Contact details
Regional Genetics Service
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Samples required
- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

Patient details
To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician.

Introduction
Metachromatic leukodystrophy (MLD) is an autosomal recessive lysosomal storage disorder caused by deficiency of the enzyme arylsulphatase A which catalyses the first step in the degradation of the sphingolipid 3-O-sulphagalactosyl ceramide (sulphatide). Accumulation of sulphatide in the brain leads to progressive demyelination of the central and peripheral nervous systems causing a variety of neurological symptoms including gait disturbances, ataxias, optical atrophy, dementia, seizures and spastic tetraparesis. Disease severity can range from mild to severe and can be broadly grouped into 3 subtypes (late-infantile, juvenile and adult). The majority of patients with arylsulphatase A deficiency and signs of MLD will have pathogenic variants in the ARSA gene; however, there is a much less common form of MLD caused by deficiency of saposin B, a non-enzymatic sphingolipid activator protein. Arylsulphatase A is also defective in multiple sulphatase deficiency due to pathogenic variants in SUMF1. The ARSA gene (22q13.31-qter) comprises 8 exons. Although many novel pathogenic variants are known, there are ‘common’ pathogenic variants within the gene, particularly the c.459+1G>A and c.1277C>T, p.(Pro426Leu) pathogenic variants, which account for around 50% of disease alleles in the Northern European population.

Pseudodeficiency of arylsulphatase A (PDASA)
Pseudodeficiency of arylsulphatase A is a condition of reduced arylsulphatase A activity (<15% normal) without clinical consequence, which can complicate the biochemical diagnosis of MLD. PDASA is caused by two sequence variants in the ARSA gene, namely PD2 (Poly A / c.*96A>G) and PD1 (NGly / p.(Asn350Ser)). PD2 is almost invariably seen on a background with PD1 but PD1 can occur independently of PD2 and its effect on causing PDASA is controversial.

Referrals
PDASA testing is used to assist the interpretation of arylsulphatase A activity results. Referrals are generally via the Enzyme Unit, Great Ormond Street Hospital however referrals may be accepted from other centers who carry out biochemical testing for arylsulphatase A. Biochemical confirmation of arylsulphatase A deficiency can only be confirmed after PDASA testing. In families with PDASA, prenatal testing by enzyme analysis can be complicated and in many cases impossible. For these families genetic testing is particularly useful but this can also mean that in some cases testing for MLD may have to be performed without biochemical confirmation. In these cases a very strong clinical picture of MLD must be present.

Prenatal testing
Prenatal testing is available for families in whom pathogenic variants have been identified or in whom appropriate family studies have been undertaken. Prenatal testing for PDASA may also be requested by the Enzyme Unit, Great Ormond Street Hospital.

Service offered
- PDASA: Testing for the presence of PD1 and PD2 by Sanger sequencing.
- MLD Level 1 analysis: testing for the common pathogenic variants c.459+1G>A and c.1277C>T, p.(Pro426Leu) by Sanger sequencing.
- MLD Level 2 analysis: Analysis of the ARSA gene by next generation sequencing (Agilent SureSelect and Illumina NextSeq). A minimum coverage of 30 reads is required to call a variant. In-house validation attributes a minimum sensitivity of 97.5% (with 95% confidence) for regions covered >30x. This assay is not currently validated to detect large deletions / duplications. All clinically relevant variants are confirmed by Sanger sequence analysis. Known benign polymorphisms and sequence variants which are unlikely to be pathogenic are not reported.

Target reporting time
4 weeks for PDASA testing and MLD routine level 1 screen in index case, 8 weeks for level 2 screen. 4 weeks for routine testing of specific pathogenic variants. Please contact the laboratory for urgent cases.

Version 10