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INTRODUCTION

- Metachromatic leukodystrophy (MLD) is a rare lysosomal storage disorder caused by an arylsulfatase A (ARSA) enzyme deficiency leading to sulfatide build-up, resulting in severe motor and cognitive dysfunction.
- Sulfatides are essential components of the myelin membrane. Sulfatide accumulation triggers inflammation and the destruction of myelin in the central and peripheral nervous systems.¹
- Sulfatides contain a sugar with a sulfate, sphingosine base, and fatty acid (Figure 1).
 - When adding an additional carbon group to sulfatide C16, it becomes C18.
 - When adding a hydroxy group to sulfatide C16, it becomes C16:0-OH.
- Disease progression of MLD is usually rapid and severe with a significant impact on quality of life, underscoring the need for early diagnosis and treatment.

Figure 1. Sulfatide structure

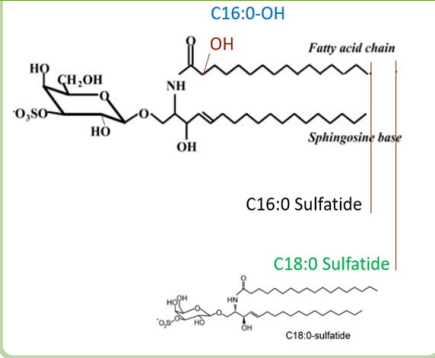


Figure adapted from Oliva P. et al. Newborn Screening for Metachromatic Leukodystrophy in Northern Germany. Presented at ICNC Annual Meeting, October 3 – 7, 2022.

- In December 2020, the European Commission approved atidarsagene autotemcel, an *ex vivo* gene therapy, for the treatment of the pre-symptomatic late infantile and early juvenile forms and the early symptomatic early juvenile form of MLD.
- Early treatment improves outcomes. However, diagnosis is often challenging.
- A newborn screening (NBS) strategy involving the measurement of sulfatides would ensure pre-symptomatic diagnosis.

METHODS

- Using a dried blood spot (DBS), a proposed NBS algorithm is being tested using a two-tier strategy:
 - (1) Sulfatides are quantified to detect elevated levels and screen out pseudodeficiencies
 - (2) ARSA enzyme activity could be employed as a second tier to eliminate any false-positives, and genetic testing will confirm positive screens

METHODS (CONTINUED)

- An internal standard C16:0 is utilized for quantification. The injection-to-injection time is 2 minutes which allows a high-throughput measurement of >400 samples in a 24-hour period (Figure 2).

Figure 2. Quantification of sulfatide species

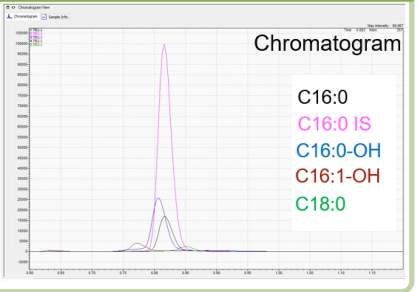


Figure adapted from Oliva P. et al. Newborn Screening for Metachromatic Leukodystrophy in Northern Germany. Presented at ICNC Annual Meeting, October 3 – 7, 2022.

- Currently, multiple active prospective screening pilots in Europe and a multiplex program including MLD in the US are ongoing as shown in Table 1. Additional studies are planned.
- All studies use the liquid chromatography tandem mass spectrometry (LC-MS/MS) method to detect elevated C16:0 and other sulfatide species.

Table 1. Ongoing newborn screening pilot programs

	Country	Design(s)
	Germany	Prospective
	United States (US)	Prospective Retrospective
	France	Prospective Retrospective
	United Kingdom (UK)	Retrospective

- The US prospective study (ScreenPlus) screens for 14 additional disorders not included on the routine screening panel (Table 2).

Table 2. Additional conditions included in ScreenPlus panel²

Acid Sphingomyelinase Deficiency	Metachromatic Leukodystrophy
Ceroid Lipofuscinosis Type 2	Mucopolysaccharidosis Type II
Cerebrotendinous Xanthomatosis	Mucopolysaccharidosis Type IIIB
Fabry Disease	Mucopolysaccharidosis Type IVA
Gaucher Disease	Mucopolysaccharidosis Type VI
GM1 Gangliosidosis	Mucopolysaccharidosis Type VII
Lysosomal Acid Lipase Deficiency	Niemann-Pick Disease Type C

RESULTS

- In Germany, a high-throughput method for sulfatide quantification from DBS was established using a LC-MS/MS method to quantify sulfatides C16:0, C16:0-OH and C16:1-OH from one 3.2 mm DBS. The method was modified and adapted.^{3,4,5}

RESULTS (CONTINUED)

- In the US, C16:0-sulfatide abundance (µM) in dried blood spots (DBS) from 15 MLD newborns (median: 0.32 µM, range: 0.18-0.47 µM) and 2,000 newborns chosen at random (median: 0.094 µM, range: 0.020-0.23 µM) were measured. In Figure 3, the solid lines represent the median of each group. The dashed line represents the screening cut-off at 0.17 µM that was established based on the results from the 15 MLD newborns.⁶

Figure 3. Sulfatide abundance in DBS

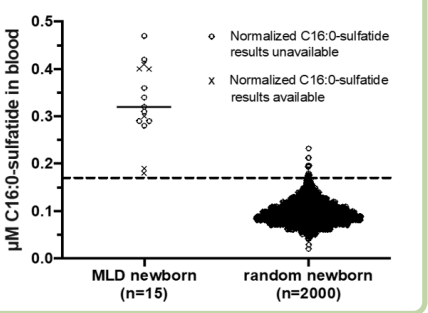


Figure adapted from Hong X. et al. Genet Med. 2021;23(3):555-561.

- In the US retrospective study, one screen positive case was confirmed as an MLD affected patient and 1 ARSA heterozygote by genetic testing.
- The prospective program in Germany has screened ~50,000 babies between October 2021 and August 2022.
 - Four ARSA heterozygote cases were identified.
 - One PSAP heterozygote and two SUMF1 heterozygotes were also identified (Table 3).

Table 3. Identified cases from Germany NBS pilot program

Mutation	Variant	Allele	Protein
ARSA	One variant	c.991G>A	p.(Glu331Lys)
ARSA	One variant	c.542T>G	p.(Ile181Ser)
ARSA	One variant	c.170G>T	p.(Gly57Val)
ARSA	One variant	c.931G>A	p.(Gly311Ser)
PSAP	One variant	c.1044G>T	p.(Met348Ile)
SUMF1	One variant	c.215G>T	p.(Arg72Leu)
SUMF1	One variant	c.215G>T	p.(Arg72Leu)

CONCLUSIONS

- The NBS algorithms in the Germany and US studies have shown to be sensitive and specific to detect MLD.
- Additionally, the growing evidence generation with these global studies shows this approach is suitable for high-throughput screening.
- MLD NBS allows for pre-symptomatic diagnosis and timely intervention that is crucial for optimal treatment outcomes.