InSiGHT Variant Interpretation Committee
Standard Operating Procedures
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1 Purpose

To provide a guide for members of the InSiGHT Variant Interpretation Committee (VIC) to follow during the process of variant interpretation. These SOPs could also be adapted by committees interpreting variants in other genes and diseases.

2 Scope

For members of the InSiGHT VIC who are administering or participating in meetings, teleconferences or online discussions for the purpose of classifying variants. This includes database curators, researchers, clinicians or other professionals. Curators can use this document to assist with running variant interpretation teleconferences. Reviewers may refer to this document to understand the process of variant interpretation. Teleconferences and/or meetings are run 3-4 times per year. Timing of teleconferences is subject to change and is determined by the curator who has to arrange the teleconference. Currently, these interpretation process guidelines apply to the Lynch Syndrome mismatch repair genes: MLH1, MSH2, MSH6 and PMS2. In the future, more genes may be included for interpretation.

3 Glossary of terms

Committee - The InSiGHT Variant Interpretation Committee (VIC)

Curator - The InSiGHT database curator.

Reviewer - A member of the InSiGHT Variant Interpretation Committee who has been assigned variants to review for a teleconference.

Chair - The chair of the InSiGHT Variant Interpretation Committee.

Database - The InSiGHT database, available at www.insight-database.org. Note that variant classifications are available at www.insight-database.org/classifications. Variants that have been reclassified are available at http://www.insight-database.org/classifications/reclassifications.html.

InSiGHT website - The official website of InSiGHT, available at www.insight-group.org.

Variant - A change in DNA sequence against a reference sequence.

VUS - Variant Of Uncertain Significance.

Segregation - Shorthand for co-segregation - denotes whether a variant is transmitted in a family along with disease.

Pathogenicity - Whether or not a variant is causative for a disease. Represented as a probability between 0 and 1, which is also mapped to a 5 tier classification system (Thompson, Bryony A., et al. 2014).

Multifactorial/Bayesian - Refers to a method of calculating the probability of pathogenicity using multiple types of evidence.

Pedigree - A representation of a family over a number of generations specifying relationships between individuals, and their associated genotype and phenotypes. This representation can be a drawing, or a digital file storing data with a recognised pedigree data structure.

4 How to read this document

The document is divided into 2 main sections corresponding to the 2 roles of Curator (teleconference administrator) and Reviewer (teleconference participant). The subsections are in order of the steps that would take place chronologically before, during and after a teleconference.

5 Responsibilities of the curator

The curator is responsible for selecting variants, assembling information or providing directions for where to access information about specific variants, and running the teleconference.

5.1 Teleconference Setup

The curator typically instigate the steps for a teleconference. Different teleconference software can be used. Currently, freeconferencecall.com is preferred because it requires only the cost of a local telephone call. Some participants in certain countries may not be able to access this service - it will need to be determined if they can call into the teleconference via an international number. The VIC usually has between 10-20 participants at each teleconference. Reviewers should be provided a minimum of 2 weeks to perform their reviews. A call for information should be performed first, and allow 2 weeks for responses. So in total, 1 month is required before the teleconference. The curator is to announce the teleconference, ask for preferred dates from participants, assign variants to Reviewers, send out available information and finally collate the reviews before running the teleconference. An agenda for the teleconference should be compiled and sent out to the committee.

5.2 Selecting variants

There are 2 different ways to select variants for a VIC meeting.

1) Email requests received from genetic counsellors, clinicians or diagnostic laboratories.
2) Selecting variants from the database.

Both methods can be used to select enough variants for a single meeting, or one method may suffice. Generally, there is a large amount of variants that require classification, so prioritization of variants is required.

5.3 Variant Prioritization

Prioritizing variants can be done in a variety of ways. One way is to check for new variant submissions. If a new submission has occurred for an already existing VUS, then this new submission may provide sufficient information to reclassify the variant. Variants with detailed information such as pedigrees, tumour testing results, and multiple reports should be given

more priority than variants with only minimal information or single reports. Variants should also be compared with other database such as ClinVar and UMD for additional reports.

Where there is a backlog of variants, the priority should be on classifying problematic variants (e.g. missense variants), especially where there appears to be substantial clinical implications eg a large family, rather than on variants that are pathogenic based on their sequence change (e.g. truncating variants).

Variants can also be prioritised based on characteristics such as what gene it is in. Some genes (e.g. MLH1) have a large number of variants requiring more information. Variants in highly conserved regions, or regions of no conservation, may also be another way to prioritize.

5.4 Requesting new information

The database curator may request information from submitters or InSiGHT members prior to a teleconference. The list of InSiGHT members is available on the membership section of the InSiGHT website: https://www.insight-group.org/members/current-members/. Information may be requested for specific variants, or request data on any new variants in general. For example, requesting information for any variants in conserved regions may see substantial responses.

5.5 Pedigree analysis / Multifactorial analysis

Segregation analysis is considered the gold standard method of variant interpretation. However, it is not always possible to obtain the necessary amount of information from a family. Briefly, segregation analysis determines the likelihood a variant is pathogenic based on pedigree information. Multifactorial analysis uses a variety of different data types, that have been calibrated to produce a Bayesian probability of pathogenicity.

Pedigree data is sent confidentially to the curator via email. The pedigree is not published. Segregation analysis is performed by experienced researchers to ensure consistency.
The data used for multifactorial analysis is received from a variety of sources including email and database submissions, published and unpublished information, and includes segregation likelihood if available.

Pedigree likelihood score and multifactorial probability are published on the database. Note that the process of analysing pedigrees and multifactorial analysis usually takes place independently of the teleconference process. However, the outcome of these analysis will need to be confirmed/approved by the VIC during a teleconference, or by email.

5.6 Appointing Reviewers

There is usually more reviewers available on the committee than necessary for a teleconference.
A simple way to select reviewers is to choose from those who have replied to the teleconference date selection poll (e.g. doodle poll). This is an indication of their availability to review variants and participate in the teleconference. Not all participants will be required to review variants (see

assigning variants to reviewers). Reviewers who are new or have not participated previously should be encouraged to perform reviews to distribute the workload as much (and as evenly) as possible.

5.7 Assigning variants to Reviewers

Variants can generally be assigned to reviewers randomly. Some reviewers may prefer certain genes over others, this should be communicated to the curator. If a reviewer is also a requestor of the variant to be reviewed, than the variant should not be assigned to that reviewer. Submitters may, on the discretion of the chair, be invited to attend as an observer.

In general, anywhere from 10 to 15 variants is a sufficient number of variants to review during a 2 hour teleconference. Variants should be assigned such that each variant is assigned to 4 independent reviewers. Currently, this is done on a spreadsheet. Note that should a reviewer not be able to complete their review, then that will leave 3 independent reviews. 2 independent reviews are the minimum required. The committee will make their decision, taking into account all the reviewers classifications and remarks.

5.8 Other meetings / Interpretation by Email

Sometimes, the committee may meet in person during conferences. The process of interpretation remain largely the same, except for the teleconference, data may be presented on a projector rather than sharing a spreadsheet.

It is also possible that variants can be approved by the committee using email only (as opposed to the teleconference process). This could be in the case of urgent requests for variant interpretation, or for speeding up the process of variant interpretation overall. Similarly to the teleconference, each variant should be assigned to 4 independent reviewers. The outcome of the email interpretation should be sent to the whole committee for approval. The precise process of interpretation by email requires further development.

5.9 Collation of variant reviews

Just prior to the teleconference, the curator will collate returned reviews into a single document (with one variant per worksheet), and send this document to the VIC (and to Reviewers).

5.10 The Teleconference

The chair of the VIC will usually chair the teleconference. If the chair is unable to participate, the curator may chair the teleconference adhering to the agenda items. The curator will need to ensure that minuting and/or recording of the discussion occurs. The teleconference system may have an option for teleconference recording. The curator should ensure that each variant's classification, the summary evidence for each classification, and any changes to the classification criteria are minuted. If there is unpublished information made available during the teleconference, this should be minuted.

A teleconference will generally take between 1 and 2 hours.

5.11 Post-teleconference

Outcomes of the VIC deliberations are published on www.insight-database.org/classifications and is dated, to ensure the classification outcome is related to the dated version of the criteria. To add the classification, the curator must edit the appropriate classification field on a google spreadsheet. For variants where the classification has changed (i.e. reclassified), the "previous classification" field will need to be updated too. The spreadsheet is located at: https://docs.google.com/spreadsheets/d/1vQ-x3R9NYdjoXR9aLdCphF0PspeCQhsyZZCnalqDMr4/. Only authorised accounts can edit this spreadsheet. Any new variants receiving a classification will need to be added to bottom of the spreadsheet. The changes made to this spreadsheet will automatically be reflected on the classifications website.

Changes made to existing classifications, for example due to new information, need special attention and notification to the submitters. There is a specific section on the website that have variants that have been reclassified: www.insight-database.org/classifications/reclassifications.html

InSiGHT New & Reclassified Variants

Criteria used for classification are available from here.
We encourage submission of relevant unpublished information to assist in the classification of variants via LOVD or this template, and such contributions are recognised by microattribution.
Contact: InSiGHT curator John-Paul Plazzer

Reclassified variants

Gene	Variant	Protein	Concensus InSiGHT Classification	Classification Date	Summary Justification	Previous Classification
MLH1	c.191A>G	p.(Asn64Ser)	Under review	Under review by the InSiGHT VIC (November 2016)	Multifactorial likelihood analysis posterior probability 0.95-0.99	Class 4 v1.9: 5/09/2013
MSH2	c.2635C>T	p.(Gln879*)	Under review	Under review by the InSiGHT VIC	Coding sequence variation resulting in a stop codon	Class 5 - v1.9: 5/09/2013
MSH2	c.2647del	p.(Ile883Leufs*9)	Under review	Under review by the InSiGHT VIC	Coding sequence variation resulting in a stop codon	Class 5 - v1.9: 5/09/2013
MSH2	c.2647dup	p.(Ile883Asnfs*16)	Under review	Under review by the InSiGHT VIC	Coding sequence variation resulting in a stop codon	Class 5 - v1.9: 5/09/2013
MSH2	c.2653C>T	p.(Gln885*)	Under review	Under review by the InSiGHT VIC	Coding sequence variation resulting in a stop codon	Class 5 - v1.9: 5/09/2013
MSH2	c.2662del	p.(Leu888Cysfs*4)	Under review	Under review by the InSiGHT VIC	Coding sequence variation resulting in a stop codon	Class 5 - v1.9: 5/09/2013
MSH2	c.2740G>T	p.(Glu914*)	Under review	Under review by the InSiGHT VIC	Coding sequence variation resulting in a stop codon	Class 5 - v1.9: 5/09/2013
MSH2	c.2785C>T	p.(Arg929*)	Under review	Under review by the InSiGHT VIC	Coding sequence variation resulting in a stop codon	Class 5 - v1.9: 5/09/2013
MLH1	c.122A>G	p.Asp41Gly	Class 3: Uncertain	Awaiting further review by VIC	RNA Analysis results from Van der Klift et al., (in preparation) conflicts with previous published results.	Class 5 - v1.9: 5/09/2013
MLH1	c.1153C>T	p.(Arg385Cys)	Class 3: uncertain	Awaiting further review by the VIC	Awaiting further review by the VIC	Class 4 - v1.9: 5/09/2013

Figure 1: View of the reclassifications listed on InSiGHT classification website.

Minutes of each meeting are formulated by the curator and circulated within 2 weeks. Update LOVD variants if their classifications have changed. Classifications are sent to ClinVar using their submission spreadsheet available at https://submit.ncbi.nlm.nih.gov/clinvar/. Note that the curator has an account on ClinVar which is linked to InSiGHT's Expert Panel status - this account should be used for submission. Contact anyone who requested variant reviews (this can be done automatically if their variant is on LOVD - they will receive an email when the

classification is updated on LOVD). All stakeholders who have an interest in variants discussed (whether the classification is changed or not) are notified of the outcomes of classification. If there are specific variants that could be classified with more information which is likely to be available, a call to the submitters, and possibly to the broader InSiGHT community, should be made.

Figure 2: View showing the InSiGHT classifications website.

6 Responsibilities of Reviewers

6.1 Funding for the operations of the VIC

Members of the VIC volunteer their time. The proper functioning of the VIC and the database requires a full time curator.

6.2 Prerequisites to become a Reviewer

Membership of InSiGHT is necessary. This is to protect the reviewer medico legally as the functions of members of InSiGHT are covered for legal liability to the extent short of criminal activity due to its incorporation. Cover allows protection to VIC members in the event of adverse health outcomes due to misclassification, despite due process. Membership of the VIC is open to all InSIGHT members. Appointments however will be made at the discretion of the chair. Commercial employees, provided they are InSiGHT members, may join the VIC.

The chair will be appointed by Council on the recommendation of the governance committee.

6.3 Understanding the Classification Criteria

The criteria by which the VIC classifies variants is made publically available and is readily available to all reviewers (see Appendix). As the criteria change from time to time, the dated version of the criteria used at any particular meeting is included in the minuted outcome. A copy of the current criteria is in the Appendix. Reviewers must have a good understanding of the current InSiGHT classification criteria.

6.4 Electing to participate in a teleconference

Reviewers are asked before a teleconference to select their preferred time and date for teleconference - this is done via email. Once a date and time is selected, reviewers will be notified via email. Reviewers have to be mindful of the time they have leading up to a teleconference - they may need several hours of time to review a small number of variants (3 to 5 variants per teleconference).

6.5 Completing the review

The curator will assign specific variants to reviewers . An email with the assigned variants and links to new information will be sent to reviewers (and to the VIC). Anyone who is not assigned variants to review may still contribute their own information via this email chain.
Reviewers should fill in the appropriate fields on the classification spreadsheet using information available to them (both published and unpublished). Reviewers should email their completed review at least 1 day prior to the teleconference, for the curator to collate the reviews into one document

6.6 The Teleconference

There is one spreadsheet that is distributed to participants on the day before the teleconference. Sometimes, new information is added to this spreadsheet by reviewers right up until the teleconference commences. Reviewers should access the latest version just prior to the teleconference - this is often available in the teleconference emails.

During the teleconference, reviewers should have access to the spreadsheet to follow the variant discussion. Any other material that the reviewer finds relevant can also be used, and where appropriate, shared with the committee beforehand.

Unpublished information available to the committee such as during the teleconference may be taken into consideration. If there is consensus across all reviewers who are nominated to review specific variants, that variant is afforded rapid passage for approval by the committee; if there is a discrepancy, these variants are considered in detail, in an attempt to reach consensus.
If no consensus is reached, the variant remains in the least pathogenic class.

A teleconference will generally take between 1 and 2 hours.

6.7 Updating the Classification Criteria

Changes to the classification criteria may be proposed or discussed during a teleconference. The committee may agree to the changes. All members of the committee should be notified of changes.

6.8 Dispute resolution

If there remains a dispute about classification, a vote is taken in the attending VIC. If there is a tie, the chair is allowed a casting vote.

6.9 Other meetings / Interpretation by Email

Sometimes, the committee may meet in person during conferences. The process of interpretation remain largely the same, except for the teleconference, data may be presented on a projector rather than sharing a spreadsheet.

It is also possible that variants can be approved by the committee using email only (as opposed to the teleconference process). This could be in the case of urgent requests for variant interpretation, or for speeding up the process of variant interpretation overall. Similarly to the teleconference, each variant will be assigned to 4 independent reviewers. Reviews are sent back to the curator for dissemination to the committee for final approval.

7 References

Thompson, Bryony A., et al. "Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the InSiGHT locus-specific database." *Nature genetics* 46.2 (2014): 107-115.

8 Appendix

The classification criteria.