature is maintained by the HGVS HGVS Variant Nomenclature Committee (HVNC) under the H Human Genome Organisation (HUGO) and is widely implemented across major genomic resources and clinical interpretation platforms. Current recommendations are detailed in Hart et al. (2) and Den Dunnen et al. (3), which formalise the syntax, reference sequence alignment, and conventions for variant expression.

Within this guideline, all variants should be reported according to the most recent HGVS Nomenclature release, aligned to an approved reference sequence (RefSeq or Ensembl transcript). Both coding (c.) and protein (p.) level annotations should be provided where possible. Genomic coordinates should follow the Genome Reference Consortium Human Build 38 (GRCh38) reference assembly.

Using standardised nomenclature ensures interoperability between laboratories, software tools, and public databases, reducing ambiguity in variant exchange and supporting precise traceability in both research and clinical reporting.

18.1.3 ACMG criteria with counter-factual evidence

The `acmg_criteria` rule interprets the condensed ACMG criteria column for each variant and flags variants that carry benign evidence. When a variant has passed upstream prioritisation tools such as Exomiser, the ACMG criteria are typically recorded in a single column as a list of applied evidence codes (for example, `PVS1`, `PM2`, `PP3`, or `BA1`). The downstream rule inspects this condensed string to determine whether it contains any benign evidence codes, recognised by the presence of the letter “B” (for example, `BA1`, `BS1--BS4`, or `BP1--BP8`). Variants with such codes are retained but marked for review, similar to those carrying gnomAD quality flags. This ensures that variants with benign evidence are not automatically excluded but instead require explicit assessment or justification in the final clinical interpretation.

Rank based scoring such as that from Tavanian offers benefits but similarly can miss the presence of counter-factual evidence that a variant might be considered benign but remain prioritised by the presence of other pathogenic flags.

variant_id	ACMG_criteria	outcome	report_action
1	PVS1, PM2, PP3	pass	include normally
2	PVS1, PM2, BA1	pass (flagged)	include with note: “contains benign ACMG evidence (BA1); review interpretation.”
3	BP4	pass (flagged)	include with note: “benign supporting evidence; verify consistency with phenotype.”
4	PS2, PM5	pass	include normally

Table S2: Example application of the `acmg_criteria` rule to patient variants. Variants containing benign ACMG evidence codes are retained but flagged for explicit review in the final report.

18.1.4 Population frequency evidence

18.1.5 Conditional evidence rules and source-dependent quality checks

Reference databases and the case sample may share variant-calling or sequencing biases. Therefore, databases which carry flagged variants should be examined through conditional automation or manual review. For instance, GnomAD is a key reference for interpreting variants in single-case analyses. The `gnomad_flags` rule ensures that

flagged variants are reviewed rather than excluded. Variants without a flag (NA) pass directly, while those with recognised gnomAD flags pass with review status.

Common flags include **AS-VQSR** (allele-specific quality recalibration), **RF** (random forest outlier), **LC pLoF** (low-confidence loss-of-function), and **SEGDU**P (segmental duplication). These indicate potential technical or annotation uncertainty rather than confirmed artefacts. We recommend that variants are retained in reporting but require justification or comment to ensure transparency and traceability.

variant_id	gnomad_flag	outcome	report_action
1	NA	pass	include normally
2	LC pLoF	pass (flagged)	include with note: “gnomAD LC pLoF; review interpretation.”
3	UnknownFlag	fail	hold for manual review

Table S4: Example application of the **gnomad_flags** rule to patient variants. Entries automatically wrap within the column width for compact layout.

18.1.6 Inheritance and segregation evidence

Version 1

When pedigree data are available, Whole Genome Sequencing (WGS) enables direct evaluation of inheritance models for each variant. Genotypes are interpreted across proband and parents using standard representations such as **REF**, **HET**, and **HOM**, or equivalent encodings used in variant data formats (0,1,2 in PLINK, 0/0, 0/1, 1/1, or phased forms such as 0|1 in VCF). From these data, the inheritance pattern for each variant is determined, such as de novo, homozygous, heterozygous, or compound heterozygous, and this information is then evaluated in relation to the known gene–disease relationship. For example, the observed segregation pattern may provide supporting or contradictory evidence for anAutosomal Dominant (AD), Autosomal Recessive (AR), or X-Linked (XL) disease mechanism.

Where genotype data are incomplete, inheritance may be inferred from clinical features, family history, or segregation information, but such cases are explicitly flagged as uncertain. Scenarios of incomplete penetrance, such as a heterozygous variant inherited from an unaffected parent, are also recorded because they influence the strength of causal interpretation. Each inheritance assessment includes both the inferred pattern and the type of supporting evidence, ensuring that interpretative

conclusions in the genetic report transparently reflect the available data and its confidence level.

Version 2

Accurate interpretation of inheritance requires integrating two complementary sources of evidence: (1) the *Mode of Inheritance (MOI)* defined by curated reference datasets that describe known gene–disease mechanisms, and (2) the *observed inheritance pattern* derived from family genotype or clinical data.

The reference MOI defines the expected transmission mechanism for a gene–disease pair, typically AD, AR, or XL. Structured datasets such as PanelAppRex (?) harmonise these annotations across thousands of curated panels. Foundational sources including Genomics England’s PanelApp and PanelApp Australia (?) provide continuously updated expert curation underpinning national infrastructures such as the NHS National Genomic Test Directory and the 100,000 Genomes Project. The MOI field thus serves as an evidence-based prior — a quantitative expectation of how pathogenic variants in a given gene are likely to segregate.

The observed inheritance pattern, by contrast, is determined from the case data. When trio or family WGS is available, inheritance can be assessed directly from genotype encodings (REF, HET, HOM, or 0/1, 1/1, 0|1). This pattern may confirm or contradict the reference MOI. For example, a de novo heterozygous variant in an AD gene supports causality, whereas biallelic variants in an AR gene are expected.

Cases with incomplete genotype data require inference from clinical or segregation information and must be explicitly flagged as uncertain. Incomplete penetrance, mosaicism, or unaffected carriers (e.g. heterozygous variants in AR genes) should be documented, as they influence the posterior probability of pathogenicity (?).

To ensure consistency, each variant interpretation should record both the reference MOI (from curated databases) and the observed inheritance pattern (from patient data). This dual recording enables probabilistic interpretation frameworks, such as Quant (?), to integrate population frequencies, genotype configurations, and inheritance priors under Hardy–Weinberg equilibrium. Together, these components quantify diagnostic confidence and prevent misclassification of variants arising from uncertain or incomplete pedigree information.

18.1.7 Functional and molecular evidence

Functional evidence from UniProt is integrated by detecting positional overlap between variant amino acid coordinates and annotated protein features recorded in UniProt GFF files. Each UniProt entry provides structured annotations describing biochemical, structural, and functional properties of the protein. For each variant, the affected residue position is compared against these annotated intervals, and any intersection is recorded as supporting evidence in the QV interpretation framework. This approach ensures that experimental and curated protein-level information contributes directly to variant interpretation and reporting (**Figure S1**).

The annotated features used as evidence sources include catalytic and binding sites, metal and nucleotide binding regions, and other experimentally defined functional motifs. Structural features such as helices, beta strands, and coiled coils provide spatial context for potential conformational disruption. Domain- and family-level annotations, including domains, motifs, and topological regions, capture conserved structural organisation and functional domains. Additional layers include post-translational modification sites, mutagenesis data, and known sequence variants curated in UniProt. Processing and localisation signals (such as signal peptides, transit peptides, and cleavage products) and cautionary sequence annotations (for example, frameshifts or sequence uncertainty) are also recorded.

By systematically linking these feature classes to variant coordinates, the framework records not only where functional or structural evidence exists, but also the type of information present—whether experimental, inferred, or computational. This enables each variant interpretation to transparently reflect the available molecular evidence supporting its classification.

18.1.8 Phenotype and gene–disease validity evidence

TNFAIP3 evidence tracks

6-137877190 T>A (GRCh38); SNV. Gene: TNFAIP3; Transcripts affected: ENST00000612899.5 (MANE Select), ENST00000237289.8, ENST00000485192.1. Consequence: stop_gained (p.Leu307Ter, c.920T>A); pLoF High-confidence. Reference population gnomAD v4.1.0: AC=1, AN=1613922, AF=6.2e-07; Homozygotes=0; Filter=PASS.

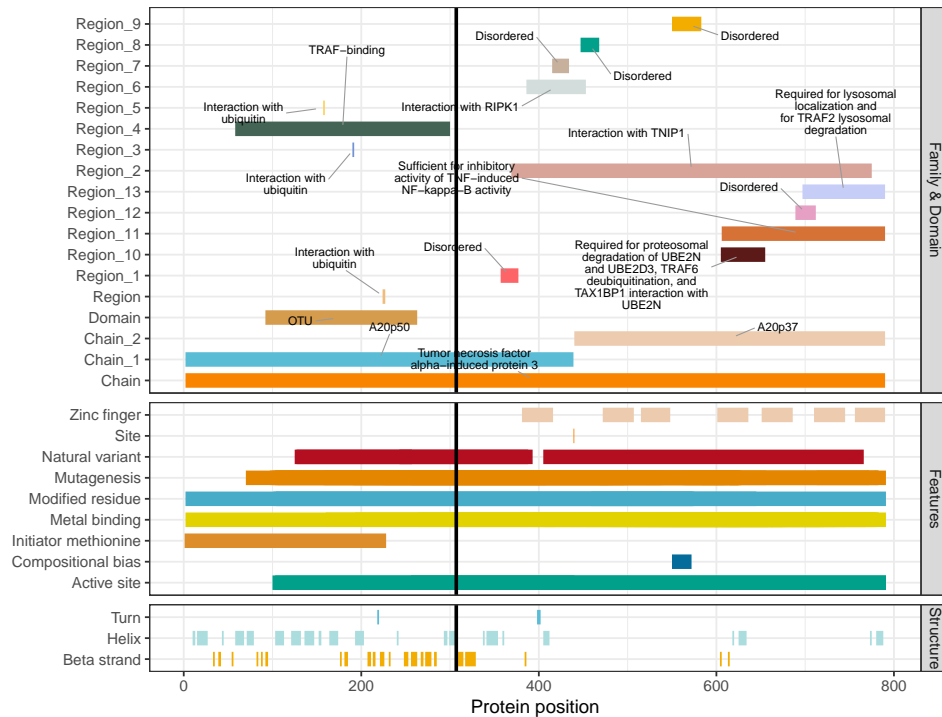


Figure S1: Functional evidence tracks from UniProt annotations. The example illustrates how protein-level features such as domains, motifs, and catalytic sites provide structured evidence supporting interpretation of coding variants. Overlaps between variant positions and curated functional regions indicate potential mechanistic relevance, while the absence of overlap suggests limited or indirect evidence. This evidence framework guides the strength of interpretation in clinical reporting, ensuring that well-supported variants are highlighted and uncertain findings are transparently qualified.