Title:	**Reclassification of GLA variants following ACMG guidelines**
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1. Purpose and Objective

This SOP aims to standardize the application of ACMG guidelines for reclassification of GLA variants detected at CENTOGENE in patients with suspicion or confirmation of Fabry disease; thus, optimizing, improving and streamlining the accuracy and reproducibility of the GLA variant interpretation process.

2. Area of Application

GLA variant reclassification process is the key element of variant reevaluation either when additional information at hand, or regularly based on the new existing evidence(s). Every GLA variant must be examined during variant reclassification process in the light of literature, publicly available clinical databases and internal observations.

3. Terms and Abbreviations

ACMG: American College of Medical Genetics

SW/IT: Software developer/Information Technology employee

VUS: Variant Uncertain Significance

CuRepo: Curation Repository

FD: Fabry disease

4. Applicable Documents

ACMG guidelines (Richards S 2015)

SOPeIT-82 Classification of GLA variants following the ACMG guidelines

SOPeR-36 Variant Classification following ACMG guidelines at Centogene

SOPeIT-40 Curation Repository High Risk changes

ACMG guidelines (Richards S 2015);

https://www.acmg.net/docs/Standards_Guidelines_for_the_Interpretation_of_Sequence_Variants.pdf

5. Responsibilities

This SOP is valid for all employees responsible for at least one of the following processes: GLA variant analysis, interpretation/annotation, review and update (i.e. reporters, medical staff and curators).

6. Reagents, materials and devices

database	**description**
CentoMD®	a variant database; product of Centogene.
Curation repository (CuRepo)	a system used to curate data produced at Centogene, and periodically feeds CentoMD-data database.
UniDB	Centogene's central variant database; includes sample information like gender, symptoms, family relations and more.
ClinVar	a freely accessible, public archive of reports of the relationships among human variations and phenotypes, with supporting evidence.

HGMD®	represents an attempt to collate all known (published) gene lesions responsible for human inherited disease.
NCBI PubMed	comprises more than 30 million citations for biomedical literature from MEDLINE, life science journals, and online books

7. Procedure

7.1 Workflow

Below is a schematic representation of the variant reclassification process:

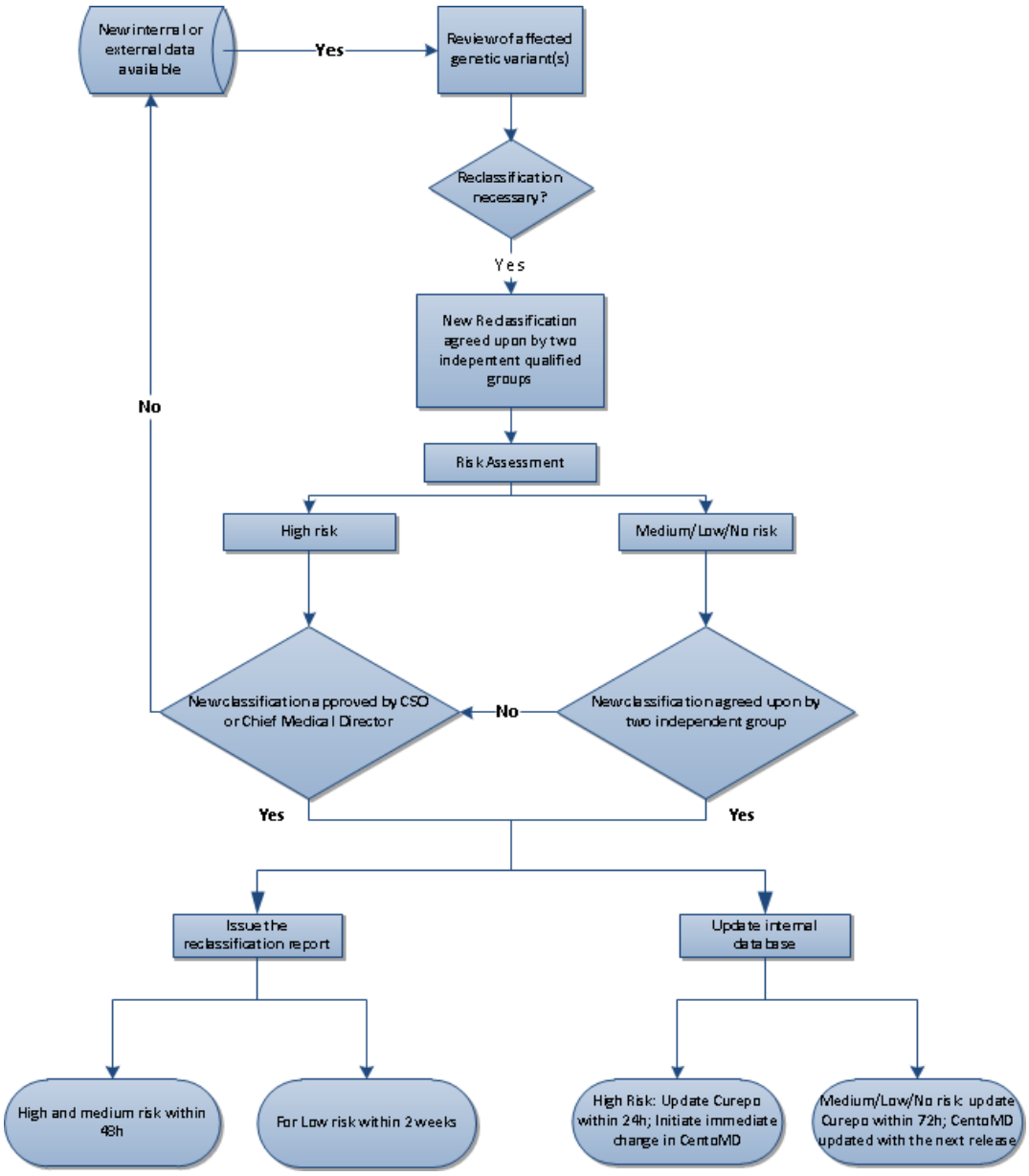

Figure 1: A schematic workflow of GLA variant reclassification process. A reclassification process is triggered when a

new data related to an affected genetic GLA variant is available. If the new data is informative for a reclassification, the risk category will be defined. High-risk reclassification needs approval of medical director or Chief Scientific Officer (CSO). For other risk groups of reclassification, two independent group of qualified employees will approve the new classification. If the reclassification takes place of a GLA variant, a reclassification reports is issued (for High and Medium risks within 48 hours). The internal databases (Curepo and CentoMD) are updated accordingly (for High-risk reclassifications within 24 hours). For a detailed description please see sections 7.2 to 7.5 of this document.

7.2 Involved departments and responsible team members

The following departments and staff are involved in GLA variant reclassification process: Integrated Bioinformatics, Medical reporting, classification team, Chief Medical Director, Chief Scientific Officer and Quality Management Officer. Reclassification of any GLA variant in the Centogene is reviewed and proceed in two independent steps: First step within integrated Bioinformatics and medical reporting and the second step within classification team.

STAFF/DEPARTMENT	PROCESS INITIATION	FINAL APPROVAL	CORRECTIVE ACTIONS
Integrated Bioinformatics, Spanish/Portuguese reporting, Standard reporting, Classification team, HTT reporting	+	+ (medium, low or no risk)	Issuing reclassification report
Data curation	-	+ (medium, low or no risk)	Updating of internal data base and CentoMD
Chief Medical Director / Chief Scientific Officer	+	+ (high risk)	Medical communication in urgent/high risk cases
Quality Management	-	-	Monitoring

Table 1: Presentation of staff and departments involved in reclassification of GLA variants. For high risk variants involvement of Chief Medical Director and Chief Scientific Officer is mandatory, while for medium risk and high-risk reclassification, two independent departments involves in the process and final approval.+: staff should act as indicated process; -: staff should not act as indicated process.

7.3.1 New data available

Revision of the clinical significance of a GLA variant must be initiated as soon as new or contradictory data becomes available. The data refers either to external sources (HGMD, ClinVar, genomAD, PubMed) or to internal observations (allele frequency, genotype- phenotype correlation; biomarker analysis) in FD-informative cases. An informative case in the FD is a male person with a hemizygous genotype in the GLA and suspected of having FD.

The internal data originating in non FD- informative cases are excluded during the reevaluation process. The reclassification process can also triggered through feedbacks from the users of the database.

Any new conflicting evidences for GLA variant classification is handled by SOPeIT-82 Classification of GLA variants following the ACMG guidelines.

For GLA gene, the most reliable evidences used for reclassification are based on Lyso-Gb3 biomarker observations. The Lyso-Gb3 biomarker measurement is performed routinely for any new FD-informative who carries informative genotype.

This can lead to reclassification of a variant that had been classified previously in the absence of Lyso-Gb3 measurement. Presence of at least of FD-informative case of informative genotype (i.e. homozygous or compound heterozygous) will lead to variant reevaluation, and ultimately variant reclassification.

In the absence of Lyso-Gb3 measurements in informative FD patients with informative GLA genotype, other evidences are used: functional characterization newly available in PubMed; discordant phenotype- genotype correlations (genomAD identifies the variant in homozygous state in healthy adult population); or the allele frequency above the threshold of the AR disease (>0.005).

In addition, whenever Lyso-Gb3 measurements is not feasible, but genotype-phenotype correlation is possible (in families with at least five family members, or two independent Trio families), segregation – based evidences are used for variant reevaluation.

Any revision of the classification (reclassification) is versioned in the database, changes are identified and maintained overtime.

7.3.2. Review of new evidence within two independent team

Review of any GLA variant affected by new evidence is initiated in the medical reporting team as first independent group reviewing new evidence. Most frequent type of evidences are as flow:

- Updated frequency data in public or internal databases (for a complete list of databases used to assess frequencies in different populations please see SOPeR-36_GLA)
- New publication available in HGMD® or Pubmed
- New ClinVar entry available
- The identification of healthy adult individuals that are hemi-/homozygous for the variant (in public or internal databases, publications, family or patient history)
- Normal lyso-Gb3 biomarker levels and normal alpha-galactosidase levels (please refer to the SOPeIT-82 for the classification of GLA variants), in naïve FD patients (not under treatment, such as enzyme replacement therapy), that are hemi-/homozygous for the variant.
- Either increased lyso-Gb3 biomarker levels (<2.8 ng/ml) in patients or decreased enzymatic level of <=15.3 µmol/l/h that are hemi-/homozygous for the variant
- The variant has been observed in cis or trans with other GLA pathogenic variant(s)
- A variant in another gene has been confirmed to cause the patient's phenotype.
- Updated patient history including biochemical testing and/or positive family history and/or segregation analysis.

7.3.3. Risk assessment of reclassification

After confirmation of reclassification by both groups, risk assessment is measured according to the new impact on patient diagnosis.

As described in figure 1 above, are two main pathways to follow: high impact path (which implies a change in diagnosis; called high risk] and lower impact (which implies no / low or unclear impact on diagnosis; called medium / low/ no risk)

HIGH RISK

The variant reclassification impacts the clinical diagnosis of the patient. The following changes are included under high risk category:

(likely) pathogenic ↔ (likely) neutral/benign

A such risk leads to immediate action (as described under 7.5.)

MEDIUM RISK

The variant reclassification may impact the clinical diagnosis of the patient. The following changes are included under medium risk category:

(likely) pathogenic ↔ VUS

VUS ↔ (likely) neutral/benign

A such risk leads to action within two weeks (as described under 7.5.)

LOW RISK

The variant reclassification does not impact the clinical diagnosis of the patient. The following changes are included under low risk category:

pathogenic ↔ likely pathogenic

A such risk leads to action within two weeks (as described under 7.5.)

NO RISK

The variant reclassification does not impact the clinical diagnosis of the patient, as the variant has not been reported before. The following changes are included under no risk category:

benign ↔ likely benign

7.3.4. Actions according to the risk of the classification change

HIGH RISK

- The classification change request form (Appendix 1 of SOPeIT-40) needs to be filled by the initiator and sent to the Chief Scientific Officer for approval.
- Approval is given by signing the form and sending it back to the person that initiated the reclassification.
- Upon approval the email needs to be forwarded to all people involved in variant classification. The subject should include the term “reclassification”, the risk category, the gene name, transcript and cDNA change.

The email should contain a short description of the classification change following the ACMG terminology and the patient IDs of patients diagnosed at Centogene that are impacted by the reclassification according to the Curation Repository.

Example:

> Dear Curation team,
>
> As approved by XXX ((please replace XXX with the abbreviation for the colleague, that confirmed the new classification)), I would like to reclassify the GLA (NM_000157.3) variant cDNA change (amino acid change) from pathogenic to likely benign.
>
> Please see attachment for details.
>
> Impacted patients for reclassification according to Curation Repository: XXX (please replace XXX with the Patient IDs).

Please send to the following email addresses:

all-medical-reporters@centogene.com

all-diagnostics-bioinformatics@centogene.com

reclassification-team@centogene.com

- A reclassification report must be written within 48 hours after informing all responsible employees for every patient for whom the variant has been reported with the now outdated classification (according to the Curation Repository). No reevaluation of the case must be done (please see the example at the end of this section).
- A member of the data curation department will take care that CuRepo is updated within 24 hours after receiving the notification email. An update of CentoMD will be initiate within the same time frame and will be done via partial transfer (i.e. IT service management).

MEDIUM, LOW RISK, NO RISK

- The new classification needs to be agreed upon by two independent members of the departments detailed in section 7.2 of this SOP (see “FINAL APPROVAL” column); at least one of them needs to be an experienced colleague (i.e. has at least one year of experience in classification of variants).
- A notification email must be sent to all people involved in variant classification. The subject should include the term “reclassification”, the risk category, the gene name, transcript and cDNA change.

The email should contain a short description of the classification change, a list of reasons for changing the classification including the ACMG evidences that are affected, and the patient IDs of patients diagnosed at Centogene that are impacted by the reclassification according to the Curation Repository.

Example:

> Dear Curation team,
>
> In agreement with XXX *[please replace XXX with the abbreviation for the colleague, that confirmed the new classification]* I would like to reclassify the GLA (NM_000157.3) *variant cDNA change (amino acid change)* from variant of uncertain significance to likely pathogenic.
>
> Previously assigned ACMG criteria: PM2, PP3
>
> Newly assigned ACMG criterion: PS4
>
> This variant has been reported in one patient (PMID: 30094525).
>
> This variant has been detected the three times in house (order IDs) in patients whose symptoms overlap with the ones described for the related disorder.
>
> Impacted patients for reclassification according to Curation Repository: XXX *[please replace XXX with the Patient ID]*.
>
> Regards,
>
> XXX

Please send to the following email addresses:

all-medical-reporters@centogene.com

all-diagnostics-bioinformatics@centogene.com

reclassification-team@centogene.com

- A reclassification report must be written for every patient for whom the variant has been reported with the now outdated classification (according to the Curation Repository). No reevaluation of the case must be done (please see example at the end of this section).

MEDIUM RISK: within 48 hours after informing all responsible people

LOW RISK: within 2 weeks after informing all responsible people

NO RISK: no reports must be issued

- A member of the data curation department will take care that CuRepo is updated within 72 hours after receiving the notification email. CentoMD will be updated with the next release/version update.

8. References

The SOP is in line with ISO15189, CAP and CLIA guidelines.

9. Appendices

No attachments.