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Insights from the LysoNeo prospective cohort study to improve newborn screening of lysosomal diseases

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Abstract

Background

Newborn screening enables early detection and treatment of serious genetic conditions before symptom onset. Lysosomal diseases, a group of more than 70 rare inherited metabolic disorders, are increasingly considered for inclusion in NBS owing to advances in pathophysiological understanding and therapy development. Early, pre-symptomatic identification offers a critical window for intervention that can improve neurodevelopmental outcomes and quality of life.

Methods

To evaluate the feasibility of high-throughput, multi-tiered newborn screening for 13 lysosomal diseases, in the LysoNeo pilot study, families of 106,609 newborns were approached between March 2021 and November 2024; 100,212 consented and 100,000 newborns were successfully screened. Dried blood spots collected shortly after birth underwent a multi-tier screening process combining first-tier biochemical testing, repeat testing of abnormal samples, second-tier reassessment, multidisciplinary review and confirmatory biochemical and molecular investigations for recalled newborns.

Results

Among 106,609 families approached, consent is obtained for 100,212 (94.0%), and screening is successfully completed for 100,000 newborns. First-tier screening identifies 75 newborns with abnormal results (screen-positive rate: 0.075%). Following second-tier reassessment and multidisciplinary review, 14 newborns are recalled (recall rate: 0.014%). Eight newborns have concordant biochemical and molecular findings consistent with lysosomal disease (confirmed case rate: 0.008%; Predictive Positive Value among recalled: 57.1% [8/14]; Predictive Positive Value among first-tier positives: 10.6% [8/75]). Two newborns initiate disease-specific therapy and six remain under structured follow-up.

Conclusion

LysoNeo demonstrates the feasibility of implementing expanded lysosomal diseases newborn screening within a regional healthcare system and provides real-world evidence on screening cascade dynamics and actionability-based governance to inform national policy.

Plain Language Summary

Newborn screening tests babies shortly after birth to find serious diseases before symptoms appear. Some rare genetic conditions, called lysosomal diseases, can cause severe health problems but may benefit from early treatment. We carried out a large pilot study in the Normandy region of France to assess whether screening for 13 of these diseases could be added safely and effectively to routine newborn screening. Between 2021 and 2024, more than 100,000 newborns were tested using a step-by-step process that combined blood tests and genetic analysis. Eight babies were confirmed to have a lysosomal disease. Two started treatments early, and six are being closely monitored. Our results show that large-scale screening for these rare diseases is possible and can identify affected children early, helping guide future decisions about expanding newborn screening programs.

Main

Newborn screening (NBS) is a major pillar of preventive pediatric medicine. The early detection of serious conditions, before clinical symptoms emerge, enables timely interventions that can substantially improve prognosis, enhance quality of life, optimize long-term healthcare trajectories, reduce diagnostic delay and disease burden. Lysosomal diseases (LDs) are a group of over 70 rare inherited metabolic conditions resulting from defects in lysosomal function. Lysosomal impairment leads not only to the pathological accumulation of undegraded substrates but also to widespread cellular dysfunction. The lysosome plays a central role in cellular homeostasis, participating in inter-organelle communication, autophagy, nutrient sensing, and signal transduction.[1,2] Furthermore, their dynamic intracellular positioning via cytoskeletal transport is critical for metabolic adaptation and proper immune and stress responses.[3-6] Consequently, lysosome disruption induces inflammation, oxidative stress, and cell death.[2,7,8] Advances in understanding the underlying pathophysiological mechanisms have led to the development of innovative therapies.[8,9] As a result, NBS has become increasingly relevant, offering the opportunity to initiate treatment during a pre-symptomatic window when it is most effective. Thus, several LDs have been incorporated into NBS initiatives in the last decade.[10] The US Recommended Uniform Screening Panel (RUSP), established on the recommendation of the Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) aims to select disorders based on evidence that supports the potential benefit of screening. Currently, the RUSP includes 4 LDs: Pompe disease, mucopolysaccharidosis type I, mucopolysaccharidosis type II, and Krabbe disease.[11]

The LysoNeo pilot study aims to evaluate the feasibility and efficacy of implementing a high-throughput, multi-tiered NBS approach for a selected subset of LDs in the Normandy Region in France. This approach is designed to address both the biological complexity of these disorders and the practical constraints of real-world screening programs. The study

seeks to inform future decisions on the expansion of national NBS panels and contribute to a growing body of evidence supporting the early detection of LDs.

Methods

Study Design and Participant

LysoNeo is a prospective, observational cohort pilot study embedded in the regional newborn-screening pathway in Normandy, France. From March 2021 to December 2024, out of the 106,609 families approached, 100,212 (94.0%) consented to participate. Testing was successfully completed for 100,000 enrolled newborns (99.8%). Written informed consent was obtained from the parents or legal guardians of all participating newborns before sample collection and inclusion in the study. Dried blood samples (DBS) from live newborns were collected from 23 maternity wards in Normandy Region (France) within 72 hours after birth. DBS transited by the Regional Newborn Screening Center in Caen then transferred by express delivery to the Department of Metabolic Biochemistry at Rouen University Hospital for testing.

In 2020, 2 LDs were selected based on specific criteria: the onset of clinical symptoms during infancy, the availability of a synthetic substrate enabling screening through tandem mass spectrometry, and the existence of ongoing clinical trials or approved treatments with a clear benefice of an early or presymptomatic treatment : this concerned Mucopolysaccharidosis I (MPS I: Hurler/Scheie Disease) and Pompe Disease (PD: Glycogen Storage Disease Type II); 9 other LDs were screened in an ancillary study to test technical feasibility: Neuronal Ceroid Lipofuscinosis type 2 (CLN2); Krabbe Disease (KD: Globoid Cell Leukodystrophy); Mucopolysaccharidosis II (MPSII, Hunter Syndrome); Mucopolysaccharidosis IIIB (MPS IIIB: Sanfilippo Syndrome B); Mucopolysaccharidosis IVA (MPS IVA: Morquio Disease A); Mucopolysaccharidosis IVB (MPS IVB: Morquio Disease B); Mucopolysaccharidosis VI (MPS VI: Maroteaux-Lamy Disease); Mucopolysaccharidosis VII (MPS VII: Sly Disease); Acid Sphingomyelinase deficiency (ASMD : Niemann-Pick Disease Types A and B). In 2022, two additional LDs: LALD (Lysosomal Acid Lipase Deficiency) and

MLD (Metachromatic Leukodystrophy) were included following the recent availability of the corresponding synthetic substrates (**Table 1**).

First-line investigations are biochemical-based, focusing on either enzyme activity or accumulated metabolites. DBS with positive results in the initial screening and re-analysis of the original DBS specimen underwent a second-tier investigation of the original DBS. All suspected cases are reviewed during a multidisciplinary meeting within the Reference Centre for Lysosomal Diseases (CRML). This multidisciplinary review serves also as a structured safeguard to ensure that clinical recall was restricted to conditions meeting predefined actionability criteria.

Recall decisions were based on predefined criteria: (1) persistence of biochemical abnormality on repeat testing and/or supportive second-tier biomarker profile and (2) clinical actionability, defined as the availability of an established and accessible presymptomatic intervention pathway during the study period. Only newborns meeting biochemical persistence criteria and for whom an actionable management pathway was available were recalled for confirmatory clinical evaluation and molecular testing. When a newborn is recalled by an inherited metabolic disease specialist from the CRML, a clinical examination and confirmatory biochemical and genetic investigations are conducted. All findings are then reviewed by the CRML to collectively define the most appropriate management strategy (treatment, clinical monitoring, or confirmation of a false positive) (**Fig. 1**).

The study has been approved by a CPP from Necker-Enfants Malades Institutional Review Board and is registered on clinicaltrials.gov (NCT04393701).

Lysosomal Enzyme Activity Assessment

The NeoLSD MSMS Kit assay system (Revvity) was used to measure enzyme activities of acid sphingomyelinase (SMPD1) for Niemann-Pick disease A/B (ASMD)**,** β-galactocerebrosidase (GALC) for Krabbe disease**,** α-L-iduronidase (IDUA) for MPS I (Hurler syndrome)**,** and acid α-glucosidase (GAA) for Pompe disease**.** For the NeoLSD kit, dried blood spot (DBS) samples and quality controls (3.2 mm) were placed into 96-well plates,

with each well receiving 30 µL of incubation cocktail. The plates were then incubated for 18 hours at 37 °C and 400 rpm in a shaking incubator. To stop the enzymatic reaction, 100 µL of quench solution was added to each well. The mixture was pipetted up and down 10 times before being transferred to a deep-well plate. For liquid-liquid extraction, 400 µL of NeoLSD Extraction Solution and 200 µL of water were added, followed by thorough mixing with a pipette. The samples were then centrifuged at 700 g for 5 minutes, and 50 µL of the upper organic layer was carefully transferred to a new 96-well plate. The extracted samples were dried under nitrogen and subsequently resuspended in 100 µL of flow solvent. Using QSight 225 MD (Revvity), flow injection analysis–MS/MS was used to quantify internal standards and enzyme-generated products using multiple reaction monitoring. To determine enzyme activity, the mean blank value from each plate was subtracted from the measured sample values. The liquid tandem mass spectrometry parameters (QSight 225 MD; Revvity) are detailed in **eTable 1** of **Supplement 1**.

Additionally, the 7-plex MSMS Kit assay system (Revvity) was used to measure iduronate-2-sulfatase (IDS) for MPS II (Hunter syndrome)**,** α-N-acetylglucosaminidase (NAGLU) for MPS IIIB (Sanfilippo syndrome B)**,** galactosamine-6-sulfatase (GALNS) for MPS IVA (Morquio A syndrome)**,** β-galactosidase (GLB1) for MPS IVB (Morquio B syndrome)**,** arylsulfatase B (ARSB) for MPS VI (Maroteaux-Lamy syndrome)**,** β-glucuronidase (GUSB) for MPS VII (Sly syndrome)**,** and tripeptidyl peptidase 1 (TPP1) for CLN2 (neuronal ceroid lipofuscinosis type 2)**.** The sample preparation begins with the addition of 30 µL of extraction cocktail to a 2 mm DBS punch in a 96-well plate. The sample is then incubated at 37°C for 2 hours with continuous shaking at 400 rpm. Following incubation, 100 µL of quenching solution (50:50 MeOH:EtOAc) is added and mixed thoroughly using a pipette. Subsequently, 40 µL of the mixture is transferred to a 96 deep-well plate, where 600 µL of an extraction solution (400 µL EtOAc followed by 200 µL of 0.5M NaCl) is added and mixed. The mixture is then centrifuged at 700 × g for 5 minutes to facilitate phase separation. After centrifugation, 200 µL of the top layer is carefully transferred to a sampling plate. The solvent is evaporated at 40°C for 10–15 minutes until the sample is

fully dried. The dried sample is then reconstituted in 100 µL of an 80:20 H_2O:ACN solution containing 0.1% formic acid, followed by continuous shaking at 400 rpm for 10 minutes at room temperature (25°C). Finally, a 10 µL aliquot of the reconstituted sample is injected into the liquide chromatography coupled with tandem mass spectrometry (LC-MS/MS) system for analysis (QSight 225 MD, Revvity). Chromatographic separation is performed using a Waters® X-Select™ CSH C18 column (50 × 2.1 mm, 3.5 µm) with a Waters® Vanguard™ CSH pre-column (10 × 2.1 mm, 3.5 µm) at a column temperature of 35°C. The mobile phases consist of 0.1% formic acid in water (Phase A) and 0.1% formic acid in acetonitrile (Phase B). The flow rate is set at 0.5 mL/min with the following gradient: 0.01 min at 70% A and 30% B, 0.50 min at 70% A and 30% B, 1.00 min at 60% A and 40% B, 2.00 min at 60% A and 40% B, 2.50 min at 15% A and 85% B, 3.00 min at 15% A and 85% B, 3.01 min at 70% A and 30% B, and 3.50 min at 70% A and 30% B. Enzyme activity was determined by subtracting the mean blank value of each plate from the measured sample values. Detailed mass spectrometry parameters are provided in **eTable 1** of **Supplement 1.**

Lysosomal acid lipase (LIPA) was measured for Lysosomal Acid Lipase Deficiency (LALD)**,** as previously described[12]**.** Breifly, DBS samples (3 mm punches) were placed in a 96-well deep-well plate and extracted using 200 µL of MilliQ water with shaking at 400 rpm for 1 hour at room temperature. After extraction, 10 µL of the sample extract was transferred to a new 96-well plate, followed by the addition of 30 µL of LIPA assay cocktail containing substrate and internal standards. The plate was incubated at 37ºC with shaking at 400 rpm for 3 hours to allow enzymatic reactions to proceed. The reaction was quenched by adding 80 µL of MilliQ water and 400 µL of LC-MS-grade ethyl acetate**,** followed by thorough mixing and centrifugation at 3000 g for 5 minutes to separate the aqueous and organic phases. 120 µL of the upper organic layer was carefully transferred to an autosampler-compatible 96-well plate, and the solvent was evaporated under a nitrogen flow manifold. The dried extracts were reconstituted with 200 µL of reconstitution solvent (0.1% formic acid in 50:50 water:acetonitrile)**,** thoroughly mixed, and analyzed by LC-

MS/MS. The enzyme-generated product and internal standard were quantified using multiple reaction monitoring (MRM), with specific transitions optimized for LIPA substrate, product, and internal standard. Enzymatic activity was calculated based on the product-to-internal standard peak area ratio, normalized using a predefined formula to determine specific activity in µmol/h/L.

For Metachromatic Leukodystrophy (MLD), arylsulfatase A activity assessment was done as previously described[13]. Briefly, DBS samples (3 mm punches) were placed in a 96-well, 500 µL polypropylene plate, and 50 µL of 0.23% ammonium solution in ARSA buffer was added to each well using a multi-channel pipettor. The plate was sealed and incubated at room temperature with shaking for 4 hours. During this extraction period, a gel filtration filter plate was prepared by adding Sephadex-G25 gel to the wells of a MultiScreen Column Loader. Excess gel was scraped off, and the filter plate was conditioned by adding 300 µL of purified water to each well. The plate was wrapped in aluminium foil and left to hydrate at room temperature for 3 hours. After conditioning, the filter plate was placed on a 96-well collection plate and was centrifuged at 800 x g for 1 minute. 200 µL of Assay Buffer was added dropwise to each well, and the plate was centrifuged three times to ensure proper conditioning. Next, the DBS extract was mixed by pipetting up and down and transferred (30 µL) to each well of the filter plate, followed by the addition of 15 µL of Assay Buffer. The plate was centrifuged as described, and the filtrates were visually inspected under bright light for any yellow color, which would indicate contamination with low molecular weight fractions from the blood. A colorless filtrate was essential to avoid inhibition of the ARSA reaction. For the assay, 10 µL of Assay Cocktail was added to each well of the receiver plate, and the plate was sealed and centrifuged at 200 x g for 30 seconds to collect all material at the bottom of the wells. The plate was then incubated at 37°C with orbital shaking at ~200 rpm for 16 hours. After incubation, the reaction was quenched by adding 300 µL of methanol to each well, and the contents were mixed by pipetting up and down five times. The plate was sealed and centrifuged at 3,000 x g for 5 minutes to separate the supernatant. 150 µL of the supernatant from each well was

carefully transferred to a 96-well autosampler-compatible plate, avoiding any particulate matter from the bottom. The autosampler plate was sealed and analyzed by LC-MS/MS. The LC analysis was performed using a LC-MS/MS analysis on a QSight 225 MD system (Revvity, Corp.) equipped with a CSH FluoroPhenyl column (1.7 µm, 2.1 x 50 mm, Waters Corp., Cat. 1860005351) maintained at 30°C. The mobile phase consisted of acetonitrile/water (50/50) with 0.1% formic acid (mobile phase A) and isopropanol/acetonitrile (50/50) with 0.1% formic acid (mobile phase B). The solvent gradient program was as follows: 0-0.5 min at 0% B, followed by a linear gradient from 0.5 to 1.0 min to 90% B, then a linear gradient from 1.0 to 1.5 min to 100% B. The mobile phase was held at 100% B from 1.5 to 1.95 min before returning to 0% B from 1.95 to 2.0 min. The flow rate was set to 0.7 mL/min. Enzymatic activity was calculated based on the product-to-internal standard peak area ratio, normalized using a predefined formula to determine specific activity in µmol/h/L**.**

Sulfatides' assessment

Sulfatides were assessed as previously described[13,14]. DBS samples (3 mm punches, 3.2 µL of blood) were placed in 96-well deep-well polypropylene plates and extracted with 300 µL of methanol containing the internal standard (d5-16:0-sulfatide, sourced from Enfanos, LLC). The plate was incubated at 37°C with orbital shaking at 400 rpm for 4 hours. After extraction, the samples were centrifuged at 3000 x g for 5 minutes, and 200 µL of the supernatant was transferred to a new 96-well shallow-well plate for further analysis. In addition to the DBS samples, methanol containing C16:0-sulfatide (external calibrators) was included in triplicate on each plate and processed in parallel. The internal standard working solution (300 µL per well) was dispensed into the plate using a multi-channel pipettor. Following a 4-hour extraction, the plate was subjected to LC-MS/MS analysis on a QSight 225 MD system (Revvity, Corp.). The analyte and internal standard were detected using multiple reaction monitoring (MRM) in electrospray ionization (ESI) negative mode. The solvent program for LC included: 0 min at 50% mobile phase B, a linear gradient to

95% B over 1 minute, followed by a linear gradient to 100% B over 0.5 min, then a jump to 50/50 at 1.5 min, holding until 2 min. The flow rate was set at 0.5 mL/min, and the column (ACQUITY UPLC, HSS T3, 1.8 uM, 2.1x50mm Waters Corp. Cat. 186003538) was maintained at 40°C. Sulfatide species were identified based on their parent and fragment m/z values. Sulfatide concentrations in DBS were calculated by comparing the MRM peak area of the analyte to that of the internal standard, then multiplying by the internal standard concentration in the sample. The relative response factor for all sulfatide species was assumed to be unity.

Biochemical Marker Assays

Biochemical markers were assessed using tandem mass spectrometry (MS/MS) on DBS, including glycosaminoglycans (GAGs) for the diagnosis of mucopolysaccharidoses (MPS),[15] psychosine for KD and lysosphingomyeline (LysoSM) for ASMD.[16] The methods used for these assays are detailed in the referenced articles.

Molecular Analysis

Target next-generation sequencing was carried out on newborns with suspected disease using a genetic diagnosis panel[17]. Genomic DNA was extracted from peripheral whole blood using QIAamp DNA Blood Mini Kit® (Qiagen) or the QuickGene-610L platform (Kurabo Biomedical, FujiFilm). The following genes: *GAA* (NM_000152.3), *SMPD1* (NM_000543.4), *GUSB* (NM_000181.3), *GALC* (NM_000153.3), *LIPA* (NM_000235.2), *ARSA* (NM_000487.5), *SUMF1* (NM_182760.3), *PSAP* (NM_002778.2), *IDUA* (NM_000203.3), *IDS* (NM_000202.5), *NAGLU* (NM_000263.3), *GALNS* (NM_000512.4), *ARSB* (NM_000046.3), *GLB1* (NM_000404.2), and *TPP1* (NM_000391.3) were sequenced using LysoGene panel, which includes 52 lysosomal genes[17]. This next-generation sequencing-based method is implemented on an Illumina® platform (San Diego, CA, USA). As previously described[18], bioinformatics pipeline including CASAVA suite v1.8 (Illumina®, CA, USA) and BWA-GATK 2.2.5 (Genome Analysis ToolKit, Broad Institute, Cambridge, MA, USA) has been used for

mapping and variant calling, Alamut Batch (Sophia Genetics, Switzerland) for variant annotation, and CanDiD database for prioritizing and filtering variants of interest. The CANOES algorithm (copy number variants with an arbitrary number of exome samples) was use for the detection of copy number variants such as deletions or duplications. The variants were named according to conventional nomenclature recommendations (http://www.hgvs.org/mutnomen). Variants were classified according to ACMG standards and guidelines.[19]

Statistics and reproducibility

The cutoff value of the 13 enzyme activities was set at 10% of the median value of each test in healthy population. For sulfatides, the 99th percentile was used as cut-off. We used 3 quality controls with different concentrations (low, medium, and high) provided by Revvity, which were added to each plate to ensure the day-to-day validity of results. Mass spectrometry data were analyzed using Simplicity 3Q MD software (Revvity). Statistical analysis and visualisation were performed using R programming core. Data were analysed from March 2021 through November 2024.

Results

We conducted a comprehensive NBS program targeting 11 LDs among 100,000 newborns. A subset of 66,000 newborns was screened for 13 LDs including MLD and LALD. The overview statistics are presented in **supplementary table 1.** All enzymatic activities and sulfatide measurements were successfully validated and cutoffs were established. Quality control samples at low, medium, and high concentrations showed consistent and acceptable performance across all assay plates, ensuring the reliability of the results (**Fig. 2**). Among 100,000 screened newborns, 48,600 (48.6%) were female. The mean (SD) gestational age was 39.0 (1.2) weeks, and the mean (SD) birth weight was 3231 (685) g. Initial DBS analysis identified 75 newborns with positive results across various LDs, including 21 (28.0%) for MPS I, 16 (21.3%) for KD, 11 (14.7%) for PD, 8 (10.7%) for MLD ,

5 (6.7%) for MPS II, 6 (8.0%) for LALD, 2 (2.7%) for MPS VI, 2 (2.7%) for MPSIV A, 2 (2.7%) for MPS VII, 1 (1.3%) for ASMD, and 1 (1.3%) for MPS IIIB (**Table 1**). Reassessment of first DBS and second-tier analysis (when available) were conducted on 34 newborns. Twenty of these newborns have not been recalled for additional evaluation, including 16 for KD, 2 for MPS IVA, 1 for ASMD, and 1 for MPS IIIB. For these 20 newborns, the biochemical suspicion concerned conditions for which no established presymptomatic intervention pathway was available at the time of screening. As these conditions were included within the ancillary arm of LysoNeo to evaluate technical feasibility for diseases with therapies in active clinical development, no clinical recall was initiated and no information was communicated to families or primary care physicians. Cases were adjudicated within the research governance framework of the pilot as requested by the Research Ethics Committee. However, 14 newborns were recalled, including 4 for MPS II, 4 for MPS I, 2 for MPS VII, 2 for LALD, 1 for MLD, and 1 for PD. Subsequent analyses identified three false positives. Two newborns who initially showed decreased LAL activity on the first DBS demonstrated normal levels on a second DBS. One newborn with elevated sulfatides and decreased ARSA activity was found to carry a heterozygous variant. One family refused further participation for their newborn with MPS II. Finally, 10 newborns (13% of the 75 screened) with consistently positive results on both first and second-tier tests, defined by abnormal biochemical findings and the presence of two variants in the implicated gene, were referred to the CRML for specialized care, including 4 for MPS I, 3 for MPS II, 2 for MPS VII, and 1 for PD. One PD and 2 MPS I were false positive. Ultimately, seven true positive newborns were managed. Treatment was initiated for 1 newborn with MPS I and 1 with MPS II. Ongoing follow-up is being conducted for five newborns; 1 with MPS I, 2 with MPS II, 2 with MPS VII. The following case series describes the biochemical, molecular and clinical spectrum among these newborns and the resulting management strategies (**Fig. 3**). In summary, following multidisciplinary review, 14 newborns were recalled for confirmatory evaluation (recall rate: 0.014%). Eight newborns had concordant biochemical and molecular findings consistent with lysosomal disease (confirmed case rate: 0.008%).

The positive predictive value (PPV) among recalled newborns was 57% (8/14). When calculated from all first-tier positives, overall PPV was 10.6% (8/75). Two newborns-initiated disease-specific therapy, and six remain under structured longitudinal follow-up (Table 2, Table 3, Fig. 3). The median age at first-tier screening was 17 days, the median age at confirmatory sampling (whole blood/urine) was 31 days. The age at the treatment initiation was 53 and 93 days for Patient 1 and Patient 4 respectively.

Patient 1 is a male born prematurely at 29 weeks and 5 days of gestation, with a birth weight of 2340 g. Lysosomal NBS identified severely reduced α-L-iduronidase activity (0.10 μmol/h, N>1). Confirmatory enzymatic and biochemical studies showed an enzyme activity level and a urinary GAG profile consistent with MPS I: chondroitin sulfate 10.9 μmol/mmol (N<9.63), dermatan sulfate 7.4 μmol/mmol (N<0.94), heparan sulfate 6.7 μmol/mmol (N<0.19), and total GAGs 24.4 μmol/mmol (N<10.39).

Molecular testing revealed compound heterozygosity for two *IDUA* variants:

The first variant, NM_000203.3: c.298A>G - p.(Arg100Gly), has been previously reported in two compound heterozygous cases: one patient presenting with a severe phenotype[20], and another identified through NBS with an indeterminate phenotype.[21] Based on current ACMG guidelines[22], this variant is classified as a variant of uncertain significance (VUS). The second variant, c.1205G>A - p.(Trp402*), is a well-known pathogenic truncating variant.[23] He was clinically asymptomatic, with normal echocardiography, brain MRI, skeletal survey, and ophthalmologic evaluation. Given the potential for a severe Hurler phenotype, enzyme replacement therapy (ERT) with laronidase was initiated at 6 months of age. Surprisingly, family studies revealed that his 3.5-year-old sister carried the same genotype with similar biochemical abnormalities (urinary GAG profile: chondroitin sulfate 14.2 μmol/mmol (N<20.15), dermatan sulfate 2.74 μmol/mmol (N<0.74), heparan sulfate 2.55 μmol/mmol (N<0.21), and total GAGs 19.46 μmol/mmol (N<21.05)). All investigations for MPS 1 have been performed and are found normal including detailed clinical assessment (growth, skeletal, ophthalmologic, cardiac and respiratory evaluation).

The propositus remains under active medical follow-up. He continues to show normal growth and development at 2 years and is followed with regular clinical and imaging assessments.

Patient 2 is a male born at 37 weeks of gestation, following an unremarkable perinatal course. Lysosomal NBS revealed reduced α-L-iduronidase activity (0.48 µmol/h, N>1). Confirmatory biochemical analysis showed borderline elevated urinary GAGs: dermatan sulfate 1.9 µmol/mmol (N<0.94), heparan sulfate 0.8 µmol/mmol (N<0.19), and total GAGs 8.9 µmol/mmol (N<10.39). Sequencing of the *IDUA* gene identified two heterozygous variants. The first one, NM_000203.3: c.1577T>C - p.(Leu526Pro), has been previously reported as a VUS[24], and the second, NM_000203.3: c.1069C>T – p.(Pro357Ser), is novel but affects a highly conserved proline residue. Of note, another missense variant affecting the same residue, c.1070C>T - p.(Pro357Leu), is reported in ClinVar (Variation ID: 1683228), further highlighting the potential relevance of this position. In silico tools predict a deleterious effect, but due to limited evidence, it is currently classified as VUS. The clinical relevance of this genotype remains unclear. At 10 months, he showed age-appropriate development, with no dysmorphism, no joint stiffness, no hepatosplenomegaly. Imaging investigations (brain MRI, abdominal ultrasound, skeletal imaging) were normal. Given the inconclusive genotype-phenotype correlation and borderline biochemical findings, a conservative strategy with close monitoring was adopted. Due to family constraints, follow-up intervals have been extended.

Patient 3 is a girl born at 36 weeks and 5 days of gestation, by caesarean section, following a normal pregnancy. Her birth weight was 3040 g and Apgar score was 10. Lysosomal NBS showed a reduced acid α-glucosidase (GAA) activity (0.667 µmol/h; N>1.4). At 3 months, her clinical exam, electrocardiogram, and echocardiography were normal. The enzyme deficiency was confirmed on follow-up testing (0.767 µmol/h).

Analysis of the *GAA* gene revealed three heterozygous variants: NM_000152.3: c.2065G>A - p.(Glu689Lys), c.1726G>A - p.(Gly576Ser), and c.-32-13T>G - (p?). The first

two variants, c.2065G>A and c.1726G>A, are found in cis and form a haplotype commonly observed in individuals of Asian descent. This haplotype is associated with acid α-glucosidase pseudodeficiency.[25] The third variant, c.-32-13T>G (p?), is a well-documented pathogenic splice-site mutation and has been associated with late-onset Pompe disease.[26] According to ACMG classification, this variant is considered pathogenic. The presence of c.-32-13T>G in trans with the pseudodeficiency allele explains the reduced acid α-glucosidase activity observed in the patient. This genotype has been previously described in the literature and is not consistent with the classic infantile form of Pompe disease.[27] Given the genetic findings, and the absence of clinical symptoms, the follow-up has been discontinued.

Patient 4 is a male born at 36 weeks of gestation. Lysosomal NBS flagged reduced iduronate-2-sulfatase (IDS) activity (0.06 µmol/h, N>0.8). Biochemical confirmation showed elevated urinary GAGs consistent with MPS II: dermatan sulfate 1.7 µmol/mmol (N<0.94), heparan sulfate 3.3 µmol/mmol (N<0.19), and total GAGs are normal, 9.5 µmol/mmol (N<10.39). *IDS* gene sequencing identified a hemizygous variant, NM_000202.5: c.1438C>T - p.(Pro480Ser). This novel variant has not been previously reported. Multiple in silico prediction tools (Align-GVGD, PolyPhen-2, SIFT, and MutationTaster) support a deleterious impact on protein function. It affects a highly conserved nucleotide and amino acid, underscoring its potential functional importance. Notably, other variants affecting the same codon (Pro480) have been described: p.(Pro480Leu) and p.(Pro480Gln) were observed in patients with moderate phenotypes, while p.(Pro480Arg) was associated with a severe phenotype (Froissart et al., 1998). Given the current lack of direct functional or clinical data for this specific change, it is classified as VUS. Clinical examination was normal, and a umbilical hernia noted at birth resolved spontaneously by 2 months. Given the risk of progression and the benefits of early intervention, ERT with idursulfase was initiated in May 2024. At 14 months, this case demonstrates normal development. Abdominal ultrasound identified bilateral

nephromegaly with suspected incomplete renal duplication, which is being monitored. He remains under multidisciplinary care.

Patient 5 is a male born at 32 weeks and 3 days of gestation from a dichorionic diamniotic twin pregnancy. His birth weight was 1930 g. He was admitted to the neonatal unit for prematurity. Lysosomal NBS revealed a reduced IDS activity (0.23 μmol/L/h; N>0.8). At 2 months of age, confirmatory biochemical testing showed elevated urinary GAGs: chondroitin sulfate 10.1 μmol/mmol (N<9.63), dermatan sulfate 2.4 μmol/mmol (N<0.94), heparan sulfate 1.3 μmol/mmol (N<0.19), and total GAGs of 13.8 μmol/mmol (N<10.39). Genetic testing of *IDS* gene identified a hemizygous NM_000202.5: c.1438C>T - p.(Pro480Ser) variant, also identified in Patient 4. Apart from a pronounced scaphocephalic cranial shape due to sagittal synostosis confirmed by CT, physical examination, brain MRI, skeletal assessment and abdominal ultrasound were normal. The variant was identified in heterozygous state in his mother. To further support the classification of this variant, we extended the family study to include two maternal uncles, aged 36 and 28 years, respectively. The older uncle showed decreased (near absent) IDS enzymatic activity, intellectual disability, and dysmorphic features. Although Down syndrome appears in his medical history, the diagnosis lacks formal clinical or genetic documentation. Genetic and clinical investigations are currently ongoing to clarify the underlying etiology. The younger uncle is asymptomatic and exhibited normal IDS enzyme activity. The treatment decision for Patient 5 (initiation) and Patient 4 (continuation) will depend on the results obtained from the extended family study.

Patient 6 is a male born at 40 weeks of gestation after an uneventful pregnancy and delivery. His birth weight was 3270 g. He is the third child of a non-consanguineous couple with no relevant family history. Lysosomal NBS revealed significantly reduced IDS activity (0.13 μmol/L/h; N>0.8). Confirmatory urinary GAG analysis demonstrated a markedly abnormal profile: chondroitin sulfate 12.5 μmol/mmol (N<9.63), dermatan sulfate 11.3 μmol/mmol (N<0.94), heparan sulfate 13 μmol/mmol (N<0.19), and total GAGs of 32.8

μmol/mmol (N<10.39). Genetic analysis identified a novel hemizygous variant in the *IDS* gene: NM_000202.5: c.143G>A - p.(Arg48His), classified as VUS. In silico predictive tools support a deleterious effect of this substitution on protein function. Although this specific variant has not been previously reported, it affects arginine at position 48, a highly conserved residue across species. Notably, other substitutions at this same codon have been described in the literature and associated with attenuated forms of Hunter syndrome: (i) c.143G>C (p.Arg48Pro) variant has been classified as likely pathogenic and associated with a mild phenotype of Hunter syndrome[28] ; (ii) c.142C>T (p.Arg48Cys) variant has also been classified as likely pathogenic and linked to an attenuated form of the disease.[29] These findings support the potential pathogenicity of this variant. This variant was found in the patient's mother and maternal grandmother. Clinical evaluation at 9 months showed normal growth and development, with no dysmorphism, no hepatosplenomegaly, and normal neurodevelopmental milestones. Brain MRI and abdominal ultrasound were unremarkable, aside from a horseshoe kidney. Cardiologic assessment was normal aside from possible bicuspid aortic valve. Following a discussion at the national board a decision was made to not initiate treatment. He remains under close clinical and biochemical monitoring.

Patient 7 is a female newborn and fifth child of a consanguineous couple. The family history includes three healthy older sisters and a brother who died of a brain tumor associated with a *de novo* pathogenic variant. She was born at 37 weeks and 3 days of gestation with a birth weight of 3135 g. Lysosomal NBS showed markedly reduced β-glucuronidase activity. Confirmatory testing revealed an enzyme activity of 0.48 μmol/h (N>3.8 μmol/h) and a significantly abnormal urinary GAG profile: chondroitin sulfate 9.6 μmol/mmol (N<9.63), dermatan sulfate 57.8 μmol/mmol (N<0.94), heparan sulfate 1.95 μmol/mmol (N<0.19), and total GAGs of 69.3 μmol/mmol (N<10.39). The molecular analysis of the *GUSB* gene identified a homozygous NM_000181.3: c.1014C>T - p.(Ile338=) variant, located in exon 6, predicted to disrupt the splicing process. This variant

has been classified as VUS. Despite the biochemical and molecular findings, clinical examination at 7 months showed normal growth and neurodevelopment, no organomegaly, and unremarkable brain MRI and abdominal ultrasound. A multidisciplinary transplant board concluded that, in the absence of symptoms and due to the uncertain pathogenicity of the variant, hematopoietic stem cell transplantation (HSCT) was not indicated. At 2 years, she remains asymptomatic with normal developmental milestones and continues under regular clinical and biochemical monitoring.

Patient 8 is a female born at 39 weeks and 6 days of gestation following an uncomplicated pregnancy. Her birth weight was 3505 g. Lysosomal NBS showed reduced β-glucuronidase activity (2.47 µmol/h, N>3.8). Confirmatory studies revealed persistently low enzyme activity of 2.49 µmol/h (N>3.8) and a highly elevated urinary GAG concentrations: chondroitin sulfate 211 µmol/mmol (N < 9.63), dermatan sulfate 19.3 µmol/mmol (N < 0.94), heparan sulfate 1.3 µmol/mmol (N < 0.19), and total GAGs 231.7 µmol/mmol (N < 10.39). Molecular investigations identified two heterozygous variants in the *GUSB* gene: a novel variant NM_000181.3: c.169C>T - p.(Arg57Trp) and a previously reported pseudodeficiency variant c.454G>A - p.(Asp152Asn).[30] At 7 months, she showed normal neurological and somatic development with no dysmorphic features nor hepatosplenomegaly. Despite the marked elevation of urinary GAG excretion, the normal clinical findings and molecular ambiguity led to the decision not to initiate treatment. She remains under regular follow-up.

Discussion

The LysoNeo project successfully established a comprehensive ecosystem for NBS of LDs, demonstrating feasibility for translation to a national scale. The entire process, from sample collection in maternity wards to the validation of results, has been thoroughly established. All biochemical investigations necessary for screening have been successfully

validated. During the first phase of the LysoNeo project (100,000 inclusions), which is the focus of this publication, we adopted a strategic algorithm for screening, including biochemical investigation on the first DBS and complementary explorations with the genetic study upon the newborn recall. We decided to pursue the LysoNeo project through a second phase focusing on four key lysosomal diseases (MPSI, PD, MLD, and LALD), using an updated algorithm that incorporates genetic testing on the first DBS (**Fig. 4**). The primary goal of this change is to reduce false positives, thereby minimizing unnecessary recalls for newborns and alleviating the associated burden on families and healthcare professionals. The second phase has already been implemented and will continue over the next two years, while awaiting national implementation of the program.

Historically, NBS has relied on targeted biochemical assays to identify metabolic disorders, particularly inborn errors of metabolism (IEMs). Tandem mass spectrometry (LC–MS/MS) enables high-throughput, multiplex biochemical analyses using DBS with strong sensitivity and specificity. This cost-effective strategy has been widely adopted, allowing timely detection and limiting the use of genetic testing to only biochemically positive cases.[31] To improve diagnostic precision, combined biochemical and genomic screening approaches have been increasingly adopted. Several studies have demonstrated the value of such integrated strategies. Tangeraas *et al.* demonstrated that the integration of biochemical and genetic data obtained from the DBS as part of first-line investigation, analyzed with post-analytical tools, substantially increased the positive predictive value (PPV) from 26% to 54% and broadened the range of detectable conditions. Furthermore, this method allowed for the confirmation of nearly all true positive cases within three days.[32] A multicenter Chinese study on nearly 30,000 newborns revealed that parallel MS/MS and NGS screening enhanced the overall detection of IEMs and facilitated diagnostic confirmation.[33] Fecarotta *et al.* further demonstrated that integrating biochemical testing with the targeted sequencing of 105 genes led to a definitive diagnosis in all cases studied, with nearly 38% requiring both data types to establish an

accurate diagnosis.[34] We are currently witnessing the emergence of high-throughput genomic sequencing as first-line investigation in the context of NBS, enabling the identification of a broad spectrum of rare diseases, including those for which no biochemical biomarker is currently detectable in blood. Current strategies generally recommend further investigation when variants are classified as pathogenic (class V) or likely pathogenic (class IV), according to the guidelines of the American College of Medical Genetics and Genomics (ACMG). However, the lack of functional confirmation for these variants can result in false positives, while the exclusion of variants of uncertain significance (VUS) may lead to false negatives. The integration of large-scale genomics into NBS introduces significant ethical challenges, particularly concerning informed consent, data privacy, and the management of incidental findings. In this context, a combined approach that integrates both biochemical and genetic data, when feasible, offers several advantages. It enhances diagnostic robustness by capturing both the genomic variant and its functional consequence, and it can be more cost-effective by reducing unnecessary follow-ups and enabling more precise interpretation of results.

The evaluation of disease incidence was not the primary objective of this study. Rather, the LysoNeo project was designed to assess the technical, organizational, and clinical feasibility of integrating lysosomal disease screening into routine neonatal care. This project is a pilot generating the empirical operational inputs required before formal cost-effectiveness modelling. It provides an opportunity to test and refine decision-making flowcharts in real-world conditions, from initial biochemical screening to confirmatory testing, multidisciplinary review, and clinical management. The results from this first phase clearly demonstrated the feasibility, scalability, and value of national-level LD screening. Regarding the positive cases, 75 newborns were identified with abnormal results in first-tier biochemical screening. Among them, 14 were recalled for confirmatory evaluations. Of these, eight individuals demonstrated both biochemical and genetic profiles consistent with a LD.

The implementation of early diagnosis through NBS raises important scientific, clinical and ethical considerations. Thus, Patient 1 provides a compelling illustration: a male newborn was found to carry both the biochemical and genetic hallmarks of MPS I. Given these findings, we initiated ERT. However, subsequent testing of his 3.5-year-old sister, who is clinically asymptomatic, revealed an identical biochemical and genetic profile. This discordance between biomarker/genotype and phenotype prompts reflection on the variable expressivity of MPS I. These findings raise questions regarding treatment thresholds in the presymptomatic phase.

Similarly, Patients 7 and 9 were consistent with MPS VII, as evidenced by markedly elevated urinary GAGs. Yet, at the time of evaluation, these patients have no clinical signs suggestive of MPS VII. Given that MPS VII is typically associated with a severe clinical phenotype [35-37], these findings highlight the challenge of interpreting biochemical and genetic abnormalities in the absence of symptoms and underline the need for longitudinal follow-up and refined stratification algorithms.

This series underscores the importance of neonatal screening for early LD detection, reducing diagnostic delays, and enabling timely intervention during peak cerebral plasticity. However, it also demonstrates the complexities inherent in managing patients identified through such programs. In recent years, several pilot studies have demonstrated the feasibility and clinical value of incorporating LDs into NBS programs, using advanced biochemical and genetic tools (**Table 4**). Within this growing landscape, the LysoNeo project represents a key initiative, contributing important evidence on screening strategies.

The LysoNeo project faced certain limitations. The principal contributors to false-positives cases included preanalytical factors (e.g., insufficient/poor-quality DBS), borderline enzyme activities near assay cut-offs, pseudodeficiency alleles associated with reduced in vitro enzyme activity, heterozygous carrier status, and variants of uncertain significance without concordant second-tier biochemical profiles. The absence of first-tier genetic analysis may have contributed to a number of false positives and delayed some

confirmatory diagnoses. Furthermore, important aspects such as the long-term clinical outcomes of early-diagnosed cases, the psychosocial impact on families, and the economic viability of expanded screening still require further investigation. While the first phase of LysoNeo focused exclusively on biochemical screening, it laid the groundwork for a transition toward more integrated, precision-based NBS. The planned implementation of combined biochemical and genetic testing in the second phase of the project reflects a growing consensus that such strategies can enhance diagnostic accuracy, reduce unnecessary follow-up procedures, and optimize resource use. LysoNeo exemplifies how national screening initiatives can evolve through innovation and evidence-based adaptation, setting a strong precedent for the future of rare disease detection in early life.

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Data Availability statement

Due to the study protocol restrictions approved by the institutional ethics committee and applicable data protection regulations, individual-level participant data and sequencing data cannot be shared publicly. Anonymized data underlying the results reported in this article are available from the corresponding author upon reasonable request and subject to approval by institutional ethics committee. The source data for Figure 2 can be accessed from Supplementary Data 1.

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Figure legends

Fig. 1. Decision algorithm used for the LysoNeo project.
Fig. 2. First-tier enzyme activity levels.
Fig. 3. Flowchart of the LysoNeo project.
Fig. 4. Suggested decision algorithm for lysosomal diseases neonatal screening

Table 1. LysoNeo project multi-tier testing.

Disease	First Tier	Second Tier	Third Tier (Genotyping)
MPS I	α-iduronidase	GAGs	*IDUA*
PD	α-glucosidase		*GAA*
CLN2	Tripeptidyl-peptidase 1		*TPP1*
KD	Galactocerebrosidase	Psychosine	*GALC*
MPS II	Iduronate-2-sulfatase	GAGs	*IDS*
MPS IIIB	N-acetylglucosaminidase	GAGs	*NAGLU*
MPS IVA	N-acetylgalactosamine 6-sulfatase	GAGs	*GALNS*

MPS IVB	β-galactosidase	GAGs	*GLB1*
MPS VI	Arylsulfatase B	GAGs	*ARSB*
MPS VII	β-glucuronidase	GAGs	*GUSB*
ASMD	Acid sphingomyelinase	LysoSM	*SMPD1*
MLD	C16:0 and C16 :1-OH sulfatides	Arylsulfatase A	*ARSA, SUMF1, PSAP*
LALD	Lysosomal Acid Lipase		*LIPA*

CLN2: Neuronal Ceroid Lipofuscinosis type 2; GAGs: Glycosaminoglycans; KD: Krabbe Disease (Globoid Cell Leukodystrophy); LALD: Lysosomal Acid Lipase Deficiency; LysoSM: lysosphingomyelin; MLD: Metachromatic Leukodystrophy; MPS I: Mucopolysaccharidosis I (Hurler Syndrome); MPS II: Mucopolysaccharidosis II (Hunter Syndrome); MPS IIIB: Mucopolysaccharidosis IIIB (Sanfilippo Syndrome B); MPS IVA: Mucopolysaccharidosis IVA (Morquio Syndrome A); MPS IVB: Mucopolysaccharidosis IVB (Morquio Syndrome B); MPS VI: Mucopolysaccharidosis VI (Maroteaux-Lamy Syndrome); MPS VII: Mucopolysaccharidosis VII (Sly Syndrome); ASMD: Acid Sphingomyelinase Deficiency (Niemann-Pick Disease Types A and B); PD: Pompe Disease (Glycogen Storage Disease Type II).

Table 2. Screening cascade and adjudication outcomes in the LysoNeo project.

Step	n	% of screened	Notes
Newborns screened	100 000	100%	Successfully completed screening
First-tier positive	75	0.075%	Abnormal first-tier enzymatic/biochemical result
Second-tier reassessed (original DBS ± secondary biomarkers)	34	0.034%	Repeat measurement on original DBS and/or second-tier biomarkers when available
Recalled for confirmatory evaluation	14	0.014%	Actionable recall after multidisciplinary review
Confirmed biochemical + molecular diagnosis	8	0.008%	Concordant biochemical + genetic findings consistent with lysosomal disease
Initiated disease-specific therapy	2	0.002%	Treatment initiated after individualized clinical decision
Structured follow-up	6	0.006%	Follow-up for attenuated/uncertain genotype–phenotype correlation

Table 3. Biochemical, Genetic data of Patients in LysoNeo project.

Case	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Sex	Male	Male	Female	Male	Male	Male	Female	Female
Disease	MPS I	MPS I	PD	MPS II	MPS II	MPS II	MPS VII	MPS VII
Enzyme activity cut off (µM/h)	1	1	1,4	0,8	0,8	0,8	3,8	3,8
1st assessment	0,105	0,486	0,667	0,0676	0,239	0,0657	1,44	2,47
2nd assessment	0,143	0,399	0,767	Insufficient sample	0,270	0,0348	1,71	2,49
Chondroitin (µmol/mmol créatinine) (N<9,63)	10,9	6,2		4,6	10,1	12,5	9,6	211
Dermatan (µmol/mmol creatinine) (N<0,94)	7,4	1,9		1,7	2,4	11,3	57,8	19,3
Heparan (µmol/mmol creatinine) (N<0,19)	6,7	0,8		3,3	1,3	13	1,95	1,3
Total GAG (µmol/mmol creatinine) (N<10,39)	24,4	8,9		9,5	13,8	32,8	69,3	231,7
Gene	*IDUA*	*IDUA*	*GAA*	*IDS*	*IDS*	*IDS*	*GUSB*	*GUSB*
Variant 1 (cDNA)	c.298A>G	c.1577T>C	c.32-13T>G	c.1438C>T	c.1438C>T	c.143G>A	c.1014C>T	c.169C>T
Variant 1 (Protein)	p.(Arg100Gly)	p.(Leu526Pro)	p.?	p.(Pro480Ser)	p.(Pro480Ser)	p.(Arg48His)	p.(Ile338=)	p.(Arg57Trp)
Variant 1 (ACMG classification)	Likely pathogenic	Likely pathogenic	Pathogenic	VUS	VUS	VUS	VUS	VUS
Variant 2 (cDNA)	c.1205G>A	c.1069C>T	c.1726G>A*					C.454G>A
Variant 2 (Protein)	p.(Trp402*)	p.(Pro357Ser)	p.(Gly576Ser)					p.(Asp152Asn)
Variant 2 (ACMG classification)	Pathogenic	VUS	Benign					VUS
Variant 3 (cDNA)			c.2065G>A*					
Variant 3 (Protein)			p.(Asp689Asn)					
Variant 3 (ACMG classification)			Benign					
CRML Decision	Treated	Follow-up	Follow-up	Treated	Follow-up	Follow-up	Follow-up	Follow-up

*These *variants* are found in cis

Table 4. Overview of ongoing newborn screening studies including lysosomal diseases.

Project	Country/Region	Method	Number/Group of Diseases	Start Date	Expected End Date	Number of Expected Babies	Reference
PERIGENOMED	France (Dijon, Besançon, Rennes, Nantes, and Angers)	Genomic	Several hundred	2025	2031 (5 years)	2,500 (CLINICS phase 1), then 19,000	[38]
BABYSEQ	United States	Genomic	Thousands	2025	Ongoing	500 (pilot phase), planned extension	[39]
BEGIN NGS	United States	Genomic	400	2024	2029	10,000	[40]
GUARDIAN	United States	Genomic	Group 1: 350; Group 2: 100	2022	2029	100,000	[41]
BABY-DETECT	Belgium (Flanders)	Genomic	165	2022	Ongoing	60,000	[42]
GENERATION-STUDY	United Kingdom	Genomic	200	2024	2025	100,000	[43]
NEOSEQ	China	Genomic	75	2020	Ongoing	100,000	[44]
LYSONEO	France (Normandy)	Biochemistry Genomic	LDs: 13 then 4	2022	2026	160,000	[45]
ScreenPLUS	États-Unis (New York)	Biochemistry Genomic	LDs: 14	2021	Ongoing	175,000	[46]
MLD	Germany (Hannover, Tübingen)	MLD	1	2021	2023	109,259	[47]

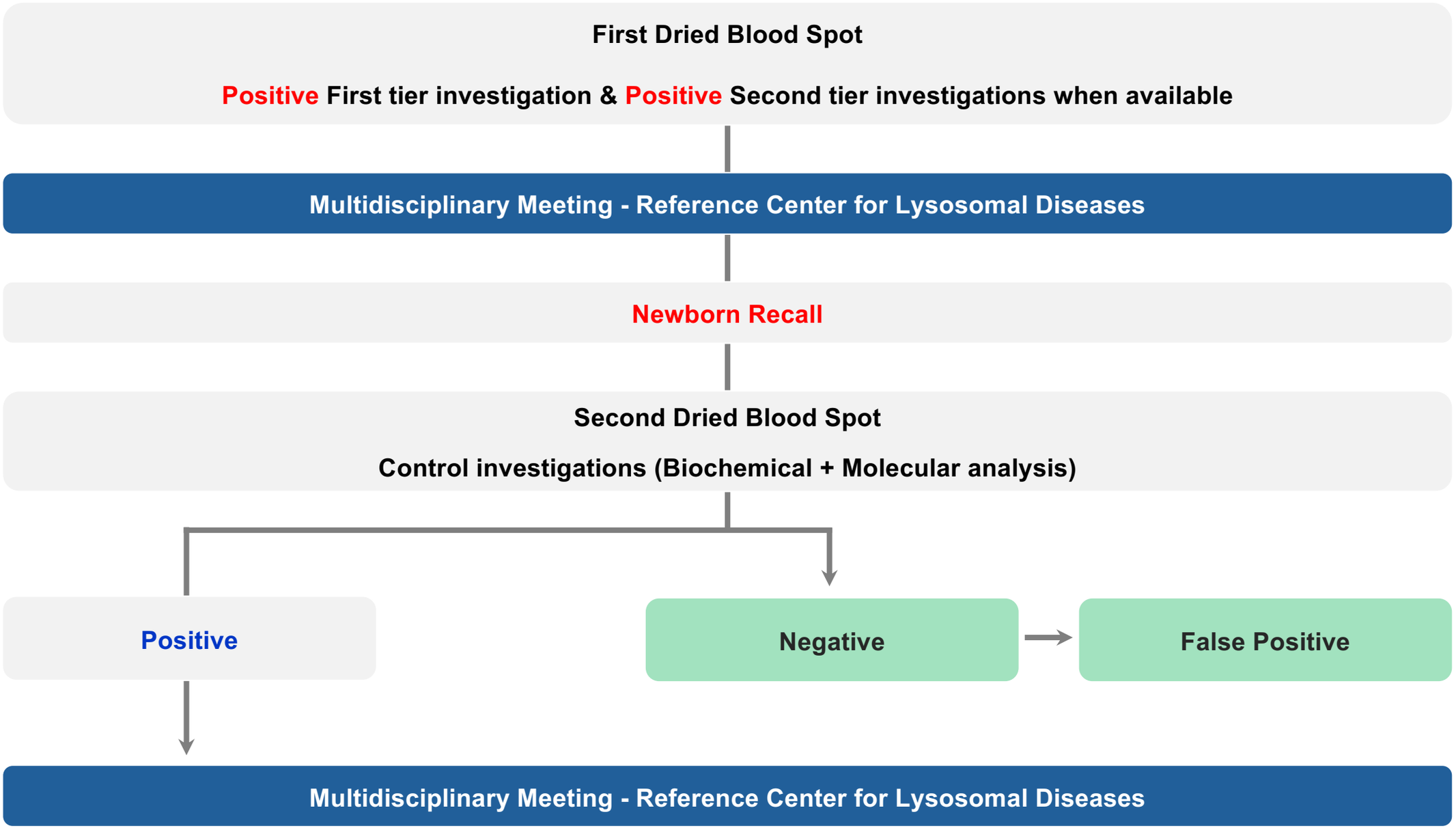
LDs: Lysosomal Diseases; MLD: Metachromatic Leukodystrophy

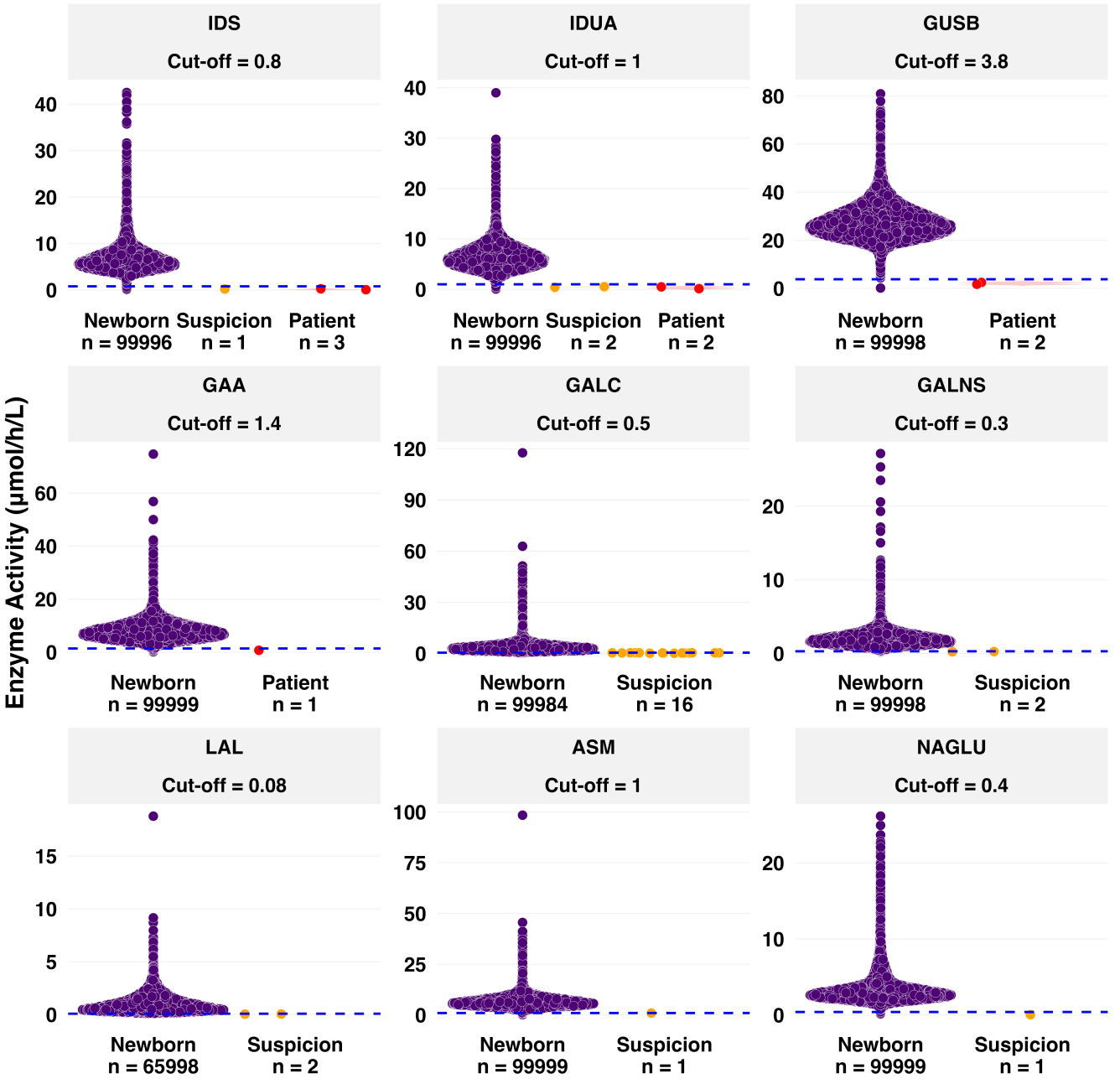
Editorial Summary :

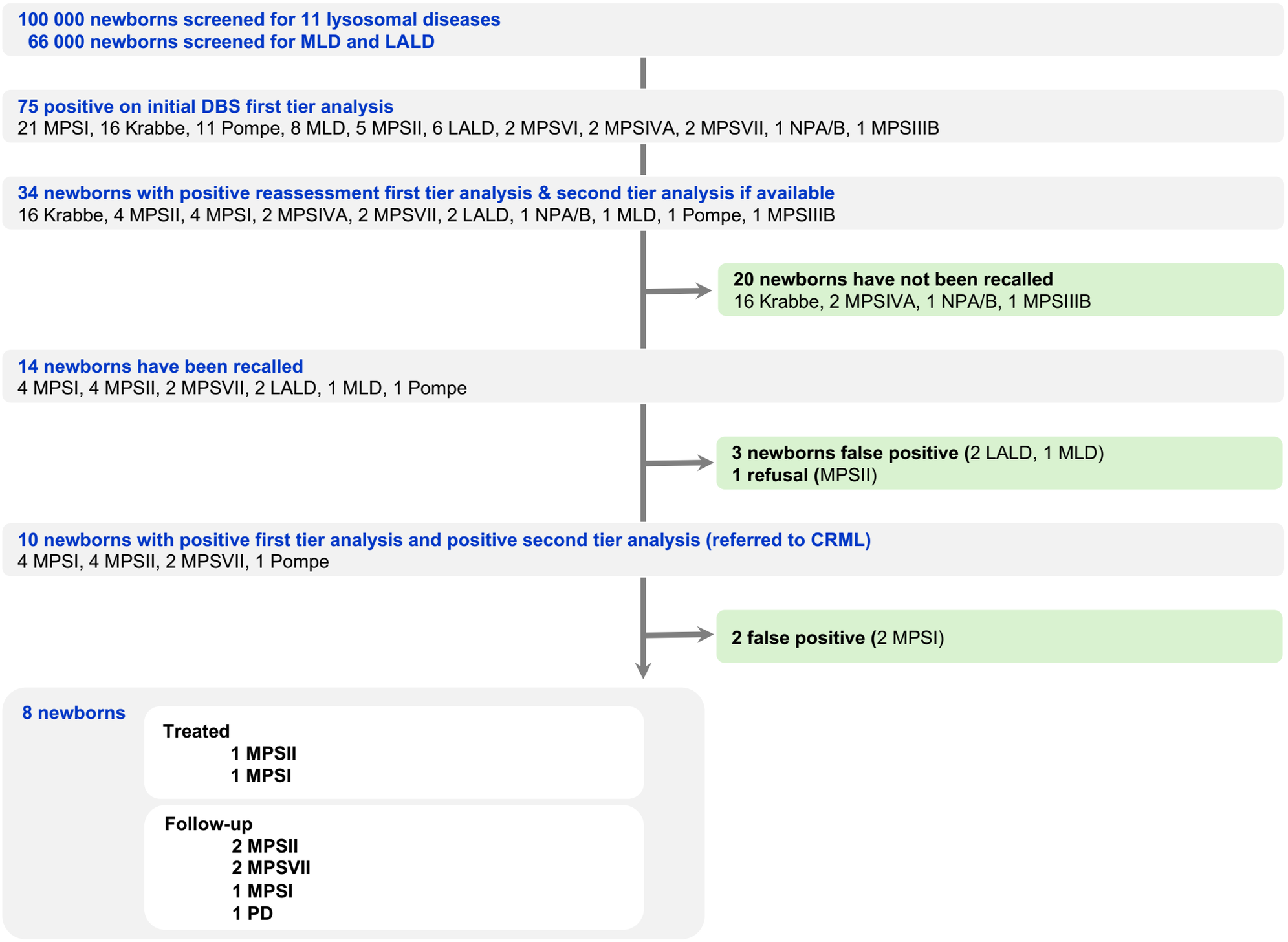
Tebani et al., evaluates the feasibility of high-throughput, multi-tier newborn screening for 13 lysosomal diseases in 100,000 newborns using biochemical, molecular and multidisciplinary assessment. It shows feasible implementation with low recall rates and moderate predictive value, identifying confirmed cases and enabling early treatment in some infants.

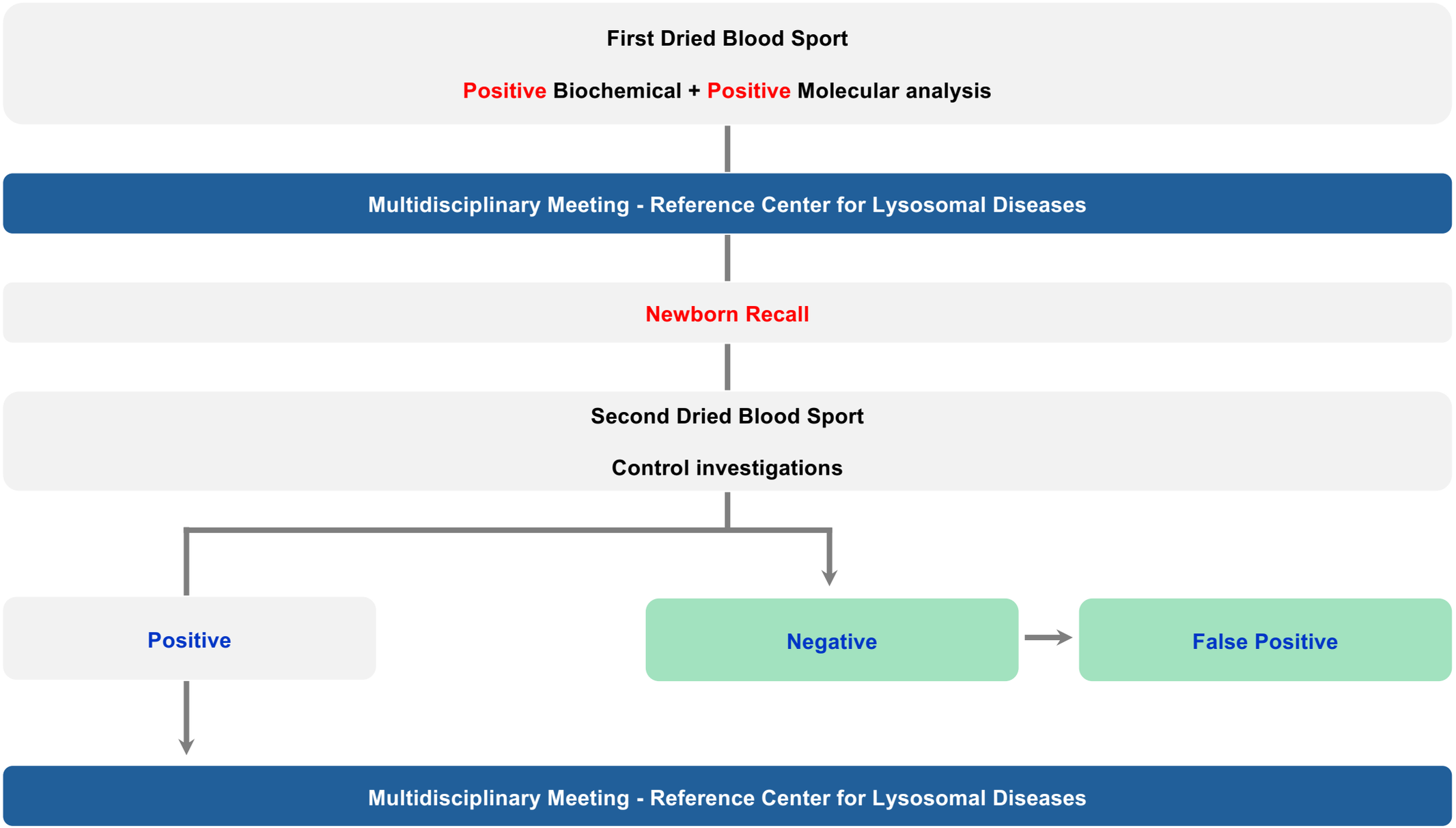
Peer review information:

Communications Medicine thanks Cristóbal Colón and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.


First Dried Blood Spot
Positive First tier investigation & Positive Second tier investigations when available
Multidisciplinary Meeting - Reference Center for Lysosomal Diseases
Newborn Recall
Second Dried Blood Spot
Control investigations (Biochemical + Molecular analysis)
Positive
Negative
False Positive
Multidisciplinary Meeting - Reference Center for Lysosomal Diseases


IDS
Cut-off = 0.8
Newborn n = 99996
Suspicion n = 1
Patient n = 3
IDUA
Cut-off = 1
Newborn n = 99996
Suspicion n = 2
Patient n = 2
GUSB
Cut-off = 3.8
Newborn n = 99998
Patient n = 2
GAA
Cut-off = 1.4
Newborn n = 99999
Patient n = 1
GALC
Cut-off = 0.5
Newborn n = 99984
Suspicion n = 16
GALNS
Cut-off = 0.3
Newborn n = 99998
Suspicion n = 2
LAL
Cut-off = 0.08
Newborn n = 65998
Suspicion n = 2
ASM
Cut-off = 1
Newborn n = 99999
Suspicion n = 1
NAGLU
Cut-off = 0.4
Newborn n = 99999
Suspicion n = 1
Enzyme Activity (µmol/h/L)


100 000 newborns screened for 11 lysosomal diseases
66 000 newborns screened for MLD and LALD
75 positive on initial DBS first tier analysis
21 MPSI, 16 Krabbe, 11 Pompe, 8 MLD, 5 MPSII, 6 LALD, 2 MPSVI, 2 MPSIVA, 2 MPSVII, 1 NPA/B, 1 MPSIIIB
34 newborns with positive reassessment first tier analysis & second tier analysis if available
16 Krabbe, 4 MPSII, 4 MPSI, 2 MPSIVA, 2 MPSVII, 2 LALD, 1 NPA/B, 1 MLD, 1 Pompe, 1 MPSIIIB
20 newborns have not been recalled
16 Krabbe, 2 MPSIVA, 1 NPA/B, 1 MPSIIIB
14 newborns have been recalled
4 MPSI, 4 MPSII, 2 MPSVII, 2 LALD, 1 MLD, 1 Pompe
3 newborns false positive (2 LALD, 1 MLD)
1 refusal (MPSII)
10 newborns with positive first tier analysis and positive second tier analysis (referred to CRML)
4 MPSI, 4 MPSII, 2 MPSVII, 1 Pompe
2 false positive (2 MPSI)
8 newborns
Treated
1 MPSII
1 MPSI
Follow-up
2 MPSII
2 MPSVII
1 MPSI
1 PD


First Dried Blood Sport
Positive Biochemical + Positive Molecular analysis
Multidisciplinary Meeting - Reference Center for Lysosomal Diseases
Newborn Recall
Second Dried Blood Sport
Control investigations
Positive
Negative
False Positive
Multidisciplinary Meeting - Reference Center for Lysosomal Diseases