



Review article

A perspective on research, diagnosis, and management of lysosomal storage disorders in Colombia



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ABSTRACT

Lysosomal storage diseases (LSDs) are a group of about 50 inborn errors of metabolism characterized by the lysosomal accumulation of partially or non-degraded molecules due to mutations in proteins involved in the degradation of macromolecules, transport, lysosomal biogenesis or modulators of lysosomal environment. Significant advances have been achieved in the diagnosis, management, and treatment of LSDs patients. In terms of approved therapies, these include enzyme replacement therapy (ERT), substrate reduction therapy, hematopoietic stem cell transplantation, and pharmacological chaperone therapy. In this review, we summarize the Colombian experience in LSDs through the evidence published. We identified 113 articles published between 1995 and 2019 that included Colombian researchers or physicians, and which were mainly focused in Mucopolysaccharidoses, Pompe disease, Gaucher disease, Fabry disease, and Tay-Sachs and Sandhoff diseases. Most of these articles focused on basic research, clinical cases, and mutation reports. Noteworthy, implementation of the enzyme assay in dried blood samples, led to a 5-fold increase in the identification of LSD patients, suggesting that these disorders still remain undiagnosed in the country. We consider that the information presented in this review will contribute to the knowledge of a broad spectrum of LSDs in Colombia and will also contribute to the development of public policies and the identification of research opportunities.

1. Introduction

Lysosomal storage diseases (LSDs) are a group of about 50 inborn errors of metabolism (IEM) characterized by the lysosomal accumulation of partially or non-degraded molecules. LSDs are caused by the impairment of lysosomal function due to mutations in proteins involved in lysosomal degradation of macromolecules (e.g. lysosomal hydrolases), transport of products from the lysosome lumen to other cell compartments, lysosomal biogenesis (e.g. lysosomal membrane proteins, proteins in charge of posttranslational modification of lysosomal hydrolases and their intracellular traffic) or modulators of lysosomal environment [1, 2].

LSDs are usually classified on a biochemical basis, taking into account the type of accumulated substrate or the physiopathology. Thus, LSD may be classified in sphingolipidoses, mucopolysaccharidoses (MPS),

oligosaccharidosis, mucopolipidoses, defects of lysosomal membrane proteins, and others. Table 1 shows a list of some LSDs classified in based on the accumulated substrate. In general, most LSD are inherited in an autosomal recessive manner, although this group includes also X-linked inheritance diseases (dominant and recessive). According to some authors, LSD might be the most common group of IEM affecting 1:5,000 newborns, with individual incidences ranging from 1:60,000 to 1:100,000 [2, 3, 4].

In general, LSDs may present at any age and are characterized by a chronic and progressive compromise with a clinical onset usually during childhood. Clinical manifestations may be multisystemic or organ-specific. Some clinical features that suggest a LSD may include hepatosplenomegaly, disostosis multiplex, corneal opacities, macular cherry red spot, neuroregression, angiokeratomas, coarse facial features,

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Table 1. Classification of LSDs and some examples of the diseases in each group.

LSD group	Disease	OMIM	Defective Protein	Lysosomal alteration
Sphingolipidoses	Gaucher	230800	β -Glucosidase	Lysosomal hydrolase
		230900		
		231000		
	GM1 Gangliosidoses	230500	β -galactosidase	Lysosomal hydrolase
		230600		
		230650		
	Variant AB of GM2 Gangliosidoses	272750	GM2 activator protein	Activator protein
	Sandhoff disease	268800	β -hexosaminidases A and B	Lysosomal hydrolase
	Tay–Sachs disease	272800	beta-hexosaminidase A	Lysosomal hydrolase
Fabry's disease	301500	α -galactosidase	Lysosomal hydrolase	
Krabbe disease	245200	Galactosylceramidase	Lysosomal hydrolase	
Metachromatic leukodystrophy	250100	Arylsulfatase A	Lysosomal hydrolase	
Mucopolysaccharidoses	Hurler syndrome (MPS I)	607014	α -L-iduronidase	Lysosomal hydrolase
	Hunter syndrome (MPS II)	309900	iduronate-2-sulfatase	Lysosomal hydrolase
	Sanfilippo syndrome (MPS III)	252900	Heparan N-sulfatase	Lysosomal hydrolase/transferase
		252920	α -N-acetylglucosaminidase	
		252930	Acetyl-CoA:alpha-glucosaminide	
		252940	N-acetyltransferase	
	Morquio syndrome (MPS IV)	253000	N-acetylgalactosamine-6-sulfatase	Lysosomal hydrolase
		β -galactosidase		
Maroteaux Lammy (MPS VI)	253200	Arylsulfatase B	Lysosomal hydrolase	
Sly syndrome	253220	β -glucuronidase	Lysosomal hydrolase	
Oligosaccharidosis	α -Mannosidosis	248500	α -Mannosidase	Lysosomal hydrolase
Mucopolidoses (ML)	ML type I	256550	Sialidase	Lysosomal hydrolase
	ML types II/III	252500	N-acetylglucosamine-1-fosforiltransferase	Lysosomal hydrolases trafficking
		252600		
ML type IV	252650	Mucopilin 1	Membrane protein	
Lysosomal membrane proteins	Danon disease	300257	LAMP2	Membrane protein
	Cystinosis	219750	Cystinosine	Membrane transporter
Other	Pompe	232300	α -glucosidase	Lysosomal hydrolase
	Multiple sulfatase deficiency	272200	Formylglycine generating enzyme	Posttranslational modification of lysosomal hydrolases

among others. A common feature among LSDs is the high clinical heterogeneity with different clinical phenotypes described according to age of onset, severity, central nervous system (CNS) involvement, and clinical progression. To mention some, GM2 gangliosidoses (Sandhoff and Tay-Sachs disease) present infantile, juvenile, and adult forms. In a similar way, MPS type I is classified according to the clinical presentation in Hurler (MPS IH), Hurler-Scheie (MPS I H/S), and Scheie (MPS IS). The first one is the earlier and most severe form characterized by CNS involvement and high mortality in the first decade, while the second one, MPS IS, is the mildest form without CNS involvement, minor clinical manifestations and normal lifespan [5, 6, 7].

Diagnosis of a LSD is a challenge for clinicians considering the clinical variability, the low frequency of these diseases, and lack of specificity of the signs and symptoms. Therefore, diagnostic approximation for LSDs requires the assessment of clinical and paraclinical test results (peripheral blood smears, radiological and neurophysiological findings, etc.). For instance, some patterns of grey/white matter involvement in cerebral nuclear magnetic resonance (NMR) might lead to the study of specific sphingolipidosis. On the other hand, certain skeletal abnormalities (e.g. platyspondily, paddle-shape ribs, J-shaped sella) are highly suggestive of MPS; while evidence of “foamy cells” in bone marrow is a characteristic finding of some sphingolipidosis, such as Gaucher and Niemann-Pick diseases.

Specific biochemical studies for LSDs include detection in biological fluids of undegraded stored material (e.g. glycosaminoglycans for MPS, oligosaccharides for sphingolipidoses, etc.). These biomarkers are available only for some LSDs and in general present a high sensitivity but low specificity. Therefore, biochemical confirmation relies in the assessment of enzyme biological activity, which can be measured from

different sources such as dried blood samples (DBS), leukocytes, and fibroblasts. DBS has gained importance due to its high stability that facilitates transport and storage, but still it is considered a screening sample. In most of the cases, enzyme confirmation is performed in leukocytes since, compared to fibroblasts, its isolation and handling manipulation is easier. In general, it is considered that values below 30% of residual activity are diagnostic of an LSD. It is important to recall that not all LSDs are caused by an enzymatic defect and some specific enzymatic tests are not widely available. Therefore, molecular diagnosis should be considered during the diagnosis of some LSD, such as neuronal ceroid lipofuscinosis [8]. Although molecular diagnosis is considered the theoretical confirmation of any IEM, including LSDs, the interpretation of results of such analyses is difficult considering the lack of complete understanding of the clinical implications of recently reported variants, and the interactions among them [2, 5, 9].

Therapy for LSDs has been a challenge since their initial descriptions. Many options have been proposed and up to date therapies available are focused on: 1) promoting storage clearance by administering an exogenous functional enzyme, the so called enzyme replacement therapy (ERT), which is currently available for 11 diseases [Gaucher, Fabry and Pompe diseases, late infantile neuronal ceroid lipofuscinosis type 2 (CLN2), acid lipase deficiency, alpha-mannosidosis, and MPS type I, II, IVA, VI, and VII] with around 15 different molecules; 2) preventing the lysosomal accumulation of substrates by inhibiting their synthesis, which is called substrate reduction therapy (SRT) and has two approved molecules; 3) potentiating the enzyme activity *in vivo* by administering small molecules called pharmacological chaperones, which are capable of improving the enzyme intracellular processing and folding; and 4) bone marrow or stem cell transplantation, especially in those diseases with

CNS involvement [10, 11, 12, 13, 14, 15, 16]. Although all the above-mentioned therapies can improve patients survival and quality of life, most of them present limited effects in some tissues such as bone and CNS. Moreover, pharmacological chaperones and SRT may need combination with other therapies to achieve better results. In addition, therapeutic strategies are available only for few LSDs and currently they do not lead to a disease cure. Thus, great efforts are being done in novel therapies including gene therapy (both by addition or editing) and ERTs with improved biodistribution, half-life, and efficacy [12].

In Colombia, resources for establishing diagnosis of IEM have been accessible for more than 30 years. In addition, important efforts have been done to increase the divulgation and knowledge of these disorders among health professional and health care providers along these years. Moreover, patient needs have driven efforts to improve the diagnosis, accessing to therapies, and the development of basic and clinical research in LSDs by different research groups. Although the incidence of LSDs in Colombia remains unknown, some reports for MPS and other LSDs have begun to offer some information about it. This has been driven, at least in part, by the Colombian Orphan Diseases Act (Law 1392 of 2010), which established: 1) the obligation of the government to create a national registry of patients with rare diseases, 2) to create a system to import and distribute orphan drugs aiming to get fair access to all the patients, and 3) to create a network of specialized centers for diagnosis, treatment, and orphan drugs distribution. This Act also covers the education of human talent on these diseases at all educational levels. As a result of this Act, the National Public Health Surveillance System (SIVIGILA), from the National Institute of Health, has begun to offer valuable information about the epidemiology of the rare diseases in Colombia [17, 18]. For instance, from 2016 to 2018, MPS IVA and Fabry disease were the LSDs with the highest number of patients in Colombia, with 115 and 92 patients, respectively [18]. Nevertheless, significant efforts have to be done

to improve the report of rare diseases, which will allow the design of proper health policies and to prioritize the research, treatment, and diagnosis resources. The reader is referred to some recent LSDs reviews, to obtain a more detailed information about the molecular bases, diagnosis, and treatment of this group of diseases [19, 20, 21, 22, 23, 24]. In this review, we summarize the Colombian experience in LSDs through the evidence published. For this purpose, an exhaustive review of the literature on the subject of LSDs carried out by Colombian researchers or physicians.

Databases like PubMed, Embase, Web of science, Medigraphic, Scopus, Scielo, Ebscohost, Springer link, Redalyc were consulted in English and Spanish from 1995 to 2019. The search was conducted as follows: (TITLE-ABS-KEY (lysosomal AND disease OR "Lysosomal disease*") AND TITLE-ABS-KEY (review OR "literature review" OR "meta-analysis" OR "meta-analysis" OR meta-analysis OR "systematic review")) AND (LIMIT-TO (LANGUAGE, "English") OR LIMIT-TO (LANGUAGE, "Spanish")) AND (LIMIT-TO (AFFILCOUNTRY, "Colombia")). Figure 1 shows the distributions of the 115 articles found in this search, which were mainly focused in MPS [n = 25], Pompe disease [n = 7], Gaucher disease [n = 8], Fabry disease [n = 12], and Tay-Sachs and Sandhoff diseases [n = 3], among others (Figure 1A). Among MPS reports, MPS IVA represented the most studied LSD in Colombia with 35 reports, followed by MPS II [n = 15], MPS I [n = 3], VI [n = 3], and MPS III [n = 1]; while no reports for MPS VII were found (Figure 1B). Finally, articles were mainly focused on basic research, followed by clinical cases and mutation reports. In addition, Colombia researches have participated in an important number of reviews and disease guidelines and registries (Figure 1C). We consider that the information presented in this review will contribute to the knowledge of a broad spectrum of the situation of LSDs in Colombia, which may also contribute to the development of public policies and the identification of research opportunities.

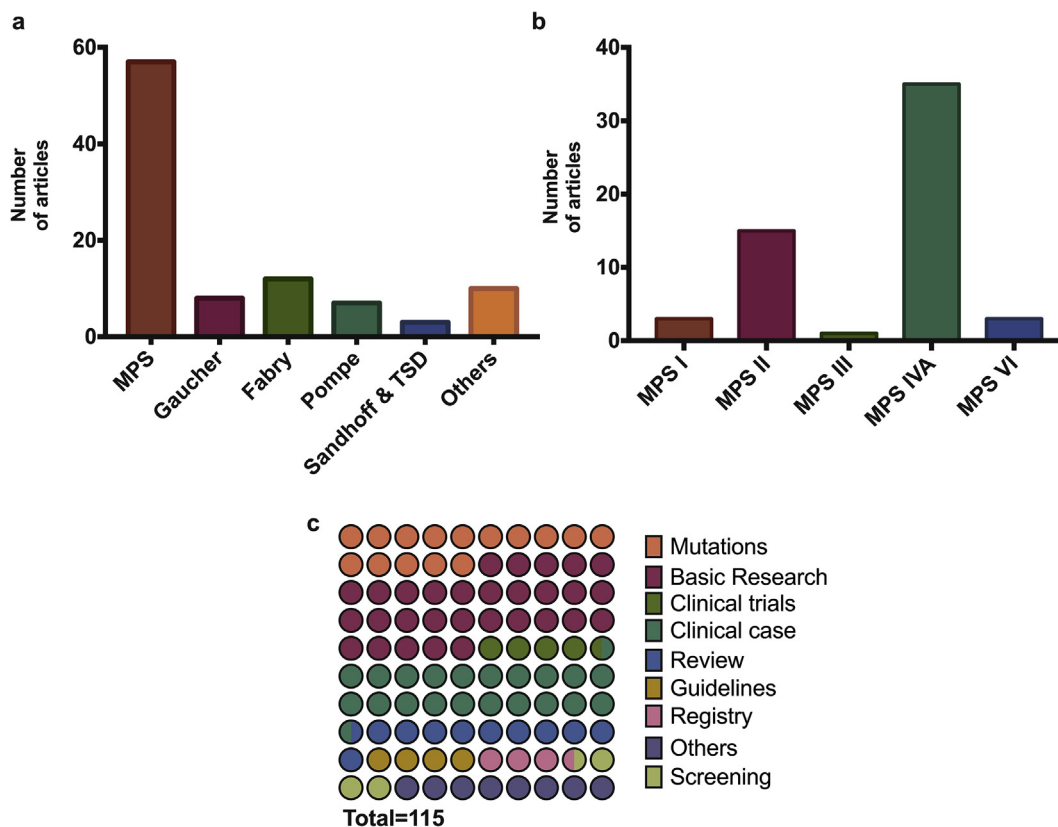


Figure 1. Articles found on databases about LSDs in Colombia classified by the LSD group, not including review articles (a), type of MPS (b) and number of articles by topic (c).

2. Fabry disease

Johannes Fabry and William Anderson first described Fabry or Anderson Fabry disease (OMIM 301500) in 1898. This is an X-linked LSD caused by the deficiency of alpha-galactosidase (α -GLA, EC 3.2.1.22) [26, 27]. This deficiency leads to the lysosomal accumulation of glycosphingolipids, mainly globotriaosylceramide (GL-3) [26]. There are more than 600 mutations reported for Fabry disease including small deletions and insertions, splicing, missense, and nonsense mutations [27, 28, 29]. It is the second most common LSD in the world with an estimated global incidence of 1:40,000 men and 1:117,000 in the general population [26, 27].

As reported for other populations, Colombian Fabry patients present mild pain and proteinuria in childhood and adolescence; kidney disease, stroke, and cardiovascular manifestations in adulthood; and cutaneous and gastrointestinal manifestations that may begin in adolescence. The appearance of angiokeratoma, which occurs as results of glycosphingolipids accumulation at the vascular level, has also been reported in this population [28, 30, 31]. Ophthalmological features of Fabry disease have been described in Colombian patients not only in the anterior segment, but also in the posterior pole of the eye. The manifestations of the anterior segment include aneurysmal vessels in the bulbar conjunctiva, vertical cornea, and lens opacification. In the posterior segment, the most common features are vessel tortuosity and retinal vascular occlusions [32]. Recently, it was reported the case of a female patient affected with systemic sclerosis and Fabry disease. In addition to the systemic sclerosis clinical manifestations, the patient was characterized by chest pain, a second-degree A-V block, and restrictive cardiomyopathy (without cardiovascular risk factors) [33].

A multidisciplinary approach, comprised of neurologists, ophthalmologists, nephrologists, cardiologists, otorhinolaryngologists, pediatricians, geneticists and dermatologists is necessary for the management of Fabry patients [26, 27]. Therefore, a global database was constructed with the clinical information collected from 3752 patients. It includes guidelines for the evaluation of patients, thus being an educational support for patients [26]. The Fabry Registry in Latin America includes patients from Argentina, Brazil, Chile, Colombia, Mexico, Peru, Uruguay, and Venezuela [27]. Although, specific data for Colombia patients was not presented in the paper, the results at that moment showed: 1) higher percentage of children and a mean age younger than in the rest of the world (ROW); 2) Latin America patients were less likely to receive ERT than patients in the ROW; 3) women patients were less likely to receive ERT than men; 4) 31% of men and 22% of women experienced a clinical event during the natural history period, which is similar to ROW patients; 5) 15% and 2% of men and women, respectively, required renal replacement therapy, similar to patients in the ROW; 6) a small percentage of patients reported stroke (5% of men and 2% of women) [27]. A recent study analyzed the clinical profiles and natural history of Latin America Fabry female patients ($n = 169$) on ERT ("ERT-recipients", $n = 93$) and without treatment ("ERT-naïve", $n = 76$) [34]. Five Colombian patients (3%) were included in this study, from which 2 and 3 were at the ERT-recipients and ERT-naïve groups, respectively. Taking in consideration all studied patients, it was observed that the first manifestation occurred at an age of 12.7 years and experienced a neurologic symptom as their first Fabry disease manifestation (mostly peripheral pain) in the second decade of life. In addition, the median time from the first Fabry disease manifestation to diagnosis was 10.3 years, and the time from disease onset to ERT initiation had a mean of 21.3 years [34].

Two different ERT-based treatments have been approved for Fabry disease: agalsidase alfa (Replagal®) and agalsidase beta (Fabrazyme®) [32, 35]. A report from a 47 year old Colombia Fabry patient, showed that this therapy reverses many of the main clinical manifestations, including pain decrease, improvement of kidney disease and ventricular hypertrophy, and generally improvement in the patient quality of life [36, 37].

3. Gaucher disease

Gaucher disease is an autosomal recessive LSD caused by partial or total deficiency of the lysosomal enzyme acid β -glucosidase (GBA, EC. 3.2.1.45) or saposin C protein, which acts as an activator of GBA [38]. The deficiencies of GBA or saposin C produce the accumulation of glucocerebroside within the lysosomes of macrophages and monocytes. This causes an increase in cell size and subsequent progressive accumulation in liver, spleen, bone marrow, and the CNS. Accumulation impairs multisystemic functions and produces great damages that can lead to early death of the patients [38, 39, 40]. Gaucher disease is the most common LSD affecting all ethnicities, with a frequency of 1:40,000 to 1:100,000 live births; while Ashkenazi Jewish can have frequencies between 1:1,200 to 1:2,000 live births [39, 41, 42]. However, no incidences have been reported for Gaucher disease in Colombia, and it was not among the top 20 rare diseases in the last SIVIGILA report [17, 18].

Clinically, Gaucher disease is classified in three forms based on the absence (type I, OMIM 230800) or presence of neurological involvement (types II, OMIM 230900, and type III OMIM 231000). Gaucher disease type I may present on infancy or adulthood. Gaucher disease type I can have a slow or fast progression, with hepatosplenomegaly, pancytopenia, (or isolated anemia or thrombocytopenia), bone pain, osteopenia and osteoporosis with no compromise of CNS. Gaucher disease type II is an acute neuronopathy that presents in infants with seizures, hypertension, apnea, anemia, thrombocytopenia, hepatosplenomegaly, developmental delay, and neuroregression with a lifespan around two years. Lastly, Gaucher disease type III is the chronic neuronopathic form with an onset in infancy or adolescence. Patients show hepatosplenomegaly, myoclonus, dementia, and oculomotor apraxia [38, 39, 40, 42, 43]. Mejía-Turizo et al., reported the first case in Colombia of a Gaucher disease type III patient with vitreous condensation and macular edema [42].

Pomponio et al. [43], described GBA mutations from 25 Colombian patients with Gaucher disease. These mutations were c.595_596delCT, c.898delG and c.1255G > C in exons 6, 7, and 9 of the GBA gene, respectively. In addition, they found a double mutation (p.L483P + p.E355K) in three patients with Gaucher disease type I. This double mutation was previously reported in a patient with Gaucher disease type II [43]. Another study showed the high prevalence of the p.L483P in Spanish patients which suggested Hispanic ancestry among patients with this mutation [44]. Wilches et al. [45], determined a high grade of association between five microsatellites (5GC3.2, ITG6.6.2, D1S2777, D1S2624, and D1S305) and the N370S mutation. This is the most frequent mutation, among other GBA mutations, in the Colombian population, more specifically, in the Cundinamarca and Boyacá region. This study showed that these microsatellites are in linkage disequilibrium with the p.N370S mutation, which suggests not only that this particular arrangement of microsatellites (haplotype) may be predictive of mutation the p.N370S, but also that p.N370S has been conserved since it was introduced in this particular population from an ancestral haplotype. Nevertheless, it was not possible to determine the origin of such ancestral haplotype [45].

One of the most studied biomarkers for Gaucher disease is chitotriosidase (EC. 3.2.1.14); a chitin-degrading enzyme that presents elevated activity in Gaucher disease. A study of Pacheco and Uribe [46], evaluated the use of chitotriosidase in serum and DBS samples as a viable biomarker in the monitoring of Gaucher disease in Colombia, especially in rural areas where health services are not easily available. The results showed that chitotriosidase activity in serum from healthy volunteers ranged from 0 to 94.7 nmol/mL/h, while Gaucher patients showed activities between 2325.5 and 35266.01 nmol/mL/h. On the other hand, DBS healthy volunteers showed activities between 0 and 106.2 nmol/mL/h; while Gaucher patients had activities between 150.9 and 2568.5 nmol/mL/h [46].

Several ERTs have been approved for Gaucher disease. The first ERT was described in 1991, aglucerase (Ceredase®), which was derived from placenta macrophages. The Food and Drug Administration (FDA)

approved Miglucerase (Cerezyme®), which replaced aglucerase, and it is an analog of the GCCase produced in Chinese Hamster Ovary (CHO) cells. In 2010 velaglucerase-alfa (Vpriv®) was approved, an enzyme produced in human fibroblast cell lines. In 2012, the FDA approved taliglucerase alfa (Elelyso®), which is produced in carrot cells [42]. Evaluation of miglucerase in Colombian Gaucher patients was reported by Lozano [40], showing amelioration of hematological and biochemical manifestations. In another case, a male patient with Gaucher disease type III was treated with ERT during 11 years [47]. After the patient died, the autopsy showed some pathological features that were identical to those of untreated patients. However, liver, spleen, and bone marrow responded to the treatment. Similarly, a study carried out by Mistry et al. [48], demonstrated that orally administered eliglustat (SRT) to a Colombian Gaucher disease patient, resulted in a clinically meaningful 30% reduction in spleen size compared with the placebo. Improvement in hemoglobin level, liver size, and platelet count was also observed. In addition, there was improvement in bone marrow burden, as well as reductions of monosialodihexosylganglioside (GM3) and glucosylceramide (GL-1) levels in plasma. In addition, chitotriosidase and macrophage inflammatory protein (MIP-1 β) were substantially reduced.

4. Pompe disease

Pompe disease, or glycogen-storage disease type II (OMIM 232300), is an autosomal recessive disorder caused by the deficiency of the lysosomal enzyme acid α -glucosidase (GAA, EC 3.2.1.20) [49]. GAA catalyzes the hydrolysis of the α -1,4 and α -1,6-glucosidic bonds of glycogen, and its deficiency leads to the lysosomal accumulation of glycogen in several tissues, especially muscle [50, 51]. It is reported that the prevalence for Pompe disease is 1:40,000 live births worldwide [52]. Although there are no data about the prevalence of this disease in Colombia, until 2013, 24 cases were reported as well as the mutations p.E176fsx45, p.L355P, p.W746R, p.G828_N882del, and p.R854X [51].

Treatment options for Pompe patients is mainly based on ERT by using the recombinant enzyme alglucosidase alfa. Symptoms can manifest early on life and if the disease is left untreated the probability of death increases. Early ERT treatment can lead to better outcomes such as significant reduction of the cardiomegaly and the improvement of patients quality of life [53]. A report on the use of ERT in Colombia showed positive results in a 60 year old patient receiving the treatment [54]. However, several cases where the therapy has been used in Colombian patients demonstrate the importance of early diagnosis to initiate successful ERT [52, 55, 56].

5. The mucopolysaccharidoses

The MPS are a group of 11 LSDs caused by mutations on the genes that encode for lysosomal enzymes in charge of glycosaminoglycans (GAGs) catabolism. The absence or reduction in the activity of these enzymes leads to the accumulation of partially degraded GAGs heparan sulfate (HS), dermatan sulfate (DS), keratan sulfate (KS), chondroitin sulfate (CS), or hyaluronan within the lysosomes of several tissues [57]. MPS can be categorized according to the lysosomal enzyme that is deficient (Table 1). MPS are multisystemic disorders commonly presented with organomegaly, corneal opacity, bone deformities, and/or mental impairment depending on the stored material [57]. Treatment options for MPS are currently limited to ERT and hematopoietic stem cell therapy (HSCT). However, approaches such as gene therapy, genome editing, and pharmacological chaperones are being studied to overcome the constraints of traditional therapy options [58].

5.1. Mucopolysaccharidosis type I (Hurler, Hurler–Scheie, and Scheie syndromes)

MPS I is an autosomal recessive LSD where the α -L-iduronidase (IDUA, EC 3.2.1.76) that is required for the hydrolysis of DS and HS, has a

deficiency [59]. This deficiency leads to accumulation of GAGs in bone, connective tissues, and the CNS [60]. Three novel missense mutations have been reported in Colombian population (p.Y625C, p.P385L, p.R621L) [59]. Birth prevalence of MPS I is higher in Northern European countries like Norway, Denmark, Sweden and Ireland where Norway has a prevalence of 1.85 per 100000 live births [61]. First cases of Hurler syndrome reported in Colombia date back to 1995 [62] and a temporal cluster of MPS I was reported in the region of Sucre, suggesting a founder effect [63]. In the regions of Boyacá and Cundinamarca a prevalence of 0.45 per 100,000 live births was reported between 1998 to 2007 [64]. Diagnosis for MPS I is initially done by a clinical suspicion, followed identification of GAGs in urine and enzyme activity assay [65]. As reported by Pineta et al. [59], molecular identification of MPS I patients is deficient in Colombia, leading to unawareness of the mutational profile of the *IDUA* gene. This might represent a limitation in diagnosis of the disease when the biochemical analysis is not conclusive.

Treatment for MPS I includes the use of HSCT and ERT [58, 65], but there are not reports showing the efficacy of these treatments in Colombian patients. As an alternative to these approaches, Simonaro et al. [60] demonstrated that the use of pentosan polysulfate to treat MPS I H/Sleads to the reduction of GAGs and inflammation that can work as an adjunct or stand-alone treatment.

5.2. Mucopolysaccharidosis type II (Hunter syndrome)

MPS II is an X-linked LSD caused by the deficiency of iduronate-2-sulfatase (IDS, EC 3.1.6.13), necessary for the catalysis of the sulfate group of the iduronic acid present in HS and DS [66]. The deficiency of IDS leads to the accumulation of GAGs in lysosomes of different organs including the CNS [67]. Galvis et al. [68] reported 13 mutations in a group of 18 MPS II patients (Figure 2), from which six were novel (c.548_564dup16, c.477insT, c.595_607del12, c. 549_562del13, c.182delC, and a complete deletion of exon 7). In addition, as an effort to understand the structure of the protein and to evaluate dynamics of the enzyme in the presence of mutations, Saenz et al [69] modeled the tridimensional structure of the human IDS. Hunter syndrome has a prevalence between 0.5 and 0.99 per 100,000 live births in Europe and there is a very high incidence in East Asia due to a common mutation [61]. In Colombia, for the regions of Cundinamarca and Boyacá it was reported a prevalence of 0.45 per 100,000 live births [64].

Cardona et al. [70], demonstrated the distribution of IDS and its interactome in the brain of wild-type mice. This gives an insight into the potential implications of IDS in cell growth, molecular adhesion, regulation of enzymatic activity, intracellular traffic, biogenesis of myelin, and glycolytic pathway on the CNS. These results may have a significant impact to the further understanding of the molecular and cellular basis of Hunter syndrome [70].

Diagnosis of MPS II, according to the clinical guide reported by Giugliani et al. [67], includes a review of the medical history of family and the patient as well as early signs of the disease. A biochemical analysis should include identification of GAGs in urine, enzyme assay of IDS, and the molecular analysis of *IDS* gene [67, 71]. Alternatively, a low cost immunoquantification technique using IgY antibodies against IDS, could be used to quantify the enzyme in human plasma samples [66, 72].

Management of MPS II patients depends on the severity of the disease, and may include cardiac valve replacement surgery, ophthalmological evaluations, the use of hearing aids and continuous positive airway pressure devices, surgery for abdominal hernias, and physical therapy [67]. On the other hand, treatment involves ERT with a recombinant enzyme produced in mammalian cells, which leads to the improvement in the six-minute-walke test, forced vital capacity, liver and spleen volumes, joint range of motion, and urine GAGs levels [73, 74]. Results from Colombian patients receiving ERT showed improvement in the six-minute-walke test, pulmonary function, and it was well tolerated [75, 76, 77]. The production of recombinant IDS in *Pichia pastoris* and *Escherichia*

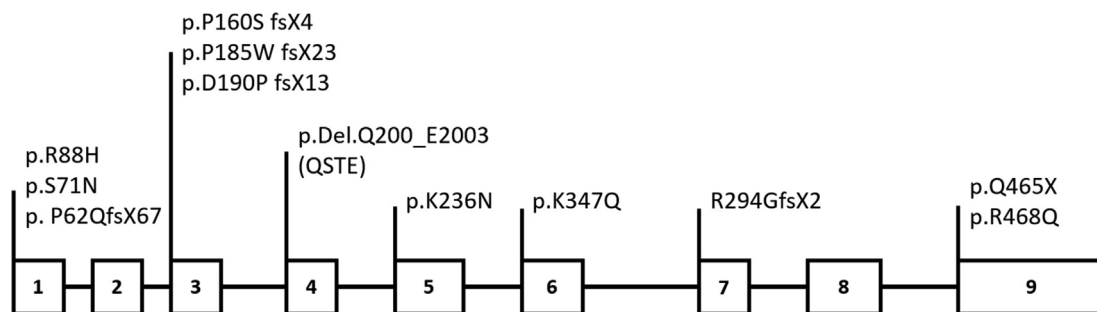


Figure 2. Location of mutations in the *IDS* gene identified in Colombian MPS II patients. The exons are represented by boxes.

coli has been evaluated as an alternative for the production of the enzyme used for ERT [78, 79, 80, 81, 82, 83].

5.3. Mucopolysaccharidosis type III (Sanfilippo syndrome)

MPS III is an autosomal recessive LSD classified into four types depending on the enzyme affected. Defects on any of the enzymes, causes accumulation of HS in lysosomes of different tissues [61, 84] (Table 1). Prevalence of the disease in Colombia has been reported as 0.17 per 100,000 live births, making MPS III the least frequent MPS. The highest prevalence was found in the Boyacá, where high levels of endogamy are reported, and a novel nonsense mutation (p.G454X) related to MPS IIIC is present in several individuals [64, 85]. In the 2013–2014 period, Velasco et al [86] evaluated the incidence of various diseases in different regions of Boyacá, Colombia (Cocuy, Soatá, Tunja, Chiquinquirá, Garagoa and Sogamoso). They found five patients positive for MPS III over 152 patients suspicious of having a genetic disease. Four of these patients belonged to the same family from which they found other six subjects with MPS III. The estimated incidence was 2.8 in 100,000, which is higher in comparison to previous values reported [64, 86].

5.4. Mucopolysaccharidosis type IVA (Morquio A syndrome)

Mucopolysaccharidosis type IVA (MPS IVA, Morquio A syndrome) is a LSD caused by mutations in the gene encoding for the enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS, EC 3.1.6.4) [87]. GALNS deficiency leads to the lysosomal accumulation of the KS and chondroitin-6-sulfate (C6S) [87, 88]. Worldwide incidence rates of MPS IVA range from 0.09 to 0.14 per 10,000 at birth [89]. In Colombia, a prevalence of 0.68 per 100,000 was reported in the provinces of Cundinamarca and Boyacá, showing the highest prevalence among other MPS [64]. Recent studies have confirmed the high frequency of MPS IVA in our country [90, 91], suggesting that Colombia is one of the countries with the highest prevalence of this disease worldwide [89]. In addition, human figures with similar features of MPS IVA have been described in pottery collections from Tumaco's-La Tolita culture, which suggest the presence of this disease in the pre-Hispanic times [92, 93]. Fourteen mutations have been described in Colombian MPS IVA patients (Figure 3), with p.G301C, p.R386C, and p.S162F being the most frequent mutations, present in about 51%, 16%, and 13% of the alleles,

respectively [90, 91, 94, 95, 96]. GALNS genotype-phenotype correlations have predicted that mutations may affect the hydrophobic core, salt bridges, ligand affinity, solvent-accessible surface, and N-glycosylation sites [97, 98, 99, 100, 101]. To improve the phenotype-genotype correlation for MPS IVA, 124 missense GALNS mutations were analyzed using a modeled 3D protein structure [102]. Molecular docking and dynamics analysis showed that sulfate group from KS and C6S interacted with Ca^{+2} , Cys79, Arg83, His142, and Asp288/Asn289; while Ala102 and Tyr108 or Tyr181 interacted with the substrate carbon chain, predicting the importance of these residues in the enzyme-substrate interaction. The bioinformatics analysis predicted that: 1) missense mutations within the active cavity reduce GALNS-substrate affinity; 2) severe mutations are associated with changes in highly conserved residues, non-conservativeness changes, and low values of Atomic Accessible Surface Area; and 3) attenuated mutations are associated with intermediate amino acid conservation, conservativeness changes, surface position, high values of Atomic Accessible Surface Area, and energy minimization close or lower to that of the wild type enzyme [102]. Finally, a computational analysis of the active cavity of human GALNS showed that 85% of amino acids are highly conserved among species. In addition, this active cavity has a positive partial charge, which correlates with the negative charge of GALNS substrates [103].

Clinically, MPS IVA patients are characterized by severe growth restriction, corneal clouding, hypoplasia of the odontoid process, *pectus carinatum*, valvular heart disease, mild hepatomegaly, laxity of joints, kyphoscoliosis, and *genu valgum*, without cognitive compromise [87, 88, 104]. Clinical characteristics of Colombian MPS IVA patients are similar to those reported for other populations, with osteoarticular-deformities, short stature, and gait alterations as the main clinical manifestations of the disease [90, 91, 96]. Compared to the reference growth charts [105], 53% male and 57% female of these patients have a height between percentiles 10 and 25 and 25 and P50, respectively [90]. Additional studies showed that Colombian MPS IVA patients have an average height of 97 cm (92–104 cm), as well as an average weight and body mass index (BMI) of 26 kg and 27.6 kg/m², respectively [91]. A study of quality of life in 28 Colombian MPS IVA patients (age 3.5–27.3 years) showed that 68% could perform self-care activities without any difficulty, while 14% were unable to do it. Similarly, 53% of patients could perform mobility activities without any difficulty, while 29% were unable to do it. Nevertheless, 43% of patients required caregiver assistance to perform

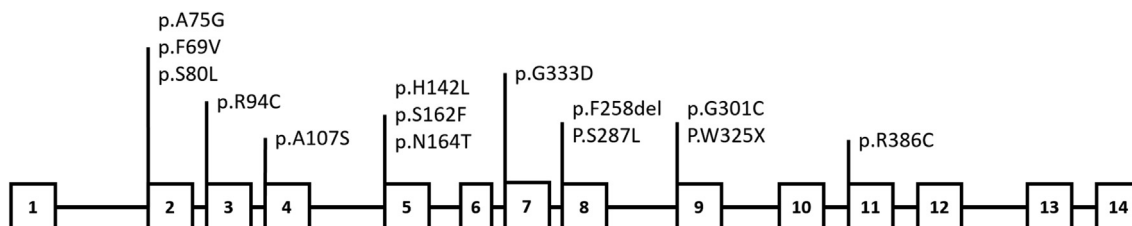


Figure 3. Location of mutations in the *GALNS* gene identified in Colombian MPS IVA patients. The exons are represented by boxes.

these activities [106]. An evaluation of school skills and neuropsychological functioning in four MPS IVA adolescent patients reported slow reading and writing speed, difficulties in fine praxis and visual tracking, and low performance in numerical management, reading comprehension, and logical reasoning [107]. Although MPS IVA patients do not appear to have serious CNS alterations, neuropathological features in cerebral cortex, Ammon's horn, and thalamic nuclei have been reported [108]. These results might represent an opportunity to design education and school inclusion policies for these patients, as well as to make adaptations to the academic curriculum. A study including 102 MPS IVA patients and their families (a total of 81 families), showed that mothers are the main caregivers, usually in a full-time manner that limits their labor market participation [109]. This study showed a high satisfaction in factors related with internal family dynamics, such as family interactions and parental role. On the other hand, families were less satisfied with factors related to external elements, such as access to health services, lack of opportunities to reach family goals, and limited support for people with disabilities [109]. These results represent valuable information to design tools that increase the quality of life of MPS IVA patients and their families, especially on issues beyond those directly related with the disease.

Contributions from Colombia in basic and applied research for MPS IVA have included production and characterization of recombinant GALNS in bacteria [110, 111, 112, 113, 114] and yeast [115, 116], the design of viral vectors for gene therapy [117, 118, 119, 120, 121, 122, 123, 124], the identification of pharmacological chaperones [125], and the generation of induced pluripotent stem cell lines [126]. Results from recombinant GALNS showed that N-glycosylations are not necessary for production of active enzyme but for cell uptake [112], and that recombinant GALNS produced in the yeast *P. pastoris* is taken up by cultured cells and reach the lysosomes, in which it can mediate the reduction of KS level [116]. Gene therapy vectors have been mainly based in the use of adeno-associated virus (AAV) derived vectors. Studies with these vectors have included the evaluation of the effect of the promoter region in GALNS expression and SUMF1 co-expression [117, 118], the *in vivo* evolution using a MPS IVA mouse model [127], and the design of a bone-tagged vector by insertion of a short acidic amino acid peptide within the viral capsid [122]. Recently, the first pharmacological chaperones for MPS IVA were reported, showing that these molecules can increase the yield of recombinant GALNS and be used as a monotherapy or combination therapy to improve the therapeutic efficacy of MPS IVA ERT [125]. In addition, significant contributions have been done in the identification and characterization of biomarkers [128, 129, 130], the evaluation of elosulfase alfa [131, 132], and the design of guidelines for the management and treatment of MPS IVA [133, 134].

5.5. Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome)

MPS IV, Maroteaux-Lamy syndrome, is a LSD caused by mutations in the gene encoding for the enzyme N-acetylgalactosamine-4-sulfatase

(arylsulfatase B, ARSB, EC 3.1.6.12). ARSB deficiency leads to the lysosomal accumulation of DS and chondroitin-4-sulfate (C4S) [135]. Clinically, MPS VI patients are characterized by short stature, hepatosplenomegaly, dysostosis multiplex, stiff joints, corneal clouding, cardiac abnormalities, and facial dysmorphism, with normal cognitive skills [135]. Worldwide incidence rates of MPS VI range from 0.36 to 1.3 per 100,000 live births [61]. In Colombia, a prevalence of 0.23 per 100,000 was reported in the region of Cundinamarca and Boyacá [64]. Human figures with suggestive features of MPS VI have been also described in pottery collections from Tumaco's-La Tolita culture [136]. The figures represent young people or infants with phenotypical features like skeletal dysplasia, macrocephaly, mildly coarse facial features, broad mouth, prominent sternum, kyphosis, and scoliosis. Due to the high number of figures with these characteristics (20 ceramic pieces), it is suggested a high prevalence of the disease in that time [136]. In fact, several cases of MPS VI have been confirmed in native groups from Colombia [137], which may confirm the presence of this disease in pre-Hispanic Latin-American cultures.

Giraldo et al. [138] reported 14 mutations (Figure 4) in a similar number of MPS VI patients, including 9 missense, 2 nonsense, 2 deletions, and 1 intronic mutation [138]. Eight mutations were described for the first time in MPS VI patients: p.H111P, p.C121R, p.H147P, p.D300E, p.S334I, p.G446S, p.Y513X, p.X534W. The most frequent mutations were p.H111P and p.G302R, accounting for 31% of the mutated alleles.

6. GM2 gangliosidosis

Tay-Sachs (OMIM 272800) and Sandhoff (OMIM 268800) diseases are autosomal recessive genetic neurodegenerative disorders caused by the accumulation of non-degraded glycolipids in lysosomes [139]. They are classified as GM2 gangliosidosis. β -hexosaminidases (Hex) are heterodimeric enzymes that exist in three isoforms: hexosaminidase A (Hex A, EC 3.2.1.52), hexosaminidase B (Hex B, EC 3.2.1.52) and hexosaminidase S (Hex S), which are involved in lysosomal degradation of glycolipids and glycans [139, 140]. They consist of α and β subunits encoded by the *HEXA* and *HEXB* genes, respectively. Mutations in these genes lead to the diseases of Tay-Sachs or Sandhoff, respectively [139, 140].

Diagnosis of a Colombian patient with Sandhoff disease include physical and ophthalmologic examination, magnetic resonance imaging, and analysis of urinary GAGs, oligosaccharides, organic acids, and amino acids [141]. In addition, treatment of a Colombian patient with miglustat and a ketogenic diet, improved the cardiac manifestations of the disease [141].

Contributions from Colombia in basic and applied research for Tay-Sachs and Sandhoff Disease include the production of recombinant Hex-A and Hex-B in the yeast *P. pastoris*, showing the potential of this host in the development of an ERT for these disorders [140]. In addition, Colombian researchers were involved in the development of induced

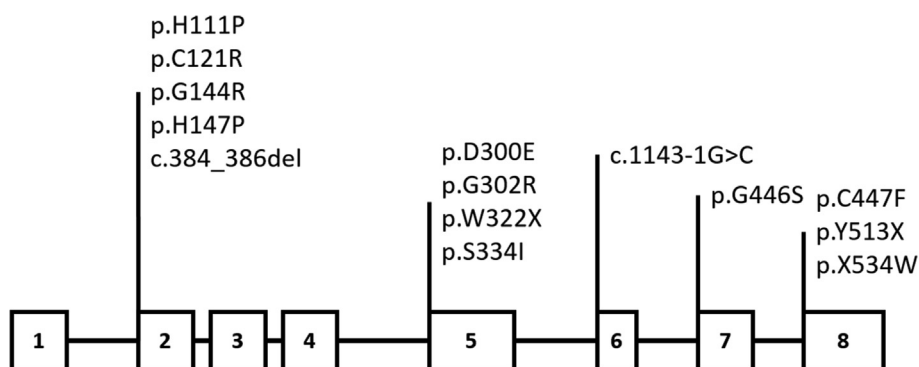


Figure 4. Location of mutations in the ARSB gene identified in Colombian MPS VI patients. The exons are represented by boxes.

pluripotent stem cells (iPSCs) derived from Tay-Sachs disease patients fibroblasts to produce a cellular model of the disease based on neuronal stem cells [139]. These cells were used to evaluate the effect of a recombinant human Hex-A protein and two small molecular compounds: 2-hydroxypropyl- β -cyclodextrin and δ -tocopherol. Overall, neuronal stem cells showed a significant increase in lipids, which were normalized after treatment with the recombinant protein and the small molecules [139].

7. Other lysosomal storage diseases studied in Colombia

7.1. Niemann-Pick

Niemann-Pick type C (NPC, OMIM, 257220) is an autosomal recessive neurodegenerative disease caused by mutations in the genes NPC1 and NPC2. These mutations lead to a deficiency in the intracellular transporter of non-esterified cholesterol, and the subsequent accumulation of cholesterol within the lysosomes [142]. Pardo et al [142]. evaluated skin biopsies from 73 patients with suspected NPC disease. In this study, pediatric and adult patients presented variable symptoms and showed cholesterol deposition at a perinuclear location. Additionally, it was also found that the accumulation of cholesterol in patient fibroblasts leads to cell apoptosis, suggesting that the accumulation causes structural alterations in neurons leading to neurotransmission damage. Furthermore, NPC patients also display lysosomal accumulation of vitamin-E, causing decreased antioxidant activity in cells and increasing the products derived from cholesterol oxidation as oxysterols in plasma [142].

7.2. Lysosomal acid lipase deficiency

Lysosomal acid lipase (LAL, OMIM, 613497) deficiency is an autosomal recessive disease caused by the deficiency of LAL glycoprotein activity producing the accumulation of lipids as ester-cholesterol and triglycerides within lysosomes of liver, blood vessels, spleen, adrenal glands, hematopoietic system, lymph nodes, and gastrointestinal tract. Diagnostic approximation of LAL deficiency is done by liver ultrasound and biopsy. A study of Botero et al [143] described the first LAL deficiency patient in Colombia, in a 14-year-old male who presented absence of LAL enzyme activity and the homozygous mutation c.894G > A. Although ERT with sebelipase alpha was available at the time of diagnosis, the patient was treated only with statins, which are considered ineffective in patients with LAL deficiency disease and did not result in improvement of the hepatic involvement. The reasons for which the patient was not treated with the ERT were the administrative problems for the approval of ERT administration, which represent also a major problem for other LSDs in Colombia.

7.3. Metachromatic leukodystrophy

Metachromatic leukodystrophy (OMIM, 250100) is an autosomal recessive demyelinating disease caused by deficiency of the lysosomal enzyme arylsulfatase A (ARSA, EC 3.1.6.8), that is involved in the metabolism of sulfatides, which are essential constituents of myelin sheaths. ARSA deficiency leads to the accumulation of metachromatic lipid material and galactosylceramide sulfates in the white matter of the central and peripheral nervous system, as well as in other organs [144, 145]. Álvarez-Pabón et al [144]. described a 24-month-old female patient with extensive periventricular hyperintense lesions and fluid attenuated inversion recovery (FLAIR) compatible with leukodystrophy. Moreover, the patient also had a decreased ARSA activity (11.92 nmol/h/mg vs. control 37.08 nmol/h/mg) and the molecular testing revealed two heterozygous mutations in ARSA gene: p.N352S and p.T393S. On the other hand, Echeverri-Peña [146] reported a Colombian female patient that presented a slight decrease in ARSA activity levels and showed perinatal noxa in brain images with possible

hypoxic-ischemic origin. However, metachromatic leukodystrophy was discarded, and it was not possible to establish a definite diagnosis.

7.4. Galactosialidosis

Galactosialidosis (OMIM, 256540) is an autosomal recessive LSD caused by partial or total deficiency of the cathepsin A protective protein (CAPP), that produces a combined phenotype of GM1 gangliosidosis and sialidosis. This protein is required for the integrity of β -D-galactosidase and neuraminidase lysosomal enzymes [147]. Prada et al [147]. mentioned that for Galactosialidosis, whole exome sequencing (WES) might be employed to make the molecular diagnosis of approximately 25–30% of the unsolved cases, taking into account that the literature has only reported 15 cases of the late form of the disease. This study included the diagnostic evaluation of a 24-year-old female patient that presented coarse facial features, short stature, mild cognitive disability, mild conductive hearing loss and aortic stenosis. The results from biochemical analyses including urinary GAGs, oligosaccharides, sialic acid, amino acid test and enzyme activity of α -L-iduronidase and arylsulfatase B, that were within normal limits and molecular testing for *GLB1* and *TRPV4* genes, which were also normal. WES analysis of the patient and her parents allowed the identification of the heterozygous mutations p.Y267N and p.296_296del in the cathepsin A gene in the proband. Enzyme activity confirmation showed low activity in neuraminidase and β -galactosidase enzymes, with 8 pmol/min/mg (control 95–653 pmol/min/mg) and 0.5 nmol/min/mg (control 3.78–11 nmol/min/mg) activity levels, respectively. For this reason, the authors concluded that WES may allow the identification not only of variant phenotypes but also expands the phenotypic spectrum of the diseases, especially when the patient does not present a key phenotypic characteristics that could lead to a specific diagnosis [147].

7.5. GM1 Gangliosidosis

GM1 Gangliosidosis is an autosomal recessive LSD caused by the deficiency of the β -galactosidase enzyme, producing the subsequent accumulation of the GM1 ganglioside and other galactoconjugate compounds, mainly in the brain gray matter. Londoño et al [148]. described a 5-year-old female patient, who, at 14 months of age, presented regression in developmental milestones. During the next 4 years, the patient presented other symptoms and the enzymatic testing confirmed the diagnosis of a late childhood or juvenile GM1 gangliosidosis. The patient progressed without improvement, with microcephaly and deep severe intellectual disability. In addition, Ortiz et al [149]. described a 5-year-old male patient, who since 12 months of age, showed delay in psychomotor development. Six months later, the patient presented progressive loss of motor milestones and language and lack of interest in the environment. General and specific analyses for metabolic disorders were performed finding decreased activity of β -galactosidase (3.96 nmol/h/mg - control. 28.24 nmol/h/mg and 0.88 nmol/h/mg - control 6.36 nmol/h/mg). Therefore, this patient was diagnosed as a juvenile variant or GM1 gangliosidosis type 2.

7.6. Fucosidosis

Fucosidosis (OMIM, 230000) is a rare autosomal recessive LSD caused by the deficiency of the α -L-fucosidase enzyme (EC 3.2.1.51) coded by *FUCA1* gene, that causes accumulation of glycopeptides such as fucosyl-glycopeptides and oligosaccharides in some tissues [150]. In Colombia, a study of Valero-Rubio et al [150] analyzed in keratinocytes the effect of *FUCA1* gene knock-down to understand the pathogenesis in skin lesions that fucosidosis patients present. The authors found that 387 genes were deregulated among cells transfected with a siRNA-A targeting *FUCA1* (222 upregulated and 165 downregulated). Moreover, the dysregulated genes showed 12 functional categories involving biological process such as keratinocytes/epidermal development and immune

responses. In addition, they also found *KRT16* and *S100A7* having a potential interaction on skin lesions pathogenesis observed in fucosidosis patients. Genes such as *SPRR*, *CRABP2*, *KRT1*, *KRT4*, and *TGM1* were overexpressed and associated with regulation patterns in pathological conditions such as hyperkeratosis. In addition, the study also showed the importance of the *FOXN1* proteins as key molecules for the regulation of thymic epithelial cells and skin keratinocytes. The study did not demonstrate the direct effect of this protein in regulation of gene expression in *FUCA1* knock-out cells. Finally, the study suggested the hypothesis that the skin injuries in fucosidosis patients could be caused by molecular cascades and dysfunctions with overlapping etiology [150].

8. Screening of LSD patients in Colombia

Studies in Colombia about LSDs are mainly based on clinical criteria because there are no large-scale screening methods for these diseases. However, the remission of liquid samples (serum, plasma cell extracts, etc.), requires strict cold chain conditions during transport, which increases the costs of analysis and makes it difficult to diagnose many patients who are distant from specialized health centers. Therefore, the need to expand the coverage of high-risk screening in Colombia made it necessary to develop a methodology using DBS on filter paper. This objective was accomplished by standardizing fluorometric micro-techniques using a 1.2 mm punch (0.52 µl of blood) and artificial 4-methylumbelliferone substrates. The project implied the study of eight lysosomal enzymes: α-Galactosidase A (Fabry disease), α-Glucosidase (Pompe disease), α-L-iduronidase (MPS I), Arylsulfatase B (MPS VI), β-galactosidase (GM1 Gangliosidosis/MPS IVB), β-Glucosidase (Gaucher disease), total hexosaminidase (GM2 Gangliosidosis) and Iduronate sulfatase (MPS II). Likewise, chitotriosidase was also studied, since overexpression can be used as a diagnostic support for sphingolipid metabolism disorders. The project lasted 6 years (2005–2011) and evaluated 4,700 patients with suspected alterations in lysosomal metabolism, finding 242 cases (5.2%) of individuals affected with Fabry disease (n = 31), Pompe disease (n = 16), MPS I (n = 15), MPS VI (n = 34), GM1 Gangliosidosis (n = 10), MPS IVB (n = 1), Gaucher disease (n = 101), GM2 Gangliosidosis (n = 1), mucopolipidosis (n = 2), and MPS I (n = 31) [151]. In addition, it was found that chitotriosidase analyses (originally intended to detect patients with Gaucher disease) could be used in the diagnosis of other LSDs, since 74 patients with different LSDs showed increased chitotriosidase activity [151]. Another finding suggests that total hexosaminidase levels could be indicators of other LSDs such as MPS, GM1 Gangliosidosis, Gaucher disease, and mucopolipidosis, since increased levels of total hexosaminidase were observed in patients confirmed to have any of those disorders [151].

This first screening phase demonstrated the level of sub-diagnosis of these diseases, mainly caused by the lack of accessibility of patients to specialized analytical resources, which improved with the implementation of methodologies that involved solid phase sample analysis (DBS). These findings did not only increase the detection and diagnostic definition of patients, but also showed the presence in Colombia of other LSDs not previously identified. In addition to the above, a 22-year follow-up (1995–2016) on high-risk screening was carried out to contrast the findings of the classical methodology versus those obtained by using DBS. This study included of a total of 32,940 individuals: 3,834 (11.6%) assessed with the classical methods (urine/serum/whole blood analysis) and 29,106 (88.4%) assessed in DBS (since 2005). The results showed a marked notable increase in remissions during the evaluated period (Figure 5), which led to 5-fold increase in the identification of LSDs patients. In this sense, during the first ten years of screening (1995–2005), using the classical methods, 30 LSD patients were identified; while the inclusion of the DBS methodology allowed the identification of 622 patients in a similar time period [152]. Studies of 22 years shown a prevalence of 2% of the total number of patients referred. Overall, the following LSDs were identified: Fabry (n = 47), fucosidosis (n = 2), Gaucher (n = 196), GM1 Gangliosidosis (n = 21), Krabbe (n = 1),

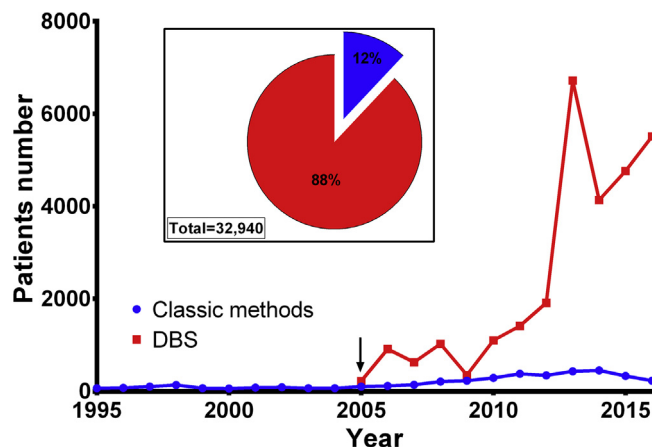


Figure 5. Annual remission of patients for studies of LSD between 1995 and 2016. Insert figure shows the percentage of samples analyzed for each methodology during the studied period. The arrow shows the year of implementation of DBS methodology.

metachromatic leukodystrophy (n = 12), mucopolipidosis (n = 8), MPS I (n = 27), MPS II (n = 43) MPS III (n = 14) MPS IVA (n = 192), MPS IVB (n = 2), MPS VI (n = 40), MPS VII (n = 1), Pompe (n = 40), Sandhoff (n = 2), Sialidosis (n = 1) and Tay Sachs (n = 3) (Table 2). Nowadays, the studies continue including new protocols of using DBS samples. This will facilitate the access to the diagnosis and to know the frequency of this group of disorders. It will also provide reasonable and sustainable costs in disorders that are not a priority for the public health programs in Colombia.

Additionally, another study evaluated the prevalence of Krabbe disease in patients with neurodegenerative compromise (NDC) from a total of 259 samples in which 110 were controls and 149 were from patients with NDC [153]. From the latter group, 46% were females and 56% were males, from which 94 (63%) were reported with white matter compromise and 30 (20%) with abnormal nervous conductivity. In addition, 13 (8.7%) were under 1-year-old, 106 (71.1%) were between 1.5 – 10.5 years of age, and 30 (20.1%) were between 11-57 years of age. One

Table 2. LSD detected in Colombian population in a high-risk screening (1995–2016).

Disease Detected	Classic Techniques 1995–2004	Dried Blood Spots 2005–2016
Fabry Disease	0	47
Fucosidosis	0	2
GM1 Gangliosidosis	4	17
Gaucher Disease	1	195
Krabbe Disease	0	1
Metachromatic Leukodystrophy	4	8
Mucopolipidosis	4	4
MPS I	5	22
MPS II	0	43
MPS III	5	11
MPS IVA	0	192
MPS IVB	0	2
MPS Type VI	4	40
MPS Type VII	0	1
Pompe Disease	0	40
Sandhoff Disease	1	2
Sialidosis	0	1
Tay-Sachs Disease	2	1
Total	30	622

patient was reported with enzyme deficiency for β -Galactosylceramidase (β -Galsil) with 3% of residual activity in comparison with the control group [153]. Three more patients were also reported with decreased levels of β -Galsil and low β -Glucosidase activity, which indicated a bias on enzymatic activity assay by the deficiency of a different enzyme also involved in the sphingolipids metabolism [153].

Uribe [154] reported a 17-year study (2000–2016) of prevalence for Gaucher disease in a Colombian population with clinical features suggestive of Gaucher disease ($n = 2073$, age: 1 month–80 years old), and including 800 control samples (healthy volunteers, age: 2 months–95 years old). In the study, 196 patients (9.5%) were reported with enzyme deficiency with values ranging from 0.0 to 3.21 nmol/mg/h in comparison with the control group (5.0–16.5 nmol/mg/h). From this group, 108 were women (55%) and 88 were men (45%) ranged between 2 months and 72 years of age [154]. Similarly, Uribe et al [155]. showed the results of a 10-years screening (2006–2015) for Pompe disease using DBS. A total of 6,522 samples from patients between 2 months-to 95 years-old were analyzed. Ninety patients were reported positive for the pathology. Nevertheless, a subsequent acarbose enzymatic assay confirmed only 40 patients. The remaining 50 patients were considered as false positives [155].

9. Conclusions and perspectives

The study of LSDs has had significant progress worldwide during the last three decades. Although, some LSDs have an approved therapy with modest to significant impacts in disease manifestations, most of the available options exhibit adverse effects and restrictions that affect the quality of life of patients, and their families, suffering from these diseases. Given this scenario, Colombia has not been the exception, since the interest in the study of these disorders has increased among health professionals and health care providers along these years.

This review reveals a significant number of researchers and clinicians who have studied, analyzed and developed biological and molecular tools that have allowed a broader knowledge of LSDs in the country. The efforts have been focused on the development of diagnostic strategies, and the study of therapeutic alternatives related to enzyme replacement therapy, gene therapy, cell therapy and more recently, the use of pharmacological chaperones to overcome the restraints of conventional therapies. It demonstrates that the most frequently studied LSDs in Colombia are MPS, particularly MPS IVA; perhaps related to the fact that an important number of MPS patients have been found in the country compared to other LSDs. This shows a great potential for the understanding of MPS and the development of better treatment and management options for these patients.

Despite this, less common LSDs are being studied to develop better diagnostic tools that ensure an early, timely, and possibly appropriate treatment for the patients. Likewise, the evaluation of potential recombinant proteins is being studied for ERT or DNA delivery strategies for gene therapy. This is done through collaborative work with international researchers specialized in the subject, that provide access to cellular or animal models to be implemented during the evaluation of the developed strategies.

Despite these efforts, it is still necessary to study many issues related to LSDs in Colombia, going from basic knowledge to the development of therapies and the installation of regulations that improve the patients' health conditions. On this matter, after many years of debate in Colombia, the Law 1980 of 2019 was recently sanctioned, which declares the execution of a newborn screening program to detect patients affected with IEM and other diseases whose early diagnosis and treatment could avoid progression, sequelae or disability. Although this newborn screening program will include at the beginning only five IEM of small molecules, we hope that in the near future a newborn screening for LSD is included, as it has been implemented in other countries [156, 157, 158, 159, 160]. In addition, although SIVIGILA has become an important source of information about rare diseases in Colombia [17, 18], we

consider that basic, applied and translational research, as well as health policy makers, will benefit from a LSDs registry that allows to have information about the number and the clinical and biochemical characteristics of Colombian LSDs patients.

Research, diagnosis, and treatment of LSDs in Colombia holds a great potential given the amount of studies done for this group of disorders. The lack of knowledge on the mechanism of some of the diseases and the amount of adverse effects of many treatments, opens an opportunity develop novel treatments, to improve existing ones, and to strengthen the basic and clinical research of LSDs in our country.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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