CENTOGENE
THE RARE DISEASE COMPANY

Title:	**GBA Gene- Gaucher Disease Association And Curation**
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1. Purpose and Objective

This Standard Operating Procedure (SOP) describes the process of association of GBA genetic variants, related biochemical results (enzymatic activity and biomarker level), and the provided clinical information for analyzed patients at Centogene AG with different types of Gaucher disease.

2. Area of Application

This SOP applies to Reporting and Curation departments at Centogene.

3. Terms and Abbreviations

GBA: b-glucocerebrosidase

GD: Gaucher disease

CuRepo: Curation repository

CNS: central nervous system

Lyso-Gb1: glucosylsphingosine

HPO: Human Phenotype Ontology

VUS: Variant of Uncertain Significance

WES: Whole Exome Sequencing

DBS: dried blood spot

G2P: Genotype – to – Phenotype

ERT: enzyme replacement therapy

IOs: Internal observations

LIMS: Laboratory information management system

OMIM:Online Mendelian Inheritance in Man

4. Applicable Documents

SOPeIT-81 GBA genetic variants classification

SOPeIT-85 GBA variant curation

SOPeIT-36 Adding new genes, transcripts and diseases in Curation Repository

SOPeIT-78 Curation of GBA cases

5. Responsibilities

This SOP applies to all employees responsible for curating gene- disease associations.

6. Reagents, materials and devices

Software:

- UniDB: http://ts0001.russ.CENTOGENE.internal/unidbweb/variantsearch
- CentoMD®: www.centomd.com

- Curation Repository: https://srv-centomd.CENTOGENE.internal/curation-repo
- OMIM:https://www.omim.org
- Gepado: https://gepado-prod.centogene.internal/Xpro/
- HPO : https://hpo.jax.org/app/
- CentoLSD: https://www.centogene.com/centolsd.html

Other websites often used during gene disease association and curation:

- Pubmed: https://www.ncbi.nlm.nih.gov/pubmed/
- GeneReviews: https://www.ncbi.nlm.nih.gov/books/NBK1116/
- Orphanet: https://www.orpha.net/consor/cgi-bin/index.php

7. Procedure

Before proceeding

A. Background:

GBA gene: is located on chromosome 1 (1q21) and encodes for acid beta-glucocerebrosidase, also known as beta-glucosidase (GBA), and is a Lysosomal enzyme that catalyzes the breakdown of the glycolipid glucosylceramide (GlcCer) to ceramide and glucose.

Gaucher's disease (GD) is a systemic, autosomal recessive disorder that can present with a various degree of systemic and neurological manifestations. According to the severity of the disease and the neurological involvement, the following types of GD have been identified:

- Gaucher disease type 1 OMIM 230800
- Gaucher disease type 2 (acute) OMIM 230900
- Gaucher disease type 3 (subacute/chronic) OMIM 231000
- Gaucher disease, perinatal-lethal form OMIM 608013
- Gaucher disease, cardiovascular form OMIM 231005

B. Workflow description

The gene- disease association and curation process implies the review of evidences from internal databases (UniDB, CuRepo, CentoMD®) and the external OMIM database for identification of appropriate GD form.

The internal evidences are collected from internally analyzed patients. These patients were referred at Centogene as:

i. Primary request to confirm the clinical suspicion of GD (targeted diagnostics)

The workflow at CENTOGENE of this test type, starts with a blood-based enzymatic test, which measures the activity of the GBA-encoded enzyme glucocerebrosidase. If this test is positive, a GBA-specific next generation sequencing (NGS)-based assay and quantification of a Gaucher disease-specific biomarker in dried blood spot (DBS)-derived samples are initiated in parallel. Identification of only one heterozygous GBA variant despite high biomarker values

entails multiplex ligation-dependent probe amplification (MLPA). The genetic test is followed by measurements of GD- specific biomarker, Lyso-Gb1. Positive test results (bi-allelic disease-causing GBA variants, pathologically low enzyme activity, and pathologically high biomarker levels), are correlated with clinical symptomatology, establishing the genetic diagnosis (section 7A) for final documentation (including reporting of genetic diagnosis). In case of G2P mismatch or non- informative clinical information, the lack of clinical correlation is stated in the case documentation.

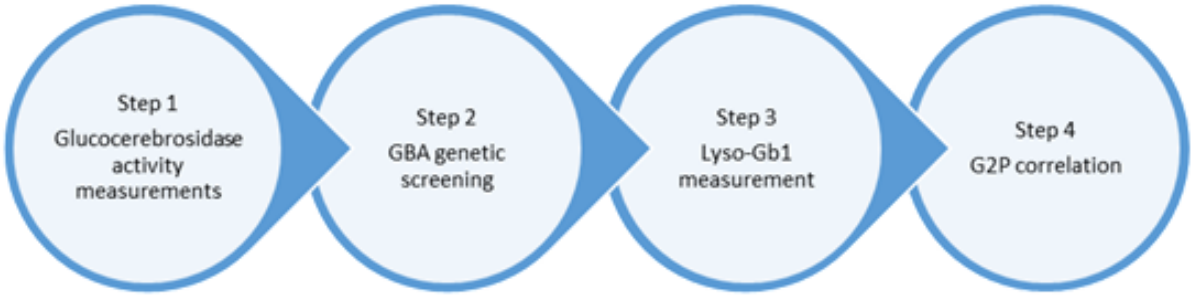


Figure: workflow representation (steps 1 to 4) for patients with clinical suspicion of GD disease

ii. Primary request was to genetically diagnose a patient for which no clinical suspicion of GD had been raised (screening diagnostics)

At CENTOGENE, the current stand-alone approach in these instances is whole exome sequencing (WES). If any GD-relevant *GBA* variant(s) identified, clarification of variant clinical class (classification according to ACMG guidelines for novel variants) and G2P correlations implies the measurements of glucocerebrosidase and Lyso-Gb1 biomarker.

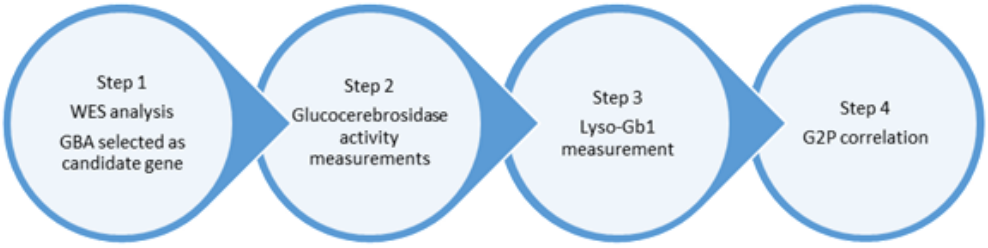


Figure: workflow representation (steps 1 to 4) for patients with no GD clinical suspicion

7.1 Review the enzymatic evidences

- The internal beta-glucocerebrosidase enzymatic results are stored in Gepado (CENTOGENE's LIMS system), UniDB and CuRepo.
- At CENTOGENE the screening method for beta-glucocerebrosidase deficiency is based on the quantitative determination of beta-glucocerebrosidase activity in DBS. The quantitation is performed by fluorimetry. The current reference is >=4.1 µmol/l/h. The enzymatic levels below the 4.1 µmol/l/h are interpreted as pathological and supportive evidences for presence of GD.

7.2 Genetic evidences

- Genetic evidences are stored in UniDB and CuRepo
- In order to associate GBA gene with GD, the patients must present clinically relevant variants (likely pathogenic and pathogenic variants) in homozygous state, or in compound heterozygosity.
- The GBA gene is analyzed by an amplicon based next-generation sequencing approach. The amplicons cover the entire coding region and the highly conserved exon-intron splice junctions. To detect gross rearrangements within GBA gene, quantitative PCR assay (qPCR) or multiplex ligation-dependent probe amplification (MLPA) is performed by using 10 gene-specific amplicons encompassing the coding exons 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 (or part of it) of the GBA gene.

7.3 Biomarker evidences

- The concentration of the biomarker Lyso-Gb1 in dried blood spot is measured using tandem mass spectrometry.
- Lyso-Gb1 (glucosylsphingosine) is a well validated and highly reliable marker in GD, reflecting the severity and progress of the disease (Rolfs et al, PLoS One. 2013). The sensitivity of the marker for GD for a concentration of > 10 ng/ml is >99.9%.
- The method for measuring the concentration of Lyso-Gb1 in DBS, EDTA-blood, plasma or serum is CE IVD labeled and only offered from CENTOGENE AG/Germany
- The Lyso-Gb1 levels higher than 10 ng/ml are evaluated as pathological and supportive evidence for presence of GD.

7.4 G2P correlation

- Genotype-to-phenotype (G2P) correlations in GD are imperfect in regards with the clinical subtypes outlined in section 7A. Significant overlap in the clinical manifestations found between individuals with the various genotypes precludes specific counseling about prognosis in individual cases.
- The disease can manifest early in childhood but it may remain undiagnosed until adulthood when the phenotype is mild (see Table 1)
- Physician must provide sufficient and specific clinical information, which is stored in internal database (UniDB, CuRepo) following HPO terminology to support diagnosis of specific GD forms.
- Additionally, where available, informative family members are used for segregation observations to determine the G2P correlation. We consider families with at least three family members being supportive.

Table 1: Phenotypic description of major symptoms for the most important clinical subtypes of GD.

Age	Subtype / OMIM	Primary CNS Involvement	Bone Disease	Other	Description

Age	Subtype / OMIM	Primary CNS Involvement	Bone Disease	Other	Description
Adult	Type 1/ 230800	No	Yes	Splenomegaly Hepatomegaly Cytopenia Pulmonary disease	GD type 1 (GD1) is the most common form of GD. Although symptoms of GD1 may vary greatly, the major symptoms include enlargement of the liver and spleen (hepatosplenomegaly), a low number of red blood cells (anemia), easy bruising caused by a decrease in blood platelets (thrombocytopenia), chronic fatigue, lung disease, and bone disease such as bone pain, fractures, and arthritis.

Age	Subtype / OMIM	Primary CNS Involvement	Bone Disease	Other	Description
Infancy - early childhood	Type 2 (acute or infantile)/ 230900	Bulbar signs Pyramidal signs Cognitive impairment	No	Hepatomegaly Splenomegaly Cytopenia Pulmonary disease Dermatologic changes	GD type 2 (GD2) is an acute neuronopathic form of the disorder with onset in infancy and death often by 2 years of age. Patients are usually normal at birth, but develop hepatosplenomegaly, developmental regression, and growth arrest within a few months of age. Neurologic deterioration proceeds rapidly, with cranial nerve and extrapyramidal tract involvement (Stone et al., 2000).
Childhood	Type 3 (subacute; juvenile)/ 231000	Oculomotor apraxia Seizures Progressive myoclonic epilepsy	Yes	Hepatomegaly Splenomegaly Cytopenia Pulmonary disease	GD type 3 (GD3) is also associated with the clinical and biological signs of ''systemic'' disease, such as frequent asthenia, growth retardation or delayed puberty, splenomegaly and hepatomegaly. Bone anomalies may also be present and manifest as deformations, osteopenia,

Age	Subtype / OMIM	Primary CNS Involvement	Bone Disease	Other	Description
					which sometimes leads to pathological fractures or vertebral compression, bone infarctions or even aseptic osteonecrosis. Involvement of other organs (rarely symptomatic pulmonary, renal and cardiac) is less common. Pancytopenia is frequent and involves varying degrees of thrombocytopenia (sometimes severe), anemia and, less frequently, leukoneutropenia. Polyclonal hypergammaglobulinemia is often present and is sometimes complicated by monoclonal gammopathy.

Age	Subtype / OMIM	Primary CNS Involvement	Bone Disease	Other	Description
Perinatal	Perinatal-lethal form/ 608013	Pyramidal signs	No	Ichthyosiform or collodion skin changes Nonimmune hydrops fetalis	This form is very rare with an incidence of less than 5% of GD cases. This form is particularly severe. The disease manifests in the fetus with a decrease or absence of fetal movements, fetal and placental anasarca, hepatosplenomegaly, ichthyosis, arthrogryposis, facial dysmorphism and fetal thrombocytopenia. Death usually occurs in utero or shortly after birth (<3 months). (Mignot et al., 2003).
Cardiovascular-predominant variant	Cardiovascular form/ 231005	Oculomotor apraxia	Yes	Calcification of mitral & aortic valves Corneal opacity Mild splenomegaly	Another form of GD is known as the cardiovascular type because it primarily affects the heart, causing the heart valves to harden (calcify). People with the cardiovascular form of GD may also have eye abnormalities, bone disease, and mild enlargement of the spleen (splenomegaly).

7.5 Validity of GBA – GD association

- For a valid GBA- GD association, the curator approves biochemical- genotype- phenotype correlations. Otherwise, an "inconclusive" dataset is documented and labelled as such (see section 7.6).
- Thus, pathological beta-glucocerebrosidase levels must associate with pathological GBA genotype (homozygote or compound heterozygote), pathological Lyso-Gb1 levels and suggestive GD phenotype.
- When no clinical information is provided, but enzyme activity, genotype and Lyso-Gb1 are correlated, GBA-GD association is still valid, however, the unknown GD form is documented (see section 7.6).

7.5.1. Summary of GBA- GD type 1 association

Pathogenic variants in the GBA gene are associated with Gaucher disease (GD), an autosomal recessive disorder. The clinical manifestations of this disease are highly variable. GD type 1 (90% of cases) is the chronic and non-neurological form associated with organomegaly (spleen, liver), bone anomalies (pain, osteonecrosis, pathological fractures) and cytopenia.

The beta-glucocerebrosidase activity are lower than 4.1 µmol/l/h (IOs: 0.94 +/-2.4 µmol/l/h; n=328 GD type 1 patients) and levels of Lyso-Gb1 biomarker above 10 ng/ml (IOs: 352.2 +/- 303 ng/ml; n=328 GD type 1 patients).

The internal age at diagnosis of GD type 1 patients is 19.8 +/- 19.3 yrs.

58.23% of the GD type 1 patients are homozygote for disease causing GBA variants; 40.85 % are compound heterozygote (carrying two heterozygous GBA disease causing variants in trans) and 0.91% have a complex GBA genotype (carrying at least three GBA disease causing variants).

7.5.2. Summary of GBA- GD type 2 association

Pathogenic variants in the GBA gene are associated with Gaucher disease (GD), an autosomal recessive disorder. The clinical manifestations of this disease are highly variable. Type 2, the acute neurological form, is characterized by early onset, rapidly progressing brainstem dysfunction, associated with organomegaly and leading to death before the age of 2.

The beta-glucocerebrosidase activity are lower than 4.1 µmol/l/h (IOs: 0.5 +/-0.93 µmol/l/h; n=45 GD type 2 patients) and levels of Lyso-Gb1 biomarker above 10 ng/ml (IOs: 451 +/- 329 ng/ml; n=45 GD type 2 patients).

The internal age at diagnosis of GD type 2 patients is 4.8 +/- 5.9 yrs.

73.33% of the GD type 2 patients are homozygote for disease causing GBA variants; 22.22 % are compound heterozygote (carrying two heterozygous GBA disease causing variants in trans) and 4.44% have a complex GBA genotype (carrying at least three GBA disease causing variants).

7.5.3 Summary of GBA- GD type 3 association

Pathogenic variants in the GBA gene are associated with Gaucher disease (GD), an autosomal recessive disorder.

The clinical manifestations of this disease are highly variable. Type 3, the subacute neurological form, affects children or adolescents and is characterized by progressive encephalopathy (oculomotor apraxia, epilepsy and ataxia) with the systemic manifestations seen in type 1.

The beta-glucocerebrosidase activity are lower than 4.1 µmol/l/h (IOs: 1.2 +/-1.5 µmol/l/h; n=34 GD type 3 patients) and levels of Lyso-Gb1 biomarker above 10 ng/ml (IOs: 370,5 +/- 319.5 ng/ml; n=34 GD type 3 patients).

The internal age at diagnosis of GD type 3 patients is 22.5 +/- 23.9 yrs.

67.65% of the GD type 3 patients are homozygote for disease causing GBA variants; 32.35 % are compound heterozygote (carrying two heterozygous GBA disease causing variants in trans). No GBA complex genotype was internally observed in GD type 3 patients.

7.5.4 Summary of GBA- GD perinatal- lethal form association

Pathogenic variants in the GBA gene are associated with Gaucher disease (GD), an autosomal recessive disorder. The clinical manifestations of this disease are highly variable. The perinatal- lethal form is particularly severe. The disease manifests in the fetus with a decrease or absence of fetal movements, fetal and placental anasarca, hepatosplenomegaly, ichthyosis, arthrogryposis, facial dysmorphism and fetal thrombocytopenia. Death usually occurs in utero or shortly after birth (<3 months).

7.5.5 Summary of GBA- GD cardiovascular form association

Pathogenic variants in the GBA gene are associated with Gaucher disease (GD), an autosomal recessive disorder. The clinical manifestations of this disease are highly variable. This form is characterized by progressive calcification of the aorta, and of the aortic and/or mitral valves. Other common features include mild splenomegaly, corneal opacities, and supranuclear ophthalmoplegia.

7.6 Inconclusive data

During GBA – GD association and curation, unclear or contradictory observations can be identified. In these situations GBA and GD association remains inconclusive.

Examples of inconclusive or unresolved GBA - GD associations:

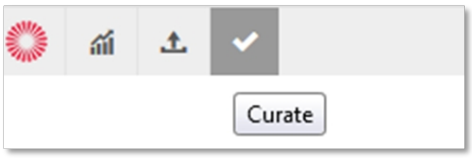
- beta-glucocerebrosidase and / or Lyso-Gb1 are within pathological range, but genetic GBA screening revealed only one or no GBA clinically relevant variant: These cases are labelled as “GD without genetic confirmation” and monitored regularly (quarterly) against new knowledge (DNA changes newly associated with GD; it includes deep intronic, promotor and other regulatory regions)
- beta-glucocerebrosidase is altered due to pre-analytical failures. The activities of beta-glucocerebrosidase and the internal control enzymes were decreased. Thus, the result of the decreased measurement of enzyme activity cannot be considered as clinically relevant: These cases are labelled as “pre-analytical failure”, and a recommendation to receive another sample for measurement repeat is communicated to the sender physician.

- No beta-glucocerebrosidase and / or Lyso-Gb1 could be performed due to not enough material, or due to inappropriate sample type (DNA only): These cases are labelled as "GD without biochemical confirmation" and recommendation to receive an appropriate sample type (i.e. blood) for biochemical confirmation is communicated to the sender physician.
- beta-glucocerebrosidase and / or Lyso-Gb1 are within normal range despite the supportive GBA genotype. In this situation, clarification if patient is already on ERT is required. These cases are not processed for CentoLSD unless clarified.
- The clinical information (healthy, asymptomatic) does not correlated with biochemical and genetic results. In this situation, clarification with physician is initiated. These cases are not processed for CentoLSD unless clarified.
- Patient is suspected/ affected and age not provided: when physician does not provide the CI of the patient or no information on the age, then GD type 1 (as being most frequent form) is used as default. These cases are labelled as "GD without clinical details".

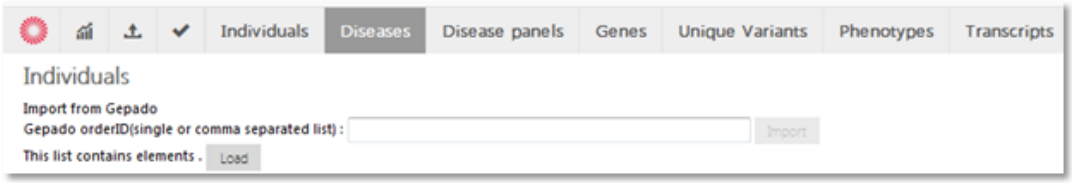
7.7 Storage and management of GBA- GD associations

- GBA gene is associated with different types of GD in CuRepo system as master data under ***Diseases module***. A detailed description of how genes and diseases are submitted and edited in CuRepo is indicated in SOPeIT- 36 Adding new genes, transcripts and diseases in Curation Repository.
- Under ***Diseases module***, the gene and disease associations are documented into a structured format, and only the approved associations are used for curation by case processes (see SOP Case curation: Curation of GBA screened individuals).
- Only curators responsible for gene- disease curation can approve Gene- Disease-MOI associations. Once associations approved, the responsible curators can add changes/ updates
- CuRepo system tracks automatically all applied changes, and display them under the ***History*** option.
- To review the current status of the GBA- GD types associations go under https://srv-centomd.centogene.internal/curation-repo/ and log in

- Select the curation symbol (dark grey below):

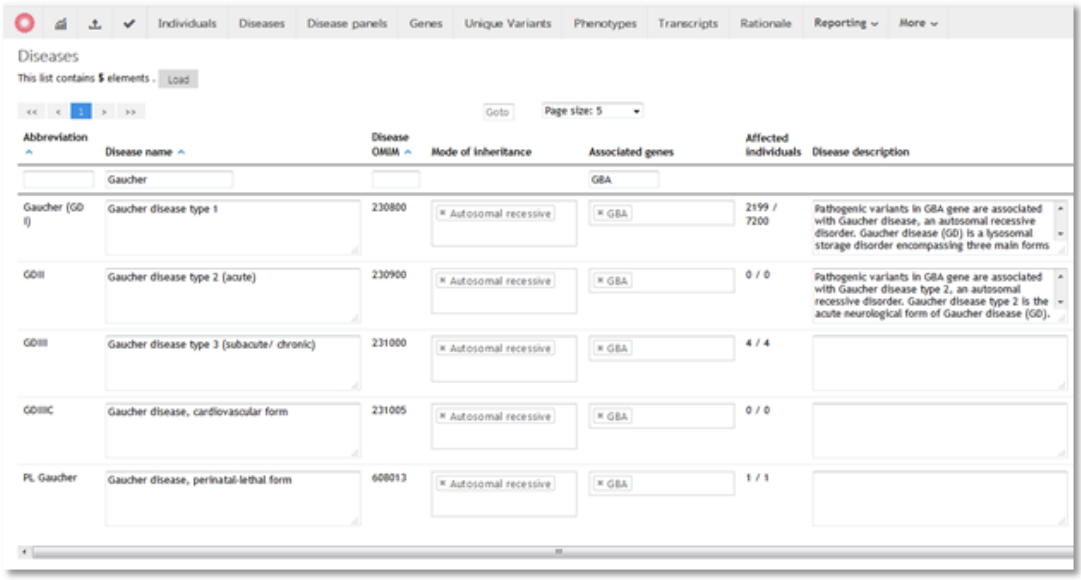


- Select ***Diseases*** Module (dark grey below):

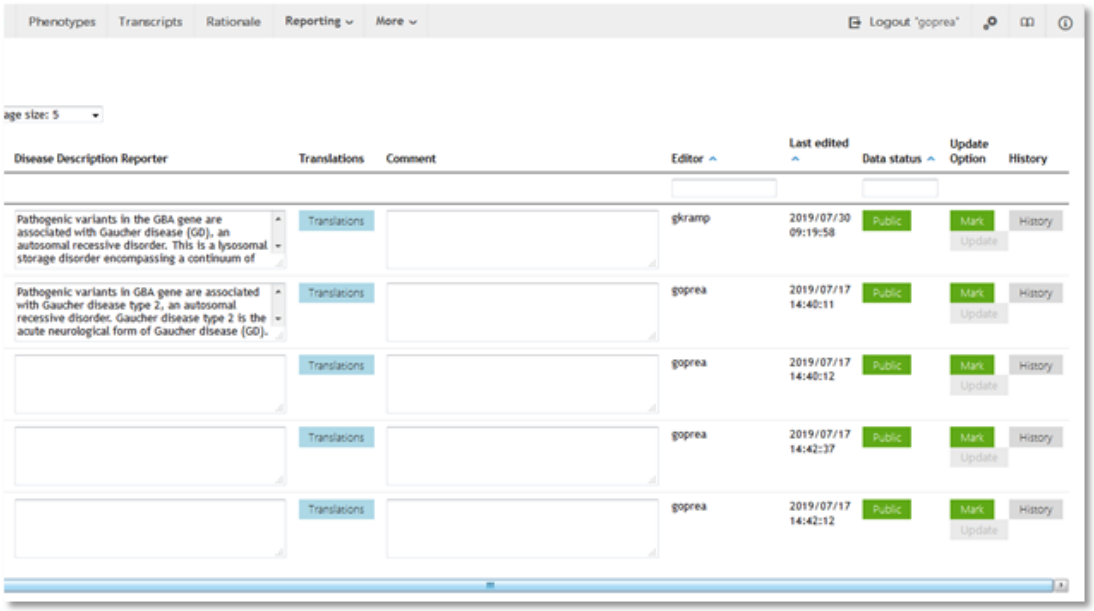


- By default, the structure of this module is indicated as following: ***Abbreviation, Disease name, Disease OMIM, Mode of inheritance, Associated genes, Affected individuals, Disease description, Disease description reporter, Translations, Comment, Editor, Last edited, Data Status, Update option, History.*** The option to add a new disease (***Add disease***) is available
- To initiate a search, add under Disease name ***Gaucher***; under Associated genes add ***GBA,*** and press ***Load*** *(see* screenshot above)
- All GBA- GD associated types are displayed. Each GBA- GD form is represented by one row in the database and is linked with its own status. Thus during curation by case process, only pre-linked and approved associations are available for selection.

Left side of the screenshot (represented are: ***Abbreviation, Disease name, Disease OMIM, Mode of inheritance, Associated genes, Affected individuals, Disease description)***



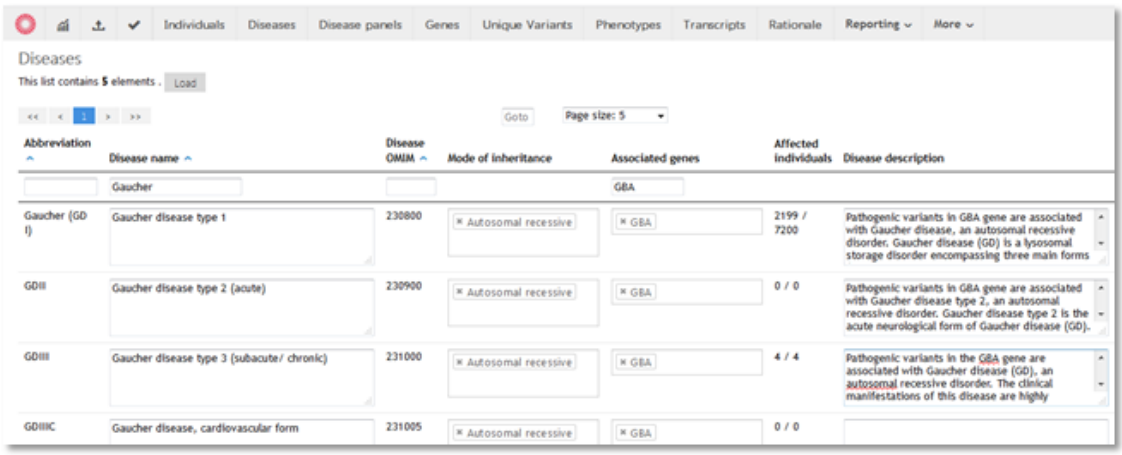
Right side of the screenshot (represented are: ***Disease description reporter, Translations, Comment, Editor, Last edited, Data Status, Update option, History***). Note that all associations are approved (Data status is ***Public;*** see screenshot below)



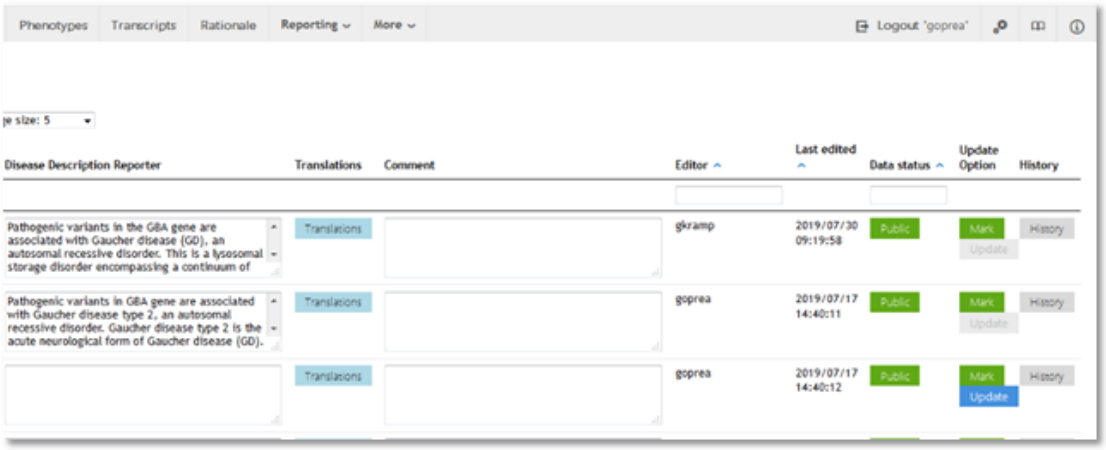
- Curator can edit associations on approved (***Public***) status. Any item subjected to change (i.e. ***Disease name, Mode of inheritance, Associated genes, Disease descriptions, Disease Description Reporter, Comment***) leads to activation of ***Update*** option (by default inactive, grey color; see the screenshot above). Only by pressing ***Update*** option, changes are saved by the system and used downstream (for example during GBA case curation)

Example of change: Add description of GD type 3 disease

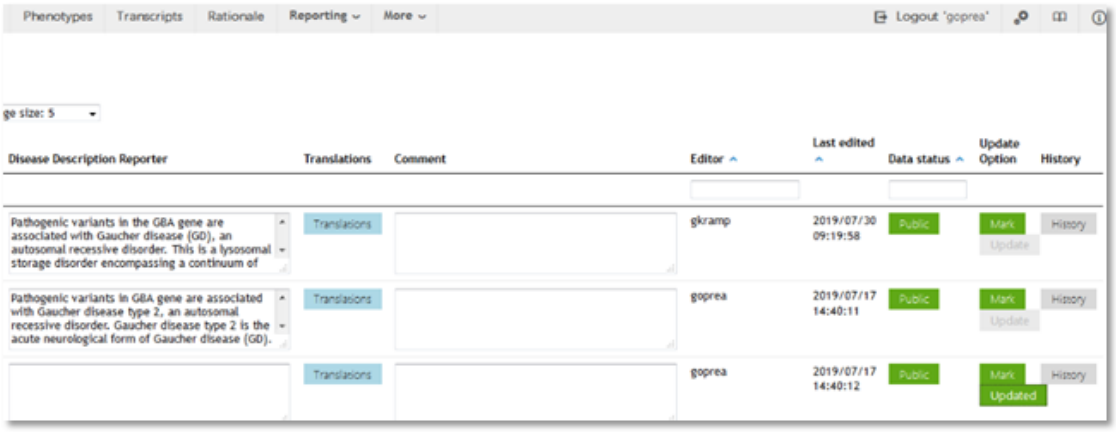
Left side of the screenshot: curator adds the description under Disease description for GD type 3 (in the screenshot below see GDIII row)



Right side of the screenshot: Once Disease description field processed, ***Update*** option becomes automatically active (blue color; see the screenshot below).



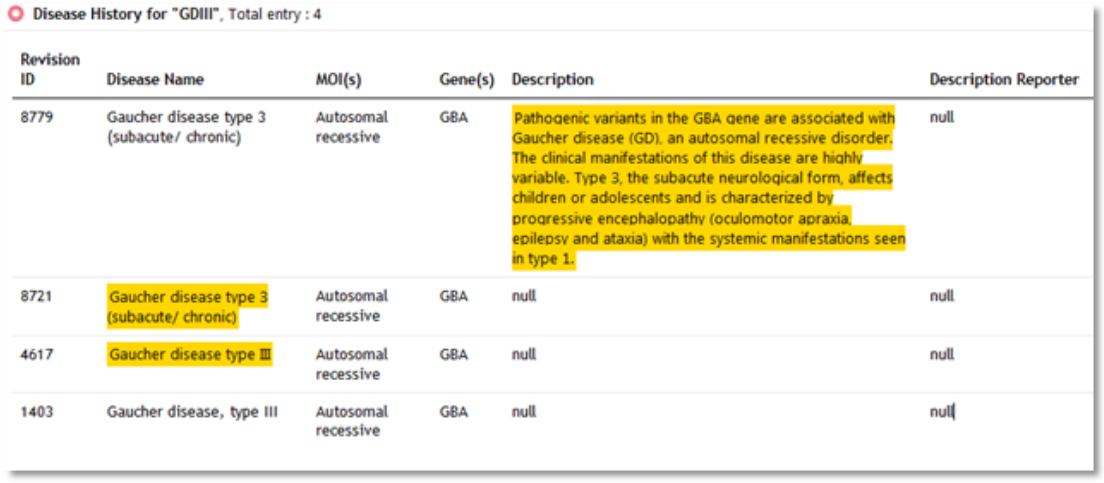
- Press ***Update*** (the blue Update option in the screenshot above turns into green; see the screenshot below)



- Under ***History*** all applied changes to an GBA- GD form associations are indicated (see gray arrow in the screenshot above). The changes are highlighted (yellow color). Under History window the following details are indicated: ***Revision ID*** (unique number, automatically generated by the system for every saved change); ***Disease name, MOI(s), Gene(s), Description, Description Reporter, Comment, Submitter, Editor, Last edited, Data status, Operation.***

Example of *History*window using the example above, i.e. tracking of changes under GBA- GD type 3 associations.

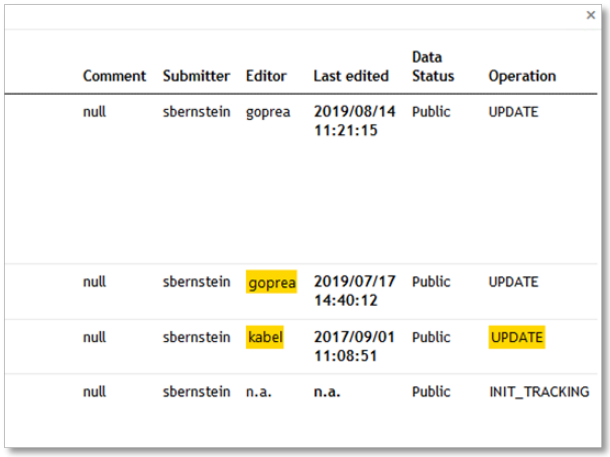
Left side of the screenshot (including: ***Revision ID, Disease name, MOI(s), Gene(s), Description, Description Reporter***). The revisions display the most up to date one on the top. Note that changes are highlighted under Disease name and Description, respectively).



Disease History for "GDIII", Total entry : 4

Revision ID	Disease Name	MOI(s)	Gene(s)	Description	Description Reporter
8779	Gaucher disease type 3 (subacute/ chronic)	Autosomal recessive	GBA	Pathogenic variants in the GBA gene are associated with Gaucher disease (GD), an autosomal recessive disorder. The clinical manifestations of this disease are highly variable. Type 3, the subacute neurological form, affects children or adolescents and is characterized by progressive encephalopathy (oculomotor apraxia, epilepsy and ataxia) with the systemic manifestations seen in type 1.	null
8721	Gaucher disease type 3 (subacute/ chronic)	Autosomal recessive	GBA	null	null
4617	Gaucher disease type III	Autosomal recessive	GBA	null	null
1403	Gaucher disease, type III	Autosomal recessive	GBA	null	null

Right side of the screenshot (including: ***Comment, Submitter, Editor, Last edited, Data status, Operation).*** System indicates for each revision the editor (i.e. who performed the change), when (expressed as date- year/month/day and time- hours:minues:seconds)



Comment	Submitter	Editor	Last edited	Data Status	Operation
null	sbernstein	goprea	2019/08/14 11:21:15	Public	UPDATE
null	sbernstein	goprea	2019/07/17 14:40:12	Public	UPDATE
null	sbernstein	kabel	2017/09/01 11:08:51	Public	UPDATE
null	sbernstein	n.a.	n.a.	Public	INIT_TRACKING

- The GBA- GD associations are used for the following processes:
  - Curation by case
  - Reporting
  - Data transfer for digital products (like CentoLSD, CentoMD®)

8. References

Rolfs A, Giese AK, Grittner U, Mascher D, Elstein D, Zimran A, Böttcher T, Lukas J, Hübner R, Gölnitz U, Röhle A, Dudesek A, Meyer W, Wittstock M, Mascher H.: *Glucosylsphingosine Is a Highly Sensitive and Specific Biomarker for Primary Diagnostic and Follow-Up Monitoring in Gaucher Disease in a Non-Jewish, Caucasian Cohort of Gaucher Disease Patients*;PLoS One. 2013 Nov 20;8(11):e79732.

Stone, D. L., Tayebi, N., Orvisky, E., Stubblefield, B., Madike, V., Sidransky, E. *Glucocerebrosidase gene mutations in patients with type 2 Gaucher disease*. Hum. Mutat. 15: 181-188, 2000

Mignot, C., Gelot, A., Bessieres, B., Daffos, F., Voyer, M., Menez, F., Fallet Bianco, C., Odent, S., Le Duff, D., Loget, P., Fargier, P., Costil, J., Josset, P., Roume, J., Vanier, M. T., Maire, I., de Villemeur, T. B. *Perinatal-lethal Gaucher disease*. Am. J. Med. Genet. 120A: 338-344, 2003.

9. Appendices

1. SOPeIT-76 APPX1 Training module GBA gene Gaucher disease curation pba.xlsx