

BASELINE ANNOTATION TEMPLATE PROTOCOL REVIEW

Title: [Gene of interest] Annotation Protocol:

This protocol is to outline the evidence, tags, and procedures to follow for the annotation of variants associated with the gene X (provide OMIM hyperlink).

Step 1: Read the ACMG variant interpretation seminal paper (Richards et al., 2015):

<https://www.ncbi.nlm.nih.gov/pubmed/25741868>

- This is to help familiarize individuals with the process of variant curation and it's importance for diagnostics.

Step 2: List important articles for the gene of interest that may inform the annotations for this gene.

- Examples:
 - o Variant specific guidelines
 - o Review papers on the gene and/or disease of interest.
- This section may be blank, and is dependent on the groups preference when generating the protocol.

Step 3: Perform the annotations.

- Table 1 shows an example of the two main annotations that will be used.
 - o The General annotation is linked to the title of the article of interest and should contain annotations indicating the PMID, gene name, and HGNC gene ID, as well as including tags for the same information.
 - Other tags of interest to possibly include are listed below.
 - o The Case report annotation is used to highlight an individual asserted to have a variant in the gene of interest.
 - This annotation should only be used if a person with a variant in the gene of interest has been reported.
 - The annotation will allow for the inclusion of demographic information, genotyping method(s), phenotypes, and more.
 - **Note:** Not all articles may include all of this information, that is okay. Annotate only what is indicated by the authors. For all other evidence categories, place an "n/a" to indicate the applicable information was not provided.
 - o It is important that when outlining the evidence asserted to use clear, concise and complete sentences.
 - o

Links for annotations:

1. Hypothesis curation group link (**Required**)
 - Provide the Hypothes.is annotation group link where all the annotations will be shared.
2. Targeted list of PMIDs (**Optional**)
 - o Groups may decide that the articles for annotation is open to the discretion of the annotator. In this case, please check the the article does not already contain annotation within your specific annotation group.
 - If it does, move on to another article.
 - o Alternatively, a specific date range may be targeted for articles of interest. Make sure to restrict your search results to this range.

- **Curators are encouraged to annotate open access articles, and thus utilize PubMed Central.**
This will ensure that all annotations and links can be observed by all members.

Note: Each Case report listed in an article should be curated as a separate annotation. For example, if there are 4 individuals reported in one paper with a variation in the gene(s) of interest, outlined in your annotation protocol, there should be 4 annotations for each report.

For case reports cited in Supplemental text, please use the “Page Note” function to annotate on the open access HTML version of an article. See the “Hypothes.is Annotation Overview” for more details.

TABLE 1: General annotation and Case report examples.

Category (tags)	Text to highlight	Information to add to annotation
<p>Article Information Annotation</p> <p>Tag with: General, HGNC gene symbol (e.g. Gene:XXX), HGNC gene ID (e.g. HGNCID:XXXX), PMID</p> <p>Additional tags as needed include: NoEvidence, InsufficientEvidence (see notes below)</p>	<p>Title of article</p>	<p>PMID: add PMID or article</p> <p>Gene: add the current HGNC approved gene name</p> <p>HGNC: add the HGNC ID, or identifying number. This allows for a standardized recognition of the gene of interest, even if the gene name changes.</p>
<p>Case-Individual Annotation</p> <p>Tag with: Each case report annotation should include the gene symbol tag, e.g. Gene:MYH7, and HGNC ID, e.g. HGNC:7577</p> <p>Further tags to use on each case-level annotation are listed below, Table 2.</p>	<p>The text surrounding the case of interest.</p> <p>Note: <i>For cases that are mentioned in the Supplement, use the “Page Note” function in Hypothes.is to annotate and indicate the Case was reported in the Supplement</i></p>	<p>Case#: Annotate with the Individual label, sex, age, ethnicity if known.</p> <p>DiseaseAssertion: Annotate with the disease assertion made by the authors for the proband/case of interest.</p> <p>FamilyInfo: Annotate with information on the family including descriptive mention in text, figure or table number, and demographic label is included.</p> <p>CasePresentingHPOs: Annotate with the HPOs presenting specifically in the proband of interest.</p> <p>CaseHPOFreeText: Annotate with phenotyping information that does not have an appropriate HPO number, or requires explanation.</p> <p>CaseNotHPOs: Annotate with the HPOs that were noted not to manifest within the proband of interest.</p> <p>CaseNotHPOFreeText: Annotate with phenotyping information that appears normal or unaltered in the patient, yet does not have an appropriate HPO number, or requires explanation.</p> <p>CasePreviousTesting: Annotate with a description of method and whether genome-wide analysis methods were used. <i>If other genes of interest to the disease were tested, list here. If they did a panel of genes, not the panel used and any reference to the gene list (i.e. table that lists genes on panel, or the name of the panel used).</i></p> <p>Variant: Annotate with the HGVS nomenclature for the variant. Note: <i>For autosomal recessive inheritance of compound mutations, you will need to separate variant entries and two separate ClinVar IDs or CA IDs.</i></p> <p>ClinVar: if known</p> <p>CAID: If no associated ClinVar ID, register the variant with the ClinGen Allele Registry (CA) and copy or create a CA ID.</p> <p>gnomAD: Annotate with the highest minor allele frequency for the variant in question if found in gnomAD, and add the link to the specific variant page.</p> <p>SupplementalData: When applicable, indicate the file, table, etc where the report information was located.</p>

Notes on specific tags to use for annotations:

1. For the disease entity, please annotate based on the assertion given by the authors. If the authors do not assert a disease entity, use **“NoDiseaseAssertion.”**
2. If there is no variant evidence, tag the General annotation note with **“NoEvidence.”** If there is insufficient evidence for the variant, i.e. variant reported for group analysis, tag with **“InsufficientEvidence”** and explain in the annotation why the evidence is insufficient.
3. For each proband, if mutations in other genes are listed, please annotate all variants in the report including the HGNC and ClinVAR or CAID if known, **however do not add these specific variant ID tags to the case tagging, instead use the tag “MultipleGeneVariants.**
 - a. Please see example annotation below to see how to annotate.
4. **Note:** If patient information is in the supplement, and there is no reference to the patient in the main text of the article, please create a Page Note to annotate the case report (Table 1 above).
 - a. Add the tag **“SupplementalData”** to the page note for the case report.
 - b. Indicate the supplemental file in which the information was found in the annotation text box.
5. If the proband has been previously published, tag with **“PreviouslyPublished”** and annotate within the annotation box the PMID is known.
 - a. In cases in which the whole cohort of individuals being published have been previously published, highlight assertion and tag with **“PreviouslyPublished.”**

TABLE 2: Annotation tags		
Required tags to add to annotation, on each proband and wherever applicable		
Category	Example Gene	Example Gene Tags
Gene Name	MYH7	Gene:MYH7
HGNC Gene ID	For MYH7 the HGNC ID is HGNC:7577	HGNC:7577
Article ID Most will be Pubmed Central (PMCID), but some may be PubMed (PMID)	PubMed Central article PMC6854592 (This article also has a PMID, 31638223)	PMCID:PMC6854592 (e.g. of PubMed ID is PMID:31638223)
Inheritance Pattern Note: For de novo, we are not requiring for you to determine this inheritance, this is based solely on the assertions of the author.	Autosomal Dominant Autosomal Recessive De novo* X-linked No inheritance assertion	InheritancePattern:AutosomalDominant InheritancePattern:AutosomalRecessive InheritancePattern:DeNovoAssertion InheritancePattern:Xlinked InheritancePattern:NoInheritanceAssertion
Disease Entity Note: if more than one is asserted for the proband please add all applicable tags if there are disease assertions beyond these listed, highlight the text and tag annotation with “DiseaseAssertion”	A list of the most well-known disease entities associated with the gene of interest can be included. Also include a tag to indicate when no assertions have been indicated, e.g. “No disease assertion”	DiseaseEntity:HCM DiseaseEntity:DCM DiseaseEntity:LVNC DiseaseEntity:RCM DiseaseEntity:LVH DiseaseEntity:ARVC DiseaseEntity:Myopathy DiseaseEntity:NoDiseaseAssertion
Mutation To indicate Germline versus Somatic according to authors assertion.	Germline Somatic	Mutation:Germline Mutation:Somatic

Zygoty	Homozygous Heterozygous Hemizygous Compound Heterozygous No zygoty assertion	Zygoty:Homozygous Zygoty:Heterozygous Zygoty:Hemizygous Zygoty:CompoundHeterozygous Zygoty:NoZygotyAssertion
Variant	(1) ClinVar ID- please annotation with the number (2) ClinGen Allele Registry ID- CAiD (3) Please also label cases in which you are unable to determine the variant ID (4) For cases that have multiple independent gene mutations listed	(1) "ClinVarID:#####" (2) "CAID:CA#####" (3) "UnregisteredVariant" (4) "MultipleGeneVariants"
Family Information Note: If the proband is part of a family with a history of the disease, and the family meets the criteria established for inclusion for scoring per the ClinGen Gene Curation SOP, tag the proband	(1) For cases that outline a family pedigree (2) For cases asserted by authors to have a family history with no pedigree or descriptive info beyond "family history" (3) For cases in which authors assert a descriptive segregation (i.e. brother has disease and variant)	FamilyInfo
Supplemental Data	If you find that the proband(s) variant information is listed strictly in the Supplemental data, tag each proband. Proband Case Report (Table 1) can be made as a Page Note in Hypothes.is on the main text.	SupplementalData
Tag for the paper or evidence specific to experimental, not to be used with proband tags		
Experimental Assay	If you find a variant associated with an experimental assay, annotate with ClinVar or CAiD.	ExperimentalAssay ClinVarID:### CAID:CA#####"
Optional Additional Tags, based on group preference		
Mutation Type	Missense Nonsense Frameshift small insertion/deletions Inframe small insertions/deletions Intronic Regulatory (e.g. 5'UTR) Large (multi-exon) insertion or deletion Complex rearrangement	Mutation:Missense Mutation:Nonsense Mutation:Frameshift Mutation:Inframe Mutation:Intronic Mutation:Regulatory Mutation:Large Mutation:Complex
Previous Testing Note: This section may or may not be applicable for every expert panel or working group. Describe any previous testing that would be appropriate for an annotator to record.	Testing performed Testing noted to not be performed Testing unknown	PreviousTesting:Performed PreviousTesting:None PreviousTesting:Undetermined

- **It is important to note that for tags, DO NOT include a space between the colon (:)** and the following word. This would alter the ability to recall information accurately.

EXAMPLE ANNOTATION:

Case-report annotations



MYH7 variant curation

Table III

Case 748: Italian, Female, 16yo

DiseaseAssertion: HCM

FamilyInfo: Family history of HCM and sudden cardiac death

CasePresentingHPOs: HP:0001712, HP:0004756, HP:0005110, HP:0001716 (left ventricular hypertrophy, ventricular tachycardia, atrial fibrillation, Wolff-Parkinson-White syndrome)

CaseHPOFreeText: maximum left ventricular wall thickness of ≥ 15 mm, LVWT = 17mm, automatic implantable cardioverter defibrillator, LV ejection fraction = 60%

CasePreviousTesting: echocardiography, Full testing (MYBPC3, MYH7, TNNT2, TNNI3, TPM1, MYL2, MYL3, ACTC1), additional testing: (GLA, LAMP2, PRKAG2), additional genes added with NGS: (TNNC1, MYH6, MYOM1, MYOZ2, ANKRD1, VCL, CALR3, CAV3)

CaseGenotypingMethod: Genomic DNA extracted from peripheral leukocytes, Sanger sequencing (73 samples), Next-gen sequencing (19 samples)

Variant: NM_000257.3(MYH7):c.5287G>A (p.Ala1763Thr)

ClinVar: 177846, <https://www.ncbi.nlm.nih.gov/clinvar/variation/177846/>

gnomAD: 0.00007423, <https://gnomad.broadinstitute.org/variant/14-23884476-C-T>

MultipleGeneVariants:

(1) LAMP2 Variant:

Variant: NM_002294.2(LAMP2):c.928G>A (p.Val310Ile)

ClinVar: 9982, <https://www.ncbi.nlm.nih.gov/clinvar/variation/9982/>

gnomAD: n/a

Less

Individual CaseInfo HCM FamilyInfo FullPreviousTesting
AdditionalPreviousTesting ClinVarID:177846 MultipleGeneVariants
AutosomalDominant

