IWGGD The International Working Group on Gaucher Disease

Guideline Activity	**Patient centered guidelines for the laboratory diagnosis of Gaucher disease (GD) type 1**
Prepared by	*Authors (in alphabetic order): Hans Aerts[1], Andrea Dardis[2], Ksenija Fumic[3], Helen Michelakakis[4], Eleonora Pavan[2], Paula Rozenfeld[5], Jasenka Wagner[6,7].* *1- Department of Medical Biochemistry, Leiden Institute of Chemistry, Leiden, Netherlands* *2-Regional Coordinator Centre for Rare Disease, university Hospital of Udine, Italy* *3- Department for Laboratory Diagnostics, University Hospital Centre Zagreb and School of Medicine, Zagreb, Croatia* *4- Department of Enzymology and Cellular Function, Institute of Child Health, Athens, Greece* *5- Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP), UNLP, CONICET, asociado CIC PBA, Facultad de Ciencias Exactas, Departamento de Ciencias Biológicas, La Plata, Argentina* *6- Department of Medical Biology and Genetics, Faculty of Medicine, J.J. Strossmayer University, Osijek, Croatia; 7- International Gaucher Alliance*
Date of preparation	16 May 2022
Due date of review	16 May 2024
Version	1.0
Overview	The **aim** of this guideline is to provide evidence-based recommendations to best meet patients' needs, ensuring timely and accurate diagnosis for GD patients. A systematic literature review on Gaucher disease biomarkers, biochemical diagnosis, acid β-glucocerebrosidase activity, molecular diagnosis and *GBA1* mutations was carried out using Medline. For the genetic testing section, only papers published in the last 30 years were considered. One hundred sixty-one papers were selected as relevant. The search was limited to English language publications only. Evidence levels were classified according to the "Evidence-based clinical practice guidelines (Burns et al., 2011)
Sections	

Biomarkers of Gaucher disease (GD) assisting diagnosis

Recommendation: chitotriosidase activity, PARC/CCL18 or glucosylsphingosine (GlcSph) concentrations in serum and/or plasma can be used as a first line test when the diagnosis of Gaucher disease (GD) is suspected.

Assaying of chitotriosidase activity will not be informative in some individuals, largely due to the presence in homozygosity of a 24 bp duplication in the *CHIT1* gene, a trait that is quite frequent in some ethnic groups. If it is the only assessed biomarker and the result is normal, the presence of 24 bp duplication in the *CHIT1* should be excluded.

However, a diagnosis of GD needs to be established by the assay of acid β-glucosidase activity (in homogenates of peripheral blood leukocytes of cultured fibroblasts), preferably supported by molecular analysis of the GBA1 gene, or by the identification of biallelic pathogenetic variants in the GBA1 gene

The use of dried blood spots (DBS) in the diagnosis of lysosomal storage disorders has become increasingly popular mainly due to its convenience (see below). Monocentric studies reported a good sensitivity of DBS GlcSph assessment in identifying GD patients. However, the outcome of extensive studies documenting specificity, stability and the impact of sample storage and shipping conditions on sensitivity of this biomarker in DBS is not yet available.

Level of evidence: II (cohort studies /case series with consistent results/ research articles)

Grade: B (Recommendation)

Remarks

A-Chitotriosidase activity

- The activity of Chitotriosidase can be assayed in plasma /serum.
- When the fluorogenic substrate 4-methylumbelliferyl-β-D-N,N',N''-triacetyl-chitotrioside (4MU-C3) is used it is important to ensure that the enzyme activity is truly proportional to the amount of chitotriosidase protein, the need to standardize the assay across laboratories is urgent and is underway through the IWGGD Biomarkers & Materials working group.
- The fluorogenic substrate 4-methylumbelliferyl-deoxychitobiose (4MU-dC2) allows a more convenient, sensitive, and accurate determination of its activity.
- Although activity well within the range observed in GD can also be detected in other conditions, including both lysosomal and non-lysosomal storage diseases, a marked elevation of chitotriosidase activity in plasma appears to be characteristic for GD.

B- PARC/CCL18

- The levels of PARC/CCL18 can be measured in either plasma or serum.
- Concentrations cannot be reliably estimated using SELDI-TOF.
- Both enzyme-linked immunosorbent assay (ELISA) and dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) can be used for reliable estimation.
- Increased levels that can overlap those found in GD have been described in patients with other conditions including both lysosomal and on lysosomal storage diseases.
- Although for the diagnosis of GD there is no added value in the concomitant evaluation of both plasma chitotriosidase activity and PARC/CCL18 levels, measurement of the PARC/CCL18 levels is particularly important for patients with zero chitotriosidase activity due to the presence in homozygosity of a 24 bp duplication in the *CHIT1* gene.

C- Glucosylsphingosine (GlcSph; lysoGL1/lysoGb1)

- The concentration of GlcSph - sometimes termed 'lysoGL1' or 'lysoGb1' in literature supported by different companies -can be measured in either plasma or serum. GlcSph can be quantified in previously frozen serum or plasma samples.
- Different techniques can be used for detection of which LC-MS/MS is presently most sensitive. Its levels cannot be reliably estimated using SELDI-TOF. Reliable determination of absolute concentrations of GlcSph by mass spectrometry requires use of an appropriate internal standard.
- Markedly increased levels of plasma GlcSph (>100 fold) are characteristic of untreated GD, but more modest elevations may be encountered in other disease conditions (e.g. AMRF, PSAP deficiency and NPC).
- The value of the demonstration of elevated GlcSph in DBS is currently being evaluated in the diagnosis of GD. Some expert centers have positively reported on the use of DBS to demonstrate elevated GlcSph in GD patients. Further confirmation and studies on this, including impact of storage and shipment conditions, should be undertaken.

D- ACE (angiotensin-converting enzyme)

- Small increases in ACE activity are observed in GD patients.
- Increased activity is not observed in all GD patients.
- Increased serum/plasma ACE has been reported in other disorders involving activation of the monocyte/macrophage lineage.
- Several genetic variants in the ACE gene have been described which result in increased ACE blood levels.
- ACE activity can be inhibited by frequently prescribed ACE inhibitors.

E-TRAP (tartrate-resistant acid phosphatase)

- TRAP is not specific to GD.
- Modest increase is observed.
- TRAP is unstable in the blood and consequently shows marked analytical variability.

F- gpNMB (glycoprotein nonmetastatic melanoma protein B)

- gpNMB is selectively overexpressed by Gaucher cells.
- Its soluble fragment into plasma that can be conveniently detected by ELISA.
- Increase has been described in GD and Niemann Pick type C disease and the protein is elevated in many other diseases.
- Limited studies.

Details: *Chitotriosidase the human analogue of chitinases from lower organisms which is massively excreted by storage cells*

Sensitivity: In terms of diagnosing GD assaying of plasma chitotriosidase activity is commonly employed in many centers as a first line screening test. The activity of chitotriosidase in plasma is elevated up to 1000-fold above normal values. In the initial chitotriosidase study, plasma activity was found to be elevated on average 641-fold (median control plasma, 20 nmol/mL/h; range, 4-76 nmol/mL/h; median GD plasma, 12 824 nmol/mL/h; range, 3122-65 349 nmol/mL/h (Hollak et al., 1994). Several subsequent reports have confirmed these findings (Deegan et al., 2005; Raskovalova et al., 2020; Stirnemann et al., 2012; van Dussen et al., 2014). In general, higher activity is observed in type 1 patients than patients with the neurological types 2 and 3. Increased activity has also been reported in asymptomatic/ presymptomatic patients identified through the screening of family members of diagnosed cases (Dimitriou et al., 2020).

The interpretation of plasma chitotriosidase levels is complicated by the occurrence of a 24-base pair (bp) duplication in the chitotriosidase gene, which prevents the formation of chitotriosidase protein. This trait is particularly common in the general population, including GD patients, where one in every three individuals is a carrier and about one in every 20 individuals is homozygous for the mutation (Boot et al., 1998). Several other mutations have been described which affect the activity of chitotriosidase (Arndt et al., 2013; Csongrádi et al., 2021; Grace et al., 2007; Lee et al., 2007; Mavrikiou et al., 2016).

Specificity: increased plasma chitotriosidase activity is not unique to GD patients. Increased activity is also found in many different lysosomal and non-lysosomal diseases such as such as Nieman Pick C disease, Acid sphingomyelinase deficiency, Alagille syndrome, hydrops fetalis due to congenital herpes virus infection, neonatal systemic candidiasis, sarcoidosis, leprosy, arthritis, multiple sclerosis,

thalassemia, chronic obstructive pulmonary disease (COPD), malaria, and atherosclerosis. In general, the increases observed are more modest than in the case of GD and although activity well within the range observed in GD can also be detected a marked elevation of chitotriosidase activity in plasma appears to be characteristic for GD (Boot et al., 1999, 2010; Boven et al., 2006; Brinkman et al., 2005; Guo et al., 1995; Hollak et al., 1994; Iyer et al., 2009; Labadaridis et al., 1998; Michelakakis et al., 2004; Vedder et al., 2006; vom Dahl et al., 1999).

Assaying of Chitotriosisdase activity: The biological material to be used is serum and/or plasma. The enzyme in plasma is stable upon storage and multiple freeze thaw cycles, (storage: stable at room temperature for 24 hours; storage at -30 after 8 month recovery 95.3-102%, data presented by Aerts at GD Biomarker Qualification Workshop, September 2010, FDA Campus). Although the use of DBS in diagnosis of lysosomal storage disorders has become increasingly popular mainly due to its convenience, at present extensive studies documenting sensitivity and specificity of assaying chitotriosidase activity in this type of biological material are not yet available (Chaves et al., 2011; Pacheco & Uribe, 2013; Rodrigues et al., 2009; Saville et al., 2020). The activity of chitotriosidase in plasma/serum can be determined using the fluorogenic substrate 4- methylumbelliferyl-β-D-N,N',N''-triacetyl-chitotrioside (4MU-C3).However, the assay is complicated by the ability of chitotriosidase to transglycosylate as well as hydrolyze this substrate rendering abnormal kinetics (Aguilera et al., 2003). It is essential therefore that special care is taken to ensure that the enzyme activity is truly proportional to the amount of chitotriosidase protein and the need to standardize the assay across laboratories is urgent. Alternatively, a far more convenient, sensitive, and accurate detection can be achieved by measuring the activity of chitotriosidase toward the fluorogenic substrate 4-methylumbelliferyl-deoxychitobiose (4MU-dC2. Chitotriosidase shows normal Michaelis–Menten kinetics with this substrate, allowing the use of saturating substrate concentrations. Thus, a more accurate and robust assay is now available (Aguilera et al., 2003; Schoonhoven et al., 2007).

PARC/CCL18: pulmonary and activation-regulated chemokine (PARC, systematic name CCL18), a member of the C-C chemokine family which is massively excreted by storage cells

Sensitivity: a 10- to 50- fold increase in the levels of PARC/CCL18 has been reported in plasma of symptomatic GD patients compared to healthy individuals (Boot et al., 2004; Deegan et al., 2005; Raskovalova et al., 2020). Increased PARC/CCL18 levels have been reported in the asymptomatic identical twin of a patient with severe disease which were however lower than in the symptomatic patient. It is stable on storage and multiple freeze thaw cycles.

Specificity: Increased levels that can overlap those found in GD have been described in patients with a-mannosidosis, Niemann Pick disease type A and B (Brinkman et al., 2005; Deegan et al., 2005). Non-

lysosomal storage diseases with increased PARC/CCL18 levels include atherosclerosis, rheumatoid arthritis, beta-thalassemia, sarcoidosis (Boot et al., 2010; Dimitriou et al., 2005; Reape et al., 1999; Struyf et al., 2003). So far, no genetic variations that could impact on the levels of PARC/CCL18 have been described.

Measurement of the levels of PARC/CCL18: The biological material to be used is serum and/or plasma. PARC/CCL18 is stable on storage and multiple freeze thaw cycles (storage: stable at room temperature for 48 hours ; storage at-30; 8 month recovery 107-109%, data presented by Aerts et al. at GD Biomarker Qualification Workshop, September 2010, FDA Campus). Its levels cannot be reliably estimated using SELDI-TOF but enzyme-linked immunosorbent assay (ELISA) and dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA) can be used for reliable estimation (Boot et al., 2004; Raskovalova et al., 2020; van Breemen et al., 2006).

Glucosylsphingosine (*a.k.a*. lysoGL1, lysoGb1):

Sensitivity: an average 180 - fold increase in the levels of GlcSph has been reported in plasma of symptomatic type 1 GD patients compared to healthy individuals (Dekker et al., 2011). A similar abnormality is noted in in mice and zebrafish with deficient glucocerebrosidase (Dahl et al., 2021; Keatinge et al., 2015; Lelieveld et al., 2019). This characteristic abnormality has been confirmed by numerous laboratories worldwide (e.g. Murugesan et al., 2016; Rolfs et al., 2013; recently reviewed in Revel-Vilk et al., 2020; van Eijk et al., 2020). GlcSph has been reported to be also increased in DBS of GD patients (Dinur et al., 2022; Polo et al., 2019; Saville et al., 2020).

Specificity: More modestly increased levels of plasma GlcSph have also been noted in patients suffering from Action Myoclonus Renal Failure syndrome with a defective LIMP-2 (Gaspar et al., 2014), patients with SapC deficiency (Mota et al, 2016) and some patients with Niemann Pick disease type C (Ferraz et al., 2016).

ACE (angiotensin-converting enzyme): a 2 – 10 fold increase in ACE has been described in serum/plasma of GD patients patients apparently originating from storage cells (Aerts & Hollak, 1997; Cabrera-Salazar et al., 2004; Danilov et al., 2018; Deegan et al., 2005; Lieberman & Beutler, 1976; Silverstein et al., 1980; Šumarac et al., 2011; van der Lienden et al., 2019). Increased serum/plasma ACE has been reported in other disorders involving activation of the monocyte/macrophage lineage. Sarcoidosis is the most frequent and the better studied of these diseases (Beneteau-Burnat & Baudin, 1991). Increased activity is not observed in all GD patients (Silverstein et al., 1980) up to 5-fold variation in blood ACE across a population can be observed and several mutations/polymorphisms in the ACE gene have been described which result in increased ACE blood levels (Danilov et al., 2021;

Nesterovitch et al., 2009). ACE activity can be repressed by frequently prescribed ACE inhibitors (Struthers et al., 1999).

TRAP (tartrate-resistant acid phosphatase): TRAP was the first biomarker to be assayed in the diagnosis of GD (Tuchman et al., 1956). TRAP is not specific to GD and the observed increase is modest. It is unstable in the blood and shows marked analytical variability (Aerts & Hollak, 1997; Deegan et al., 2005). In interpreting TRAP serum levels its increased levels in children compared to adults should be taken into consideration together with its thermoinstability (Lam et al., 1978).

gpNMB (glycoprotein nonmetastatic melanoma protein B) has been identified by proteomics analysis of laser dissected Gaucher cells from GD spleens (Kramer et al., 2016; van der Lienden et al., 2019). It is selectively overexpressed by Gaucher cells that release a soluble fragment into plasma that can be conveniently detected by ELISA. The soluble fragment of gpNMB is found to be elevated over 50-fold in plasma of patients with type 1 GD (Kramer et al., 2016)and was also found to be elevated in human NPC plasma samples (van der Lienden et al., 2019). A recent investigation confirms the value of soluble gpNMB as plasma marker of Gaucher cells and substantiates its diagnostic potential (Murugesan et al., 2018). However further studies are needed before its role as a diagnostic biomarker is established.

Enzyme activity

Recommendations: acid β-glucosidase (BGLU) activity could be measured in DBS samples as a first-line test in clinical suspected individuals. However, Gaucher diagnosis should never be relied on DBS enzyme activity measurement and samples with activity below cut-off values as well as normal activity but a highly suspicious clinical picture require confirmation by measuring BGLU activity in gold standard samples: homogenates of leukocytes or fibroblasts. GD diagnosis is made by demonstration of deficient (below 15% of mean normal activity) BGLU enzyme activity in these cell's homogenates

Level of evidence: II, III and IV (Well-designed cohort, case-control study, case reports)

Grade: B (Recommendation)

Each laboratory should establish its own Quality Management (QM) system, if possible according to ISO15189 international standards and participate in both internal and external quality assessments.

Level of evidence: V (Review, expert opinion)

Grade: D (Option)

Remarks

- *BGLU activity could be measured using artificial substrate with fluorimetric methods, tandem mass spectrometry or by digital microfluidics platform.*
- *Fluorometric method is used for the gold standard assay in leukocytes/fibroblasts.*
- *Tandem mass spectrometry or by digital microfluidics platforms are generally used for DBS samples in screening studies.*
- *Residual enzyme activity does not correlate with disease severity.*
- *Enzyme test is not suitable for diagnosis of heterozygotes of GD nor of saposin C deficiency.*

Details: The BGLU activity could be measured in different samples such as blood dried on standardized filter paper, leukocytes, fibroblasts and in case of prenatal diagnosis in chorionic villi sampling (CVS) or cultured amniocytes (Bodamer & Hung, 2010). BGLU could be measured in DBS samples as a first-line laboratory test. Pre-analytical requirements are critical for reliable BGLU results from DBS samples. DBS is obtained by application of 50-75 µL drops of blood obtained by venipuncture into heparin tubes and spotted on the Whatman®903 or S&S903 filter paper. Another option is application of the same amount of blood after fingerprick on filter paper collection device onto printed circles (Gasparotto et al., 2009; Hannon, 2013). DBS should be dried for 4 h at room temperature avoiding direct illumination, and then packed in a sealed plastic bag with desiccant, and stored at 4 °C until analysis (Adam et al., 2011). Exposure of DBS to both heat and humidity can destroy enzyme functions rapidly. Moreover, an incomplete mixed blood before spotting can result in significant variation on enzyme activity (Elbin et al., 2011).

The use of DBS as first line laboratory test offers many advantages over leukocytes or fibroblasts samples including easy collection methodologies, need of a small amount of blood, and simpler transportation as samples can be shipped via regular mail at room temperature. If the DBS sample is treated appropriately, the BGLU remain stable at least for 21 days (Ceci et al., 2011; Chamoles et al., 2002; Reuser et al., 2011). DBS has limitations for measurement BGLU activity. The volume of blood applied, hematocrit, recent blood transfusions and other preanalytical steps such as drying time, homogeneity and extraction of the analyte influences the quality of the DBS sample (Moat et al., 2020). To ensure integrity of BGLU activity and to avoid false positive results, another lysosomal enzyme should be measured as a control enzyme with approximately same stability at room temperature. The value of the control (reference) sample should generally lie between the mean +/ - 2 standard deviation (Lukacs et al., 2003).

Different studies have shown good sensitivity and specificity, above 95%. However, enzyme testing in DBS has a low positive predictive value of< 45% in average (Bender et al., 2020; Burlina et al., 2019; Elliott et al., 2016; Huang et al., 2020; Kang et al., 2017; Sanders et al., 2020; Stroppiano et al., 2014; Tortorelli et al., 2016; Wasserstein et al., 2019).

Patient leukocytes or cultured skin fibroblast homogenates are the gold standard for measurement the BGLU activity (Leukocytes as the BGLU source are obtained by separation from approximately 5-10 milliliters of blood, drawn from patient in K2EDTA or heparin tube. Moreover, skin fibroblasts should be used when patients have received blood transfusions or when discordant results are obtained with white blood cells. The shipment of blood samples to the reference laboratory should be carried out at 4°C (Kolodny & Mumford, 1976). The isolation of leukocytes from the whole blood should be completed within 24 h after blood collection using dextran sedimentation or the ammonium chloride lysis method (Karatas et al., 2020; Peters et al., 1975; Roos & Loos, 1970; Skoog & Beck, 1956). Isolated leukocytes pellet could be stored at- 20°C until enzyme measurement for at least 20 days (Karatas et al., 2020).

Cultured fibroblasts homogenates require an invasive skin biopsy and a primary cell culture of skin fibroblasts (avoiding the risk of contamination), and to wait for the growing of fibroblasts to obtain a confluent cell monolayer The shipment of cultured fibroblasts should be at room temperature in a tube, dish or sealed flask (T25 or T75) filled with culture media (Coelho & Giugliani, 2000). There are some potential interfering factors: excessive transport time, lack of viable cells, bacterial or mycoplasma contamination, exposure of the specimen to temperature extremes (freezing or >30°C).

The use of gold standard samples requires a homogenisation step with a metal tip sonicator, and total protein measurement (Bradford, 1976; Kolodny & Mumford, 1976).

BGLU activity could be measured using fluorimetric methods, tandem mass spectrometry or by digital microfluidics platforms. Fluorimetric methods are based on artificial substrate 4-methylumbelliferyl-β-D-glucopyranoside (4-MUG). They are mostly performed in microtiter plates (Chiao et al., 1980; Olivova et al., 2008; Wenger et al., 1978). The sample is put into a reaction mixture containing low pH, sodium deoxytaurocholate, and the fluorogenic substrate 4-Methylumbelliferyl β-D-glucopyranoside (4-MUG). Sodium deoxytaurocholate is added in order to inhibit the non-lysosomal isoenzyme BGLU activity (Chamoles et al., 2002; Daniels et al., 1981; Magalhães et al., 1984; Michelin et al., 2004; Shapira et al., 1989). Fluorometric enzyme assays for BGLU onto digital microfluidic platform show potential for a simple, rapid and high-throughput selective screening of BGLU activity (Camargo Neto et al., 2018; Hopkins et al., 2015; Sista, Wang, Wu, Graham, Eckhardt, Bali, et al., 2013; Sista, Wang, Wu, Graham, Eckhardt, Winger, et al., 2013). Beside digital microfluidic fluorimetry, there are other

available compact digital microfluidic platforms (e.g. electro-wetting based digital microfluidics) (Millington et al., 2018)

Tandem mass spectrometry enzyme assays with (LC-MS/MS) or without (MS/MS) liquid chromatography are based on a non-fluorometric synthetic substrates (Gelb et al., 2006; Li et al., 2004; Zhang et al., 2008). This approach may be particularly suitable for high-throughput analyses with a large number of individuals at-risk and/or for future newborn screening for GD (Elliott et al., 2016; Orsini et al., 2012; Wolf et al., 2018) All three technologies (approaches) are suitable for selective screening BGLU activity (Sanders et al., 2020) .

Enzyme determinations in DBS samples are useful screening tests in clinically suspected individuals. Samples with BGLU activity below cut-off values require confirmation by measuring BGLU activity in gold standard samples: homogenates of leukocytes or fibroblasts (Ceci et al., 2011; Wenger et al., 1978). Whenever subjects present suggestive GD symptoms they must be reassessed even in the presence of normal BGLU from DBS testing (Stroppiano et al., 2014).

An enzyme activity result of less than 15% of normal activity in homogenates of leukocytes or fibroblasts is diagnostic of GD (Stirnemann et al., 2017). Residual enzyme activity does not correlate with disease severity. Enzyme testing is not suitable for identification of carriers of GD nor of saposin C deficiency (Baris et al., 2014; Fateen & Abdallah, 2019; Michelin et al., 2004). Heterozygotes may have half-normal enzyme activity, but overlapping with activity levels of healthy controls, rendering enzymatic testing for carrier status unreliable (Butcher et al., 1989; Essabar et al., 2015; Mistry & Germain, 2006)

To ensure the quality of BGLU testing performance, each laboratory should establish its own Quality Management (QM) system according to ISO15189 and participate in both internal and external quality assessments. The internal audit program monitors operations throughout the testing process and the quality system. For quality control purposes, it is necessary to include an appropriate blank and at least one affected control and one normal control sample for each run of enzyme assays. All assays should be performed in duplicate. The cut-off range, normal range and disease range should be established by the laboratory based on its own analysis (Blau et al., 2008). The inter-laboratory variance of numerical enzyme activity could be large (Martins et al., 2009). Reproducibility was demonstrated by intra- (n = 6) and inter-assay (n = 10) results using threshold of %CV <15.Therefore, quality assurance and improvement in diagnostic proficiency have become essential in this area (Verma et al., 2015). The enzyme assay is made in house by each lab based on the original published methods. It implies differences in units (pmol/h/disk, µmol/l/h, µmol/mg/h), disease cut-off; limit of detection (LOD) and limit of quantitation (LOQ). For this reason, laboratory reports from reference

labs should include an interpretation of the result that reflects the conclusion of the result as normal or deficient, possible limitations of the test, and recommendations for additional testing if applicable

The European Research Network for Evaluation and Improvement of Screening, Diagnosis, and Treatment of Inherited Disorders of Metabolism (ERNDIM) serves as an external proficiency testing program for clinical diagnostic laboratories, providing lyophilized fibroblasts for eight LSD enzymes (http://cms.erndimqa.nl/Home/Lysosomal-Enzymes.aspx). For laboratories testing lysosomal enzymes on DBS, the Newborn Screening Quality Assurance Program (NSQAP) at Centers for Disease Control and Prevention (CDC) provides QC materials, proficiency testing (PT) services, and technical support in collaboration with the NSTRI at CDC (Fowler et al., 2008; Yu et al., 2013).

Genetic testing

Recommendations: Molecular analysis of the *GBA1* gene should always be performed when biomarker results or phenotype are at odds with the enzymology and is highly recommended in subjects with BGLU activity below normal reference intervals in cells to further support/confirm the diagnosis of GD and provide genetic counseling. Testing of familial variants and genetic counseling should be made available to at risk family members.

In all cases, molecular testing should be accompanied by a pre and post-test genetic counseling to ensure informed choices.

The high degree of homology between *GBA1* and *GBAP* makes the molecular analysis of *GBA* challenging. Therefore, adequate strategies performed in laboratories with experience in the genetic testing of GBA1 gene should be applied to avoid mis-genotyping.

Level of evidence: II and IV (retrospective cohort studies or case series with consistent results)

Grade: B (Recommendation)

Remarks

- *Although genetic testing should be performed to confirm/support biochemical data and to provide genetic counseling, it could be done as a primary test (before testing enzymatic activity). However, results should be interpreted with caution since GBA1 testing is challenging (see below) and variants of unknown significance (VUS) are often identified. Therefore, confirmation of diagnosis through the assessment of enzymatic activity in patient's cells is mandatory.*
- *DNA can be extracted from different samples such us DBS, peripheral blood, fibroblasts, tissue biopsies.*

- *Genetic testing is the most reliable method to detect heterozygous carriers and it should be made available to family members at risk of being carriers.*
- *Segregation of alleles by identifying variants in parents, should be determined.*
- *Sequencing analysis of GBA1 exons and intron exon boundaries should be performed as primary molecular test.*
- *Sequencing analysis can be performed using specific long template amplification of the GBA1 gene (avoiding the amplification of the pseudogene) followed by Sanger sequencing or NGS specifically designed to avoid reads misalignments. This strategy allows detecting point mutations and most recombinant alleles but is not suitable to detect large deletions.*
- *GBA1 could be included in gene panels analyzed by NGS. This technology allows the detection of point mutations, although false positive results have been reported. Therefore, point mutations detected by NGS methods should always be confirmed by Sanger sequencing. Standard workflows are not suitable for the detection of large deletions or recombinant alleles.*
- *The presence of homozygous pathogenetic variants not confirmed in parents, as well as the absence of pathogenetic variants (in one or both allele) after sequencing should always be questioned and additional investigations should be performed. In particular, multiplex ligation-probe amplification (MLPA) and mRNA analysis should be done to identify possible undetected recombinant/ deleted alleles or deep intronic pathogenetic variants, respectively.*
- *Segregation of alleles by identifying variants in parents, should be determined.*
- *Variants should be classified following the ACMG criteria and in case of identification of VUS, pathogenicity should be assessed by functional analysis.*
- *In the absence of pathogenetic variants in GBA1 gene in subjects with a clinical phenotype compatible with GD, increased chitotriosidase activity, increased levels of GlcSph and normal or low BGLU activity in cells a SAPC deficiency should be suspected and the PSAP gene should be analyzed* (Christomanou et al., 1989; Dekker et al., 2011; Kang et al., 2018; Rafi et al., 1993; Schnabel et al., 1991; Tylki-Szymańska et al., 2007; Vaccaro et al., 2010).
- *In the absence of pathogenetic variants in GBA1 gene in subjects with normal chitotriosidase activity, increased plasma levels of GlcSph, normal or low BGLU activity leucocytes and BGLU deficiency in fibroblasts a LIMP-2 deficiency should be suspected and SCARB-2 gene should be analyzed* (Balreira et al., 2008; Dardis et al., 2009; Gaspar et al., 2014; Mota et al., 2016; Quraishi et al., 2021; Zeigler et al., 2014).

Details:

According to The Human Gene Mutation Database (HGMD-Professional 2021.1), 540 variants of the *GBA1* gene have been reported to date, although not all of them are linked to GD. Indeed, 403 of them have been associated to GD.

All kind of variants have been reported: missense and nonsense variants, splice junction variants, deletions and insertions of one or more nucleotides and complex alleles (complex rearrangements) resulting from gene conversion or gene fusion with the downstream pseudogene *GBAP*. However, missense and nonsense variants, are the most frequently identified in GD patients worldwide (Hruska et al., 2008).

The frequency distribution of *GBA1* variants differs accross ethnic groups. While 4 pathogenetic variants (N370S; L444P, c.84-85 insG; IVS+1G>A) account for 90% of alleles within Ashkenazi Jewish, they account only for about 50-75% of alleles in non-Jewish populations. In addition, about 10% of patients present large deletions/recombinant alleles (Alfonso et al., 2001; Beutler & Gelbart, 1993; Bronstein et al., 2014; Cherif et al., 2009; Dimitriou et al., 2020; Duran et al., 2012; Emre et al., 2008; Erdos et al., 2007; Feng et al., 2018; Filocamo et al., 2002; Giraldo et al., 2000; Grabowski et al., 2015; Hatton et al., 1997; Horowitz et al., 1993; Jeong et al., 2011; Koprivica et al., 2000; Lepe-Balsalobre et al., 2020; Mattošová et al., 2015; Miocić et al., 2005; Orenstein et al., 2014; Ortiz-Cabrera et al., 2016; Sheth et al., 2019).

Long template specific PCR amplification of the *GBA1* gene (and not the pseudogene) followed by Sanger sequencing allows the identification of single base pair variants and most recombinant alleles leading to molecular diagnosis of GD about 95-98 % of cases (Alfonso et al., 2001; Dimitriou et al., 2020; Duran et al., 2012; Emre et al., 2008; Erdos et al., 2007; Feng et al., 2018; Filocamo et al., 2002; Jeong et al., 2011; Koprivica et al., 2000; Lepe-Balsalobre et al., 2020; Mattošová et al., 2015; Miocić et al., 2005; Ortiz-Cabrera et al., 2016; Sheth et al., 2019); however this method fails to detect large deletions (Beutler & Gelbart, 1994; Cozar et al., 2011; Koprivica et al., 2000; Ortiz-Cabrera et al., 2016).

GBA1 gene can also be analyzed using NGS technologies, both as a single gene or as part of targeted gene panels. In all cases, the workflow should be optimized to avoid false positive or negative results due to misalignment of reads between the gene and the pseudogene.

Strategies to specifically analyze the *GBA1* as a single gene using NGS technology have been developed (den Heijer et al., 2020, 2021; Zampieri et al., 2017). Such NGS strategies allow the identification of single base pair variants and recombinant alleles (excluding the Recdelta55) with high specificity and sensitivity (den Heijer et al., 2021; Zampieri et al., 2017). Conversely, analysis of the *GBA1* gene as part

of gene panels using well designed NGS strategies that consider the presence of the pseudogene, allows only the identification of point mutations, while fail to identify both large deletions and recombinant alleles due misalignment of reads with the homologous pseudogene (Lee et al., 2021; Málaga et al., 2019; Muñoz et al., 2020; Zampieri et al., 2021; Zanetti et al., 2020) .

However, NGS data analysis is a field in continuous and rapid evolution and new solutions to improve sensitivity and specificity are expected to be available in the near future (Spataro et al., 2017).

Indeed, the use of PacBio long-read Single Molecule Real-Time (SMRT) for *GBA* deep sequencing has recently been developed (Drelichman et al., 2021). However, this technology is still not widely available in most genetic laboratories.

Multiplex ligation-probe amplification (MLPA) kits have been developed for the identification of recombinant/deleted *GBA* alleles. However, commercially available kits do not discriminate between L444P mutant and *RecNci* alleles and do not discriminate between recombinant and deleted alleles (Amico et al., 2016; Ortiz-Cabrera et al., 2016; Zampieri et al., 2021).

Use of DBS samples for diagnosis in external laboratories

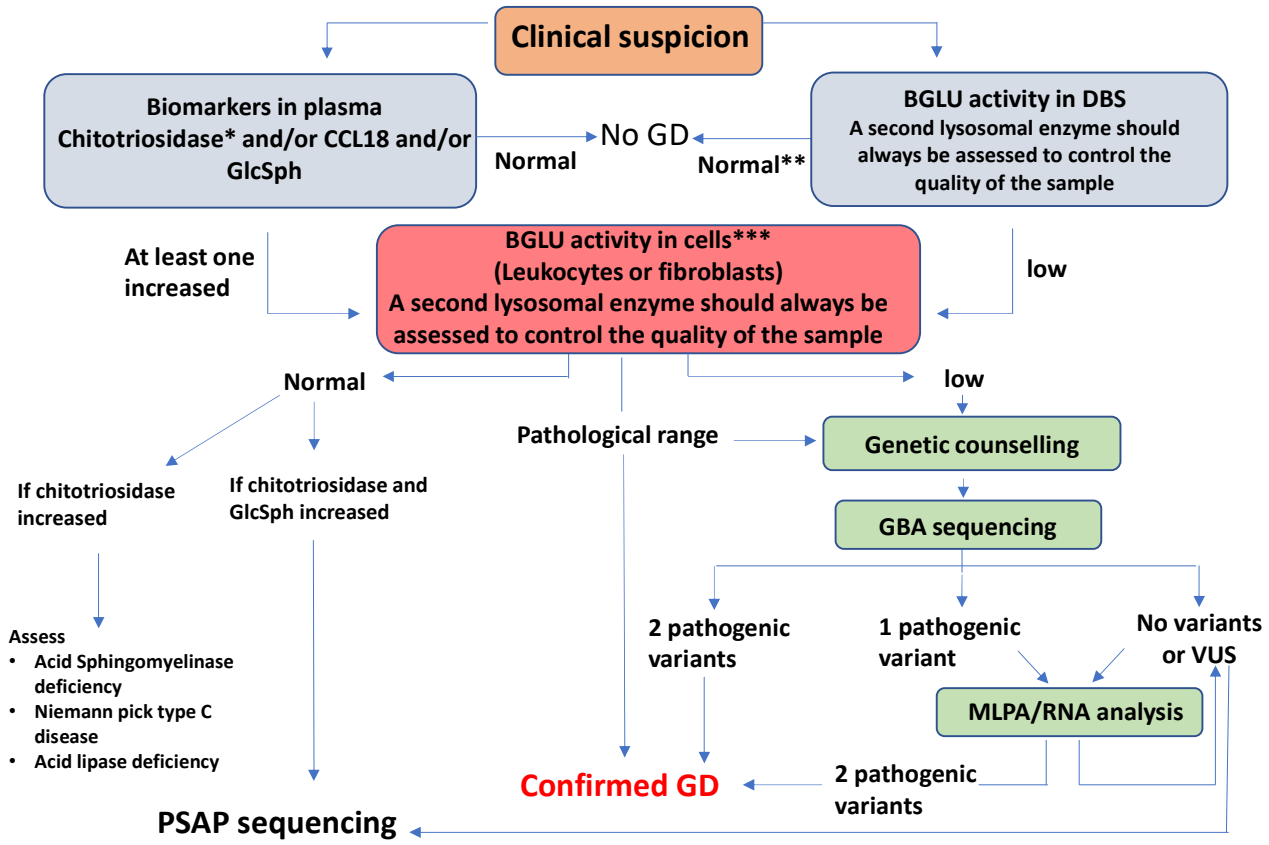
Recommendations: DBS can be used for diagnosis of GD in patients without access to in house testing. In these cases, DBS can be sent to external laboratories with expertise in GD. Pre-analytical requirements are critical for reliable results. Both BGLU and/or GlcSph can be assessed as a first line test in this type of sample. However, results have to be interpreted with caution since, the BGLU activity in DBS has a very poor positive predictive value (see enzyme activity section) and although recent monocentric studies have shown encouraging results in favour of use of DBS to assess GlcSph, a number of points still need to be clarified. In particular, stability over time of the sample (to define storage and transport time recommendations), correlation between standard and DBS assays as well as specificity and differential diagnosis (see biomarkers and enzyme activity sections) need to be tested.

Therefore, diagnosis should never be relied on these tests only and they should be confirmed by demonstration of biallelic pathogenetic variants in the GBA1 gene (see genetic testing section).

In the absence of biallelic pathogenetic variants, the assessment of BGLU activity in cells is mandatory.

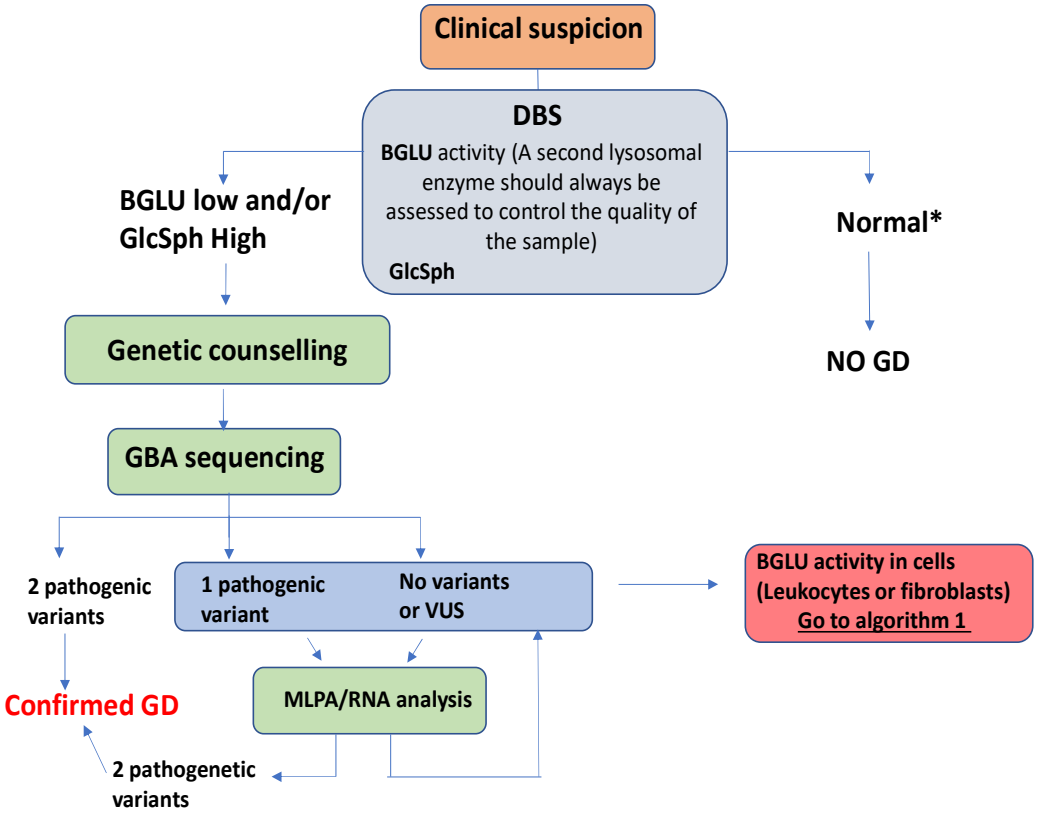
Final conclusions:

These guidelines address the laboratory workup for the diagnosis of GD type 1 and are intended to facilitate accurate and timely diagnosis regardless of their demography and access to health care. Based on the gathered evidences and the recommendations elaborated a diagnostic algorithm has been developed as shown in figure 1 (algorithm 1).



***The presence of the 24 bp deletion has to be excluded if this is the only biomarker assessed and it results normal.**
****Subjects presenting suggestive GD symptoms must be reassessed even in the presence of normal BGLU in DBS**
*****A biochemical pattern characterized by BGLU activity normal/low in leucocytes but deficient in fibroblasts associated with high levels of GlcSph and normal chitotriosidase activity are highly suggestive of Action Myoclonus renal failure Syndrome (AMRF) due to LIMP-2 deficiency. This diagnosis can be confirmed by molecular analysis of the SCARB2 gene.**

The group is aware that not all patients around the world have access to in house testing and they have to rely on external laboratories for diagnosis. In this case, dry blood spots can be used although results have to be interpreted with caution. An algorithm for diagnosis using DBS is shown in figure 2 (algorithm 2):



***Subjects presenting suggestive GD symptoms must be reassessed even in the presence of normal BGLU in DBS**

The interpretation of the test described in this workflow can be challenging and not always straight forward.

Therefore, the group recommends to expert laboratories to interpret the results in the context of the clinical picture of the patient and to include in the report a clear interpretation that reflects the conclusion of the result, possible limitations of the test, and recommendations for additional testing if applicable.

Future challenges

1, A standardization of assays of various plasma biomarkers is recommendable. A first step in this direction is undertaken by the IWGGD working group Biomarkers & Materials.

2, The use of DBS to assess biomarkers (e.g. GlcSph) should be confirmed by multiple centers with special care to the influence of storage and shipment conditions.

3, The potential application of plasma biomarkers to monitor disease progression and efficacy of therapeutic intervention warrants attention, in consultation with other IWGGD working groups.

4, Collection of more information on plasma biomarkers in other conditions in which a (partial) deficiency of GCase activity occurs: Niemann Pick type C, Action Myoclonus Renal Failure Syndrome, Saposin C deficiency.

5, Identification of biomarkers able to predict the possible neurological involvement in newly identified patients

6. Development of new methods for accurate and cost/effective analysis genetic testing of GBA

References

Adam, B. W., Hall, E. M., Sternberg, M., Lim, T. H., Flores, S. R., O'Brien, S., Simms, D., Li, L. X., de Jesus, V. R., & Hannon, W. H. (2011). The stability of markers in dried-blood spots for recommended newborn screening disorders in the United States. *Clinical Biochemistry*, *44*(17–18), 1445–1450. https://doi.org/10.1016/j.clinbiochem.2011.09.010

Aerts, J. M. F., & Hollak, C. E. M. (1997). Plasma and metabolic abnormalities in Gaucher's disease. *Bailliere's Clinical Haematology*, *10*(4), 691–709. https://doi.org/10.1016/S0950-3536(97)80034-0

Aguilera, B., Ghauharali-van der Vlugt, K., Helmond, M. T. J., Out, J. M. M., Donker-Koopman, W. E., Groener, J. E. M., Boot, R. G., Renkema, G. H., van der Marel, G. A., van Boom, J. H., Overkleeft, H. S., & Aerts, J. M. F. G. (2003). Transglycosidase Activity of Chitotriosidase: Improved enzymatic assay for the human macrophage chitinase. *Journal of Biological Chemistry*, *278*(42), 40911–40916. https://doi.org/10.1074/jbc.M301804200

Alfonso, P., Cenarro, A., Pérez-Calvo, J. I., Giralt, M., Giraldo, P., & Pocoví, M. (2001). Mutation prevalence among 51 unrelated Spanish patients with Gaucher disease: Identification of 11 novel mutations. *Blood Cells, Molecules, and Diseases*, *27*(5), 882–891. https://doi.org/10.1006/bcmd.2001.0461

Amico, G., Grossi, S., Vijzelaar, R., Lanza, F., Mazzotti, R., Corsolini, F., Ketema, M., & Filocamo, M. (2016). MLPA-based approach for initial and simultaneous detection of GBA deletions and recombinant alleles in patients affected by Gaucher Disease. *Molecular Genetics and Metabolism*, *119*(4), 329–337. https://doi.org/10.1016/j.ymgme.2016.10.008

Arndt, S., Hobbs, A., Sinclaire, I., & Lane, A. B. (2013). Chitotriosidase deficiency: A mutation update in an African population. In *JIMD Reports* (Vol. 10, pp. 11–16). Springer. https://doi.org/10.1007/8904_2012_193

Balreira, A., Gaspar, P., Caiola, D., Chaves, J., Beirão, I., Lima, J. L., Azevedo, J. E., & Miranda, M. C. S. (2008). A nonsense mutation in the LIMP-2 gene associated with progressive myoclonic epilepsy and nephrotic syndrome. *Human Molecular Genetics*, *17*(14), 2238–2243. https://doi.org/10.1093/HMG/DDN124

Baris, H. N., Cohen, I. J., & Mistry, P. K. (2014). Gaucher disease: The metabolic defect, pathophysiology, phenotypes and natural history. *Pediatric Endocrinology Reviews*, *12*(0 1), 72–81. /pmc/articles/PMC4520262/

Bender, F., Burin, M. G., Tirelli, K. M., Medeiros, F., de Bitencourt, F. H., Civallero, G., Kubaski, F., Bravo, H., Daher, A., Carnier, V., Franco, J. F. S., & Giugliani, R. (2020). Newborn screening for lysosomal disorders in Brazil: A pilot study using customized fluorimetric assays. *Genetics and Molecular Biology*, *43*(2). https://doi.org/10.1590/1678-4685-gmb-2018-0334

Beneteau-Burnat, B., & Baudin, B. (1991). Angiotensin-converting enzyme: Clinical applications and laboratory investigations on serum and other biological fluids. *Critical Reviews in Clinical Laboratory Sciences*, *28*(5–6), 337–356. https://doi.org/10.3109/10408369109106868

Beutler, E., & Gelbart, T. (1993). Gaucher disease mutations in non-Jewish patients. *British Journal of Haematology*, *85*(2), 401–405. https://doi.org/10.1111/J.1365-2141.1993.TB03185.X

Beutler, E., & Gelbart, T. (1994). Erroneous assignment of Gaucher disease genotype as a consequence of a complete gene deletion. *Human Mutation*, *4*(3), 212–216. https://doi.org/10.1002/humu.1380040307

Blau, N., Duran, M., & Gibson, K. M. (Eds.). (2008). *Laboratory Guide to the Methods in Biochemical Genetics*. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-76698-8

Bodamer, O. A., & Hung, C. (2010). Laboratory and genetic evaluation of Gaucher disease. *Wiener Medizinische Wochenschrift*, *160*(23–24), 600–604. https://doi.org/10.1007/s10354-010-0814-1

Boot, R. G., Hollak, C. E. M., Verhoek, M., Alberts, C., Jonkers, R. E., & Aerts, J. M. (2010). Plasma chitotriosidase and CCL18 as surrogate markers for granulomatous macrophages in sarcoidosis. *Clinica Chimica Acta*, *411*(1–2), 31–36. https://doi.org/10.1016/j.cca.2009.09.034

Boot, R. G., Renkema, G. H., Verhock, M., Strijland, A., Bliek, J., de Meulemeester, T. M. A. M. O., Mannens, M. M. A. M., & Aerts, J. M. F. G. (1998). The human chitotriosidase gene - Nature of inherited enzyme deficiency. *Journal of Biological Chemistry*, *273*(40), 25680–25685. https://doi.org/10.1074/jbc.273.40.25680

Boot, R. G., van Achterberg, T. A. E., van Aken, B. E., Renkema, G. H., Jacobs, M. J. H. M., Aerts, J. M. F. G., & de Vries, C. J. M. (1999). Strong induction of members of the chitinase family of proteins in atherosclerosis: Chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *19*(3), 687–694. https://doi.org/10.1161/01.ATV.19.3.687

Boot, R. G., Verhoek, M., de Fost, M., Hollak, C. E. M., Maas, M., Bleijlevens, B., van Breemen, M. J., van Meurs, M., Boven, L. A., Laman, J. D., Moran, M. T., Cox, T. M., & Aerts, J. M. F. G. (2004). Marked elevation of the chemokine CCL18/PARC in Gaucher disease: A novel surrogate marker for assessing therapeutic intervention. *Blood*, *103*(1), 33–39. https://doi.org/10.1182/blood-2003-05-1612

Boven, L. A., van Meurs, M., van Zwam, M., Wierenga-Wolf, A., Hintzen, R. Q., Boot, R. G., Aerts, J. M., Amor, S., Nieuwenhuis, E. E., & Laman, J. D. (2006). Myelin-laden macrophages are anti-inflammatory, consistent with foam cells in multiple sclerosis. *Brain*, *129*(2), 517–526. https://doi.org/10.1093/brain/awh707

Bradford, M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1006/abio.1976.9999

Brinkman, J., Wijburg, F. A., Hollak, C. E., Groener, J. E., Verhoek, M., Scheij, S., Aten, J., Boot, R. G., & Aerts, J. M. (2005). Plasma chitotriosidase and CCL18: Early biochemical surrogate markers in type B Niemann-Pick disease. *Journal of Inherited Metabolic Disease*, *28*(1), 13–20. https://doi.org/10.1007/s10545-005-4416-9

Bronstein, S., Karpati, M., & Peleg, L. (2014). An Update of Gaucher Mutations Distribution in the Ashkenazi Jewish Population: Prevalence and Country of Origin of the Mutation R496H. *Israel Medical Association Journal*, *16*(11), 683–685.

Burlina, A. B., Polo, G., Rubert, L., Gueraldi, D., Cazzorla, C., Duro, G., Salviati, L., & Burlina, A. P. (2019). Implementation of second-tier tests in newborn screening for lysosomal disorders in North Eastern Italy. *International Journal of Neonatal Screening*, *5*(2). https://doi.org/10.3390/ijns5020024

Burns, P. B., Rohrich, R. J., & Chung, K. C. (2011). The Levels of Evidence and their role in Evidence-Based Medicine. *Plastic and Reconstructive Surgery*, *128*(1), 305. https://doi.org/10.1097/PRS.0B013E318219C171

Butcher, B. A., Gopalan, V., Lee, R. E., Richards, T. C., Waggoner, A. S., & Glew, R. H. (1989). Use of 4-heptylumbelliferyl-β-d-glucoside to identify Gaucher's disease heterozygotes. *Clinica Chimica Acta*, *184*(3), 235–242. https://doi.org/10.1016/0009-8981(89)90056-9

Cabrera-Salazar, M. A., O'Rourke, E., Henderson, N., Wessel, H., & Barranger, J. A. (2004). Correlation of surrogate markers of Gaucher disease. Implications for long-term follow up of enzyme replacement therapy. *Clinica Chimica Acta*, *344*(1–2), 101–107. https://doi.org/10.1016/j.cccn.2004.02.018

Camargo Neto, E., Schulte, J., Pereira, J., Bravo, H., Sampaio-Filho, C., & Giugliani, R. (2018). Neonatal screening for four lysosomal storage diseases with a digital microfluidics platform: Initial results in Brazil. *Genetics and Molecular Biology*, *41*(2), 414–416. https://doi.org/10.1590/1678-4685-gmb-2017-0227

Ceci, R., Francesco, P. N. de, Mucci, J. M., Cancelarich, L. N., Fossati, C. A., & Rozenfeld, P. A. (2011). Reliability of enzyme assays in dried blood spots for diagnosis of 4 lysosomal storage disorders. *Advances in Biological Chemistry*, *01*(03), 58–64. https://doi.org/10.4236/abc.2011.13008

Chamoles, N. A., Blanco, M., Gaggioli, D., & Casentini, C. (2002). Gaucher and Niemann-Pick diseases - Enzymatic diagnosis in dried blood spots on filter paper: Retrospective diagnoses in newborn-screening cards. *Clinica Chimica Acta*, *317*(1–2), 191–197. https://doi.org/10.1016/S0009-8981(01)00798-7

Chaves, R. G., Coelho, J. C., Michelin-Tirelli, K., Michelin-Tirelli, K., Freitas Maurıcio, T., de Freitas Maia Chaves, E., Rômulofilgueira Maurıcio, C., le Almeida, P. C., & Cavalcanti, G. B. (2011). Successful screening for Gaucher disease in a high-prevalence population in tabuleirodo norte (Northeastern Brazil): A cross-sectional study. *Journal of Inherited Metabolic Disease*, *1*, 73–78. https://doi.org/10.1007/8904_2011_19

Cherif, W., ben Turkia, H., ben Rhouma, F., Riahi, I., Chemli, J., Kefi, R., Messai, H., Amaral, O., Miranda, M. C. S., Caillaud, C., Tebib, N., ben Dridi, M. F., & Abdelhak, S. (2009). Gaucher disease in Tunisia: High frequency of the most common mutations. In *Blood Cells, Molecules, and Diseases* (Vol. 43, Issue 2, pp. 161–162). Blood Cells Mol Dis. https://doi.org/10.1016/j.bcmd.2009.05.004

Chiao, Y. B., Glew, R. H., Driven, W., & Lee R E. (1980). Comparison of various β-glucosidase assays used to diagnose gaucher's disease. *Clinica Chimica Acta*, *105*(1), 41–50. https://doi.org/10.1016/0009-8981(80)90090-X

Christomanou, H., Chabás, A., Pámpols, T., & Guardiola, A. (1989). Activator protein deficient Gaucher's disease. A second patient with the newly identified lipid storage disorder. *Klinische Wochenschrift*, *67*(19), 999–1003. https://doi.org/10.1007/BF01716064

Coelho, J. C., & Giugliani, R. (2000). Fibroblasts of skin fragments as a tool for the investigation of genetic diseases: Technical recommendations. *Genetics and Molecular Biology*, *23*(2), 269–271. https://doi.org/10.1590/S1415-47572000000200004

Cozar, M., Bembi, B., Dominissini, S., Zampieri, S., Vilageliu, L., Grinberg, D., & Dardis, A. (2011). Molecular characterization of a new deletion of the GBA1 gene due to an inter Alu recombination event. *Molecular Genetics and Metabolism*, *102*(2), 226–228. https://doi.org/10.1016/j.ymgme.2010.10.004

Csongrádi, A., Altorjay, I. T., Fülöp, G., Enyedi, A., Enyedi, E. E., Hajnal, P., Takács, I., Tóth, A., Papp, Z., & Fagyas, M. (2021). Chitotriosidase gene polymorphisms and mutations limit the determination of chitotriosidase expression in sarcoidosis. *Clinica Chimica Acta*, *513*, 50–56. https://doi.org/10.1016/j.cca.2020.11.025

Dahl, M., Smith, E. M. K., Warsi, S., Rothe, M., Ferraz, M. J., Aerts, J. M. F. G., Golipour, A., Harper, C., Pfeifer, R., Pizzurro, D., Schambach, A., Mason, C., & Karlsson, S. (2021). Correction of pathology in mice displaying Gaucher disease type 1 by a clinically-applicable lentiviral vector. *Molecular Therapy - Methods and Clinical Development*, *20*, 312–323. https://doi.org/10.1016/j.omtm.2020.11.018

Daniels, L. B., Glew, R. H., Diven, W. F., Lee, R. E., & Radin, N. S. (1981). An improved fluorometric leukocyte β-glucosidase assay for Gaucher's disease. *Clinica Chimica Acta*, *115*(3), 369–375. https://doi.org/10.1016/0009-8981(81)90251-5

Danilov, S. M., Jain, M. S., Petukhov, P. A., Goldman, C., DiSanto-Rose, M., Vancavage, R., Francuzevitch, L. Y., Samokhodskaya, L. M., Kamalov, A. A., Arbieva, Z. H., Sturrock, E. D., Dudek, S. M., & Judson, M. A. (2021). Novel ACE mutations mimicking sarcoidosis by increasing blood ACE levels. *Translational Research*, *230*, 5–20. https://doi.org/10.1016/j.trsl.2020.07.010

Danilov, S. M., Tikhomirova, V. E., Metzger, R., Naperova, I. A., Bukina, T. M., Goker-Alpan, O., Tayebi, N., Gayfullin, N. M., Schwartz, D. E., Samokhodskaya, L. M., Kost, O. A., & Sidransky, E. (2018). ACE phenotyping in Gaucher disease. *Molecular Genetics and Metabolism*, *123*(4), 501–510. https://doi.org/10.1016/j.ymgme.2018.02.007

Dardis, A., Filocamo, M., Grossi, S., Ciana, G., Franceschetti, S., Dominissini, S., Rubboli, G., di Rocco, M., & Bembi, B. (2009). Biochemical and molecular findings in a patient with myoclonic epilepsy due to a mistarget of the beta-glucosidase enzyme. *Molecular Genetics and Metabolism*, *97*(4), 309–311. https://doi.org/10.1016/J.YMGME.2009.04.011

Deegan, P. B., Moran, M. T., McFarlane, I., Schofield, J. P., Boot, R. G., Aerts, J. M. F. G., & Cox, T. M. (2005). Clinical evaluation of chemokine and enzymatic biomarkers of Gaucher disease. *Blood Cells, Molecules, and Diseases*, *35*(2), 259–267. https://doi.org/10.1016/j.bcmd.2005.05.005

Dekker, N., van Dussen, L., Hollak, C. E. M., Overkleeft, H., Scheij, S., Ghauharali, K., van Breemen, M. J., Ferraz, M. J., Groener, J. E. M., Maas, M., Wijburg, F. A., Speijer, D., Tylki-Szymanska, A., Mistry, P. K., Boot, R. G., & Aerts, J. M. (2011). Elevated plasma glucosylsphingosine in Gaucher disease: Relation to phenotype, storage cell markers, and therapeutic response. *Blood*, *118*(16). https://doi.org/10.1182/blood-2011-05-352971

den Heijer, J. M., Cullen, V. C., Quadri, M., Schmitz, A., Hilt, D. C., Lansbury, P., Berendse, H. W., van de Berg, W. D. J., de Bie, R. M. A., Boertien, J. M., Boon, A. J. W., Contarino, M. F., van Hilten, J. J., Hoff, J. I., van Mierlo, T., Munts, A. G., van der Plas, A. A., Ponsen, M. M., Baas, F., … Groeneveld, G. J. (2020). A Large-Scale Full GBA1 Gene Screening in Parkinson's Disease in the Netherlands. *Movement Disorders*, *35*(9), 1667–1674. https://doi.org/10.1002/mds.28112

den Heijer, J. M., Schmitz, A., Lansbury, P., Cullen, V. C., Hilt, D. C., Bonifati, V., & Groeneveld, G. J. (2021). False negatives in GBA1 sequencing due to polymerase dependent allelic imbalance. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-020-80564-y

Dimitriou, E., Moraitou, M., Cozar, M., Serra-Vinardell, J., Vilageliu, L., Grinberg, D., Mavridou, I., & Michelakakis, H. (2020). Gaucher disease: Biochemical and molecular findings in 141 patients diagnosed in Greece. *Molecular Genetics and Metabolism Reports*, *24*. https://doi.org/10.1016/j.ymgmr.2020.100614

Dimitriou, E., Verhoek, M., Altun, S., Karabatsos, F., Moraitou, M., Youssef, J., Boot, R., Sarafidou, J., Karagiorga, M., Aerts, H., & Michelakakis, H. (2005). Elevated plasma chemokine CCL18/PARC in β-thalassemia. *Blood Cells, Molecules, and Diseases*, *35*(3), 328–331. https://doi.org/10.1016/j.bcmd.2005.07.006

Dinur, T., Bauer, P., Beetz, C., Kramp, G., Cozma, C., Iurașcu, M.-I., Becker-Cohen, M., Istaiti, M., Rolfs, A., Zimran, A., & Revel-Vilk, S. (2022). Gaucher Disease Diagnosis Using Lyso-Gb1 on Dry Blood Spot Samples: Time to Change the Paradigm? *International Journal of Molecular Sciences 2022, Vol. 23, Page 1627*, *23*(3), 1627. https://doi.org/10.3390/IJMS23031627

Drelichman, G., Fernández Escobar, N., Soberon, B., Basack, N., Frabasil, J., Schenone, A., Aguilar, G., Larroudé, M., Knight, J., Zhao, D., Ruan, J., & Mistry, P. K. (2021). Long-read single molecule real-time (SMRT) sequencing of GBA1 locus in Gaucher disease national cohort from Argentina reveals high frequency of complex allele underlying severe skeletal phenotypes: Collaborative study from the Argentine Group for Diagnosis and Treatment of Gaucher Disease. *Molecular Genetics and Metabolism Reports*, *29*. https://doi.org/10.1016/J.YMGMR.2021.100820

Duran, R., McNeill, A., Mehta, A., Hughes, D., Cox, T., Deegan, P., Schapira, A. H. V., & Hardy, J. (2012). Novel pathogenic mutations in the glucocerebrosidase locus. *Molecular Genetics and Metabolism*, *106*(4), 495–497. https://doi.org/10.1016/j.ymgme.2012.05.006

Elbin, C. S., Olivova, P., Marashio, C. A., Cooper, S. K., Cullen, E., Keutzer, J. M., & Zhang, X. K. (2011). The effect of preparation, storage and shipping of dried blood spots on the activity of five lysosomal enzymes. *Clinica Chimica Acta*, *412*(13–14), 1207–1212. https://doi.org/10.1016/j.cca.2011.03.012

Elliott, S., Buroker, N., Cournoyer, J. J., Potier, A. M., Trometer, J. D., Elbin, C., Schermer, M. J., Kantola, J., Boyce, A., Turecek, F., Gelb, M. H., & Scott, C. R. (2016). Pilot study of newborn screening for six lysosomal storage diseases

using Tandem Mass Spectrometry. *Molecular Genetics and Metabolism*, *118*(4), 304–309. https://doi.org/10.1016/j.ymgme.2016.05.015

Emre, S., Gürakan, F., Yüce, A., Rolf, A., Scott, R., & Özen, H. (2008). Molecular analysis of Turkish Gaucher disease patients: Identification of novel mutations in glucocerebrosidase (GBA) gene. *European Journal of Medical Genetics*, *51*(4), 315–321. https://doi.org/10.1016/j.ejmg.2008.02.004

Erdos, M., Hodanova, K., Taskó, S., Palicz, A., Stolnaja, L., Dvorakova, L., Hrebicek, M., & Maródi, L. (2007). Genetic and clinical features of patients with Gaucher disease in Hungary. *Blood Cells, Molecules, and Diseases*, *39*(1), 119–123. https://doi.org/10.1016/j.bcmd.2007.02.005

Essabar, L., Meskini, T., Lamalmi, N., Ettair, S., Erreimi, N., & Mouane, N. (2015). Gaucher's disease: Report of 11 cases with review of literature. *Pan African Medical Journal*, *20*. https://doi.org/10.11604/pamj.2015.20.18.4112

Fateen, E., & Abdallah, Z. Y. (2019). Twenty- five years of biochemical diagnosis of Gaucher disease: the Egyptian experience. *Heliyon*, *5*(10). https://doi.org/10.1016/j.heliyon.2019.e02574

Feng, Y., Huang, Y., Tang, C., Hu, H., Zhao, X., Sheng, H., Zhang, W., Tan, M., Xie, T., Zheng, J., Liu, Z., Su, X., Shao, Y., Li, X., Cheng, J., Mao, X., & Liu, L. (2018). Clinical and molecular characteristics of patients with Gaucher disease in Southern China. *Blood Cells, Molecules, and Diseases*, *68*, 30–34. https://doi.org/10.1016/j.bcmd.2016.10.026

Ferraz, M. J., Marques, A. R. A., Gaspar, P., Mirzaian, M., van Roomen, C., Ottenhoff, R., Alfonso, P., Irún, P., Giraldo, P., Wisse, P., Sá Miranda, C., Overkleeft, H. S., & Aerts, J. M. (2016). Lyso-glycosphingolipid abnormalities in different murine models of lysosomal storage disorders. *Molecular Genetics and Metabolism*, *117*(2), 186–193. https://doi.org/10.1016/J.YMGME.2015.12.006

Filocamo, M., Mazzotti, R., Stroppiano, M., Seri, M., Giona, F., Parenti, G., Regis, S., Corsolini, F., Zoboli, S., & Gatti, R. (2002). Analysis of the glucocerebrosidase gene and mutation profile in 144 Italian gaucher patients. *Human Mutation*, *20*(3), 234–235. https://doi.org/10.1002/humu.9058

Fowler, B., Burlina, A., Kozich, V., & Vianey-Saban, C. (2008). Quality of analytical performance in inherited metabolic disorders: The role of ERNDIM. In *Journal of Inherited Metabolic Disease* (Vol. 31, Issue 6, pp. 680–689). Springer Netherlands. https://doi.org/10.1007/s10545-008-1025-4

Gaspar, P., Kallemeijn, W. W., Strijland, A., Scheij, S., van Eijk, M., Aten, J., Overkleeft, H. S., Balreira, A., Zunke, F., Schwake, M., Sá Miranda, C., & Aerts, J. M. F. G. (2014). Action myoclonus-renal failure syndrome: diagnostic applications of activity-based probes and lipid analysis. *Journal of Lipid Research*, *55*(1), 138–145. https://doi.org/10.1194/JLR.M043802

Gasparotto, N., Tomanin, R., Frigo, A. C., Niizawa, G., Pasquini, E., Blanco, M., Donati, M. A., Keutzer, J., Zacchello, F., & Scarpa, M. (2009). Rapid diagnostic testing procedures for lysosomal storage disorders: alpha-glucosidase and beta-galactosidase assays on dried blood spots. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *402*(1–2), 38–41. https://doi.org/10.1016/J.CCA.2008.12.006

Gelb, M. H., Turecek, F., Scott, C. R., & Chamoles, N. A. (2006). Direct multiplex assay of enzymes in dried blood spots by tandem mass spectrometry for the newborn screening of lysosomal storage disorders. *Journal of Inherited Metabolic Disease*, *29*(2–3), 397–404. https://doi.org/10.1007/s10545-006-0265-4

Giraldo, P., Pocoví, M., Pérez-Calvo, J., Rubio-Félix, D., & Giralt M. (2000). Report of the Spanish Gaucher's disease registry: clinical and genetic characteristics. *Haematologica*, *85*(8), 792–799.

Grabowski, G. A., Zimran, A., & Ida, H. (2015). Gaucher disease types 1 and 3: Phenotypic characterization of large populations from the ICGG Gaucher Registry. In *American Journal of Hematology* (Vol. 90, Issue S1, pp. S12–S18). Wiley-Liss Inc. https://doi.org/10.1002/ajh.24063

Grace, M. E., Balwani, M., Nazarenko, I., Prakash-Cheng, A., & Desnick, R. J. (2007). Type 1 Gaucher disease: Null and hypornorphic novel chitotriosidase mutations - Implications for diagnosis and therapeutic monitoring. *Human Mutation*, *28*(9), 866–873. https://doi.org/10.1002/humu.20524

Guo, Y., He, W., Boer, A. M., Wevers, R. A., de Bruijn, A. M., Groener, J. E. M., Hollak, C. E. M., Aerts, J. M. F. G., Galjaard, H., & van Diggelen, O. P. (1995). Elevated plasma chitotriosidase activity in various lysosomal storage disorders. *Journal of Inherited Metabolic Disease*, *18*(6), 717–722. https://doi.org/10.1007/BF02436762

Hannon, H. W. (2013). *Blood Collection on Filter Paper for Newborn Screening Programs; Approved Standard* (Sixth Edition). www.clsi.org.

Hatton, C. E., Cooper, A., Whitehouse, C., & Wraith, J. E. (1997). Mutation analysis in 46 British and Irish patients with Gaucher's disease. *Archives of Disease in Childhood*, *77*(1), 17–22. https://doi.org/10.1136/ADC.77.1.17

Hollak, C. E. M., van Weely, S., van Oers, M. H. J., & Aerts, J. M. F. G. (1994). Marked elevation of plasma chitotriosidase activity. A novel hallmark of Gaucher disease. *Journal of Clinical Investigation*, *93*(3), 1288–1292. https://doi.org/10.1172/JCI117084

Hopkins, P. v., Campbell, C., Klug, T., Rogers, S., Raburn-Miller, J., & Kiesling, J. (2015). Lysosomal storage disorder screening implementation: Findings from the first six months of full population pilot testing in Missouri. *Journal of Pediatrics*, *166*(1), 172–177. https://doi.org/10.1016/j.jpeds.2014.09.023

Horowitz, M., Tzuri, G., Eyal, N., Berebi, A., Kolodny, E. H., Brady, R. O., Barton, N. W., Abrahamov, A., & Zimran, A. (1993). Prevalence of nine mutations among Jewish and non-Jewish Gaucher disease patients. *American Journal of Human Genetics*, *53*(4), 930. /pmc/articles/PMC1682396/?report=abstract

Hruska, K. S., LaMarca, M. E., Scott, C. R., & Sidransky, E. (2008). Gaucher disease: Mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). In *Human Mutation* (Vol. 29, Issue 5, pp. 567–583). Hum Mutat. https://doi.org/10.1002/humu.20676

Huang, Y., Jia, X., Tang, C., Liu, S., Sheng, H., Zhao, X., Zeng, C., & Liu, L. (2020). High risk screening for Gaucher disease in patients with splenomegaly and/or thrombocytopenia in China: 55 cases identified. *Clinica Chimica Acta*, *506*, 22–27. https://doi.org/10.1016/j.cca.2020.03.016

Iyer, A., van Eijk, M., Silva, E., Hatta, M., Faber, W., Aerts, J. M. F. G., & Das, P. K. (2009). Increased chitotriosidase activity in serum of leprosy patients: Association with bacillary leprosy. *Clinical Immunology*, *131*(3), 501–509. https://doi.org/10.1016/j.clim.2009.02.003

Jeong, S. Y., Park, S. J., & Kim, H. J. (2011). Clinical and genetic characteristics of Korean patients with Gaucher disease. *Blood Cells, Molecules, and Diseases*, *46*(1), 11–14. https://doi.org/10.1016/j.bcmd.2010.07.010

Kang, L., Zhan, X., Gu, X., & Zhang, H. (2017). Successful newborn screening for Gaucher disease using fluorometric assay in China. *Journal of Human Genetics*, *62*(8), 763–768. https://doi.org/10.1038/jhg.2017.36

Kang, L., Zhan, X., Ye, J., Han, L., Qiu, W., Gu, X., & Zhang, H. (2018). A rare form of Gaucher disease resulting from saposin C deficiency. *Blood Cells, Molecules & Diseases*, *68*, 60–65. https://doi.org/10.1016/J.BCMD.2017.04.001

Karatas, M., Dogan, S., Spahiu, E., Ašić, A., Bešić, L., & Turan, Y. (2020). Enzyme kinetics and inhibition parameters of human leukocyte glucosylceramidase. *Heliyon*, *6*(11). https://doi.org/10.1016/j.heliyon.2020.e05191

Keatinge, M., Bui, H., Menke, A., Chen, Y. C., Sokol, A. M., Bai, Q., Ellett, F., da Costa, M., Burke, D., Gegg, M., Trollope, L., Payne, T., McTighe, A., Mortiboys, H., de Jager, S., Nuthall, H., Kuo, M. S., Fleming, A., Schapira, A. H. V., … Bandmann, O. (2015). Glucocerebrosidase 1 deficient Danio rerio mirror key pathological aspects of human Gaucher disease and provide evidence of early microglial activation preceding alpha-synuclein-independent neuronal cell death. *Human Molecular Genetics*, *24*(23), 6640–6652. https://doi.org/10.1093/hmg/ddv369

Kolodny, E. H., & Mumford, R. A. (1976). Human leukocyte acid hydrolases: Characterization of eleven lysosomal enzymes and study of reaction conditions for their automated analysis. *Clinica Chimica Acta*, *70*(2), 247–257. https://doi.org/10.1016/0009-8981(76)90426-5

Koprivica, V., Stone, D. L., Park, J. K., Callahan, M., Frisch, A., Cohen, I. J., Tayebi, N., & Sidransky, E. (2000). Analysis and classification of 304 mutant alleles in patients with type 1 anti type 3 gaucher disease. *American Journal of Human Genetics*, *66*(6), 1777–1786. https://doi.org/10.1086/302925

Kramer, G., Wegdam, W., Donker-Koopman, W., Ottenhoff, R., Gaspar, P., Verhoek, M., Nelson, J., Gabriel, T., Kallemeijn, W., Boot, R. G., Laman, J. D., Vissers, J. P. C., Cox, T., Pavlova, E., Moran, M. T., Aerts, J. M., & van Eijk, M. (2016). Elevation of glycoprotein nonmetastatic melanoma protein B in type 1 Gaucher disease patients and mouse models. *FEBS Open Bio*, *6*(9), 902–913. https://doi.org/10.1002/2211-5463.12078

Labadaridis, J., Dimitriou, E., Costalos, C., Aerts, J., van Weely, S., Donker- Koopman, W. E., & Michelakakis, H. (1998). Serial chitotriosidase activity estimations in neonatal systemic candidiasis [1]. In *Acta Paediatrica, International Journal of Paediatrics* (Vol. 87, Issue 5, p. 605). Acta Paediatr. https://doi.org/10.1080/08035259850158407

Lam, W. K. W., Ted, D., Li, C.-Y., & Yam, L. T. (1978). Biochemical Properties of Tartrate-Resistant Acid Phosphatasein Serum of Adultsand Children. *Clinical Chemistry*, *24*(7), 1105.

Lee, C. yu, Yen, H. Y., Zhong, A. W., & Gao, H. (2021). Resolving misalignment interference for NGS-based clinical diagnostics. *Human Genetics*, *140*(3), 477–492. https://doi.org/10.1007/s00439-020-02216-5

Lee, P., Waalen, J., Crain, K., Smargon, A., & Beutler, E. (2007). Human chitotriosidase polymorphisms G354R and A442V associated with reduced enzyme activity. *Blood Cells, Molecules, and Diseases*, *39*(3), 353–360. https://doi.org/10.1016/j.bcmd.2007.06.013

Lelieveld, L. T., Mirzaian, M., Kuo, C. L., Artola, M., Ferraz, M. J., Peter, R. E. A., Akiyama, H., Greimel, P., van den Berg, R. J. B. H. N., Overkleeft, H. S., Boot, R. G., Meijer, A. H., & Aerts, J. M. F. G. (2019). Role of β-glucosidase 2 in aberrant glycosphingolipid metabolism: Model of glucocerebrosidase deficiency in zebrafish. *Journal of Lipid Research*, *60*(11), 1851–1867. https://doi.org/10.1194/jlr.RA119000154

Lepe-Balsalobre, E., Santotoribio, J. D., Nuñez-Vazquez, R., García-Morillo, S., Jiménez-Arriscado, P., Hernández-Arévalo, P., Delarosa-Rodríguez, R., Guerrero, J. M., & Macher, H. C. (2020). Genotype/phenotype relationship in Gaucher disease patients. Novel mutation in glucocerebrosidase gene. *Clinical Chemistry and Laboratory Medicine*, *58*(12), 2017–2024. https://doi.org/10.1515/cclm-2020-0306

Li, Y., Scott, C. R., Chamoles, N. A., Ghavami, A., Pinto, B. M., Turecek, F., & Gelb, M. H. (2004). Direct multiplex assay of lysosomal enzymes in dried blood spots for newborn screening. *Clinical Chemistry*, *50*(10), 1785–1796. https://doi.org/10.1373/clinchem.2004.035907

Lieberman, J., & Beutler, E. (1976). Elevation of Serum Angiotensin-Converting Enzyme in Gaucher's Disease. *New England Journal of Medicine*, *294*(26), 1442–1444. https://doi.org/10.1056/nejm197606242942609

Lukacs, Z., Keil, A., Peters, V., Kohlschütter, A., Hoffmann, G. F., Cantz, M., & Kopitz, J. (2003). Towards quality assurance in the determination of lysosomal enzymes: A two-centre study. *Journal of Inherited Metabolic Disease*, *26*(6), 571–581. https://doi.org/10.1023/A:1025904132569

Magalhães, J., SáMiranda, M. C., Pinto, R., Lemos, M., & Poenaru, L. (1984). Sodium taurocholate effect on β-glucosidase activity: a new approach for identification of Gaucher disease using the synthetic substrate and leucocytes. *Clinica Chimica Acta*, *141*(2–3), 111–118. https://doi.org/10.1016/0009-8981(84)90003-2

Málaga, D. R., Brusius-Facchin, A. C., Siebert, M., Pasqualim, G., Pereira, M. L. S., de Souza, C. F. M., Schwartz, I. v.d., Matte, U., & Giugliani, R. (2019). Sensitivity, advantages, limitations, and clinical utility of targeted next-generation sequencing panels for the diagnosis of selected lysosomal storage disorders. *Genetics and Molecular Biology*, *42*(1), 197–206. https://doi.org/10.1590/1678-4685-gmb-2018-0092

Martins, A. M., Valadares, E. R., Porta, G., Coelho, J., Filho, J. S., Dudeque Pianovski, M. A., Kerstenetzky, M. S., de Fátima Pombo Montoril, M., Aranda, P. C., Pires, R. F., Mota, R. M. V., & Bortolheiro, T. C. (2009). Recommendations on Diagnosis, Treatment, and Monitoring for Gaucher Disease. *Journal of Pediatrics*, *155*(4 SUPPL.). https://doi.org/10.1016/j.jpeds.2009.07.004

Mattošová, S., Chandoga, J., Hlavatá, A., Šaligová, J., & Maceková, D. (2015). Spectrum of GBA mutations in patients with gaucher disease from Slovakia: Identification of five novel mutations. *Israel Medical Association Journal*, *17*(3), 166–170. https://pubmed.ncbi.nlm.nih.gov/25946768/

Mavrikiou, G., Petrou, P., Georgiou, T., & Drousiotou, A. (2016). Chitotriosidase deficiency in the Cypriot population: Identification of a novel deletion in the CHIT1 gene. *Clinical Biochemistry*, *49*(12), 885–889. https://doi.org/10.1016/j.clinbiochem.2016.03.013

Michelakakis, H., Dimitriou, E., & Labadaridis, I. (2004). The expanding spectrum of disorders with elevated plasma chitotriosidase activity: An update. *Journal of Inherited Metabolic Disease*, *27*(5), 705–706. https://doi.org/10.1023/B:BOLI.0000043025.17721.fc

Michelin, K., Wajner, A., Goulart, L. D. S., Fachel, Â. A., Pereira, M. L. S., de Mello, A. S., Souza, F. T. S., Pires, R. F., Giugliani, R., & Coelho, J. C. (2004). Biochemical study on β-glucosidase in individuals with Gaucher's disease and normal subjects. *Clinica Chimica Acta*, *343*(1–2), 145–153. https://doi.org/10.1016/j.cccn.2004.01.010

Millington, D., Norton, S., Singh, R., Sista, R., Srinivasan, V., & Pamula, V. (2018). Digital microfluidics comes of age: high-throughput screening to bedside diagnostic testing for genetic disorders in newborns. In *Expert Review of Molecular Diagnostics* (Vol. 18, Issue 8, pp. 701–712). Taylor and Francis Ltd. https://doi.org/10.1080/14737159.2018.1495076

Miocić, S., Filocamo, M., Dominissini, S., Montalvo, A. L. E., Vlahovicek, K., Deganuto, M., Mazzotti, R., Cariati, R., Bembi, B., & Pittis, M. G. (2005). Identification and functional characterization of five novel mutant alleles in 58 Italian patients with Gaucher disease type 1. *Human Mutation*, *25*(1), 100. https://doi.org/10.1002/humu.9301

Mistry, P., & Germain, D. P. (2006). Phenotype variations in Gaucher disease. *La Revue de Médecine Interne*, *27*, S3–S6. https://doi.org/10.1016/s0248-8663(06)80002-0

Moat, S. J., George, R. S., & Carling, R. S. (2020). Use of Dried Blood Spot Specimens to Monitor Patients with Inherited Metabolic Disorders. In *International Journal of Neonatal Screening* (Vol. 6, Issue 2). MDPI Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/ijns6020026

Motta M, Tatti M, Furlan F, Celato A, Di Fruscio G, Polo G, Manara R, Nigro V, Tartaglia M, Burlina A, Salvioli R. Clinical, biochemical and molecular characterization of prosaposin deficiency Clin Genet. 2016 Sep;90(3):220-9. doi: 10.1111/cge.12753. Epub 2016 Feb 19.

Muñoz, G., García-Seisdedos, D., Ciubotariu, C., Piris-Villaespesa, M., Gandía, M., Martín-Moro, F., Gutiérrez-Solana, L. G., Morado, M., López-Jiménez, J., Sánchez-Herranz, A., Villarrubia, J., & del Castillo, F. J. (2020). Early detection of lysosomal diseases by screening of cases of idiopathic splenomegaly and/or thrombocytopenia with a next-generation sequencing gene panel. *JIMD Reports*, *51*(1), 53–61. https://doi.org/10.1002/jmd2.12078

Murugesan, V., Chuang, W. L., Liu, J., Lischuk, A., Kacena, K., Lin, H., Pastores, G. M., Yang, R., Keutzer, J., Zhang, K., & Mistry, P. K. (2016). Glucosylsphingosine is a key biomarker of Gaucher disease. *American Journal of Hematology*, *91*(11), 1082–1089. https://doi.org/10.1002/ajh.24491

Murugesan, V., Liu, J., Yang, R., Lin, H., Lischuk, A., Pastores, G., Zhang, X., Chuang, W. L., & Mistry, P. K. (2018). Validating glycoprotein non-metastatic melanoma B (gpNMB, osteoactivin), a new biomarker of Gaucher disease. *Blood Cells, Molecules, and Diseases*, *68*, 47–53. https://doi.org/10.1016/j.bcmd.2016.12.002

Nesterovitch, A. B., Hogarth, K. D., Adarichev, V. A., Vinokour, E. I., Schwartz, D. E., Solway, J., & Danilov, S. M. (2009). Angiotensin I-converting enzyme mutation (Trp1197Stop) causes a dramatic increase in blood ACE. *PLoS ONE*, *4*(12). https://doi.org/10.1371/journal.pone.0008282

Olivova, P., Cullen, E., Titlow, M., Kallwass, H., Barranger, J., Zhang, K., & Keutzer, J. (2008). An improved high-throughput dried blood spot screening method for Gaucher disease. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *398*(1–2), 163–164. https://doi.org/10.1016/J.CCA.2008.08.024

Orenstein, M., Barbouth, D., Bodamer, O. A., & Weinreb, N. J. (2014). Patients with type 1 Gaucher disease in South Florida, USA: Demographics, genotypes, disease severity and treatment outcomes. *Orphanet Journal of Rare Diseases*, *9*(1). https://doi.org/10.1186/1750-1172-9-45

Orsini, J. J., Martin, M. M., Showers, A. L., Bodamer, O. A., Zhang, X. K., Gelb, M. H., & Caggana, M. (2012). Lysosomal storage disorder 4+1 multiplex assay for newborn screening using tandem mass spectrometry: Application to a small-scale population study for five lysosomal storage disorders. *Clinica Chimica Acta*, *413*(15–16), 1270–1273. https://doi.org/10.1016/j.cca.2012.04.012

Ortiz-Cabrera, N. v., Gallego-Merlo, J., Vélez-Monsalve, C., de Nicolas, R., Mas, S. F., Ayuso, C., & Trujillo-Tiebas, M. J. (2016). Nine-year experience in Gaucher disease diagnosis at the Spanish reference center Fundación Jiménez Díaz. *Molecular Genetics and Metabolism Reports*, *9*, 79–85. https://doi.org/10.1016/j.ymgmr.2016.06.008

Pacheco, N., & Uribe, A. (2013). Enzymatic analysis of biomarkers for the monitoring of Gaucher patients in Colombia. *Gene*, *521*(1), 129–135. https://doi.org/10.1016/j.gene.2013.03.044

Peters, S. P., Lee, R. E., & Glew, R. H. (1975). A microassay for Gaucher's disease. *Clinica Chimica Acta*, *60*(3), 391–396. https://doi.org/10.1016/0009-8981(75)90083-2

Polo, G., Burlina, A. P., Ranieri, E., Colucci, F., Rubert, L., Pascarella, A., Duro, G., Tummolo, A., Padoan, A., Plebani, M., & Burlina, A. B. (2019). Plasma and dried blood spot lysosphingolipids for the diagnosis of different sphingolipidoses: A comparative study. *Clinical Chemistry and Laboratory Medicine*, *57*(12). https://doi.org/10.1515/cclm-2018-1301

Quraishi, I. H., Szekely, A. M., Shirali, A. C., Mistry, P. K., & Hirsch, L. J. (2021). Miglustat Therapy for SCARB2-Associated Action Myoclonus-Renal Failure Syndrome. *Neurology. Genetics*, *7*(5), e614. https://doi.org/10.1212/NXG.0000000000000614

Rafi, M. A., de Gala, G., Zhang, X. ling, & Wenger, D. A. (1993). Mutational analysis in a patient with a variant form of Gaucher disease caused by SAP-2 deficiency. *Somatic Cell and Molecular Genetics*, *19*(1), 1–7. https://doi.org/10.1007/BF01233949

Raskovalova, T., Deegan, P. B., Mistry, P. K., Pavlova, E., Yang, R., Zimran, A., Berger, J., Bourgne, C., Pereira, B., Labarère, J., & Berger, M. G. (2020). Accuracy of chitotriosidase activity and CCL18 concentration in assessing type I Gaucher disease severity. A systematic review with meta-analysis of individual participant data. *Haematologica*, *105*(5). https://doi.org/10.3324/HAEMATOL.2019.236083

Reape, T. J., Rayner, K., Manning, C. D., Gee, A. N., Barnette, M. S., Burnand, K. G., & Groot, P. H. E. (1999). Expression and cellular localization of the CC chemokines PARC and ELC in human atherosclerotic plaques. *American Journal of Pathology*, *154*(2), 365–374. https://doi.org/10.1016/S0002-9440(10)65283-2

Reuser, A. J., Verheijen, F. W., Bali, D., van Diggelen, O. P., Germain, D. P., Hwu, W. L., Lukacs, Z., Mühl, A., Olivova, P., Piraud, M., Wuyts, B., Zhang, K., & Keutzer, J. (2011). The use of dried blood spot samples in the diagnosis of lysosomal storage disorders - Current status and perspectives. *Molecular Genetics and Metabolism*, *104*(1–2), 144–148. https://doi.org/10.1016/j.ymgme.2011.07.014

Revel-Vilk, S., Fuller, M., & Zimran, A. (2020). Value of glucosylsphingosine (Lyso-Gb1) as a biomarker in gaucher disease: A systematic literature review. In *International Journal of Molecular Sciences* (Vol. 21, Issue 19, pp. 1–33). MDPI AG. https://doi.org/10.3390/ijms21197159

Rodrigues, M. D. B., de Oliveira, A. C., Müller, K. B., Martins, A. M., & D'Almeida, V. (2009). Chitotriosidase determination in plasma and in dried blood spots: A comparison using two different substrates in a microplate assay. *Clinica Chimica Acta*, *406*(1–2), 86–88. https://doi.org/10.1016/j.cca.2009.05.022

Rolfs, A., Giese, A. K., 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. (2013). 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*, *8*(11). https://doi.org/10.1371/journal.pone.0079732

Roos, D., & Loos, J. A. (1970). Changes in the carbohydrate metabolism of mitogenicellay stimulated human peripheral lymphocytes I. Stimulation by phytohaemagglutinin. *BBA - General Subjects*, *222*(3), 565–582. https://doi.org/10.1016/0304-4165(70)90182-0

Sanders, K. A., Gavrilov, D. K., Oglesbee, D., Raymond, K. M., Tortorelli, S., Hopwood, J. J., Lorey, F., Majumdar, R., Kroll, C. A., McDonald, A. M., Lacey, J. M., Turgeon, C. T., Tucker, J. N., Tang, H., Currier, R., Isaya, G., Rinaldo, P., & Matern, D. (2020). A comparative effectiveness study of newborn screening methods for four lysosomal storage disorders. *International Journal of Neonatal Screening*, *6*(2). https://doi.org/10.3390/ijns6020044

Saville, J. T., McDermott, B. K., Chin, S. J., Fletcher, J. M., & Fuller, M. (2020). Expanding the clinical utility of glucosylsphingosine for Gaucher disease. *Journal of Inherited Metabolic Disease*, *43*(3), 558–563. https://doi.org/10.1002/jimd.12192

Schnabel, D., Schröder, M., & Sandhoff, K. (1991). Mutation in the sphingolipid activator protein 2 in a patient with a variant of Gaucher disease. *FEBS Letters*, *284*(1), 57–59. https://doi.org/10.1016/0014-5793(91)80760-Z

Schoonhoven, A., Rudensky, B., Elstein, D., Zimran, A., Hollak, C. E. M., Groener, J. E., & Aerts, J. M. F. G. (2007). Monitoring of Gaucher patients with a novel chitotriosidase assay. *Clinica Chimica Acta*, *381*(2), 136–139. https://doi.org/10.1016/j.cca.2007.02.042

Shapira, E., Blitzer, M. G., Africk, D. K., & Miller, J. B. (1989). *Biochemical Genetics: A Laboratory Manual* (1st edition). Oxford University Press.

Sheth, J., Bhavsar, R., Mistri, M., Pancholi, D., Bavdekar, A., Dalal, A., Ranganath, P., Girisha, K. M., Shukla, A., Phadke, S., Puri, R., Panigrahi, I., Kaur, A., Muranjan, M., Goyal, M., Ramadevi, R., Shah, R., Nampoothiri, S., Danda, S., … Sheth, F. (2019). Gaucher disease: Single gene molecular characterization of one-hundred Indian patients reveals novel variants and the most prevalent mutation. *BMC Medical Genetics*, *20*(1). https://doi.org/10.1186/s12881-019-0759-1

Silverstein, E., Pertschuk, L. P., & Friedland, J. (1980). Immunofluorescent detection of angiotensin-converting enzyme (ACE) in Gaucher cells. *American Journal of Medicine*, *69*(3), 408–410. https://doi.org/10.1016/0002-9343(80)90012-1

Sista, R. S., Wang, T., Wu, N., Graham, C., Eckhardt, A., Bali, D., Millington, D. S., & Pamula, V. K. (2013). Rapid assays for Gaucher and Hurler diseases in dried blood spots using digital microfluidics. *Molecular Genetics and Metabolism*, *109*(2), 218–220. https://doi.org/10.1016/j.ymgme.2013.03.010

Sista, R. S., Wang, T., Wu, N., Graham, C., Eckhardt, A., Winger, T., Srinivasan, V., Bali, D., Millington, D. S., & Pamula, V. K. (2013). Multiplex newborn screening for Pompe, Fabry, Hunter, Gaucher, and Hurler diseases using a digital microfluidic platform. *Clinica Chimica Acta*, *424*, 12–18. https://doi.org/10.1016/j.cca.2013.05.001

Skoog, W. A., & Beck, W. S. (1956). Studies on the fibrinogen, dextran and phytohemagglutinin methods of isolating leukocytes. *Blood*, *11*(5), 436–454. https://doi.org/10.1182/blood.v11.5.436.436

Spataro, N., Roca-Umbert, A., Cervera-Carles, L., Vallès, M., Anglada, R., Pagonabarraga, J., Pascual-Sedano, B., Campolongo, A., Kulisevsky, J., Casals, F., Clarimón, J., & Bosch, E. (2017). Detection of genomic rearrangements from targeted resequencing data in Parkinson's disease patients. *Movement Disorders*, *32*(1), 165–169. https://doi.org/10.1002/mds.26845

Stirnemann, J., Belmatoug, N., Camou, F., Serratrice, C., Froissart, R., Caillaud, C., Levade, T., Astudillo, L., Serratrice, J., Brassier, A., Rose, C., Billette de Villemeur, T., & Berger, M. (2017). A Review of Gaucher Disease Pathophysiology, Clinical Presentation and Treatments. *International Journal of Molecular Sciences*, *18*(2), 441. https://doi.org/10.3390/ijms18020441

Stirnemann, J., Vigan, M., Hamroun, D., Heraoui, D., Rossi-Semerano, L., Berger, M. G., Rose, C., Camou, F., de Roux-Serratrice, C., Grosbois, B., Kaminsky, P., Robert, A., Caillaud, C., Froissart, R., Levade, T., Masseau, A., Mignot, C., Sedel, F., Dobbelaere, D., … Belmatoug, N. (2012). The French Gaucher's disease registry: Clinical characteristics, complications and treatment of 562 patients. *Orphanet Journal of Rare Diseases*, *7*(1). https://doi.org/10.1186/1750-1172-7-77

Stroppiano, M., Calevo, M. G., Corsolini, F., Cassanello, M., Cassinerio, E., Lanza, F., Stroppiana, G., Cappellini, M. D., & Filocamo, M. (2014). Validity of β-d-glucosidase activity measured in dried blood samples for detection of potential Gaucher disease patients. *Clinical Biochemistry*, *47*(13–14), 1293–1296. https://doi.org/10.1016/j.clinbiochem.2014.06.005

Struthers, A. D., MacFadyen, R., Fraser, C., Robson, J., Morton, J. J., Junot, C., & Ezan, E. (1999). Nonadherence with angiotensin-converting enzyme inhibitor therapy: A comparison of different ways of measuring it in patients with chronic heart failure. *Journal of the American College of Cardiology*, *34*(7), 2072–2077. https://doi.org/10.1016/S0735-1097(99)00439-8

Struyf, S., Schutyser, E., Gouwy, M., Gijsbers, K., Proost, P., Benoit, Y., Opdenakker, G., van Damme, J., & Laureys, G. (2003). PARC/CCL18 Is a Plasma CC Chemokine with Increased Levels in Childhood Acute Lymphoblastic Leukemia. *American Journal of Pathology*, *163*(5), 2065–2075. https://doi.org/10.1016/S0002-9440(10)63564-X

Šumarac, Z., Suvajdžì, N., Ignjatoviż, S., Majkí-Singh, N., Janí, D., Petakov, M.,ˊoŕeví, M., Mitroví, M., Dajak, M., Goluboví, M., & Rodí, P. (2011). Biomarkers in Serbian patients with Gaucher disease. *Clinical Biochemistry*, *44*(12), 950–954. https://doi.org/10.1016/j.clinbiochem.2011.05.016

Tortorelli, S., Turgeon, C. T., Gavrilov, D. K., Oglesbee, D., Raymond, K. M., Rinaldo, P., & Matern, D. (2016). Simultaneous testing for 6 lysosomal storage disorders and x-adrenoleukodystrophy in dried blood spots by tandem mass spectrometry. *Clinical Chemistry*, *62*(9), 1248–1254. https://doi.org/10.1373/clinchem.2016.256255

Tuchman, L. R., Suna, H., & Carr, J. J. (1956). Elevation of serum acid phosphatase in Gaucher's disease. *Journal of the Mount Sinai Hospital, New York*, *23*(2), 227–229. https://europepmc.org/article/med/13307230

Tylki-Szymańska, A., Czartoryska, B., Vanier, M. T., Poorthuis, B. J. M. H., Groener, J. A. E., Ługowska, A., Millat, G., Vaccaro, A. M., & Jurkiewicz, E. (2007). Non-neuronopathic Gaucher disease due to saposin C deficiency. *Clinical Genetics*, *72*(6), 538–542. https://doi.org/10.1111/J.1399-0004.2007.00899.X

Vaccaro, A. M., Motta, M., Tatti, M., Scarpa, S., Masuelli, L., Bhat, M., Vanier, M. T., Tylki-Szymanska, A., & Salvioli, R. (2010). Saposin C mutations in Gaucher disease patients resulting in lysosomal lipid accumulation, saposin C

deficiency, but normal prosaposin processing and sorting. *Human Molecular Genetics*, *19*(15), 2987–2997. https://doi.org/10.1093/HMG/DDQ204

van Breemen, M. J., Bleijlevens, B., de Koster, C. G., & Aerts, J. M. F. G. (2006). Limitations in quantitation of the biomarker CCL18 in Gaucher disease blood samples by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. *Biochimica et Biophysica Acta - Proteins and Proteomics*, *1764*(10), 1626–1632. https://doi.org/10.1016/j.bbapap.2006.08.004

van der Lienden, M. J. C., Gaspar, P., Boot, R., Aerts, J. M. F. G., & van Eijk, M. (2019). Glycoprotein non-metastatic protein B: An emerging biomarker for lysosomal dysfunction in macrophages. In *International Journal of Molecular Sciences* (Vol. 20, Issue 1). MDPI AG. https://doi.org/10.3390/ijms20010066

van Dussen, L., Hendriks, E. J., Groener, J. E. M., Boot, R. G., Hollak, C. E. M., & Aerts, J. M. F. G. (2014). Value of plasma chitotriosidase to assess non-neuronopathic Gaucher disease severity and progression in the era of enzyme replacement therapy. *Journal of Inherited Metabolic Disease*, *37*(6), 991–1001. https://doi.org/10.1007/s10545-014-9711-x

van Eijk, M., Ferra, M. J., Boot, R. G., & Aerts, J. M. F. G. (2020). Lyso-glycosphingolipids: Presence and consequences. In *Essays in Biochemistry* (Vol. 64, Issue 3, pp. 565–578). Portland Press Ltd. https://doi.org/10.1042/EBC20190090

Vedder, A. C., Cox-Brinkman, J., Hollak, C. E. M., Linthorst, G. E., Groener, J. E. M., Helmond, M. T. J., Scheij, S., & Aerts, J. M. F. G. (2006). Plasma chitotriosidase in male Fabry patients: A marker for monitoring lipid-laden macrophages and their correction by enzyme replacement therapy. *Molecular Genetics and Metabolism*, *89*(3), 239–244. https://doi.org/10.1016/j.ymgme.2006.04.013

Verma, J., Thomas, D. C., Sharma, S., Jhingan, G., Singh, A., Hsiao, K. J., Schoonderwoerd, K., Puri, R. D., & Verma, I. C. (2015). Inherited metabolic disorders: Quality management for laboratory diagnosis. *Clinica Chimica Acta*, *447*, 1–7. https://doi.org/10.1016/j.cca.2015.04.040

vom Dahl, S., Harzer, K., Rolfs, A., Albrecht, B., Niederau, C., Vogt, C., Weely, S. van, Aerts, J., Müller, G., & Häussinger, D. (1999). Hepatosplenomegalic lipidosis: What unless Gaucher? Adult cholesteryl ester storage disease (CESD) with anemia, mesenteric lipodystrophy, increased plasma chitotriosidase activity and a homozygous lysosomal acid lipase -1 exon 8 splice junction mutation. *Journal of Hepatology*, *31*(4), 741–746. https://doi.org/10.1016/S0168-8278(99)80356-0

Wasserstein, M. P., Caggana, M., Bailey, S. M., Desnick, R. J., Edelmann, L., Estrella, L., Holzman, I., Kelly, N. R., Kornreich, R., Kupchik, S. G., Martin, M., Nafday, S. M., Wasserman, R., Yang, A., Yu, C., & Orsini, J. J. (2019). The New York pilot newborn screening program for lysosomal storage diseases: Report of the First 65,000 Infants. *Genetics in Medicine*, *21*(3), 631–640. https://doi.org/10.1038/s41436-018-0129-y

Wenger, D. A., Clark, C., Sattler, M., & Wharton, C. (1978). Synthetic substrate beta-glucosidase activity in leukocytes: a reproducible method for the identification of patients and carriers of Gaucher's disease. *Clinical Genetics*, *13*(2), 145–153. https://doi.org/10.1111/J.1399-0004.1978.TB04242.X

Wolf, P., Alcalay, R. N., Liong, C., Cullen, E., Pauciulo, M. W., Nichols, W. C., Gan-Or, Z., Chung, W. K., Faulkner, T., Bentis, C., Pomponio, R. J., Ma, X., Kate Zhang, X., Keutzer, J. M., & Oliva, P. (2018). Tandem mass spectrometry assay of β-glucocerebrosidase activity in dried blood spots eliminates false positives detected in fluorescence assay. *Molecular Genetics and Metabolism*, *123*(2), 135–139. https://doi.org/10.1016/j.ymgme.2017.10.011

Yu, C., Sun, Q., & Zhou, H. (2013). Enzymatic Screening and Diagnosis of Lysosomal Storage Diseases. *North American Journal of Medicine and Science*, *06*(04), 186. https://doi.org/10.7156/najms.2013.0604186

Zampieri, S., Cattarossi, S., Bembi, B., & Dardis, A. (2017). GBA Analysis in Next-Generation Era: Pitfalls, Challenges, and Possible Solutions. *Journal of Molecular Diagnostics*, *19*(5), 733–741. https://doi.org/10.1016/j.jmoldx.2017.05.005

Zampieri, S., Cattarossi, S., Pavan, E., Barbato, A., Fiumara, A., Peruzzo, P., Scarpa, M., Ciana, G., & Dardis, A. (2021). Accurate Molecular Diagnosis of Gaucher Disease Using Clinical Exome Sequencing as a First-Tier Test. *International Journal of Molecular Sciences*, *22*(11), 5538. https://doi.org/10.3390/ijms22115538

Zanetti, A., D'Avanzo, F., Bertoldi, L., Zampieri, G., Feltrin, E., de Pascale, F., Rampazzo, A., Forzan, M., Valle, G., & Tomanin, R. (2020). Setup and Validation of a Targeted Next-Generation Sequencing Approach for the Diagnosis of Lysosomal Storage Disorders. *Journal of Molecular Diagnostics*, *22*(4), 488–502. https://doi.org/10.1016/j.jmoldx.2020.01.010

Zeigler, M., Meiner, V., Newman, J. P., Steiner-Birmanns, B., Bargal, R., Sury, V., Mengistu, G., Kakhlon, O., Leykin, I., Argov, Z., Abramsky, O., & Lossos, A. (2014). A novel SCARB2 mutation in progressive myoclonus epilepsy indicated by reduced β-glucocerebrosidase activity. *Journal of the Neurological Sciences*, *339*(1–2), 210–213. https://doi.org/10.1016/J.JNS.2014.01.022

Zhang, X. K., Elbin, C. S., Chuang, W. L., Cooper, S. K., Marashio, C. A., Beauregard, C., & Keutzer, J. M. (2008). Multiplex enzyme assay screening of dried blood spots for lysosomal storage disorders by using tandem mass spectrometry. *Clinical Chemistry*, *54*(10), 1725–1728. https://doi.org/10.1373/clinchem.2008.104711