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

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Aim. To agree a shared minimum evidence standard that any service provider can satisfy, ensuring that variant interpretation results are verifiable, comparable, and trusted across clinical, research, and commercial settings. By adopting a common set of computable rules and flags, independently developed pipelines can demonstrate that their outputs meet the national evidence format, reducing fragmentation and supporting reliable use of genomics in everyday practice.

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]

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

Contents

1 Executive summary **4**

2 Introduction **5**

3 Design principles for evidence-based variant interpretation **6**

4 Data provenance and sample quality requirements **7**

5 Evidence rule framework **7**

5.1 Structure of evidence rules 8

5.2 Versioning and audit trace 9

5.3 Optional reference implementation (QV framework) 9

5.4 Implemented rule set 10

6 Evidence flags **10**

6.1 Evidence domains 11

6.2 Final flag set 11

6.3 Interpretation of flag combinations 12

7 Structured representation and interoperability **13**

7.1 Alignment with GA4GH Variant Representation and Variant Annotation models 13

7.2 GA4GH Variant Annotation model 14

7.3 Exchange formats and machine readable outputs 15

8 Three pillar framework **15**

9 Reporting and synthesis statements **16**

10 Auditability, versioning, and reproducibility **16**

11 Clinical review checkpoints **16**

12 Limitations and scope **17**

13 Future extensions **18**

14 Conclusion **18**

15 Supplemental **20**

16 Appendix **20**

16.1 Example rule files 20

16.2 Example YAML or JSON evidence objects 24

16.3 Worked examples of flagged variants 24

17 Evidence flags **24**

17.1 Evidence domains 24

1 Executive summary

In genetic analysis, any number of service providers may detect the same variant using their own proprietary or public pipelines. Regardless of how the variant is called or prioritised, each provider can generate the national minimum evidence state by supplying the required inputs and computing the standard flags. Consider the missense variant `NM_000546.6:c.215C>G (TP53 p.Pro72Arg)`. A valid minimal evidence profile might include:

- `flag_gt_valid`: Genotype is well formed and passes basic QC.
- `flag_popfreq_common`: The variant is common in gnomAD, indicating strong counterfactual evidence against causality for a rare Mendelian disorder.
- `flag_moi_parent_gt_missing_any`: Parental genotypes are not available, so inheritance cannot be assessed.
- `flag_uniprot_hits_any_feature` The variant lies within a UniProt-annotated region.

Even this short list is sufficient to show that:

1. the variant identity is correct,
2. upstream calling is technically valid,
3. population evidence contradicts a rare disease hypothesis, and
4. all required checks have been applied consistently.

The specific pipeline does not matter. Proprietary and public systems can keep their internal methods private while still supplying the factual inputs needed for verification. What matters is that the *same* flags emerge from any compliant system, ensuring transparent, reproducible checks and a shared basis for trust across clinical, research, and commercial settings.

2 Introduction

Genome sequencing is used across clinical, research, and commercial settings, yet the evidence supporting variant interpretation is recorded and applied in incompatible ways. Many systems treat algorithmic scores or proprietary rankings as conclusions rather than inputs, and key information such as provenance, variant identity, inheritance fit, population context, phenotype alignment, and contradictory signals is often incomplete or not directly comparable. This limits reproducibility and prevents reliable exchange of results across institutions.

A related difficulty is that public research projects, clinical programmes, and commercial services often rely on their own formats and internal conventions. These differences create structural barriers that prevent data from moving between sectors, even when the underlying science is identical. As a result, large numbers of sequenced genomes cannot be reanalysed, cross checked, or shared, not because the data are technically unusable, but because the evidential structure is incompatible. Valuable public investment is lost when clinically relevant data produced in research settings cannot be integrated with industry pipelines, and commercial interpretations cannot be verified or reused by academic or hospital partners.

The Swiss Genomics Association is developing a national evidence architecture to address this gap. The aim is not to replace existing tools or pipelines, nor to expose private data to external parties, but to define a minimal evidential contract that allows independently developed systems to remain compatible and to collaborate when appropriate. This avoids the repeated remapping of semantics and complex data structures that currently separates clinical, research, and industrial workflows and prevents a shared ecosystem from forming.

A shared contract specifies how variant identity, provenance, and evidence states are represented, and how falsification tests are applied to every proposed variant. It provides a computable basis for reasoning that can be traced, audited, and

exchanged across clinical services, research groups, and commercial providers.

This guideline defines the recommended minimum requirements for evidence based interpretation of variants in Mendelian disease. It sets out the rules and evidence flags that form the uniform evidence state for each variant. These rules evaluate all upstream outputs and record the presence, absence, or contradiction of supporting evidence. Any institution may use its own sequencing workflow or prioritisation tools, provided it supplies the identifiers and evidence required for interoperability.

A national genomics system must remain durable while adapting to scientific change. Clinical records and audit trails require stable structures, whereas biological knowledge and interpretive resources evolve continuously. The guideline reflects this balance by defining the stable elements needed for long term interoperability while allowing evidence rules and knowledge sources to be updated without invalidating past interpretations. This approach supports integration with new annotations and analytical frameworks and aligns with the broader national strategy to build genomic infrastructure that is open, verifiable, and compatible with clinical, academic, and industrial use.

3 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.

4 Data provenance and sample quality requirements

Reliable variant interpretation requires a minimal level of trust in how each variant was generated. The guideline therefore assumes a small set of provenance fields that describe the sample, the sequencing assay, the reference build, and the primary calling pipeline. These identifiers do not constrain laboratories to specific technologies, but they ensure that evidence rules can be applied consistently and that interpretation remains traceable to defined inputs. Provenance must be represented using structured fields rather than free text so that downstream systems can reference them deterministically.

Sample quality must also meet basic thresholds for coverage, call completeness, and genotype confidence. Where provenance or quality information is missing, the system records explicit flags rather than inferring validity. This ensures that all downstream conclusions reflect the trustworthiness of the underlying data and that gaps remain visible to both clinical reviewers and automated pipelines.

A fuller description of the provenance framework is provided in the companion pillar on semantic sample-to-sequence lineage (SGA 2026). That framework uses SPHN-aligned semantic models and include but are not limited to RDF-based representations of sequencing events, assay metadata, instruments, files, and quality metrics, building on established work in semantic clinical-genomic harmonisation (1) and FAIR data principles (2–4). The present guideline requires only the minimal provenance contract necessary for interpretable variant-level evidence, while remaining compatible with the full semantic model for institutions that implement it.

5 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.

5.1 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 independent of any specific pipeline. Laboratories and companies may use proprietary methods or detection algorithms, but the evidence supporting their outputs must be expressible through the standardised rule set. This ensures that downstream users can verify key elements of the result, such as inheritance fit or population context, without visibility into the underlying intellectual property. By supplying the minimal inputs required for the rules to execute, providers enable a transparent and reproducible evidence state, giving users confidence that a result meets the expectations of clinical, research, and regulatory stakeholders.

5.2 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.

5.3 Optional reference implementation (QV framework)

The guideline is accompanied by an optional reference implementation based on Qualifying Variant (QV) protocols (5). 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 released with the corresponding rule set versions. Laboratories and

companies are free to implement the our rules directly or to adopt the QV framework as a ready to use reference.

5.4 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.

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/ (NOTE: we can host this QV builder separately so that there is no conflict - open source and available on Zenodo), 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.

6 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.

6.1 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.

6.2 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
```

6.3 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 need not be reduced to a single classification. Instead, they can 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.

We do not prescribe how institutions should convert flag patterns into final reports or classifications. Each laboratory or pipeline may apply its own decision logic, provided that the full evidence state is preserved. The guideline ensures that all users, regardless of workflow, have access to the same verifiable evidential signals from which their conclusions are derived.

7 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.

7.1 Alignment with GA4GH Variant Representation and Variant Annotation models

This framework aligns with the Global Alliance for Genomics and Health (GA4GH) standards for representing genomic variation and associated evidence. The Variation Representation Specification (VRS) defines computable, globally unique representations of sequence variation, enabling unambiguous exchange independent of file format or provider. The Variant Annotation (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 Binary Alignment Map (BAM)/Compressed Reference Oriented Alignment Map (CRAM) remain the primary formats for variant calls and alignments, while structured phenotype and disease descriptors use the Human Phenotype Ontology (HPO) and Orphanet Rare Disease Ontology (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.

7.2 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.

7.3 Exchange formats and machine readable outputs

The evidence framework is designed so that every output can be exchanged and reused without custom transformation. Our 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.

8 Three pillar framework

The national evidence architecture consists of three coordinated pillars that together support reproducible, interoperable genomic interpretation. Pillar 1 provides standardised sample to sequence provenance, using technologies such as SQL based clinical records and RDF based semantic metadata to ensure that every genome has an auditable and technology independent lineage. Pillar 2 defines a workflow agnostic representation of analysis variables through QV sets, allowing different pipelines, callers, and vendors to produce equivalent evidence inputs.

The present guideline specifies the core of Pillar 3. It formalises how variant level evidence is verified, recorded, and represented so that downstream interpretation is

grounded in explicit computable signals rather than implicit assumptions. The evidence model follows reverse reasoning, testing each variant for any signal that contradicts or weakens the causal hypothesis and encoding these results as reproducible evidence flags that form a uniform, auditable evidence state.

Together, the three pillars create a coherent foundation for national genomics. Pillar 1 supports those who generate data, Pillar 2 supports those who process it, and Pillar 3 enables everyone who relies on the results to trust the evidence behind them. This alignment allows industry, clinical teams, researchers, and public services to work independently yet remain compatible, giving a shared and dependable genomic environment.

9 Reporting and synthesis statements

10 Auditability, versioning, and reproducibility

11 Clinical review checkpoints

The framework is designed to minimise reliance on subjective or opinion-based review. Automated rules provide a complete, reproducible evidence state for each variant, ensuring that all falsification tests are applied consistently and transparently. Clinical review is therefore limited to tasks that cannot be resolved from available data, such as clarifying phenotype information, confirming clinical relevance, or determining whether additional investigations are warranted.

Interpretation should not depend on discretionary judgments that vary between experts. Instead, conclusions should follow directly from the structured evidence state and, where applicable, quantitative reasoning in Pillar 3. This approach promotes reproducibility, reduces unexplained variability, and prepares the ecosystem for future AI-assisted interpretation by ensuring that all downstream reasoning is grounded in explicit, computable evidence rather than informal appraisal.

12 Limitations and scope

This guideline defines the evidence rules and flag structures used to test whether observable data contradict the hypothesis that a variant causes disease. Its strength lies in detecting counterfactual evidence when it is present. Its limitation is the converse: counterfactual signals that are not recorded, not measurable, or not available to the system cannot be identified. Important sources of contradiction may therefore remain undetected, as is the case in all current practice.

The guideline does not compensate for absent pedigree data, incomplete phenotype information, limited functional evidence, or gaps in external resources. It also does not define sequencing workflows, variant calling methods, or clinical decision pathways. All outputs should be understood as the result of systematic falsification applied to the available data, not as guarantees that all relevant counter-evidence has been observed.

A central limitation arises from what we call the *non-falsification fallacy*: the mistaken assumption that the absence of counterfactual evidence implies the presence of supporting evidence. A complete absence of flags does not indicate that a variant is causal or pathogenic; it only shows that, within the minimal evidential contract, no contradictions were detected. This is a baseline state, not a conclusion. In the long term, we aim for an evidence model where truly causal variants have a recognisable flag profile, but such completeness is not achievable today. Our initial aim is to meet the standard of the best current clinical genetics in a reproducible and transparent way.

Unmeasured, unrecorded, or unavailable evidence may still contradict the causal hypothesis even when all checks pass. Interpreting a clean flag profile as confirmation of causality risks conflating non-falsification with proof. The framework therefore reports only what can be ruled out, providing the baseline upon which quantitative inference in Pillar 3 can be constructed.

13 Future extensions

14 Conclusion

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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 ...

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Acronyms

AD Autosomal Dominant 26
AR Autosomal Recessive 26
BAM Binary Alignment Map 14
CRAM Compressed Reference Oriented Alignment Map 14
GA4GH Global Alliance for Genomics and Health 13
GRCh38 Genome Reference Consortium Human Build 38 24
HGVS Human Genome Variation Society 24
HPO Human Phenotype Ontology 14
HVNC HGVS Variant Nomenclature Committee 24
HUGO Human Genome Organisation 24
MOI Mode of Inheritance 27
ORDO Orphanet Rare Disease Ontology 14
QV Qualifying Variant 9
VA Variant Annotation 13
VRS Variation Representation Specification 13
VCF variant call format 14
WGS Whole Genome Sequencing 26
XL X-Linked 26

15 Supplemental

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

16 Appendix

16.1 Example rule files

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,SEGDUP,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_geno" 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_geno" operator="==" value="HOM"
criteria not_hom_parent field="mother_geno" operator="!=" value="HOM"
criteria not_hom_parent field="father_geno" operator="!=" value="HOM"

criteria not_hemi_parent field="proband_geno" operator="==" value="HEMI
    ↪ "
criteria not_hemi_parent field="father_geno" 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_geno"
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"
````

16.2 Example YAML or JSON evidence objects

16.3 Worked examples of flagged variants

17 Evidence flags

17.1 Evidence domains

17.1.1 Counter-evidence and conflict handling

17.1.2 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. (6) and Den Dunnen et al. (7), 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.

17.1.3 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.

17.1.4 Population frequency evidence

17.1.5 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 `SEGDUP` (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.

17.1.6 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.

17.1.7 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.

17.1.8 Phenotype and gene–disease validity evidence

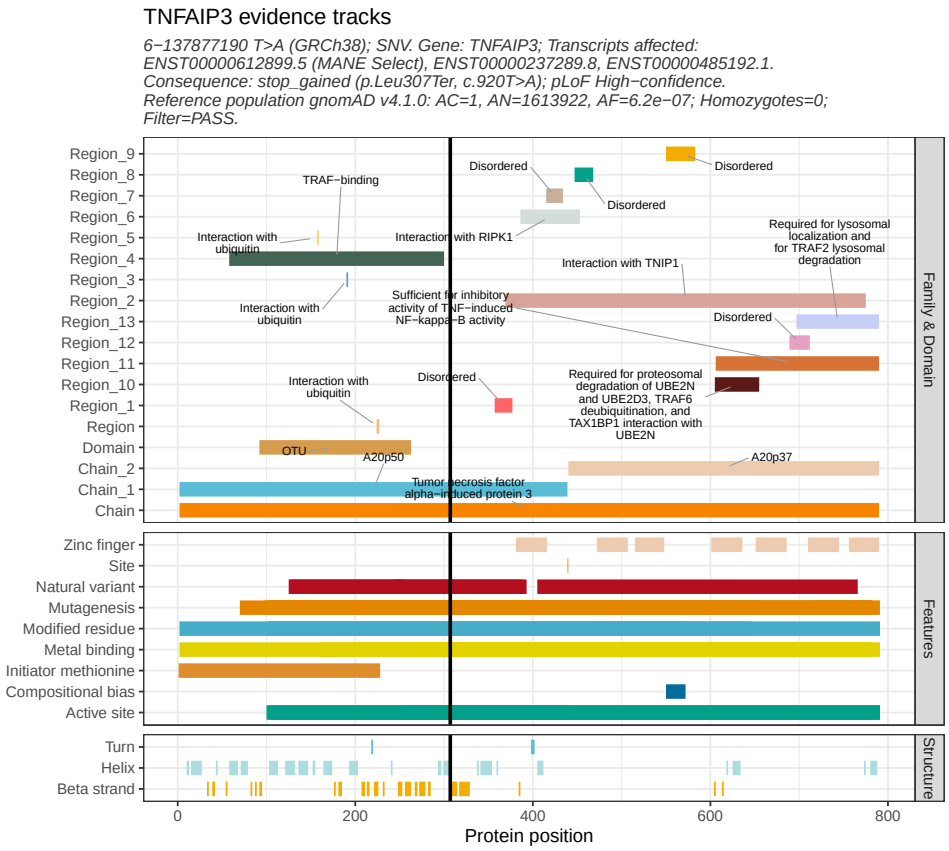

Figure S1: **Functional evidence tracks from UniProt annotations.** The example illustrates how protein-level features such as domains, motifs, and catalytic sites provide structured evidence supporting interpretation of coding variants. Overlaps between variant positions and curated functional regions 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.