

Variant curation in TruSight™ Software Suite

Tools for gathering evidence
for variant curation and
interpretation

Accelerate variant interpretation

Whole-genome sequencing (WGS) and whole-exome sequencing (WES) provide a high-resolution, unbiased view across the genome. Informatics solutions streamline analysis of the vast amounts of data produced by these methods, enabling discovery of causative variants associated with rare diseases. Interpretation of these variants requires manual curation, which can represent a significant bottleneck, as it often requires a great deal of time and repeated effort.

To address this challenge, TruSight Software Suite offers various features to aid with variant curation and determining which prioritized variants are relevant to the current case. TruSight Software Suite aggregates data from external databases, such as the [Online Mendelian Inheritance in Man \(OMIM\) catalog](#), [ClinVar](#), and others. Additionally, TruSight Software Suite integrates standard terminology for variant classification developed by the American College of Medical Genetics and Genomics (ACMG), which helps with recording variant details and associations. This technical note outlines the process for gathering evidence in TruSight Software Suite for selection and interpretation of variants.

Tools and tabs in Variant Grid

TruSight Software Suite integrates with BaseSpace™ Sequence Hub and Illumina sequencing systems to enable comprehensive, streamlined workflows for variant analysis and interpretation. After sequencing data has been imported to TruSight Software Suite and aligned to a reference, variants need to be called, filtered, and prioritized. For more information, see the [variant filtering technical note](#). The user begins the curation process by selecting a prioritized variant in the Variant Grid, which opens a series of tabs with information about the variant, gene, and any associated diseases to aid in curation (Figure 1). The curated content, including all evidence, will be saved in the Knowledge Network Service, a structured database within TruSight Software Suite and can be added to the final report.

AI Variant Prioritizer tab

The AI Variant Prioritizer tab displays a Knowledge Graph only for variants prioritized by Emedgene. This integrated genomics artificial intelligence (AI) engine prioritizes

* Emedgene supports filtering of small variants only

The screenshot shows the TruSight Software Suite interface. At the top, there's a navigation bar with 'TSS-WGS-SMA' and 'INTERPRETATION' tabs. Below that is a filter bar with various variant categories like 'ClinVar-PLP', 'CNV_Rare', 'Denovo', etc. A table of variants is displayed with columns for flags, status, linked, IGV, category, position, gene, transcript, zygosity, MYKB, overlap, length, variant type, and consequence. The 'SMN1' variant is highlighted. Below the table, the 'Gene Details' tab for SMN1 is open, showing gene information, constraint metrics, related diseases, and literature search results.

FLAGS	STATUS	LINKED	IGV	CATEGORY	POSITION	GENE	TRANSCRIPT	ZYGOSITY (FATHER)	MYKB	OVERLAP	LENGTH (BP)	VARIANT TYPE	CONSEQUENCE
		CH		Small Variant	MT : 12372	MTND5 (+1)	ENST00000361...			5	1	SNV	Synonymous
		CH		Small Variant	MT : 14766	MT-CYB (+2)	ENST00000361...			5	1	SNV	Missense
		CH		Small Variant	5 : 70247773	SMN1	NM_000344.3	Heterozygous		4	1	SNV	Synonymous
		CH		Small Variant	MT : 11467	MTND4	ENST00000361...			4	1	SNV	Synonymous
		CH		Small Variant	2 : 210640622	UNC80	NM_032504.1			3	1	SNV	Stop
		CH		Small Variant	5 : 148422281	SH3TC2	NM_024577.3	Heterozygous		2	1	SNV	Missense
		CH		Small Variant	MT : 15326	MT-CYB	ENST00000361...	Heterozygous		2	1	SNV	Missense
		CH		Small Variant	4 : 155520935	EP2	NM_021870.2	Heterozygous		1	1	CNV	Missense

The 'Gene Details' tab for SMN1 shows the following information:

- Gene Name:** SMN1
- Description:** The SMN1 and SMN2 genes lie within the telomeric and centromeric halves, respectively, of a large, inverted duplication on chromosome 5q13. These genes share more than 99% nucleotide identity, and both are capable of encoding a 294-amino acid RNA-binding protein, SMN, that is required for efficient assembly of small nuclear ribonucleoprotein (snRNP) complexes. Homozygous loss of the SMN1 gene causes spinal muscular atrophy (SMA). Absence of SMN1 is partially compensated for by SMN2, which produces enough SMN protein to allow for relatively normal development in cell types other than motor neurons. However, SMN2 cannot fully compensate for loss of SMN1 because, although SMN2 is transcribed at a level comparable to that of SMN1, a large
- Constraint Metrics (gnomad):** LOEUF: 1.93, synZ: -0.861, misZ: 0.0584, pNull: 0.504, pRec: 0.48, pLI: 0.0158
- Dosage Sensitivity (ClinGen):** No data available.
- Related Diseases:** Spinal muscular atrophy-1, Spinal muscular atrophy-2, Spinal muscular atrophy-3, Spinal muscular atrophy-4
- PrimataEAI:** No data available
- Literature Search (Indications/Total):** Yes / 2070
- Links:** OMIM, PubMed, CaseLog (17), MGI, Decipher, GeneReviews, Monarch, ZFIN, STRING

Figure 1: Variant Grid—The customizable Variant Grid displays a list of tabs with information on the variant category, chromosomal position, gene affected, phenotype overlap, consequence of the variant, and more.

pathogenic variant candidates based on phenotypic information. The Knowledge Graph shows the relationships between the variant, gene, diseases, and phenotypes that are relevant for the case (Figure 2). Users can explore the Knowledge Graph by reviewing the variant details, the respective gene in which it occurs, and associations with “clinical indications,” including confirmed and unconfirmed phenotypes. Links to supporting evidence from the scientific literature and online databases for more information are provided.

Gene Details tab

The Gene Details tab provides information about the gene in which the variant of interest has been identified (Figure 3). If more than one gene is involved, the initial view lists a summary of each gene, overlapping phenotypes, and literature search results. A partial overlapping icon will

be provided if a breakpoint occurs within the gene. Users can copy the gene list to a clipboard for interpretation, if needed.

Information available on the Gene Details tab includes:

- Gene name
- Associated disease and inheritance pattern (OMIM, ClinGen)
- Constraint metrics (gnomAD)
- Dosage sensitivity (ClinGen Dosage Sensitivity Map)
- Disease information (OMIM)
- Gene-disease relationship (ClinGen Gene-Disease Clinical Validity Curation)
- Predicted pathogenicity of missense variants (computed by PrimateAI)

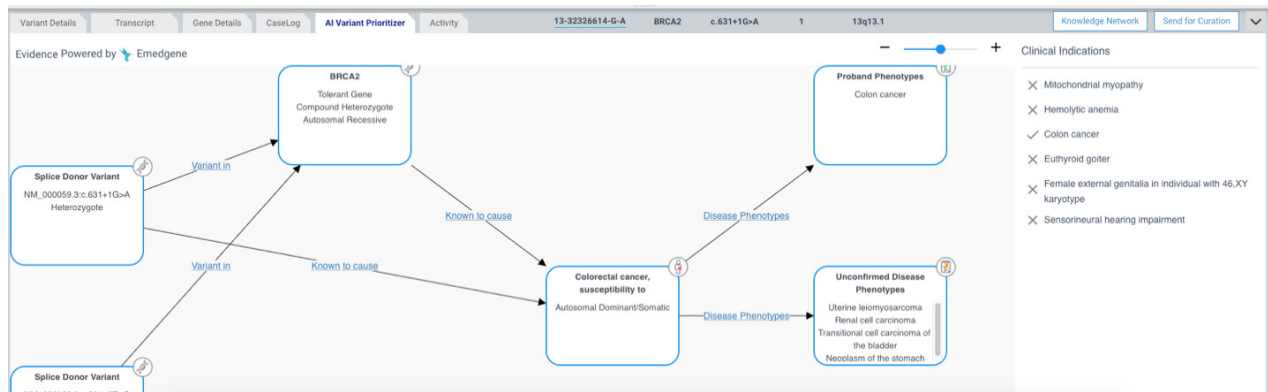


Figure 2: AI Variant Prioritizer tab—The AI Variant Prioritizer tab displays a Knowledge Graph for variants prioritized by the Emedgene genomics AI engine. The Knowledge Graph displays relationships between the variant, gene, diseases, and related phenotypes.



Figure 3: Gene Details tab—The Gene Details tab displays details about the gene containing the variant of interest. A partial overlapping icon (green) will be provided if a breakpoint occurs within the gene. The gene list can be copied to a clipboard (red) for interpretation, if needed.

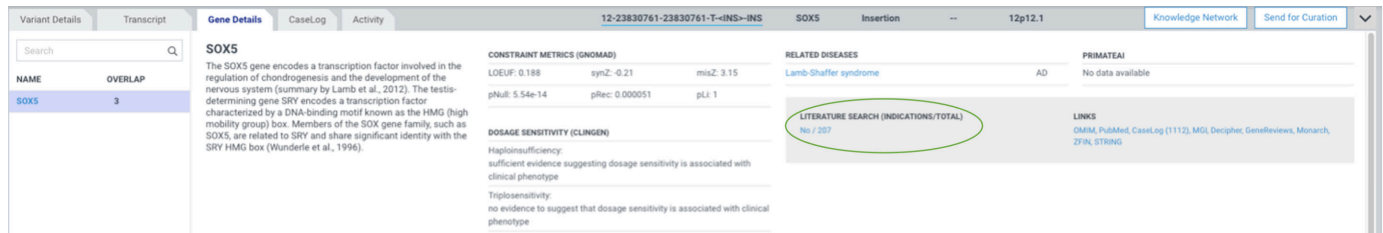


Figure 4: Gene Details literature search result—TruSight Software Suite can perform a literature search in PubMed for a selected gene and display information about each publication, including title, authors, and abstract.

A link is provided to a literature search performed by TruSight Software Suite in [PubMed](#) for the selected gene name (Figure 4). The PubMed reference number (PMID), title, authors, and abstract are displayed for each publication found in the search. The results of this search can be filtered by gene, specific keywords, or phenotypes. Users can select publications to add to a queue for review when looking for variant- or gene-level evidence. Users can also add comments to publications that will be saved with that publication and shared within the workgroup.

Variant Details tab

The Variant Details tab provides detailed information about the specific variant under curation (Figure 5), including:

- Chromosomal position
- Zygosity and metrics (genotype quality and allele depth)
- Population frequency ([gnomAD](#) or [MITOMAP](#) for mitochondrial variants)
- SpliceAI score (available depending on variant type and location)
- Visualization (including [UCSC](#), [ENSEMBL](#), STAR Alignment, and [IGV](#))
- Links to external databases, including [dbSNP](#), [ClinVar](#), [gnomAD](#), [LOVD](#), will only show if available (Figure 6)

If a user submitted any previous TruSight Software Suite cases to CaseLog from the Dashboard (“Add to CaseLog”), the Variant Details tab will show any of those cases that carry the same genetic variant, together with some details on their respective genotype, age, sex, phenotypes, affected status, and whether the variant occurs in a previous report for that case (Figure 7). In addition to TruSight Software Suite cases, CaseLog will also display any matches found against various public disease cohorts.

By default, the Variant Details tab shows the number of previous cases, alongside an allele frequency based solely on the user’s submitted cases. The display can be expanded (“Show more”) to view relevant details. If the variant has been previously curated and exists in the Knowledge Network Service, the variant associations will be displayed in the Variant Associations section.

CaseLog tab

TruSight Software Suite collects information pertaining to the selected variant and gene across all of a user’s previous cases in the CaseLog tab. The data are enriched with information from matching individuals found in various public disease cohorts. Additional documentation on CaseLog can be found in [TruSight Software Suite Online Help](#).

CaseLog needle plot

In the CaseLog tab, a needle plot displays all protein coding variants found in the user’s and public cases, together with their respective consequence and allele frequency from gnomAD (Figure 8). Every needle represents a variant found in at least one cohort: a fully colored needle pin indicates a user’s variants, while open needle pins indicate variants observed in a public disease cohort. If the same variant is found in multiple cohorts, the needle pins will be stacked on top of each other. Moving the mouse over a needle pin will bring up a table displaying information on the variant, gnomAD allele frequency, sample counts, and the five phenotypes most often found in cases carrying that variant.

The needle plot also shows protein domains from Pfam and InterPro (Figure 8, horizontal, colored bars). The selected variant will be highlighted by a black triangle. Based on consequence type and proximity, users can decide if

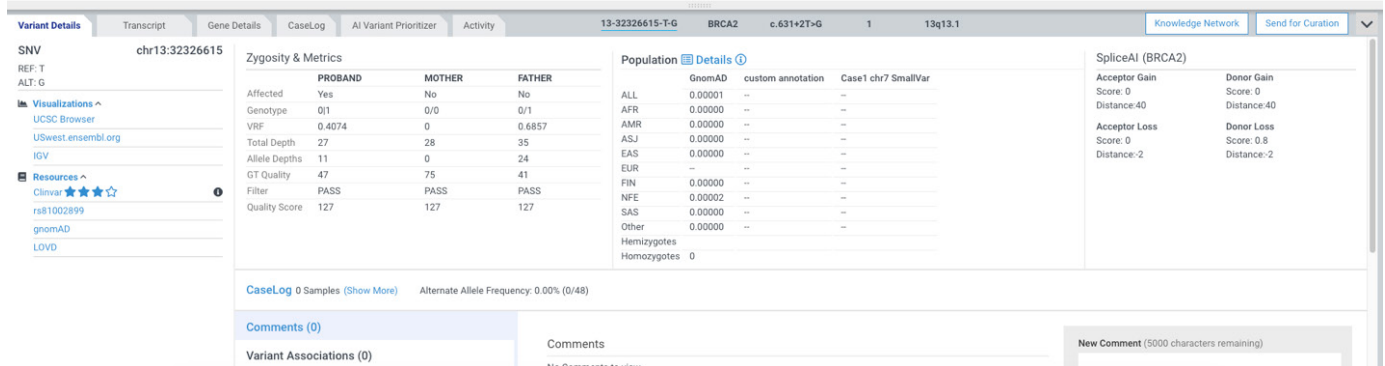


Figure 5: Variant Details Tab—The Variant Details tab provides detailed information about a variant of interest, including chromosomal position, zygosity and metrics, population frequency, visualization, and links to external databases to aid in variant curation.

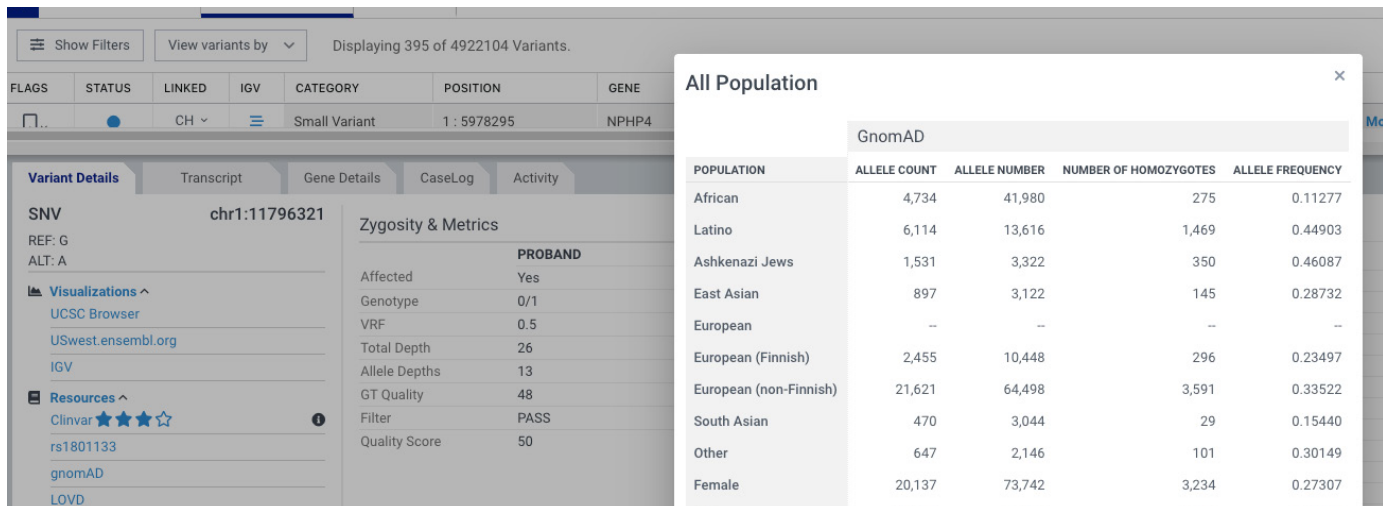


Figure 6: External databases in the Variant Details tab—TruSight Software Suite links to external databases, including dbSNP, ClinVAR, gnomAD, and LOVD, if available for the variant of interest. The Variant Details tab displays relevant information, such as population frequency metrics from gnomAD and/or MitoMap.

different variants observed in other cases are potentially relevant for curation and interpretation.

In a scatter plot located below the needles and protein domains, the needle plot shows the PrimateAI score for the most impactful amino acid change at every protein position. These can help determine whether particular regions, such as protein domains, tend to be intolerant to variation.

To inform users about matching or similar variants, the needle plot shows all pathogenic ClinVar variants, as well as any variants submitted by the user to the Knowledge

Network Service, underneath the PrimateAI scatter plot. Triangles represent ClinVar variants, circles represent Knowledge Network Service variants, all using the same color coding to differentiate consequence types. To zoom into particular protein regions, especially for larger proteins exhibiting extensive genetic variation, users can drag each of the left and right red handles on the exon track towards the region of interest.

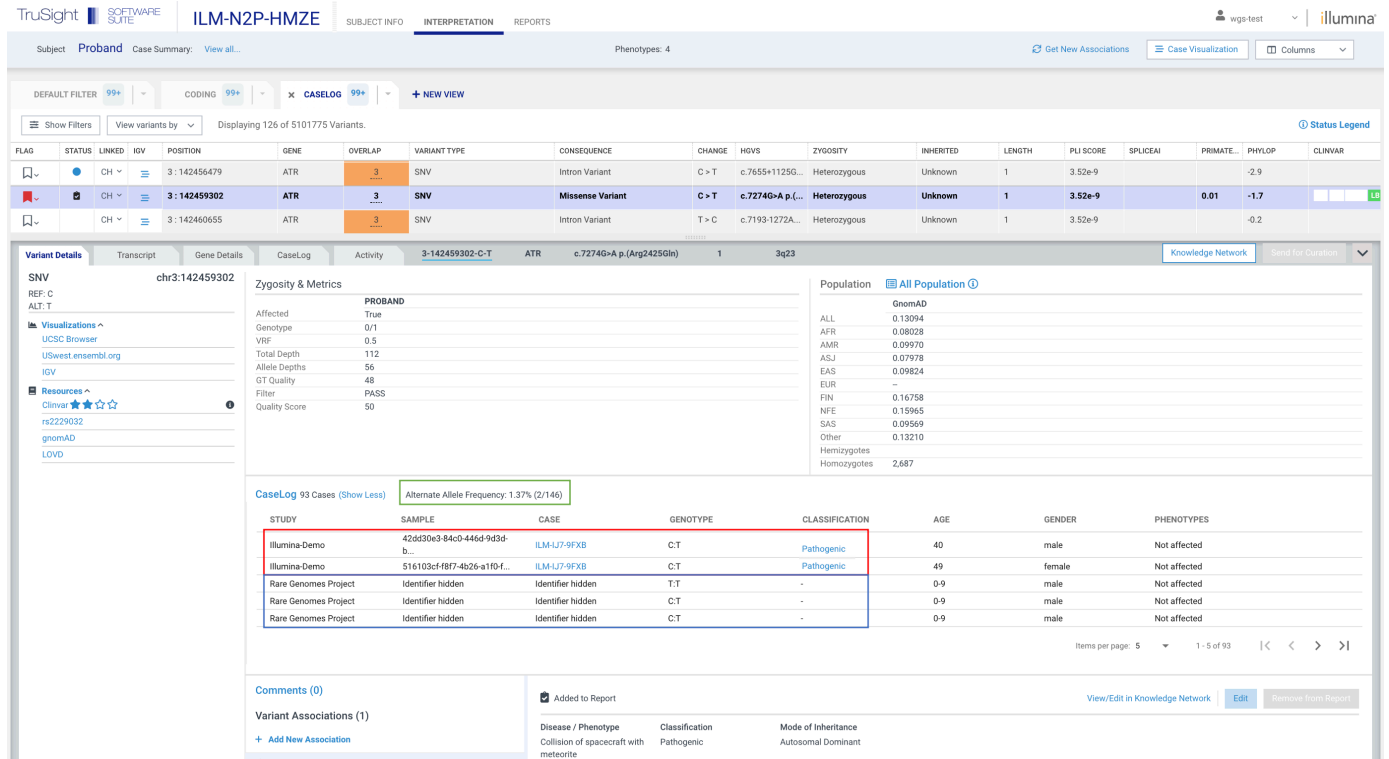


Figure 7: Previously curated variants in the Variant Details tab—Previous cases and reports processed in TruSight Software Suite submitted to CaseLog from the Dashboard will be displayed in the Variant Details tab (red) if they carry the same genetic variant, and include pertinent details such as genotype, age, gender, affected status, and more. Any matches found in various publicly available disease cohorts (blue) will also be displayed, along with an allele frequency based on the submitted cases (green).

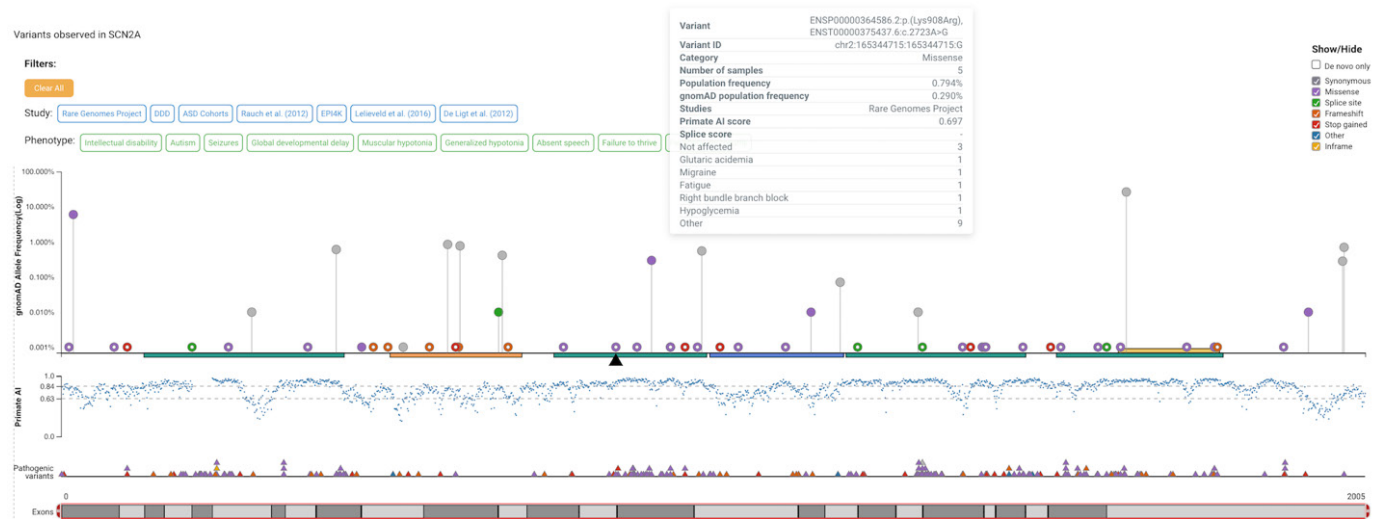


Figure 8: CaseLog needle plot—The CaseLog tab includes needle plots that display all protein-coding variants found in both internal and public (external) cases, their respective consequence, and gnomAD allele frequency. Protein sequence is on the X-axis and gnomAD frequency is on the Y-axis in log scale. Each needle pin is an identified variant, with colored and open needles corresponding to internal and external variants, respectively.

CaseLog genetic burden test

In addition to the graphical overview of a gene’s coding variants, CaseLog computes phenome-wide association study (PheWAS) scores across all available cohorts using *de novo* variants observed in cases with each phenotype. The data are displayed in a table showing all contributing studies, expected counts of *de novo* variants (based on sample and protein size), observed counts, and Bonferoni-adjusted P-values for each relevant phenotype.

CaseLog list of variants

In addition to the needle plot, the CaseLog tab provides a tabular output of all variants observed inside the selected gene’s region, up to 5 kb up- and downstream. Variants are grouped by cohort (user or public) and show protein change, CDS change, consequence, and SpliceAI or

PrimateAI scores (where applicable), and all phenotypes observed in any case carrying that variant.

Gene expression bar chart

The CaseLog tab can display a bar chart plotting gene expression across more than 50 different tissues, according to GTEx v8 (Figure 9).

Transcript tab

The Transcript tab lists all available transcripts and concomitant consequences for the given variant (Figure 10). *In silico* predictive scores from SIFT, PolyPhen, REVEL, and N-Species AA are also listed per transcript. Users can explore more than one transcript for curation; however, the transcript should be selected before curation as this will affect the literature search result.

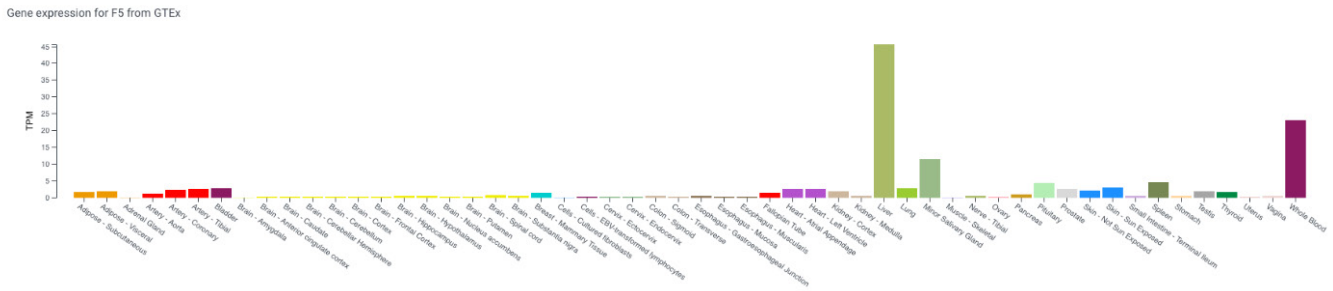


Figure 9: Gene expression bar chart—The CaseLog tab can display gene expression data as a bar chart for more than 50 different tissues.

Variant Details		Transcript	Gene Details	CaseLog	AI Variant Prioritizer	Activity	1-45334493-G-C MUTYH c.55C>G p.(Arg19Gly)		1	1p34.1	Knowledge Network	Send for Curation
TRANSCRIPT	GENE	SIFT	SIFT PREDICTION	POLYPHEN	POLYPHEN PREDICTION	REVEL	N-SPECIES AA	EXON	HGVS CDNA	HGVS AA	CONSEQUENCE	
<input checked="" type="radio"/> NM_001128425.1	MUTYH	0	deleterious - low confidence	0.55	possibly damaging	0.087		2/16	c.55C>G	p.(Arg19Gly)	Missense Variant	
<input type="radio"/> NM_001048171.1	MUTYH	0	deleterious - low confidence	0.6	possibly damaging	0.087		2/16	c.55C>G	p.(Arg19Gly)	Missense Variant	
<input type="radio"/> NM_001048172.1	MUTYH	0	deleterious - low confidence	0.255	benign	0.087		2/16	c.13C>G	p.(Arg5Gly)	Missense Variant	
<input type="radio"/> NM_001048173.1	MUTYH	0	deleterious - low confidence	0.6	possibly damaging	0.087		2/16	c.13C>G	p.(Arg5Gly)	Missense Variant	
<input type="radio"/> NM_001048174.1	MUTYH	0	deleterious - low confidence	0.6	possibly damaging	0.087		2/16	c.13C>G	p.(Arg5Gly)	Missense Variant	
<input type="radio"/> NM_001293190.1	MUTYH	0	deleterious - low confidence	0.58	possibly damaging	0.087		2/16	c.55C>G	p.(Arg19Gly)	Missense Variant	
<input type="radio"/> NM_001293191.1	MUTYH	0	deleterious - low confidence	0.32	benign	0.087		2/16	c.13C>G	p.(Arg5Gly)	Missense Variant	
<input type="radio"/> NM_001293192.1	MUTYH					0.087		2/16	c..200C>G		5 Prime UTR Variant	
<input type="radio"/> NM_001293195.1	MUTYH	0	deleterious - low confidence	0.6	possibly damaging	0.087		3/17	c.13C>G	p.(Arg5Gly)	Missense Variant	
<input type="radio"/> NM_001293196.1	MUTYH					0.087		2/16	c..200C>G		5 Prime UTR Variant	

Figure 10: Transcript tab—The Transcript tab lists all available transcripts for a variant of interest, and various *in silico* predictive scores for each transcript. A mark indicates a Canonical transcript and a indicates the default transcript.

Activity tab

The Activity tab lists flag and review activity for the variant in the case.

Variant curation workflow in TruSight Software Suite

The variant curation workflow involves creating a new variant and gene association, adding evidence, and saving to the Knowledge Network Service (Figure 11).

Add gene associations

Two types of gene associations can be added in the Gene Details tab:

- **Gene Information Association** includes information on the gene and encoded protein
- **Gene Association** describes the relationship between a gene and a disease

To add a new gene association, users provide a disease, mode of inheritance, classification, and, optionally, mechanism of disease and penetrance, if known. The user can import an existing gene–disease relationship from ClinGen or OMIM (Figure 12). To add additional evidence to the association, users can click and open the “Add evidence” window.

Different types of evidence can be added, including data from publications and information from web pages or other sources. After the evidence type is selected, and the source given, the evidence itself can be added in the “Evidence Summary” box (Figure 12). If adding PMIDs, the citation is automatically pulled through to the “Citation” box. The user can attach evidence from the literature search queue, and citations and PMID will be autopopulated. Notes can be added in the “Curator Notes box.” These will be saved, but not pulled through onto the report. When all of the evidence have been added, press “Save” to save all curated content in the Knowledge Network Service and return to the “Gene Details” page. The disease/phenotype field is ontology-based, and the user can add custom terms, if desired.

To add an association to the report, select the association and then click on “Add to Report.” It is recommended to add a variant association to the report before a gene association for correct population of the pathogenicity in the report. When an association is added to the report, the curation summary is automatically populated in the interpretation summary section of the report and all citations linked to that association are added to the reference section. Users can edit the interpretation summary in the report and add information about the variant specific to a particular case, if needed. Edits made in the report page are only saved for a specific report and will not be saved in the Knowledge Network Service.

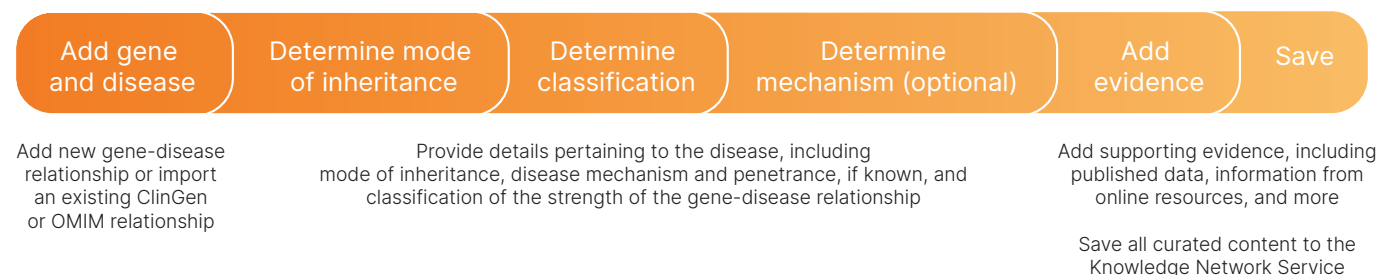


Figure 11: Variant curation workflow—Variant curation in TruSight Software Suite begins with adding a new gene–disease association, followed by determining relevant details pertaining to the disease of interest. Supporting evidence is added and all curated content is saved to the Knowledge Network Service.

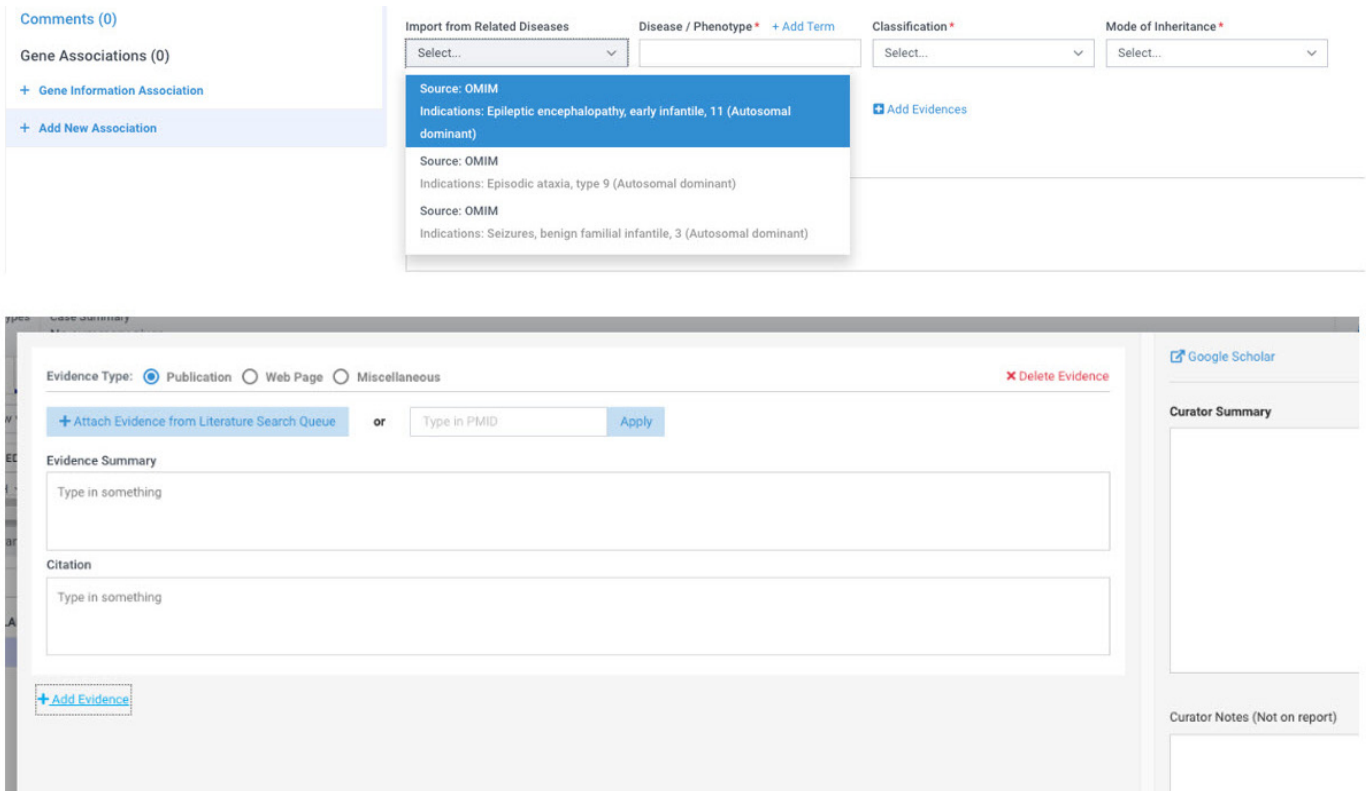


Figure 12: Adding gene associations—Begin the variant curation workflow by adding a gene association. The user provides a disease, mode of inheritance of the associated disease, classification, and mechanism of disease and penetrance (optional). The user can import an existing gene–disease relationship from ClinGen or OMIM. Different types of evidence can be added, including data from publications and information from web pages or other sources.

Add variant associations

To add a new association for a variant, the user inputs a disease/phenotype and selects a mode of inheritance and classification. If information on the gene in which the variant is found already exists in the Knowledge Network Service, it can be used to prepopulate the disease and mode of inheritance for the variant. If a variant has been previously curated and exists in the Knowledge Network Service, the variant associations will be displayed in the variant associations section. Users can edit an existing association or add the already curated content directly to a report.

To add evidence and apply ACMG criteria to classify small SNVs and indels, users click on the link to the ACMG Calculator. To add evidence to large variants (CNVs, SNVs,

ROH, or STRs), click on “Add Evidence.” In the ACMG Calculator window, there is a link to a literature search in Google Scholar for the variant under curation using the Human Genome Variation Society (HGVS) nomenclature.

Users can choose to apply criteria by clicking one or more ACMG criteria boxes at the top of the ACMG Calculator (Figure 13). The strength of the evidence and, therefore, the level at which the individual criteria is applied can be selected from the dropdown menu next to the Evidence Strength. For each criterion applied, users can provide the rationale for application as free text. As each of the criteria is applied, the pathogenicity of the variant will be recalculated and the ACMG criteria boxes will be filled in, with the depth of color representing the strength of evidence.

Different types of evidence can be added to support the

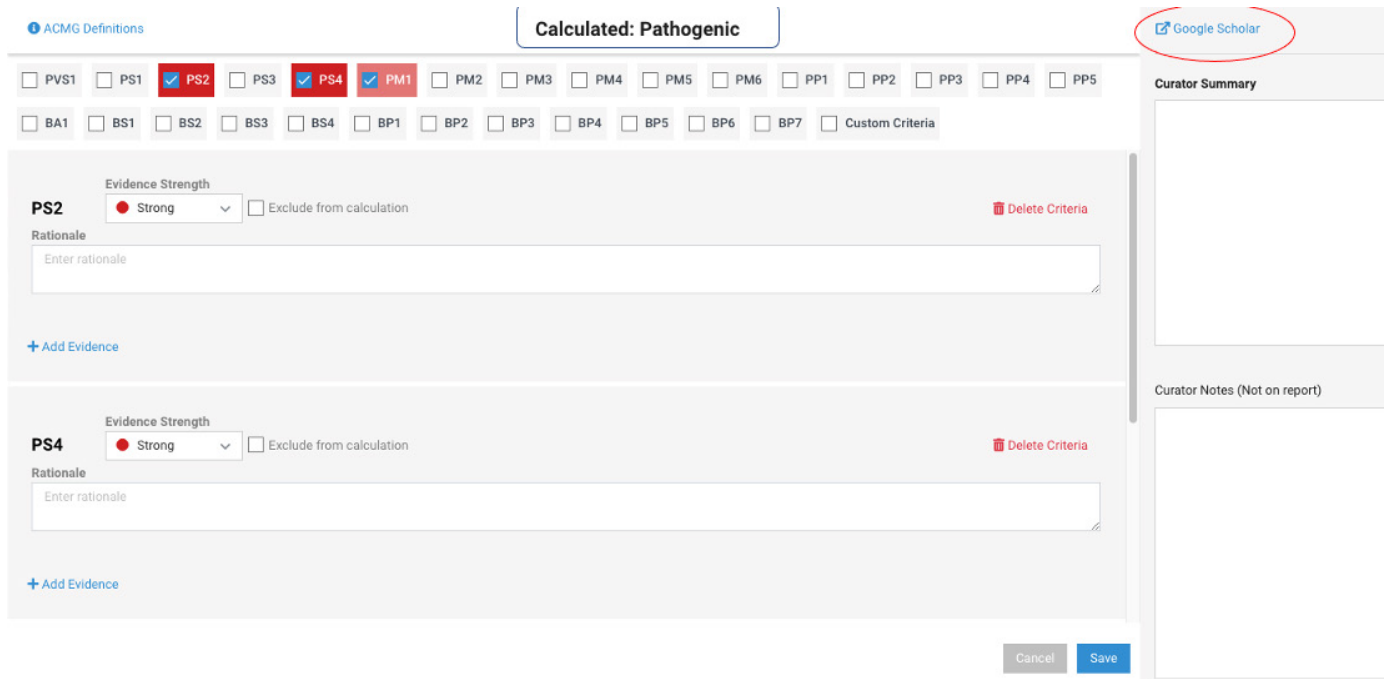


Figure 13: Adding evidence by ACMG criteria—Users can apply ACMG criteria to classify SNVs and indels by clicking boxes in the ACMG Calculator. The strength of the evidence and the level at which the individual criteria is applied can be selected from the drop-down menu next to the Evidence Strength. As each of the criteria is applied, the pathogenicity of the variant will be recalculated (top of the window) and the ACMG criteria boxes will be filled in, with the depth of color representing the strength of evidence.

selected ACMG criteria. Users can add evidence directly or attach evidence from a literature search queue. Multiple pieces of evidence can be added for each criteria. At least one piece of evidence is required per selected ACMG criteria.

After all criteria are applied and evidence added, users save the calculation in the ACMG Calculator and return to the Variant Details tab. The calculated pathogenicity and curator summary will be automatically updated in the Variant Details tab. After curation is complete, the user can press “Save” and the curation and supporting evidence is saved in the Knowledge Network Service.

Add associations to report

To add a gene or variant association to a report, select the association and then click on “Add to Report.” It is recommended to add a variant association to the report before a gene association to allow the pathogenicity of the variant to populate correctly in the

report. When an association is added to the report, the curation summary will automatically populate in the interpretation summary section of the report and all citations linked to that variant will be automatically added to the reference section. The user can edit the interpretation summary in the report and add any information about that variant that is specific to the particular case. Edits made in the report page will only be saved for the specific report and will not be saved in the Knowledge Network Service.

"Send for Curation" feature

The TruSight Software Suite "Send for Curation" feature allows the user to select a variant for review or create a list of variants for curation in bulk (Figure 14). After a variant is sent for curation, the user can assign the variant a due date in the Knowledge Network Service. Variant information will be exported to the Knowledge Network Service

along with any Case Manager notes, where the user can edit or complete the curation of the variant. After a variant is curated and approved, it will be visible in the “Variant Details” or “Gene Details” tab in TruSight Software Suite.

Knowledge Network Service

Knowledge Network Service is a database structured to save curated associations at the gene and variant level, along with any supporting evidence. The content in the Knowledge Network Service captures the relationships between the variant, gene, and disease; the variant classification; and evidence supporting the classification. For small variants, the Knowledge Network Service also stores the specific ACMG criteria applied as part of the variant classification. Data stored in the Knowledge Network Service can be updated by users and versioned with a record of modification displayed in the detail page of each association.

All variants curated and saved in TruSight Software Suite will be automatically saved and enabled in the user’s private Knowledge Network Service. All variants created

or uploaded into the Knowledge Network Service need to be reviewed. A reviewer (Curation Manager) can approve variants one at a time or in bulk. Only approved (“Enabled”) variants will be shown in TruSight Software Suite. The user also has the ability to bulk download variants in a format that could be easily uploaded to ClinVar.

Adding associations in Knowledge Network Service

A user can add a new association directly in the Knowledge Network Service or bulk upload their existing internal database (Figure 15). To add an association in the Knowledge Network Service, the user first specifies a “curation level.” The two curation levels currently supported by the Knowledge Network Service are gene level curation and position level curation.

To store gene-level curation data, select “Gene” under the “curation level.” Users add the gene name and select a transcript and association type. Two association types are supported for gene-level curation: Gene information and Mendelian Gene disease. Under “Gene information,” the user can add a summary of curated information about the gene. The “Mendelian Gene Disease” association captures information about the gene-disease relationship. Users

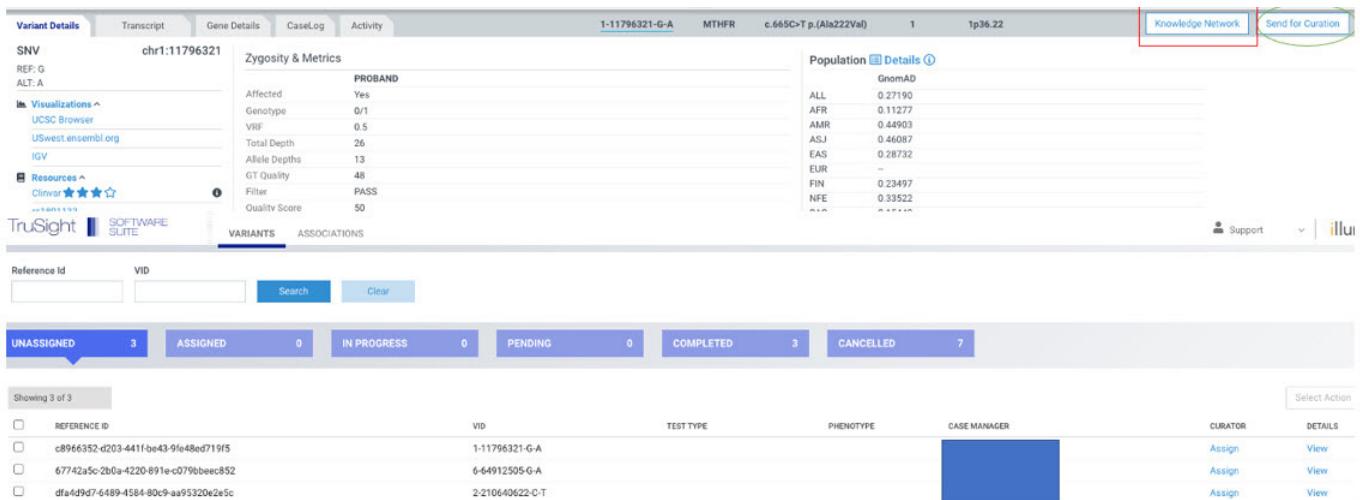


Figure 14: “Send for Curation” feature—TruSight Software Suite includes a “Send for Curation” feature that allows users to send cases for curation or review, either internally or externally, giving the task assignee limited access to the user’s instance of TruSight Software Suite.

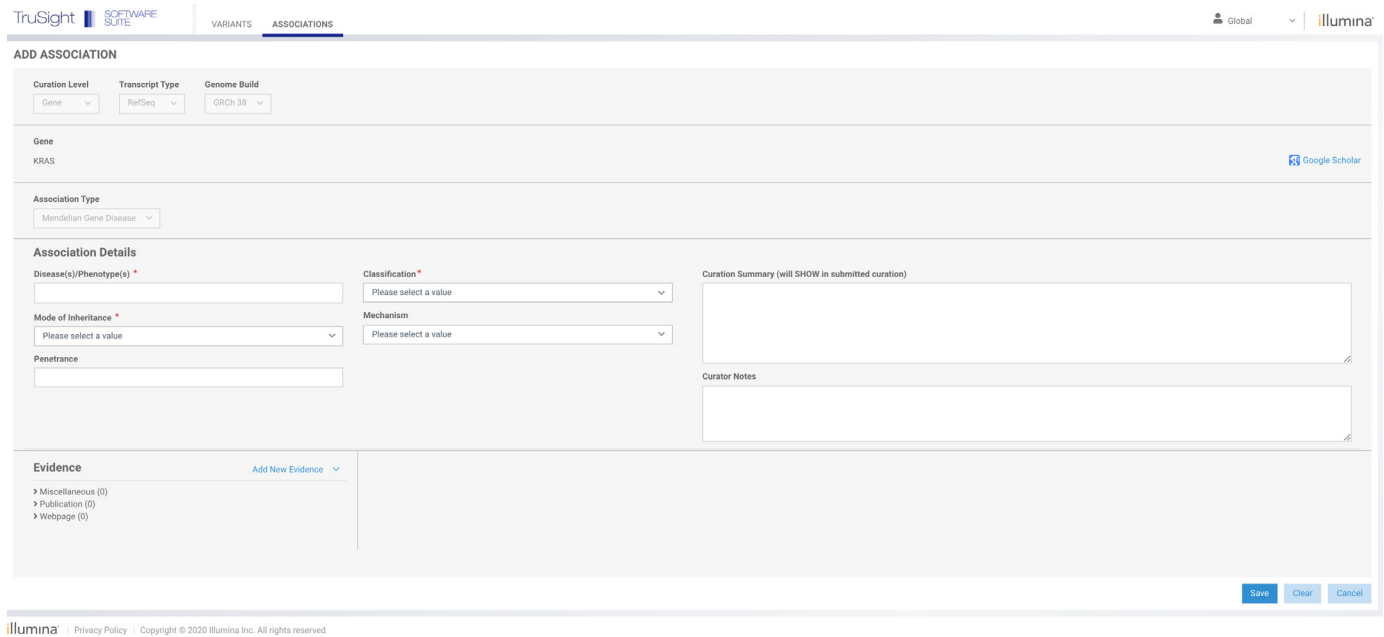


Figure 15: Adding associations in Knowledge Network Service—A user can add a new association directly in Knowledge Network Service or bulk upload their existing internal database.

can add an associated disease, mode of inheritance, the classification of the strength of that gene–disease relationship, and, optionally, mechanism of disease and disease penetrance. There is a free field for the user to add “Curator Notes” that is not displayed in the Gene Details page, and a “Curation Summary” box to add information that will be displayed in the Gene Details page.

To store position-level curation data, select “Position” under the “curation level.” Users select “biomarker type” and then fill in the required fields for each biomarker type. After filling in the required fields, users can click on the “verify” button and the system will autopopulate additional information of the variants to be saved in the system (eg, cDNA, amino acid change, cytoband). Users can then add supporting evidence as described before. For small variants, users can select ACMG criteria and add evidence to support the selection.

To bulk upload a list of variants in the Knowledge Network Service, click “Upload Log” on the landing page. The Upload Log will display all previous activity. The user can click on the “Upload” button to upload a new file. The user specifies an association type and genome build, and then

uploads a file using the format provided in the system. The user can download the sample template by clicking the “Download .CSV template” button. After the upload, the system will provide a log with the status of all records. The user can check the log by clicking the “Details” link in the Upload Log page.

All variants saved or uploaded to the Knowledge Network Service will be automatically assigned an “in progress” status and can only be viewed in TruSight Software Service after approval (Enabled). To approve an association, click on the “Approve” button within the association or bulk select a list of variants in the landing page and select “Approve.”

To edit an association, click on the “Create Draft” button in the association page. Users can “Edit” the association as needed. After the edit is done, users can “Save” the change and “Approve” the association again. If a user saves the change but does not “Approve” it, the draft will be saved in the background and the association will NOT be updated in TruSight Software Suite. Users can go back to the draft by clicking the “Show Draft” button to continue editing; all updates will show in TruSight Software Suite

after clicking “Approve.” To disable an association so it will no longer show up in TruSight Software Suite, users can click on the “Reject” button in the association page or bulk “Reject” a list of variants in the landing page.

Summary

Variant interpretation relies on manual curation, requiring a great deal of time and repeated effort. TruSight Software Suite offers various features and tools as part of an integrated workflow to aid with, and accelerate, variant curation and interpretation to help determine which prioritized variants are relevant to a case.

Learn more

TruSight Software Suite,
illumina.com/trusight-software-suite

Appendix

A quick guide to resources in TruSight Software Suite used in application of ACMG criteria for variant curation is provided. This is not an exhaustive list of resources used in curation, but refers to those resources available in TruSight Software Suite.

Quick guide to resources in TruSight Software Suite used in the application of ACMG criteria

ACMG criteria	Information used to apply criteria/ data source	Where to find the information/data
PVS1: Null variant (nonsense, frameshift, canonical +/- 1 or 2 splice sites, initiation codon, single- or multi-exon deletion) in a gene where loss of function (LoF) is a known mechanism of disease	Related Diseases (ClinGen+OMIM)	Gene Details tab
	gnomAD constraint metrics	
	ClinGen Dosage Sensitivity	Pathogenicity and Review status filter in the Variant Grid; link to ClinVar record will be provided in the Variant Details tab if the variant is present in ClinVar
	ClinVar	
Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP; PubMed search results for the gene and disease can be found in the Gene Details tab	
PS1: Same amino acid change as a previously established pathogenic variant regardless of nucleotide change	ClinVar	Link will be provided in the Variant Details tab, if the variant is present in ClinVar
	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
	Previously observed pathogenic variants	CaseLog needle plot in the CaseLog tab
PS2: <i>De novo</i> (both maternity and paternity confirmed) in a patient with the disease and no family history	Case data for proband - inheritance information for the variant under curation	Variant Details tab—Zygosity and Metrics
	Literature search	Google Scholar link found within the ACMG calculator for a search based on gene, its HGVS nomenclature and/or RefSNP
PS3: Well-established <i>in vitro</i> or <i>in vivo</i> functional studies supportive of a damaging effect on the gene or gene product	Literature search	Google Scholar link found within the ACMG calculator for a search based on gene, its HGVS nomenclature and/or RefSNP
PS4: The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls	Case-specific allele frequency	CaseLog section within the Variant Details tab
	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
PM1: Located in a mutational hotspot and/or critical and well-established functional domain (eg, active site of an enzyme) without benign variation	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
	Protein domain with previously observed pathogenic variant	CaseLog needle plot in the CaseLog tab

Quick guide to resources in TruSight Software Suite used in the application of ACMG criteria, cont.

ACMG criteria	Information used to apply criteria/ data source	Where to find the information/data
PM2: Absent from controls (or at extremely low frequency if recessive) in gnomAD	Population frequency with detail (gnomAD)	Variant Details tab
	Case-specific allele frequency	CaseLog section within the Variant Details tab
PM3: For recessive disorders, detected <i>in trans</i> with a pathogenic variant	Literature search	Google Scholar link found within the ACMG Calculator.
	Previously observed pathogenic variants	CaseLog needle plot in CaseLog tab
PM4: Protein length changes due to inframe deletions/insertions in a nonrepeat region or stop-loss variants	gnomAD	Gene Details tab
	UCSC Browser	Variant Details tab
PM5: Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
	Previously observed pathogenic variants	CaseLog needle plot in CaseLog tab
PM6: Assumed <i>de novo</i> , but without confirmation of paternity and maternity	Inheritance	Variant Details tab—Zygosity and Metrics
	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
PP1: Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease	Inheritance	Variant Details tab—Zygosity and Metrics
	Case data (family information)	Variant Details tab
	Literature search	Google Scholar link can be found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
PP2: Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease	ClinVar	Link to ClinVar record provided in the Variant Details tab if the variant is present in ClinVar
	Previously observed pathogenic variants	CaseLog needle plot in CaseLog tab
	Constraint metrics for the gene (gnomAD)	Variant Details tab
PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)	PrimateAI	Gene Details tab
	Polyphen2, SIFT, REVEL, N-Species AA, Consequence	Transcript tab
	SpliceAI	Variant Details tab (if available)

Quick guide to resources in TruSight Software Suite used in the application of ACMG criteria, cont.

ACMG criteria	Information used to apply criteria/ data source	Where to find the information/data
PP4: Patient phenotype or family history is highly specific for a disease with a single genetic etiology	Case summary	Available at the top of the Variant Grid
PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation	ClinVar	Pathogenicity and Review status filter in the Variant Grid; link to ClinVar record will be provided in the Variant Details tab if the variant is present in ClinVar
BA1: Allele frequency is > 5% in gnomAD	Population frequency with detail (gnomAD)	Variant Details tab
BS1: Allele frequency is greater than expected for disorder	Population frequency with detail (gnomAD)	Variant Details tab
	Disease prevalence and penetrance	Gene Details tab
BS2: Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age	Population frequency with detail (gnomAD)	Variant Details tab
BS3: Well-established <i>in vitro</i> or <i>in vivo</i> functional studies show no damaging effect on protein function or splicing	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
	Case data (family information)	Variant Details tab
BS4: Lack of segregation in affected members of a family	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
	Previously observed pathogenic variants	CaseLog needle plot in CaseLog tab
BP1: Missense variant in a gene for which primarily truncating variants are known to cause disease	gnomAD constraint metrics	Gene Details tab
	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
BP3: Inframe deletions/insertions in a repetitive region without a known function	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
	Protein domain with previously observed pathogenic variants	CaseLog tab

Quick guide to resources in TruSight Software Suite used in the application of ACMG criteria, cont.

ACMG criteria	Information used to apply criteria/ data source	Where to find the information/data
BP4: Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	PrimateAI	Gene Details tab
	Polyphen2, SIFT, REVEL, N-Species AA, Consequence	Transcript tab
	SpliceAI	Variant Details tab (if available)
BP5: Variant found in a case with an alternate molecular basis for disease	Literature search	Google Scholar link found within the ACMG Calculator for a search based on gene, HGVS nomenclature, and RefSNP
	Previously observed pathogenic variants	CaseLog needle plot in CaseLog tab
BP6: Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	ClinVar	Pathogenicity and Review status filter in the Variant Grid; link to ClinVar record will be provided in the Variant Details tab if the variant is present in ClinVar.
BP7: A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved	SpliceAI	Variant Details tab (if available)



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