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Description of Lysosome Accumulation Disorders, Priorities of Family Screening in the Diagnosis of Fabry Disease

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Abstract: Alpha galactosidase A (GLA) deficiency causes Fabry disease (FD), an X-linked lysosomal storage disorder. Multiple organ involvement occurs in affected males and, to a lesser extent, in affected females due to the progressive, intralysosomal buildup of neutral glycosphingolipids in endothelial cells and podocytes. GLA analysis in leukocytes or dried blood spots (DBS) is used to diagnose FD in males, but GLA activity in females may be within the normal range. The usual enzymology fluorometric approach applied to dried blood spots made this achievable. High-throughput multiplexable tests were then created, including tandem mass spectrometry and digital microfluidics. Some nations have recently started using DNA-based techniques for newborn screening. Numerous newborn screening pilot projects and initiatives have been put into place globally using these techniques. However, there are still a number of issues, and not everyone agrees that newborns should be screened for Fabry disease. Specifically, a significant number of afflicted females are missed by enzyme-based approaches. High-throughput screening of at-risk groups and neonates has been made easier with the introduction of fluorometric and mass spectrometry techniques for enzyme analysis in DBS. However, genetic investigation of the GLA gene continues to be the primary diagnostic method for FD females. Furthermore, a significant percentage of babies have variations of unknown significance or later onset types, which raise ethical concerns. Long-term monitoring of those identified through birth screening will advance our understanding of the illness's natural course, phenotypic prediction, and patient care, enabling a more accurate assessment of the advantages and disadvantages of newborn Fabry disease screening. The comprehensive analytical procedure for measuring GLA activity in DBS using tandem mass spectrometry will be provided in the next unit.

Keywords: Alpha galactosidase A, neutral glycosphingolipids, dried blood spots, globotriaosylceramide, autophagy–lysosomal pathway, cell-based processes.

Introduction. Women's engagement in the labor force increased significantly as a result of the Industrial Revolution in Europe in the 18th century, and this increase was exacerbated in the years following World War II, which led to a major increase in the number of women employed. As a result of their involvement in the workforce, family structures have changed, moving away from extended families that take on the role of child care when parents are not present and toward a nuclear family structure [1-5]. Preschool facilities have become more well-known for meeting the basic needs of children whose parents work as a result of these advancements. Preschool education's main goals are to foster children's self-care abilities and to assist their cognitive, linguistic, motor, and social-emotional development. In keeping with this goal, they

receive health-related information and awareness, and positive attitudes and actions are encouraged. The number of children attending preschools has further expanded as a result of the public's growing awareness of the value of early childhood education [6-11]. In order to prevent the spread of infectious diseases, this study aimed to determine the prevalence of respiratory tract infections, diarrheal diseases, and rashes as well as the underlying causes of their transmission in preschool education institutions. By conducting a thorough investigation that included all variables pertaining to teachers, children, parents, and institutions, the current study aimed to contribute to the development of intervention programs [12-17]. Fabry disease (FD, OMIM 301500) is an X-linked lysosomal disorder that is caused by a deficiency of α-GalA, which is encoded by the GLA gene and causes a progressive accumulation of globotriaosylceramide (Gb3) and related glycosphingolipids. The clinical spectrum of FD is broad; patients with a classic phenotype present with angiokeratomas, neuropathic pain, hypohidrosis, hearing loss, and gastrointestinal symptoms. These symptoms can occur in early childhood before the age of five years old, particularly neuropathic pain and gastrointestinal symptoms; in adulthood, the patients may show severe involvement of the kidney, heart, central nervous system (primarily cerebrovascular disease), and peripheral nervous system [17,18,19]. Patients with the later onset subtypes present with cardiac, renal, and neurological involvements, varying in clinical severity. Numerous studies conducted in the last ten years have advanced our understanding of lysosomal biology and offered fresh, significant perspectives on the pathophysiology of LSD. According to these research, lysosomes are extremely dynamic organelles that may respond to external stimuli and are engaged in a variety of cellular processes, including signaling. Important developments have also made it easier to comprehend the metabolic and molecular processes that underlie LSDs and to create novel treatment approaches for these conditions [20,21,22]. Recent findings on the cellular and organismal effects of lysosomal dysfunction, new methods and technology for studying LSDs, and developing facets of lysosomal biology will be the main topics of this review. The long-term follow-up of individuals detected by newborn screening will improve our knowledge about the natural history of the disease, the phenotype prediction, and the patients' management, allowing a better evaluation of the risks and benefits of newborn screening for Fabry disease. However, a number of concerns remain, and newborn screening for Fabry disease is still not universally accepted. In particular, enzyme-based methods miss a significant number of affected females, and there are ethical issues due to the large number of infants with later onset forms or variants of uncertain significance [19,23,24].

The main purpose of the presented manuscript is to describe the disease of lysosomal accumulation, to conduct a brief analysis of the literature in the context of the basic principles of familial diagnosis of Fabry disease related to these diseases.

More than two hundred lysosomal-resident proteins contribute to the biology and function of lysosomes, which are membrane-limited, ubiquitous, intracellular organelles involved in multiple cellular processes. Of these, about 60 are acidic hydrolases, most of which act as exoglycosidases or sulfatases and are localized to the lysosomal lumen. The remaining proteins are localized at the lysosomal membrane and have a variety of functions, including the formation of a glycocalyx-like layer, transport across the membrane, acidification, membrane stability, and mediating interactions between lysosomes and other cellular structures. Apart from lysosomal-resident proteins, other proteins interact with the lysosome and contribute to lysosomal function by being dynamically recruited to the lysosomal surface under specific conditions [17,18,21]. These include the transcription factor EB (TFEB), the mechanistic target of rapamycin complex 1 (mTORC1), the mTORC1 regulator tuberous sclerosis complex (TSC), folliculin (FLCN) and FLCN-interacting protein (FNIP), the energy-sensing complex AMP-activated kinase (AMPK), and the signal transducer and activator of transcription-3 (STAT3). The first function of normal lysosomes to be recognized is turnover of cellular constituents. Lysosomes are involved in the degradation of a wide range of structurally diverse compounds, including proteins, glycosaminoglycans, sphingolipids, oligosaccharides, glycogen, nucleic acids, and complex lipids [22,23,24]. Lysosomes are part of a more complex pathway called the autophagy–

lysosomal pathway (ALP), which involves multiple steps, including autophagosome formation, cargo recruitment, and autophagosome–lysosome fusion. Importantly, autophagic function is entirely dependent on the lysosome's ability to degrade and recycle autophagy substrates. Cellular and extracellular materials and substrates destined for degradation reach lysosomes through various routes (endocytosis, phagocytosis, and autophagy), or by direct transport [25,26].

Cellular modeling and new technologies are being used to investigate lysosomal function in both health and disease. The creation of diagnostic instruments for patients suspected of having LSD, the study of lysosome biology, and the discovery and confirmation of novel treatment targets have all been greatly impacted by novel technology. In many instances, these methods rely on high-throughput procedures in conjunction with bioinformatic processing of a substantial amount of data (metabolomic, genomic, and proteomic methods). Additionally, novel approaches include automated robotic-based, miniaturized, or cell-based processes (high-content imaging), as well as cutting-edge methods that give rise to in vitro and in vivo disease models and genetic information modification [24-30].

NGS, a powerful diagnostic tool based on technological platforms that allow sequencing of millions of small fragments of DNA in parallel, has been used both through untargeted approaches based on whole-exome sequencing and through targeted strategies with gene panels. Given the challenges in the diagnostic work-up and the overlapping clinical phenotypes in LSDs, patients with a clinical suspicion of these disorders are excellent candidates for the application of an NGS-based diagnosis. In particular, NGS has made significant progress in the diagnostic approach to LSDs. Prior to the advent of NGS, the conventional method for diagnosing LSDs was based on a progressive, step-by-step procedure that began with physical examination, moved on to the identification of metabolites in biological fluids, and ended with the precise diagnosis by identifying mutations in a particular gene and demonstrating an enzymatic deficiency (or a lysosomal function deficiency) [21,24,27,29]. NGS-based techniques are considerably modifying this sequential procedure. While the functional study of the defective enzyme (or a non-enzymatic protein) provides a supplementary method to conclusively validate disease diagnoses, molecular analysis and the search for mutations in LSD-related genes can be carried out as soon as there is a clinical suspicion of LSD,[28,29,30].

The diagnosis of type 1 classic phenotype can be made clinically by doctors who recognize the characteristic findings of corneal dystrophy in childhood or adolescence, absent or decreased sweating, typical skin lesions, and episodic pain in the extremities. The disease progresses to renal insufficiency and/or heart and cerebrovascular disease in adulthood. Many males with type 2 later-onset phenotype have been diagnosed by screening patients in hemodialysis, cardiac, and stroke clinics. The diagnosis of both type 1 and type 2 males is confirmed by proving the enzyme deficiency and by identifying the specific GLA gene mutation. Heterozygous females can have α-GAL A enzymatic activity that ranges from significantly reduced to values within the normal range. Therefore, the only way to accurately diagnose heterozygous females is to show the specific α-galactosidase A (GLA) gene mutation. Early prenatal diagnosis at approximately 10 weeks of pregnancy can be made by analyzing the α-Gal A enzyme and GLA mutation of villi obtained by chronic villus sampling, or by amniocentesis at approximately 15 weeks of gestation by determining the α-Gal A enzyme activity and demonstrating the family-specific GLA mutation. Preimplantation genetic diagnosis is available when the familial mutation in the GLA gene is known. Newborn screening studies have identified affected males by showing the reduced α-Gal A activity in dried blood spots followed by GLA gene sequencing [27-32].

Methods of screening. From a technological standpoint, a number of analytical techniques may make high-throughput newborn screening for FD possible. Due of their ability to multiplex with commercially available reagents, digital microfluidics (DMF) and tandem mass spectrometry (MS/MS) are the most commonly employed of these.Immune quantification measures the quantity of each protein using fluorescence detection and microbead array technology. It needs

protein-specific antibodies, which aren't on the market yet. Furthermore, if a non-functional protein is produced, the approach is useless.A fluorogenic substrate (4-methylumbelliferyl-D-galactopyranoside) is used in the fluorometric enzymatic test. The fluorescence of the enzyme product 4-MU (4-methylumelliferone) is measured following an overnight incubation period. Fluorometric enzyme activity assays are the foundation of the multiplex DMF enzymatic assay. Through a process called electrowetting, sub-microliter quantities of sample and enzyme assay components are transported across an array of electrodes in digital microfluidics while being subjected to an electric field [21-27]. The "spatial multiplexing" DMF technique enables each LSD enzyme reaction to be carried out in a separate droplet under conditions that are specifically optimized for it. It allows for same-day result reporting and is currently the fastest way available. An assay mixture comprising the substrate and internal standard is used in the MS/MS enzymatic assay. After overnight incubation, and remotion of detergents, salts and excess substrate, the samples are introduced to a tandem mass spectrometer [28-32].

The implementation of FD newborn screening is still controversial because, as previously discussed, there are reliable and effective methods for screening on DBS. The disease is more prevalent than previously clinically estimated. Newborn screening allows for the early diagnosis and treatment, which improves the prognosis. In 1968, Wilson and Jungner described ten principles that should be met before introducing a screening program. According to this algorithm, FD reaches a score of 8. However, it should be noted that most LSDs do not reach this threshold. Additionally, it raises the price of follow-up appointments and diagnostic laboratory tests. To comprehend the natural history of the disease, which includes the presentations of the many phenotypes, and the effects of early treatment, these infants' long-term follow-up will be crucial [25-28]. Nonetheless, there may be a number of benefits to diagnosing the later onset variants early. Many patients are still misdiagnosed or go years without a diagnosis. This "diagnostic odyssey" might be avoided with the use of NBS, enabling prompt treatment and improved results. Additionally, their identification enables doctors to discover undiagnosed relatives and do cascade genotyping in at-risk family members. Notwithstanding these drawbacks, FD patients have a positive opinion of NBS. A number of studies examined FD patients' (n = 88) opinions regarding NBS for FD (and other later onset diseases), and the majority of participants agreed with NBS. They believed that NBS could lead to better current health, eliminate diagnostic odysseys, result in more timely and effective treatment, and lead to different life-decisions, including lifestyle, financial, and reproductive decisions. Genetic healthcare providers, on the other hand, expressed a different opinion, according to Lisi et al.: FD was viewed less favorable than other LSDs due to its later age of onset (possibility of medicalization, stigmatization, and psychological burden) and lack of certainty regarding prognosis [31-36].

Discussion. A class of metabolic disorders known as lysosomal storage diseases is brought on by deficits in a number of lysosomal function components. Lysosomal hydrolases, which break down and recycle a wide range of complex chemicals and cellular structures, are most frequently impacted. Over time, our knowledge of lysosomal biology has steadily increased. These days, lysosomes are seen as highly dynamic components of the autophagic-lysosomal system, participating in a variety of cellular processes, including signaling, and capable of adapting to environmental stimuli. They are no longer thought of as organelles solely involved in catabolic pathways. Our comprehension of the pathophysiology of lysosomal illnesses has been significantly influenced by this improved understanding of lysosomes [18-21]. We currently lack an ideal disease-specific biomarker for Fabry disease. Plasma lysoGb3 has been established as a good diagnostic biomarker for Fabry disease, but it is not as sensitive and specific as lysoGb1 for NBS in Gaucher disease. LysoGb3 correlates well with the classic form, male sex, but normal levels cannot rule out a later-onset form. Ideally, the FD NBS program should include: (1) a combined enzymatic and genetic approach to perform a comprehensive screening of all patients (males and females); and (2) an improved biomarker to use as a second tier test. Additionally, we need more information on values during infancy, as the majority of the literature on lysoGb3

refers to measurements in adult Fabry patients; (3) a newborn screening program for FD should be linked to a long-term follow-up program, as only such a clinical follow-up could ascertain the impact of this early diagnosis in the real-life management [25-30]. The characterization of lysosomal biology or lysosomal dysfunction has been greatly aided by novel technologies, most of which are based on high-throughput approaches. It is now well established that substrate accumulation sets off complex pathogenetic cascades that cause disease pathology, including aberrant vesicle trafficking, autophagy impairment, signaling pathway dysregulation, calcium homeostasis abnormalities, and mitochondrial dysfunction. These technologies may also make it easier to identify new therapeutic targets and streamline diagnostic procedures [31-37].

Conclusions. The lack of a second-tier test that can cover all forms of the disease and lower the recall rate; the biochemical detection of heterozygous females; the clinical interpretation of unclassified variants and VUS; the impact of early diagnosis on patients with later onset forms; and the need for long-term follow-up data related to functional characterization of the controversial variants, studies of biomarkers and modifier genes to a better phenotype prediction and patient management will be crucial to address important ethical issues. In conclusion, both the advantages and risks of NBS merit further research, highlighting the need for long-term follow up.

Even with the advancements in our understanding of the disease, there is still a significant delay in diagnosing FD, making the introduction of new diagnostic techniques necessary. Current promising therapies demonstrate efficacy directly related to their early initiation, which would prevent the irreversible cellular damage that occurs in advanced FD. Lastly, the clearance of Gb3 deposits and the development of strategies to reduce antibodies against the recombinant enzyme are dependent on the ERT being started before the onset of organ damage.

This has been done in cases of other LSDs, including Pompe disease. As a result, it is extremely important to screen and study as many people as possible after a case has been diagnosed in a family, not only to allow for. It is, therefore, of enormous importance to research and screen as many persons as possible once a case has been found in a family, not only to allow for early treatment, but also to permit appropriate genetic counselling to prevent new cases of this fatal disease.

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