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# Biochemical profiling to predict disease severity in metachromatic leukodystrophy

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ABSTRACT

Metachromatic leukodystrophy is a neurodegenerative disease that is characterized by a deficiency of arylsulfatase A, resulting in the accumulation of sulfatide and other lipids in the lysosomal network of affected cells. Accumulation of sulfatide in the nervous system leads to severe impairment of neurological function with a fatal outcome. Prognosis is often poor unless treatment is carried out before the onset of clinical symptoms. Pre-symptomatic detection of affected individuals may be possible with the introduction of newborn screening programs. The ability to accurately predict clinical phenotype and rate of disease progression in asymptomatic individuals will be essential to assist selection of the most appropriate treatment strategy. Biochemical profiling, incorporating the determination of residual enzyme protein/activity using immune-based assays, and metabolite profiling using electrospray ionization-tandem mass spectrometry, was performed on urine and cultured skin fibroblasts from a cohort of patients representing the clinical spectrum of metachromatic leukodystrophy and on unaffected controls. Residual enzyme protein/activity in fibroblasts was able to differentiate unaffected controls, arylsulfatase A pseudo-deficient individuals, pseudo-deficient compound heterozygotes and affected patients. Metachromatic leukodystrophy phenotypes were distinguished by quantification of sulfatide and other secondarily altered lipids in urine and skin fibroblasts; this enabled further differentiation of the late-infantile form of the disorder from the juvenile and adult forms. Prediction of the rate of disease progression for metachromatic leukodystrophy requires a combination of information on genotype, residual arylsulfatase A protein and activity and the measurement of sulfatide and other lipids in urine and cultured skin fibroblasts.


## Introduction

Metachromatic leukodystrophy (MLD[3]) is a neurodegenerative disease that is inherited in an autosomal recessive manner. The primary defect results from the decreased catalytic action of arylsulfatase A (ASA) on 3-*O*-sulfogalactosylceramide (sulfatide), resulting in its accumulation in the lysosomal network of cells in several peripheral organs, notably the nervous system. Whilst there is no pathophysiological effect from this accumulation in most peripheral tissues, the nervous system exhibits progressive demyelination that leads to severe impairment of neurological function with a fatal outcome [1]. MLD is classified into four clinical sub-types based on the age of onset. Clinically, the most severe sub-type or rapidly progressing type is the late-infantile form, in which death ensues 1- to 7-years after diagnosis. The early- and late-juvenile types have a more protracted clinical course while the adult form is the slowest to develop neurological symptoms. The presenting clinical features differ in each of the four types of MLD even though they share the same pathophysiological defect in the nervous system [2].

Biochemical diagnosis of MLD is usually achieved through the measurement of ASA activity in peripheral blood leukocytes and/or cultured skin fibroblasts [2]. However, diagnosis based on enzyme activity alone is complicated by the high frequency of ASA pseudo-deficiency (ASA-PD). The difficulty in diagnosis is further compounded by the low sensitivity and specificity of conventional enzymatic assays. Due to the complexity in interpreting results, a definitive diagnosis is usually only obtained after extensive testing with an array of supplementary laboratory assays [2].

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[3] *Abbreviations used:* MLD, metachromatic leukodystrophy; ASA, arylsulfatase A; ASA-PD, ASA pseudo-deficiency; ASA-PD/MLD, ASA-PD compound heterozygote; PC, phosphatidylcholine; Cer, ceramide; GC, glucosylceramide; $G_{M2}$, monosialoganglioside; LC, lactosylceramide, PG, phosphatidylglycerol; PI, phosphatidylinositol; rhASA, recombinant human ASA; CTH, ceramide trihexoside; SM, sphingomyelin; BMP bis(monoacylglycero)phosphate.

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In common with most lysosomal storage disorders, the prognosis for MLD patients is poor because diagnosis is usually made after the onset of substantial clinical symptoms. Currently, the only effective treatment available for MLD is hematopoietic stem cell transplant [3] which has an associated high mortality rate. Positive outcomes have been reported in the adult and juvenile forms of MLD following successful engraftment [4,5]. Results from hematopoietic stem cell transplants also showed that the highest efficacy was obtained when the transplant was carried out before the onset of clinical symptoms; this may be possible with a newborn screening program, as suggested previously [6,7]. However, early and accurate diagnosis for MLD, as well as the prediction of disease severity, will be important in the selection and timing of treatment strategies. To address this need we evaluated measurements of ASA activity and protein as biochemical markers using immune-based assays. We also established a profile of sulfatide and other lipid species in the urine and skin fibroblasts from the major forms of MLD, ASA-PD, ASA-PD compound heterozygotes (ASA-PD/MLD), and unaffected controls that have been subjected to sulfatide loading. This approach has enabled discrimination between the late-infantile MLD (severe form), adult-onset and ASA-PD and shows promise for improved clinical assessment of MLD.

## Materials and methods

### *Patient samples*

Urine and skin fibroblasts from MLD patients, ASA-PD and ASA-PD/MLD individuals were provided by the National Referral Laboratory for the Diagnosis of Lysosomal, Peroxisomal and Related Genetic Disorders, SA Pathology [Women's and Children's Hospital campus], Adelaide, SA, Australia. Urine samples from 11 MLD patients, six ASA-PD individuals and 18 unaffected controls (six samples each from adults, juveniles and infants) were analyzed. Samples from healthy controls were obtained with informed consent. The use of all samples was approved by the Research Ethics Committee of the Women's and Children's Hospital. Details and biochemical data of patients are shown in Table 1. Skin fibroblasts from 18 unaffected controls, two ASA-PD, two ASA-PD/MLD, three adult MLD, five juvenile MLD and five late-infantile MLD patients were used in this study. Details and biochemical data of these patients are shown in Table 2. Genotyping was carried out in the National Referral Laboratory for the Diagnosis of Lysosomal, Peroxisomal and Related Genetic Disorders.

**Table 2**
Skin fibroblast cell lines used to evaluate multiple parameters for the prediction of clinical severity in MLD.

| Cell line | Age (years) | Type | Genotype[b] |
|---|---|---|---|
| S1 | NA[a] | Control | |
| S2 | NA | Control | |
| S3 | NA | Control | |
| S4 | NA | Control | |
| S5 | NA | Control | |
| S6 | NA | ASA-PD | ASA-PD/ASA-PD |
| S7 | NA | ASA-PD | ASA-PD/ASA-PD |
| S8 | NA | ASA-PD/MLD | ASA-PD/T274M |
| S9 | NA | ASA-PD/MLD | ASA-PD/D169N |
| S10 | 31 | Adult | SDEX2/I179S |
| S11 | 19 | Adult | P426L/unknown |
| S12 | 17 | Adult | SDEX2/unknown |
| S13 | 8 | Juvenile | SDEX2/P426L |
| S14 | 7 | Juvenile | G345C/T274M |
| S15 | 11 | Juvenile | R244C/R288C |
| S16 | 8 | Juvenile | SDEX2/unknown |
| S17 | 6 | Juvenile | SDEX2/P426L, Y429S |
| S18 | 1 | Late-infantile | T274M/T274M |
| S19 | 3 | Late-infantile | DelCCT(EX7)/DelCCT(EX7) |
| S20 | 2 | Late-infantile | SDEX2/SDEX2 |
| S21 | 3 | Late-infantile | SDEX2/SDEX2 |
| S22 | 4 | Late-infantile | D335V/P377L |

[a] NA, age not available.
[b] SDEX2, single deletion at exon 2; delCCT(EX7), deletion of CCT at exon 7.

### *Reagents*

All solvents were of HPLC grade. Sulfatide (bovine brain) and phosphatidylcholine (PC 28:0) were purchased from Sigma–Aldrich, St. Louis, MO, USA. Ceramide (Cer 17:0), deuterated gluco-

**Table 1**
Biochemical data of individuals providing urine samples in this study.

| Sample[a] | Sex | Age (years) | Type | ASA activity[b] | Creatinine (mM) | Genotype |
|---|---|---|---|---|---|---|
| UA1–6 ($n$ = 6) | | 1–4 | Control adult | | | |
| UJ1–6 ($n$ = 6) | | 10–16 | Control juvenile | | | |
| UI1–6 ($n$ = 6) | | 27–51 | Control infant | | | |
| U1 | M | 23 | ASA-PD | 0.34 | 5.9 | ASA-PD/? |
| U2 | F | 27 | ASA-PD | 0.66 | 8.8 | ASA-PD/ASA-PD |
| U3 | M | 31 | ASA-PD | 0.66 | 3.0 | ASA-PD/ASA-PD |
| U4 | M | 9 | ASA-PD | 0.45 | 1.2 | ASA-PD/T391S |
| U5 | M | 29 | ASA-PD | 0.63 | 12.0 | ASA-PD/ASA-PD |
| U6 | M | 26 | ASA-PD | 0.60[c] | 8.1 | ASA-PD/ASA-PD + Y39C |
| U7 | F | 5 | Late-infantile | 0.30 | 1.6 | T274M/T274M |
| U8 | M | 4 | Late-infantile | 0.04 | 2.4 | A212 V/Y39C |
| U9 | F | 9 | Late-infantile | 0.30 | 6.6 | Not done |
| U10 | F | 2 | Late-infantile | 0.15 | 4.7 | Not done |
| U11 | M | 2 | Late-infantile | 0.11 | 1.4 | R84Q/R114X |
| U12 | M | 2 | Late-infantile | 0.14 | 3.7 | SDEX2/1027delC[d] |
| U13 | F | 12 | Juvenile | 0.12 | 4.7 | SDEX2/P426L |
| U14 | F | 10 | Juvenile | 0.38 | 9.4 | Not done |
| U15 | M | 11 | Juvenile | NA[e] | 10.7 | Y306H/1622G > A(IVS5) |
| U16 | F | 4 | Juvenile | 0.20 | 0.9 | InsG97–99/P426L |
| U17 | M | 13 | Juvenile | NA[e] | 11.3 | Y306H/1622G > A(IVS5) |

[a] Urine samples from: UA1–6, unaffected adults; UJ1–6, unaffected juveniles; UI1–6, unaffected infants; U1–U6, ASA-PD individuals; U7–U12, late-infantile MLD; and U13–U17, juvenile MLD.
[b] Blood leukocyte ASA activity measured using artificial substrate (control range 1–5 nmol/min/mg protein).
[c] ASA activity (nmol/min/mg protein) measured in skin fibroblasts using artificial substrate (control range 6–50 nmol/min/mg protein).
[d] SDEX2, single deletion at exon 2.
[e] NA, not available.