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Five years of newborn screening for Pompe, Mucopolysaccharidosis type I, Gaucher, and Fabry diseases in Oregon

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ABSTRACT

In October 2018, the Oregon newborn screening program began screening for four lysosomal storage disorders (LSDs) Pompe, Mucopolysaccharidosis Type I (MPSI), Gaucher, and Fabry. The laboratory used two different methodologies, digital microfluidics and tandem mass spectrometry, to measure the four LSD enzyme activities. Accuracy and precision were improved and lower false positive rates were observed with use of the Revvity NeoLSD assay on the mass spectrometry platform, as compared to the Baebies digital microfluidics method. All newborn specimens with screen positive results were reflexed to a second-tier molecular assay to identify variants in the target gene. Over the first five years of screening, 139 cases were referred for confirmatory testing and clinical evaluation due to presence of pathogenic/likely pathogenic variant(*s*)or variant(s) of unknown significance. These identified newborns were evaluated at the Oregon Health & Science University (OHSU) metabolic clinic in Portland, Oregon. However, due to the COVID-19 pandemic, clinicians had to pivot from in-person to virtual visits and triage on acuity, which impacted the time to diagnosis. Of referred babies, 3 are currently receiving treatment for their detected LSD. Over 50 babies have an inconclusive or possible late onset diagnosis with uncertain timeline for development of symptoms.

1. Introduction

Newborn bloodspot screening (NBS) is a coordinated public health system that identifies infants with medical conditions requiring early treatment to prevent death or other irreversible harm. It began in the early 1960s with a single condition, phenylketonuria (PKU), and has expanded to include over 37 conditions in accordance with the Health Resources and Services Administration (HRSA) Recommended Uniform Screening Panel (RUSP) [1]. Disorders on the RUSP require approval by the Secretary of Health and Human Services (HHS) and are chosen based on the net benefit of screening, the ability of states to accurately test for and identify newborns with the condition, and the availability of effective treatments [2].

1.1. Newborn screening for Lysosomal Storage Disorders (LSDs) on the RUSP – Pompe and MPSI

Pompe disease (PD) is a lysosomal storage disorder (LSD) and a glycogen storage disorder (GSD) Type 2, that results from deficient lysosomal enzyme, alpha-glucosidase (GAA) and insufficient glycogen metabolism in lysosomes [3]. The spectrum of disease ranges from an infantile form with cardiac symptoms present at birth to a late onset form with musculoskeletal symptoms in later decades of life. Treatment is available for all types with enzyme replacement therapy (ERT)

Abbreviations: ALT, Alanine transaminase; AST, Aspartate aminotransferase; CK, Creatinine Kinase; DMF, Digital Microfluidics; ERT, Enzyme replacement therapy; FD, Fabry disease; GAA, Acid alpha-glucosidase enzyme; Gags, Glycosaminoglycans; GCase, Glucocerebrosidease enzyme; GD, Gaucher disease; GSD, Glycogen storage disorder; GLA, Alpha galactosidase A enzyme; Hex4, Glucotetrasaccharides; HHS, Health and Human Services; HRSA, Health Resources and Services Administration; HSCT, Hematopoietic stem cell transplantation; IDUA, Alpha-L-iduronidase enzyme; IOPD, Infantile onset Pompe disease; LOPD, Late onset Pompe disease; LP, Likely pathogenic variant; LSD, Lysosomal storage disorders; MPSI, Mucopolysaccharidosis Type I; MN, Multiples of normal; MSMS, Mass spectrometry; NREs, Urinary heparan sulfate non-reducing ends; PD, Pompe disease; PV, Pathogenic variant; NBS, Newborn bloodspot screening; OHSU, Oregon Health & Science University; RUSP, Recommended Uniform Screening Panel; SRT, Substrate reduction therapy; US, United States; VUS, Variant(s) of unknown significance.

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infusions [4]. The first pilot screening program was conducted in Taiwan in 2005 [5] and PD was first screened in the U.S. in Missouri in 2013 [6]. It was added to the RUSP in 2015 and as of this publication, 47 U.S. states and programs screen for PD [7].

Mucopolysaccharidosis Type I (MPSI) is an LSD resulting from deficient lysosomal enzyme alpha-L-iduronidase (IDUA) and accumulation of glycosaminoglycans (Gags). The spectrum of disease ranges from a severe form (sometimes called "Hurler") with a multisystemic presentation including coarse features, dysostosis multiplex, organ involvement and intellectual disability to an attenuated form (sometimes called "Scheie") with mild bone malformations in childhood or adulthood, normal development and intellect. Hematopoietic stem cell transplantation (HSCT) is available for patients with severe disease [8]. Enzyme replacement therapy infusions are available for all types, even post-transplant in some cases [9]. The first pilot screening was conducted in Taiwan in 2008 [10] and MPSI was first screened in the U.S. in Missouri in 2013 [6]. MPSI was added to the RUSP in 2016 [7]. As of this publication, 44 U.S. states and programs screen for MPSI.

Two additional LSDs are included on the RUSP, Mucopolysaccharidosis Type II (MPSII) and Early Infantile Krabbe Disease. These were not on the state's newborn screen panel during this study period and were not evaluated in this study.

1.2. Newborn screening for non-RUSP LSDs – Gaucher and Fabry diseases

Gaucher disease (GD) results from deficient lysosomal enzyme beta-glucocerebrosidase (GCase) and accumulation of glucosylceramides. The spectrum of disease ranges from a severe, untreatable form, present in infancy and resulting in a shortened lifespan (Type 2) to a disease with musculoskeletal involvement that may not occur until adulthood (Type 1). Type 3 disease is similar in symptomatology to Type 1, but also includes neurological features. Treatment is effective for type 1 and 3 disease either by oral substrate reduction therapy (SRT) or regular ERT infusions [11,12]. GD was first screened in the U.S. in Missouri in 2013 with subsequent pilots in Illinois and New York. New York detected the highest rates of disease with 1:4374 affected individuals, compared with 1:43,000 in Illinois and 1:61,000 in Missouri [6,13,14]. While it has not been added to the RUSP, as of this publication 6 states and programs screen for GD, including Oregon [15].

Fabry disease (FD) results from deficient lysosomal enzyme, alpha-galactosidase A (GLA) and subsequent accumulation of glycosphingolipids in multiple organ systems. Unlike most other LSDs, Fabry is an X-linked disorder. Males with classic disease are expected to show early symptoms which include neuropathic pain, hypohidrosis, cardiac and renal disease. Non-classic disease can include some or all these symptoms but typically presents later in life. Females can be affected with classic or non-classic disease. Available treatment includes oral chaperone therapy for certain amenable gene variants or ERT infusions [16]. Pilot studies screening for Fabry started as early as 2003 in Europe and FD was first screened in the U.S. in Missouri in 2013 [6]. Other U.S. pilots or retrospective studies have been conducted [13,17,18] with reported incidence ranging from 1:394 to 1:15,558 males [19]. These are higher than clinically ascertained estimates of classic disease in males of around 1:40,000 [20]. Fabry has not been added to the RUSP but as of this publication 8 U.S. states or programs screen for Fabry, including Oregon [15].

1.3. Oregon geography

Oregon is a geographically large state of almost 100,000 mile^2 (254,803 km^2) with multiple mountainous regions and a significant rural area. The Northwest Newborn Screening Program, located in Hillsboro, Oregon, is in the northwest corner of the state. It performs the screening and reporting for all babies born in Oregon. Screening for four LSDs (PD, MPSI, GD and FD) began on October 1, 2018. The program contracts with OHSU, the states only metabolic center, to provide follow-up consultation for all infants with detected metabolic disorders including the LSDs. Almost all referrals for infants identified by screening must travel to Portland, Oregon for care.

1.4. Newborn screening for LSDs

Initially the Northwest Newborn Screening Program used the Baebies digital microfluidics platform (DMF) to measure the enzyme activities for the four LSDs extracted from the blood spots, based upon the experience of the Missouri screening program [21]. The benefits of this platform included the overall simplicity of the device, minimal volumes of reagent and sample, scalability to handle hundreds of samples in a single day, and the greatly reduced time to generate results [22]. However, in August of 2021, the program switched to the tandem mass spectrometry (MSMS) method developed by Revvity (NeoLSD). At the time, the Northwest Newborn Screening Program struggled with a large number of screened positive results from the Baebies platform and was looking for a way to improve screening accuracy. Studies suggested that the tandem mass spectrometry method would decrease false positive rates [23]. However, in a more recently published head-to-head comparison of both methods, the Baebies DMF was determined to perform better than MSMS with fewer cases below established cutoffs but may have an increased risk of false negative, or missed cases [17].

This five-year retrospective study summarizes and compares the screening outcomes from the two platforms (DMF and MSMS) and provides data on the clinical course, including treatment, of identified individuals. This study also highlights considerations for long-term follow-up for confirmed patients.

2. Materials and methods

2.1. Specimens

During the five-year study period (October 1, 2018 – September 30, 2023) 117,399 babies were screened for the four LSDs using the Baebies DMF method and 85,330 babies were screened using the Revvity NeoLSD MSMS method, for a total of 202,729 screened babies. It is mandated in statute and rules that every baby born in Oregon receive two screens; the first specimen collected between 24 and 48 h of life and the second specimen collected between 10 and 14 days of life. Premature babies require a third specimen at one month of life. Screening for LSDs is only performed on the first specimen. Greater than 95 % of the first specimens tested on the 202,729 babies were collected within the recommended time frame (24–48 h). In this cohort of newborns, 51 % were reported as male (49 % female) and 6.7 % of the babies had a birth weight less than 2500 g.

2.2. Screening algorithms

The first-tier screening for LSDs measures the enzyme activity of GAA, IDUA, GCase, and GLA. Specimens with out-of-range results due to low enzyme activity are reflexed to full gene sequencing for the given condition and copy number variation. If the activities of two or more enzymes were reduced in a specimen, the results were deemed inconclusive, and a repeat specimen was requested.

When using the Baebies DMF method, fixed cutoffs were set by the enzyme activity measured and were not initially adjusted for the time of collection (1st screen versus 2nd screen). However, after the first five months of screening, lower thresholds were implemented for GLA activity in specimens collected at 7 days of life or greater. After 14 months of screening, a lower threshold was also implemented for GCase activity in specimens collected after the first week. Any specimen collected early, prior to 20 h of life, required recollection during the recommended time frame (24–48 h). In premature babies, LSDs screening was not performed until the second specimen collection at greater than 312 h of life

(13 days).

Switching to the Revvity NeoLSD method required a reassessment of the cutoff values for each enzyme. Because of seasonal differences, fluctuations of instruments, and lot-to-lot variation of reagents, floating cutoffs were implemented. Each day, the multiple of the median (MoM) expressed as a percent was calculated across all samples. The cutoff was set at a given percent MoM. Initially, no age or birth weight adjustments to the thresholds were made for the any of the enzyme activities but were later implemented for GLA.

Second-tier sequencing assays were performed by Baebies when the DMF screening method was in use. Following the switch to Revvity's NeoLSD MSMS assay, second-tier sequencing transitioned to Revvity. Babies, who screen positive for PD, MPSI, or GD with multiple pathogenic variant(s), likely pathogenic variant(s) or variant(s) of unknown significance (VUS), are referred to the metabolic specialists at OHSU for confirmatory testing and clinical evaluation. Babies determined to be carriers of heterozygous variants (pathogenic/likely pathogenic/VUS), pseudodeficiency alleles, or no variants detected, are reported as having a normal LSD screen. For FD, all males and females with a pathogenic, likely pathogenic, or VUS detected are referred to the metabolic specialists.

2.3. Baebies DMF assay

The digital microfluidics assay simultaneously measures the activity of four enzymes (GAA, IDUA, GCase, GLA). From a 3.2 mm punch of the specimen, the enzymes are extracted and then loaded onto the disposable cartridge. The instrument manipulates droplets of specimens, substrates, and buffers in the cartridge to allow for product formation. The enzyme activity is calculated from the emitted fluorescence from the reaction product and is quantitatively compared to the calibration curve to determine the final value. Results are available the same day as the assay is initiated [21].

2.4. Revvity NeoLSD MSMS assay

Similarly the Revvity NeoLSD assay uses a 3.2 mm punch from the specimen and enzymes are extracted and incubated with substrates for the four conditions [24]. The kit was modified and validated using a 3-h incubation for the enzymatic assays, instead of the recommended 18 h. After incubation, a liquid extraction is performed, followed by a dry down step and reconstitution with flow solvent. The sample is then injected into the tandem mass spectrometer for analysis of enzyme activities. Unlike the DMF assay, a calibration curve is not used for quantification. Results are not available until the following day.

2.5. Confirmatory testing for each condition

2.5.1. Pompe disease

After referral from the program, the determination of visit acuity and priority for follow-up testing is made based on the screening enzyme activity, presence of any clinical symptoms and the *GAA* genotype. Because Pompe is a time critical condition, infants with a screening enzyme activity $\leq$5 % of the daily median are referred prior to the completion of sequencing. This urgent referral cutoff was used for both the Baebies DMF and Revvity NeoLSD methods. All infants with critical low enzyme and/or pathogenic variants receive office visits (when feasible) and laboratory testing, including the assessment of GAA activity in leukocytes, creatinine kinase (CK), aspartate aminotransferase (AST), alanine transaminase (ALT) and urine glucotetrasaccharides (Hex 4) values. An echocardiogram, EKG, and/or chest x-ray is typically obtained as soon as possible.

For well infants with presence of the known late onset variant (c.-32-13 T > G), visits are offered for next available clinic slot and can be virtual. Testing is focused on confirmation of disease by GAA activity in leukocytes along with CK, AST, ALT and urine Hex 4 for baseline evaluation. Cardiac evaluation may be deferred until diagnosis is confirmed.

2.5.2. MPSI

Confirmatory testing and clinical evaluation for babies who screen positive for MPSI is based on the presence of any clinical symptoms and the *IDUA* genotype available from screening. For infants with potential symptoms of concern and/or the presence of clear pathogenic or likely pathogenic variants, office visits (when feasible) are required as physical examination and skeletal/abdominal imaging can be used to immediately confirm or rule-out infantile onset disease. Confirmatory testing with the assessment of IDUA activity in leukocytes, urinary heparan sulfate non-reducing ends (NREs), and glycosaminoglycans (Gags) in blood and urine are also ordered. Clinicians may pursue abdominal imaging, echo, EKG, skeletal films, brain imaging and referral to ophthalmology.

For well infants with presence of one or more variant(s) of uncertain significance, non-urgent visits are offered for next available clinic slot and can be virtual. Confirmatory testing includes IDUA activity in leukocytes, NREs and Gags in blood and urine.

2.5.3. Gaucher disease

Generally, follow-up determinations are made with the genotype information available from screening. Testing is focused on confirmation of disease by GCase activity in leukocytes, along with complete blood count (CBC), chitotriosidase, glucosylsphingosine (lyso GL-1) and abdominal imaging for baseline evaluation.

2.5.4. Fabry disease

Fabry referrals are not considered urgent due to the prolonged period in which symptoms are expected to develop. Families are offered the opportunity for either a virtual clinic visit or in the office visit to answer questions. A complete family history is obtained during the visit, given the potential for identifying other affected individuals who may benefit from intervention. Confirmatory testing in newborn males includes GLA activity in leukocytes and plasma globotriaosylsphingosine (lyso GL-3) for baseline evaluation. This testing is strongly encouraged for all individuals, even those with *GLA* variants of uncertain significance, including the common p.Ala143Thr variant. For heterozygous females, confirmatory testing is often deferred as it is not expected to change management or follow-up.

Male infants with diagnostic GLA activity within the normal range are discharged from care. For male infants with low or "indeterminate" GLA activity along with normal lyso GL-3 and negative family history, clinicians engage in shared decision making with families to determine whether regular follow-up clinic visits throughout childhood are appropriate. If preferred by the family, a return to clinical care upon development of signs and symptoms is discussed. Familial testing is offered for interested family members if they reside in Oregon or Washington, where our providers are licensed.

3. Results

3.1. Screening results

During the five-year study period, 202,729 babies were screened for the four LSDs; roughly 58 % using the Baebies DMF method and 42 % using the Revvity NeoLSD method. The results are summarized within Table 1. Of the screened infants, 138 (0.07 %) were referred and seen for a clinical visit at OHSU. Thirty nine of the 138 (27.5 %) referred cases were confirmed to have a LSD, 62 of the 138 cases (45.7 %) were determined to have a possible disease though their status is inconclusive as of this writing, and 37 of the 138 (26.8 %) were determined to be unaffected (false positives) and were discharged from clinic.

Table 1
Outcomes of screening by disorder and platform, 2018–2023.

Method		Pompe			MPSI			Gaucher			Fabry	
		Baebies DMF	NeoLSD MSMS		Baebies DMF	NeoLSD MSMS		Baebies DMF	NeoLSD MSMS		Baebies DMF	NeoLSD MSMS
Babies Screened		1,17,399	85,330		1,17,399	85,330		1,17,399	85,330		1,17,399	85,330
Outcomes of Screening	Referrals	29	9	Referrals	11	4	Referrals	6	0	Referrals	43 M, 8 F	23 M, 5 F
	Carriers	304	23	Carriers	37	6	Carriers	48	0			
	Pseudo deficiency	438	11	Pseudo deficiency	25	2	No Variants	236	0	No Variants	345 M, 246 F	56 M, 40 F
	No Variants	427	10	No Variants	30	0						
	Total	1198	53	Total	103	12	Total	290	0	Total (M/F)	388 M, 254 F	79 M, 45 F
	PPV	2.4 %	17.0%	PPV	10.7 %	33.3%	PPV	2.1 %	0	PPV (M/F)	11.1 % M, 3.1 % F	29.1 %M, 11.1 % F
Outcomes of Diagnostic Testing/Evaluation	Confirmed Cases	18 (1 IOPD, 17 LOPD)		Confirmed Cases	6		Confirmed Cases	3		Confirmed Cases	8 M, 4 F	
	Unaffected	20		Unaffected	8		Unaffected	2		Unaffected	5 M, 2 F	
	Inconclusive	0		Inconclusive	1		Inconclusive	1		Inconclusive	53 M, 7 F	
	Indeterminant			Indeterminant			Indeterminant			Indeterminant		
	Total	38		Total	15		Total	6		Total	66 M, 13 F	
	PPV	47.4 %		PPV	40.0%		PPV	50.0 %		PPV	12 % M, 30.7 % F	

3.2. Pompe disease

For Pompe disease, 1251 babies screened positive on the first-tier assay and 38 were identified on second-tier sequencing to have two variants warranting confirmatory testing and evaluation. The positive predictive value (PPV) of the first-tier assay to identify newborns with two variants was significantly higher for the NeoLSD assay, as compared to the DMF assay (17 % and 2.4 %, respectively). After diagnostic testing and clinical evaluation, 18 of the 38 babies were confirmed to have Pompe disease: 1 with infantile onset and 17 with late onset. The other 20 babies were determined to be unaffected. Second-tier PPV was also improved from the Baebies method of 34 % with the NeoLSD method to 89 % resulting in an overall 47 % PPV for the two-tiered screening algorithm. At present, only 2 of the 18 babies are being treated for Pompe disease; one with IOPD and the other with LOPD.

The infant with IOPD was confirmed shortly after referral based upon abnormal cardiac findings. Treatment was initiated at day-of-life 32 with immune modulation and enzyme replacement therapy (ERT) infusions of 40 mg/kg weekly. Within population of babies with LOPD, only one individual is currently receiving treatment as of November 2024. This baby is compound heterozygous for the pathogenic c.-32-13 T > G variant and another pathogenic variant, p.Leu552Pro. At the time of diagnosis, their GAA activity in leukocytes was 0.56 nmol/h/mg [Reference $\geq$1.5]. The determination to treat was made at age 15 months due to abnormal motor findings and laboratory values including CK of 663 U/L [Ref < 135 U/L] and urinary Hex 4 of 15 mmol/mol Ct [Ref $\leq$ 4.0]. ERT infusions were initiated at 18 months at a dose of 40 mg/kg every two weeks. At 2 years, 7 months of age, their laboratory values had normalized and there were no abnormal findings on her motor exam per two examiners, one metabolic genetics clinician and a pediatric physical therapist.

3.3. MPSI

A total of fifteen babies with two variants were referred for MPSI confirmatory testing of the 115 babies who screened positive with a low enzyme activity. MPSI first tier screening using the NeoLSD assay yielded a three-fold higher PPV (33.3 %), as compared to the DMF assay (10.7 %), for the identification of babies with two variants. However, PPV on basis of second-tier testing went from 36 % using Baebies to 25 % with NeoLSD.

After clinical evaluation and confirmatory testing, six of the fifteen babies were confirmed to have attenuated MPSI. The oldest detected individual with attenuated disease recently demonstrated mild, bilateral coxa valga, at age five years on x-ray, which is the first symptom attributable to MPSI. As of November 2024, none of the six attenuated cases have started treatment. In one of the 15 referred cases, no formal classification was made before the family was lost to follow-up so they are considered “inconclusive”, though this was likely an attenuated case. Eight of the referred newborns were considered to be unaffected after confirmatory testing.

3.4. Gaucher disease

A total of 290 babies screened positive for low GCase activity as identified by the DMF assay and only 6 were referred for confirmatory testing because of the detection of two variants. Interestingly, the NeoLSD assay did not detect any cases of low GCase activity so no comparison in PPV on the basis of second-tier results could be generate. From the six referred cases, three babies were confirmed to have disease, one baby remains indeterminant as the family moved from the area before a formal classification could be considered, and two babies were determined to be unaffected.

Of the three confirmed cases, one baby is homozygous for a well-described variant, p.Asn409Ser, which is expected to result in type 1 disease. Another infant has one pathogenic variant in combination with

a VUS and is asymptomatic as of last clinical evaluation at 3 years, 10 months of age. The third baby was symptomatic in infancy. This baby is compound heterozygous for a pathogenic variant, p.Arg502Cys, and a variant of uncertain significance, p.Ser146Leu. Their diagnostic GCase enzyme was low at 1.4 nmol/h/mg [Ref: 4.6–12] and they demonstrated mildly low hemoglobin and elevated chitotriosidase. By age 14 months, they had low hemoglobin of 9.5 g/dL [Ref: 10.5–13.5], low platelets of 170 K/cu mm [Ref: 250–600], increasing chitotriosidase of 15,000 nmol/h/mL [Ref: 4–120], spleen size 9 multiples of normal (MN) and liver 2.5 MN. Treatment was initiated at age 14 months. As of last clinical evaluation the child was age 5 years, 7 months with normal hemoglobin, platelets, height velocity and no hepatosplenomegaly. Currently they receive enzyme replacement therapy infusions of 80 units/kg every two weeks. This case is considered to have type 1 disease with no neurological signs or symptoms to date, however, they are monitored closely for any changes in their status.

3.5. Fabry disease

During the five-year study, 766 babies screened positive for a low GLA activity; 642 were identified from the Baebies DMF assay and 124 from the NeoLSD platform. Following second-tier sequencing, 79 referrals to the metabolic clinic were made (66 males and 13 females) due to an identified variant of pathogenic or likely pathogenic significance, or a variant of unknown significance.

During the evaluation of cutoffs, the laboratory noted the inverse relationship between the birth weight and GLA activity, which may be due to the differences in leukocyte counts for premature and full-term babies, as previously described [25]. Therefore, the laboratory adjusted cutoff values stratified by birth weight. However, it was unclear whether an affected low birth weight baby would have decreased activity, as compared to other premature newborns, or decreased activity as compared to the population of healthy, full-term newborns. In our evaluation of 66 referred males, five had a birth weight less than 2500 g and enzyme activities ranging from (0.68 to 6.49 umol/L/h). The GLA activities in the remaining 61 babies with a birth weight greater than 2500 g ranged from 2.13 to 9.79 umol/L/h. It was concluded that affected babies will have significantly reduced GLA activity, regardless of their birth weight and cutoff stratification has since been removed.

Of the 79 referrals received, eight males and four females had a pathogenic or likely pathogenic variant expected to confer a high risk for developing signs and symptoms of Fabry disease. These cases are classified as 'confirmed', though the timeline for development of symptoms remains uncertain. To date, no newborn with confirmed disease has had symptoms resulting in treatment initiation.

Of the remaining referrals, 53 males and seven females are classified as 'inconclusive' with the expectation that they may develop late onset disease or no disease. The majority of inconclusive cases are attributed to the presence of the common p.Ala143Thr variant with 40 male babies (of the 53) and 3 female babies (of the 7) having this variant. While the lab performing second-tier genetic testing currently classifies this variant as "likely pathogenic", the OHSU metabolic specialists approached it as a "variant of uncertain significance (VUS)". Another (*non*-p.Ala143Thr) variant of uncertain significance is present in the remaining 13 males and 4 females classified as inconclusive. Table 2 provides a summary of all the variants identified in affected individuals and those with inconclusive, possibly late onset disease.

3.6. Distance from care and time to diagnosis

The average distance from OHSU metabolic clinic in Portland, Oregon for all referred infants was approximately 70 miles, however, at least 47 of the 138 (34 %) live over 70 miles from OHSU. In person office visits were conducted for 57 of the 138 infants (41.3 %) and 79 infants had a virtual visit either by video (47.8 %) or by telephone (9.4 %). Of note, the average distance from clinic for those seen in office was 80.8 miles compared with only 27.8 miles for those seen by phone. For infants with a suspected risk of an infantile or classic disease presentation, office visits were the priority whenever possible.

Table 2
Variants detected and expected outcome in newborns referred for Fabry disease.

FABRY REFERRALS - MALE		
Count	Variant	Outcome
4	p.Ile198Thr (LP)	classic/confirmed
2	p.Met296Val (P)	classic/confirmed
1	p.Tyr207Cys (LP)	classic/confirmed
1	p.Asn215Ser (P)	classic/confirmed
1	p.Asp313Tyr (VUS)	false positive
1	p.Pro305 = (VUS)	false positive
2	p.Arg332Glu (VUS)	false positive
39	p.Ala143Thr (LP)	inconclusive/possible late onset
5	p.Arg118Cys (VUS)	inconclusive/possible late onset
3	p.Tyr123Cys (VUS)	inconclusive/possible late onset
2	p.Lys140Thr (VUS)	inconclusive/possible late onset
1	p.Trp24Arg (VUS)	inconclusive/possible late onset
1	p.Pro60Ser (VUS)	inconclusive/possible late onset
1	p.Arg356Gln (VUS)	inconclusive/possible late onset
1	p.Trp24Arg; p.Asn419Asp (VUS/VUS)	inconclusive/possible late onset
1	p.Trp277Cys (VUS)	lost to follow-up
FABRY REFERRALS - FEMALE		
Count	**Variant**	**Outcome**
2	p.Arg363His (LP)	classic/confirmed
1	p.Arg356Trp (P)	classic/confirmed
1	p.Asn215Ser (P)	classic/confirmed
2	p.Asp313Tyr (VUS)	false positive
4	p.Ala143Thr (LP)	inconclusive/possible late onset
1	p.Met290Leu (LP)	inconclusive/possible late onset
1	p.Arg356Gln (VUS)	inconclusive/possible late onset
1	p.Arg118Cys (VUS)	inconclusive/possible late onset

Table 3 summarizes the average number of days for each of the screening milestones for referred newborns ($n = 138$): abnormal sample collection, reported screening results (both first- and second-tier), intake visit with specialist and diagnostic lab results completed. The intake visits with the specialists were conducted at similar time frames, regardless of whether it occurred virtually or in the office. However, the diagnostic testing which represents time to diagnosis was longer for patients seen virtually. In particular, the late onset Pompe case experienced a significant delay in diagnosis due to the lack of access to diagnostic testing in community settings.

4. Discussion

Over the five-year period, the Oregon NBS program referred 138 babies for clinical evaluation and diagnosis of four LSDs. Thirty-eight babies from this cohort were confirmed to have disease: 18 babies with Pompe, 8 babies with Fabry, 6 babies with attenuated MPSI, and 3 babies with Gaucher. Fabry disease presented the greatest challenge for the NBS program, as over 50 babies with inconclusive results were also identified.

Table 3
Average age in days of screening milestones by visit type (virtual or office visit).

Average Age in Days	All LSD Cases (+/-SD)	Cases with Office Visit	Cases with Virtual Visit
Abnormal specimen collected*	3.9 (+/− 9.6)	5.4	3.0
Screen positive results available after both first- and second-tier testing	19.7 (+/− 11.4)	21.9	18.1
Intake visit with specialist	70.8 (+/− 95.5)	74.0	68.9
Diagnosis by diagnostic lab result	104.4 (+/− 120.9)	75.5	130.2

* *This may be the first screen or a second/repeat screen.*

The Baebies DMF assay was easier to set-up and validate than the NeoLSD assay. The DMF instruments cost considerably less money and required significantly less daily maintenance and optimization. There was also less manual effort by the laboratory staff in performing the DMF assay and it produced results within four hours, allowing for a faster turn-around time of reports. However, the large number of first-tier screened positive results pushed the laboratory to consider the NeoLSD assay using tandem mass spectrometry. Data from the NeoLSD assay showed better precision and a lower positivity rate. The benefit of improved assay specificity outweighed the increased hands-on time for staff to perform the NeoLSD assay and the overnight delay in obtaining results. The use of second-tier molecular testing, both gene sequencing and deletion / duplication analysis, further improved the specificity of screening and drastically reduced the number of referrals.

Setting cutoffs for these four LSD assays was challenging, and the laboratory changed cutoffs more than 7 times during the five-year period. Because Oregon was an early adopter of LSD screening and unique in including Fabry and Gaucher, cutoffs were set very conservatively at first. Additionally, the NBS program also didn't appreciate all the factors that can affect measured enzyme activities such as lot to lot variation, seasonal variation, birth weight, timing of collection, and race and ethnicity differences [25]. To address these variables, age and birth weight specific cutoffs were introduced and modifications to the calculation of results, from fixed cutoffs to multiple of the daily median, was implemented. Presently, the laboratory is actively assessing the cutoff values for GCase because there were no screened positive cases for Gaucher identified from the NeoLSD platform. Interestingly, the outcome data generated from this study suggests that babies affected with Fabry disease will have significantly reduced GLA activity, regardless of their birth weight. Cutoff stratification based upon birth weight has since been removed.

4.1. Pompe disease

Infantile onset Pompe disease requires immediate initiation of treatment, however the large proportion of screened positive cases were late-onset disease. Protocols for how to identify the newborns at greatest risk depend upon confirmatory laboratory testing and cardiac imaging to make acuity determinations, but access to this testing, outside of the metropolitan regions of Oregon, is very limited. At diagnosis, the infantile case was approximately 200 miles and the treated late onset case was approximately 100 miles from OHSU. In the treated LOPD case, laboratory evaluations coordinated by primary care office were unsatisfactory or invalid on three attempts and could not successfully be completed until age 11 months which delayed the detection.

Treatment initiation for LOPD remains controversial. Presently, OHSU follows-up with identified individuals frequently in the first two years of life to closely monitor motor development. Clinic visits include a pediatric physical therapist (PT) and a metabolic dietitian, who promotes a high-protein, limited simple carbohydrate diet. Treatment decisions are based upon a combination of physical exam, including PT input, laboratory values and parental interest, however, this is largely expert opinion-based and may be approached differently by another provider.

Once a diagnosis is established, the access to recommended services like pediatric PT and pediatric infusion centers also remains a challenge outside of the metropolitan areas. Like many, Oregon lacks sufficient pediatric PTs with adequate comfort or experience in monitoring Pompe cases. OHSU has a pediatric physical therapist to see these children at the time of their metabolic clinic appointment, however for families unable to come to Portland or with insurance barriers limiting access to that service, there are very few options for monitoring. Oregon has less than a half dozen hospital-based pediatric infusion centers; however, these can be up to 2 h' drive one-way from patients in certain locations, representing a major challenge for patients and their families.

4.2. MPSI

The diagnosis of MPSI in newborns referred for screen positive results depends upon physical examinations, x-rays, and urine laboratory evaluations, which are all easier to obtain throughout the state than in Pompe monitoring described above. However, establishing a diagnosis of attenuated disease has its own challenges. At initiation of screening, urinary and serum Gags were the primary diagnostic marker. However, OHSU has since transitioned to using urine NREs to make a determination. If urine NREs are abnormal, even with consistently normal Gags, the baby is considered to have an attenuated form of MPSI. Anecdotally, other programs and providers have reported that this may be an "overly sensitive" approach that captures individuals who would never come to clinical detection. Weighing the benefits of early treatment while exercising caution not to over-medicalize healthy individuals is an ongoing balance.

At a diagnosis of MPSI of any type, the risks and benefits of treatment with enzyme replacement therapy are discussed. Based on more recent reports [26,27], OHSU metabolic providers have shifted to emphasize the benefits of ERT on bone disease when started in the first 1–2 years of life, even for those with attenuated disease. However, a shared decision-making model with both parents and providers is paramount. To date, we have not initiated treatment prior to onset of detectable symptoms. The biggest barriers identified are the surgical placement of a portacath for ERT administration, the time commitment of regular infusions including long drives to a pediatric infusion center, and the uncertainty on timeline of development of symptoms. Some individuals detected may not ever develop actionable symptoms and this has a major impact on parental investment in treating for life.

4.3. Gaucher disease

Over the five-year study period, there was an extremely low referral burden for Gaucher disease which fell to 0 after the transition to MS/MS. Due to the prolonged lack of first-tier screen positive results using the Revvity NeoLSD method, the program was concerned about the likelihood of false negative cases and has since increased the first-tier enzyme cutoff. This has resulted in additional second-tier testing and diagnosed cases. They are also investigating a transition of second-tier testing to glucosylsphingosine (lyso GL-1) levels, instead of molecular testing, which has been suggested to be more specific for disease [28].

The program identified three cases of Gaucher and the one treated case is a great example of the benefits of early detection. However, determining classification of disease type between 1 and 3 is a challenge in absence of natural progression of clinical symptoms. While the treated child is currently classified as type 1 disease, type 3 cannot be ruled out and this patient is continually monitored for neurological signs or symptoms.

4.4. Fabry disease

The referral burden of Fabry has been the highest of all the LSDs. As with the other disorders, obtaining laboratory or other recommended follow-up evaluations in community settings is difficult for those patients outside of the Portland metropolitan area. A unique challenge in this population has been a higher risk for loss to follow-up. This is understandable given the lag in timeline or perceived risk for developing signs or symptoms. For all families, but particularly those with a classic variant detected, counseling and family cascade testing is offered, however uptake is not high despite the actionability of results. None-theless, at least one symptomatic parent was detected and chose to initiate treatment based on the child's result.

The primary challenge for Fabry screening continues to be the iceberg of uncertain variants, including A143T which make up most of the detected population [29], and the uncertainty of developing disease for female heterozygotes. A shared decision-making approach with

families has been used to resist overmedicalization and to promote follow-up and monitoring in this population, who may greatly benefit from treatment eventually.

4.5. Time to diagnosis

Initiation of LSD screening occurred shortly before the onset of the COVID-19 global pandemic, which had a major impact on the follow-up for babies with screen positive results. Prior to April 2020, no virtual visits were available for families, regardless of where they lived in the state. Because the pandemic prevented the ability to conduct in-person clinic visits, virtual visits became available almost overnight. Virtual visits are now considered best practice for well infants in locations that make OHSU too distant or difficult to reach. Telephone visits are the least optimal visit type but were often relied upon for families with technological or other barriers or during the height of COVID-19 lock-downs or surges, even when they were located in the Portland area. When visits were conducted virtually there was an almost 2-month delay in diagnosis, typically due to challenges with obtaining or collection errors of the specialty diagnostic laboratories in community settings. This is an ongoing challenge for the metabolic clinic and screening program, even post-pandemic.

5. Conclusion

Three children with LSDs detected by newborn screening in Oregon have started treatment and are doing well without progression or worsening of their disease. Overall, the screening burden has been manageable to the program and metabolic specialists. Engagement of families in determination of how and when to follow-up and if or when to start treatment is ongoing as we work to balance treating those who will benefit while reasonably and appropriately monitoring those who may be at-risk.

CRediT authorship contribution statement

Sarah Viall: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Patrice Held:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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