Saposins A, B, C, and D in Plasma of Patients with Lysosomal Storage Disorders

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Background: Early diagnosis of lysosomal storage disorders (LSDs), before the onset of irreversible pathology, will be critical for maximum efficacy of many current and proposed therapies. To search for potential markers of LSDs, we measured saposins A, B, C, and D in patients with these disorders.

Methods: Four time-delayed fluorescence immunquantification assays were used to measure each of the saposins in plasma from 111 unaffected individuals and 334 LSD-affected individuals, representing 28 different disorders.

Results: Saposin A was increased above the 95th centile of the control population in 59% of LSD patients; saposins B, C, and D were increased in 25%, 61%, and 57%, respectively. Saposins were increased in patients from several LSD groups that in previous studies did not show an increase of lysosome-associated membrane protein-1 (LAMP-1).

Conclusion: Saposins may be useful markers for LSDs when used in conjunction with LAMP-1.

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Lysosomal storage disorders (LSDs)5 are a group of >40 genetic diseases, with a combined incidence of ~1:5000 births. Each disorder results from a deficiency in a lysosomal enzyme, cofactor, transporter, or protein involved in lysosomal biogenesis or function (1). The deficiency leads to the accumulation of substrates usually degraded within the lysosome, and can be seen as an increase in the size and number of lysosomes within the cell.

Predominantly affecting young children, LSDs can present with a wide range of clinical symptoms that depend on the genotype involved, including mental retardation, skeletal abnormalities, organomegaly, corneal clouding, and coarse facial features (1, 2). In recent years, treatments for several LSDs have become possible, e.g., cystinosis is treated with cysteamine (3, 4), several LSDs have been responsive to bone marrow transplants (5–7), and Gaucher disease currently is being treated by enzyme replacement therapy (8), which like bone marrow transplantation, is theoretically applicable to a wide range of LSDs (9). Studies in animal models have shown that maximum efficacy, in most cases, is achieved when treatment is given at an early stage of pathology (10–12).

Most individuals are brought to attention only after the presentation of clinical symptoms. The diagnosis of most LSD-affected individuals is carried out using a range of enzymatic assays, performed on urine, blood, and skin fibroblasts. These assays are time-consuming, costly, and invasive. The possibility of newborn screening for LSDs has been discussed (13), and a strategy with a two-tier system has been proposed (14).

For newborn screening for LSDs to be economically viable, screening markers are required that will enable the detection of most LSDs in a single procedure. In a previous study, the plasma concentrations of lysosome-associated membrane protein-1 (LAMP-1) were above the 95th centile of the control population in 72% of LSD-affected individuals (13). Because the remaining LSDs not identified by LAMP-1 were predominantly those that stored sphingolipids, we have studied the sphingolipid activator proteins, or saposins, as potential complementary markers.

The saposins are four small, heat-stable glycoproteins,
tored saposins A, B, C, and D; all are derived from a single 73-kDa precursor protein, called prosaposin. The mature saposins have specific roles in activating and enhancing the activities of their respective lysosomal hydrolases (15, 16). Saposins are critical in the control of the glycosphingolipid flux through the lysosomal hydrolytic pathway (16), and genetic defects in sphingolipid hydrolases and/or saposins have been associated with the storage of sphingolipids (17). Conversely, saposin accumulations have been reported in the tissues of several LSD-affected individuals. Increases as much as 80-fold have been observed in the spleens, livers, and brains of patients affected with Gaucher disease, Niemann-Pick disease (type 1), fucosidosis, Tay-Sachs disease, and Sandhoff disease (18, 19).

In this study, we evaluated the frequency of increases in saposins A, B, C, and D in LSDs by measuring the concentrations of these proteins in plasma samples taken from unaffected and LSD-affected individuals.

Materials and Methods

Patient samples

Plasma samples were produced from whole blood (EDTA or lithium heparin) samples submitted to the National Referral Laboratory (Women's and Children's Hospital, Adelaide, Australia) for LSD screening and processing for routine biochemistry. Plasma was prepared as described previously (20). Whole-blood samples were obtained from healthy volunteers within the laboratory and fractionated into plasma, white cells, and red cells by the same method.

Polyclonal antibodies

Polyclonal antibodies against saposins A, B, C, and D were produced and characterized as described previously (16). Each antibody was purified on a 2-mL Hitrap™ Protein G column (Pharmacia Biotech) and quantified by absorbance at 280 nm (absorbance = 1.4 for 1.0 g/L).

Europium labeling of polyclonal antibodies

Purified anti-saposin polyclonal antibodies were labeled with Eu³⁺, using the DELFIA® labeling kit (Wallac). Labeled antibodies were purified on a 1.5 × 30 cm Superose 12 fast-phase liquid chromatography column (Pharmacia Biotech) as described previously (13). The amount of Eu³⁺ conjugated to each antibody molecule was determined by the fluorescence of a known antibody concentration compared with a 1 nmol/L Eu³⁺ solution.

Preparation of calibrators and quality-control materials

Liquid calibrators for the immunoquantification of saposins were prepared using purified saposin A, B, C, and D proteins (21). Purified saposin A protein was diluted in DELFIA assay buffer to obtain final concentrations of 2.43, 1.21, 0.61, 0.3, and 0.15 μg/L. For the saposin A immunoquantification assays, low and high quality-control samples were prepared by diluting saposin A protein to 0.24 and 2.43 μg/L, respectively. Purified saposin B protein was diluted in the same buffer to obtain final concentrations of 6.68, 3.34, 1.67, 0.83, and 0.42 μg/L. The saposin B low and high quality-control samples were prepared by diluting saposin B protein to 0.67 and 6.68 μg/L, respectively. Purified saposin C protein was diluted to obtain final concentrations of 1.88, 0.94, 0.47, and 0.24 μg/L. The saposin C low and high quality-control samples were prepared by diluting saposin C protein to 0.47 and 1.88 μg/L, respectively. Purified saposin D protein was diluted to obtain final concentrations of 0.92, 0.46, 0.23, and 0.11 μg/L. The saposin D low and high quality-control samples were prepared by diluting saposin D protein to 0.23 and 0.92 μg/L, respectively.

Calibrators and controls for saposins C and D were supplemented with a volume of control plasma equal to that being assayed to compensate for the inhibitory effects of plasma on the saposin C and D assays. All liquid saposin calibrators and controls were stored at 4 °C.

Immunoquantification of saposins

Saposin concentrations were determined by time-delayed fluorescence immunoassays as described previously (13). Briefly, microtiter plates (ImmunoMouse 4; Dynatech Laboratories) were coated overnight at 4 °C with anti-saposin polyclonal antibody (2.5 mg/L diluted in 0.1 mol/L NaHCO₃, pH 8.8; 100 μL/well), and then prewashed once with DELFIA wash buffer. Samples were diluted in DELFIA assay buffer (100 μL/well), shaken for 10 min at 20 °C, and incubated (4 h at 4 °C). Plates were then washed six times, and Eu³⁺-labeled anti-saposin polyclonal antibody added (0.25 mg/L diluted in DELFIA assay buffer; 100 μL/well) and incubated overnight at 4 °C. The plates were washed six times; DELFIA enhancement solution (200 μL) was then added to each well, and the plates were shaken (10 min at 20 °C). The fluorescence was read on a Wallac 1234 DELFIA Research Fluorometer. For each saposin assay, the corresponding saposin calibrators were located across the first row of the microtiter plate, with the quality-control samples dispersed randomly. All saposin calibrators, quality controls, and samples were assayed in duplicates. Saposin concentrations in plasma samples were calculated using linear regression.

Results

Optimization of saposin immunoquantification assays

Europium labeling of anti-saposin polyclonal antibodies gave recoveries of 75–100% with four to seven Eu³⁺ atoms bound to each antibody molecule. The final conditions of the immunoquantification assays were optimized by generating a series of calibration curves under different test conditions. The conditions that were optimized included the concentrations of the coating and Eu³⁺-labeled antibodies, and the incubation times of the antibodies and samples. Minimization of cost and time required, as well
as the reproducibility of each assay, were considered when selecting the final assay conditions.

The optimized saposin A, B, and C calibration curves gave linear responses over the range of the calibrators ($R^2$ values $>0.99$), with the exception of the saposin D calibration curve, which had a reduced linear range ($R^2 = 0.9575$; Fig. 1). When control plasma was added to the saposin C and D calibration curves, a reduction in signal intensity was observed, 2 μL of plasma gave a 20% reduction (data not shown). To compensate for the inhibitory effect of plasma on the saposin C and D immunoquantification assays, control plasma at an equal volume to that being assayed was added to the calibrators. The assumption was made that LSD-affected plasma would inhibit the assays in the same manner as control plasma.

We evaluated the cross-reactivity between the saposin assays and found that it was $<3\%$ for the saposin A, B, and C assays, whereas the saposin D assay showed between 6% and 13% cross-reactivity with the other saposins (Table 1).

Fig. 1. Calibration curves for the saposin A, B, C, and D immunoquantification assays. Optimum conditions were used to generate calibration curves for the use of saposin A (A), saposin B (B), saposin C (C), and saposin D (D) in the immunoquantification of the respective proteins. Microtiter plates were coated with primary anti-saposin polyclonal antibody (2.5 mg/L, 4 °C, overnight), after which calibrators were incubated (4 °C, 4 h) and detected with Eu$^{3+}$-labeled anti-saposin polyclonal antibody (0.25 mg/L, 4 °C, overnight).
SAPOSIN CONCENTRATIONS IN THE PLASMA OF CONTROL AND LSD-AFFECTED INDIVIDUALS

To evaluate the suitability of each of the saposins as screening markers for LSD, the concentrations of saposins A, B, C, and D were measured in the plasma samples from 111 control individuals (median age, 7 years; range, 0–66 years) and 334 LSD-affected individuals, representing 28 different disorders (Table 2). The 95th centiles of saposin concentrations in the control population are listed in Table 2, together with the total proportion of LSD-affected individuals having plasma saposin concentrations above this value. Saposins A, C, and D showed a tight distribution in the plasma samples of the control population, whereas a wider range of saposin B was observed (Fig. 2). A significant proportion of LSD-affected individuals were found to have saposin concentrations above the 95th centile of the control population, with some individuals having increases of up to 10-fold above the median concentration of the control population. When the disorders were taken separately, it was noted that in 15 of the 28 disorders, >80% of individuals had one or more saposins above the 95th centile of the control population.

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**Table 1. Cross-reactivity of the saposin immunoquantification assays.**

<table>
<thead>
<tr>
<th>Saposin Immunoquantification Assay</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.00</td>
<td>0.03</td>
<td>0.01</td>
<td>0.13</td>
</tr>
<tr>
<td>B</td>
<td>0.03</td>
<td>1.00</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>C</td>
<td>0.01</td>
<td>0.00</td>
<td>1.00</td>
<td>0.06</td>
</tr>
<tr>
<td>D</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>1.00</td>
</tr>
</tbody>
</table>

*a* Each saposin calibrator (1.0 μg/L) was quantified using each of the four saposin immunoquantification assays. Results are expressed as μg/L.

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**Table 2. Saposin concentrations in plasma from control and LSD-affected individuals.**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>n</th>
<th>Age,* years</th>
<th>Median saposin concentration,b μg/L (% with increased concentrations)c</th>
<th>Median saposin concentration,b μg/L (% with increased concentrations)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>111</td>
<td>7 (0–66)</td>
<td>12.2 (54.8)</td>
<td>14.6 (23.8)</td>
</tr>
<tr>
<td>Cystinosis*</td>
<td>9</td>
<td>6 (1–24)</td>
<td>26.7 (56)</td>
<td>57.7 (22)</td>
</tr>
<tr>
<td>Fabry disease*</td>
<td>27</td>
<td>27 (4–47)</td>
<td>33.9 (93)</td>
<td>82.5 (48)</td>
</tr>
<tr>
<td>Galactosialidosis</td>
<td>1</td>
<td>16</td>
<td>15.1 (0)</td>
<td>48 (0)</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>52</td>
<td>52 (0–73)</td>
<td>60.3 (96)</td>
<td>124.2 (81)</td>
</tr>
<tr>
<td>GM I-gangliosidosis</td>
<td>12</td>
<td>4 (0–15)</td>
<td>32.5 (83)</td>
<td>72.3 (33)</td>
</tr>
<tr>
<td>Mucolipidosis II/III</td>
<td>16</td>
<td>16</td>
<td>27.3 (69)</td>
<td>47.5 (6.3)</td>
</tr>
<tr>
<td>Krabbe disease*</td>
<td>11</td>
<td>11</td>
<td>20.6 (63)</td>
<td>54.1 (0)</td>
</tr>
<tr>
<td>α-Mannosidosis</td>
<td>5</td>
<td>5</td>
<td>15.9 (0)</td>
<td>51 (0)</td>
</tr>
<tr>
<td>MLD*</td>
<td>32</td>
<td>32</td>
<td>14.1 (16)</td>
<td>49.5 (0)</td>
</tr>
<tr>
<td>MPS I</td>
<td>18</td>
<td>18</td>
<td>23 (67)</td>
<td>56.2 (17)</td>
</tr>
<tr>
<td>MPS II</td>
<td>24</td>
<td>24</td>
<td>20.6 (54)</td>
<td>42.7 (4.2)</td>
</tr>
<tr>
<td>MPS IIIA</td>
<td>19</td>
<td>19</td>
<td>14.4 (0)</td>
<td>29 (0)</td>
</tr>
<tr>
<td>MPS IIIB</td>
<td>16</td>
<td>16</td>
<td>14.3 (0)</td>
<td>35 (0)</td>
</tr>
<tr>
<td>MPS IIIC</td>
<td>3</td>
<td>3</td>
<td>16.1 (0)</td>
<td>24.5 (0)</td>
</tr>
<tr>
<td>MPS IIID</td>
<td>3</td>
<td>3</td>
<td>17.2 (33)</td>
<td>40.7 (0)</td>
</tr>
<tr>
<td>MPS IVA</td>
<td>16</td>
<td>16</td>
<td>21.2 (63)</td>
<td>50.1 (0)</td>
</tr>
<tr>
<td>MPS VI</td>
<td>10</td>
<td>10</td>
<td>26.6 (70)</td>
<td>55.3 (0)</td>
</tr>
<tr>
<td>Multiple sulphatase deficiency</td>
<td>2</td>
<td>2</td>
<td>16.1 (0)</td>
<td>43.8 (0)</td>
</tr>
<tr>
<td>Neuronal ceroid lipofuscinosis (infantile)*</td>
<td>1</td>
<td>1</td>
<td>8.6 (0)</td>
<td>4.7 (0)</td>
</tr>
<tr>
<td>Niemann-Pick disease (A/B)*</td>
<td>9</td>
<td>26 (2–44)</td>
<td>51.5 (89)</td>
<td>121.2 (89)</td>
</tr>
<tr>
<td>Niemann-Pick disease (C)*</td>
<td>10</td>
<td>13 (0–40)</td>
<td>24.2 (80)</td>
<td>49.3 (30)</td>
</tr>
<tr>
<td>Pompe disease*</td>
<td>5</td>
<td>0.3 (0.1–0.5)</td>
<td>19.5 (40)</td>
<td>44.7 (20)</td>
</tr>
<tr>
<td>Sandhoff disease</td>
<td>6</td>
<td>1 (0.9–1.3)</td>
<td>33.5 (100)</td>
<td>71.4 (50)</td>
</tr>
<tr>
<td>Sialic acid storage disease</td>
<td>2</td>
<td>2 (0–3)</td>
<td>20.9 (50)</td>
<td>59.6 (0)</td>
</tr>
<tr>
<td>Tay-Sachs disease*</td>
<td>21</td>
<td>4 (0–27)</td>
<td>24.1 (71)</td>
<td>59.6 (9.5)</td>
</tr>
<tr>
<td>Tay-Sachs disease (A/B variant)*</td>
<td>2</td>
<td>2</td>
<td>18.8 (0)</td>
<td>50.8 (0)</td>
</tr>
<tr>
<td>Wolman disease*</td>
<td>2</td>
<td>2</td>
<td>35 (50)</td>
<td>52.5 (0)</td>
</tr>
<tr>
<td>Total LSD patients</td>
<td>334</td>
<td></td>
<td>114.8 (100)</td>
<td>110.5 (100)</td>
</tr>
</tbody>
</table>

*a* Median age (range) of individuals within the group.

*b* Median saposin concentrations of individuals within the group.

*c* Percentage of individuals within the group with saposin concentrations above the 95th centile of the control population.

*d* Saposin concentration at the 95th centile of the control population (μg/L).

*e* Disorders in which LAMP-1 and LAMP-2 were not increased.

*f* Hexosaminidase activator deficiency.
All saposin assays included low and high quality-control samples to determine the interassay coefficient of variation (CV) for each assay performed (Table 3), with the number of replicates ranging from 17 to 24. The interassay CVs of the saposin A assays for both the low and high quality-control samples were ≤11%. The saposin B, C, and D assays had CVs ≤10% for the high quality-control samples and CVs of 11–20% for the low quality-control samples. A separate experiment was carried out in which 24 replicate samples of the high and low quality-control samples were assayed on a single plate to calculate the intraassay CV (Table 3). The intraassay CVs were ≤16% for the low quality-control samples and ≤10% for the high quality-control samples.

Because the LAMP-1 concentrations in the same plasma samples had been assayed previously (13), direct comparisons between the saposin and LAMP-1 concentrations could be carried out. Strong Pearson correlations were observed between all saposins ($P < 0.01$), with the exception of saposin B, which showed low correlation with both saposin C and D (Table 4). Weak correlations were observed between the saposins and LAMP-1 ($P < 0.05$), whereas no correlations were seen between saposin concentrations and age, in the control population. The correlation between age and the saposin concentrations was also examined in Fabry disease, Gaucher disease, metachromatic leukodystrophy (MLD), mucopolysaccharidosis (MPS) II, and Tay-Sachs disease, where there were ≥20 patients in each group. Of these groups, only the Gaucher disease group showed a slight but significant ($P$...
<0.05) negative correlation between age and saposin C and D concentrations (Pearson correlation of −0.39 and −0.35, respectively).

**Saposin Concentrations in Whole Blood**

Whole-blood samples from six unaffected individuals were fractionated, and the distribution of saposins was determined in plasma, white cells, and red cells (Table 5). It was found that the dominant saposin in whole blood was saposin D, at a concentration of 75 µg/L, whereas a greater proportion of saposin B (77%) was found in the plasma fraction compared with the other saposins. The distributions of saposins A, C, and D in plasma, white cells, and red cells were approximately equal.

**Discussion**

Saposins were evaluated in this study for their potential use as screening markers, following reports on LAMP-1 and a related protein, LAMP-2. LAMP-2 increased in the majority of LSDs (13), and although LAMP-2 concentrations were also increased in many LSDs (14), it did not detect additional disorders and therefore was of limited value as an additional marker. The LSDs that did not show an increase in LAMP-1 were predominantly of the sphingolipidosis subgroup. Because saposins are sphingolipid activator proteins, involved in the degradation of sphingolipids, they are potential candidates for additional LSD markers. Moreover, saposins have been reported to be increased in various tissue samples from LSD patients (18, 19).

The saposin D calibration curve (Fig. 1D) had a reduced linear range and low signal compared with the other saposin calibration curves (Fig. 1, A–C), indicating that the anti-saposin D polyclonal antibody had a relatively low affinity. The higher affinity of the anti-saposin A antibody was reflected in the lower inter- and intraassay CVs obtained for the saposin A assay (Table 3), where the CV values were ≤11% for both the high and low quality-control samples. The intra- and inter-assay CVs of saposin B for the low quality-control samples were 11% and 20%, respectively, reflecting the lower affinities of these antibodies. Upon investigation of the cross-reactivity of the antibodies to the other saposin proteins, we found that the polyclonal antibodies were of high specificity for their respective saposins with the exception of saposin D, which had a slight cross-reactivity to the other saposins (Table 1).

In a previous study, plasma caused a reduction in the fluorescent signal in the LAMP-2 immunoquantification assay (14). We therefore investigated this effect for saposins and observed a slight reduction in signal for the saposin C and D assays. Three possibilities involving plasma proteins may contribute to this inhibitory effect. First, proteins present in the plasma may bind to saposins C and D, altering their conformation and rendering them unrecognized to their antibodies. Second, plasma proteins may competitively occupy the saposin-binding sites of the antibodies, inhibiting them from binding the saposins. Third, plasma proteins may bind and aggregate the saposins or their antibodies, thus reducing the signals obtained.

The goal of this study was to identify additional protein markers for use in a newborn-screening program for LSDs. However, the lack of suitable diagnostic samples from newborns with LSDs necessitated the use of plasma samples from LSD-affected individuals taken at the time of diagnosis. The age of patients at diagnosis varied considerably with disorder (Table 2) and was generally <10 years of age. The control population was age-matched to the LSD-affected group. Comparison of fresh control samples with those stored frozen for many years showed no difference in the median values, indicating the stability of the saposins in these samples.

We measured the saposin concentrations in plasma from LSD-affected individuals and compared these to values in plasma from control individuals. In 59% of patients, saposin A was increased above the 95th centile of the control population, and saposins B, C, and D were increased in 25%, 61% and 57% of patients, respectively (Table 2). Of the 28 disorders represented in our study, the
saposin A concentrations were above the 95th centile of the control group in >80% of individuals in 6 disorders, saposin B concentrations were increased in 2 disorders, and saposins C and D were increased in 10 disorders (Table 2). Together the saposins identified >80% of individuals in 15 of the 28 LSD groups; increased LAMP-1 concentrations had not been observed previously in 6 of these LSDs (13), namely cystinosis, Fabry disease, Niemann-Pick disease (types A/B and C), Pompe disease, and Wolman disease. The remaining LSDs in which LAMP-1 was not increased (Krabbe disease, MLD, and Tay-Sachs disease) had significant numbers of patients (44–71%) with increases in saposin concentrations (Table 2). We calculate that up to 85% of LSD-affected individuals can be detected using a combination of LAMP-1 and saposins as screening markers.

It is worth noting that 13 of the LSD plasma samples were from newborns (<6 weeks of age), representing Gaucher (n = 3), Krabbe (n = 1), MLD (n = 2), MPS VI (n = 2), mucolipidosis II/III (n = 1), Niemann-Pick C (n = 2), Pompe (n = 1), and Tay-Sachs (n = 1) disorders. Of these, 11 (85%) showed an increase in one or more of the saposins above the 95th percentile of the control population, Tay-Sachs and Krabbe disorders being the exceptions.

The ratios of individual saposin concentrations in the plasma from the LSD group were also examined and compared with the control population to determine whether the ratio value was a better marker for LSD detection. However, because the correlations between the saposins were strong (Table 4), the ratios of any two saposins gave a lower predictive value than the individual saposins.

Theoretically, because saposins originate from the same precursor, their rate of synthesis should be equal. In this study, saposin B was by far the predominant saposin in the plasma of control individuals (Table 2). Analysis of the distribution of saposins in fractions of whole blood (Table 5) revealed that saposin D was the most abundant saposin in whole blood, at 75 μg/L. A greater proportion of saposin B (77%) was found in the plasma fraction compared with saposins A, C, and D (38%, 23%, and 30%, respectively), which may explain the high concentrations observed in the plasma samples in this study.

Studies on the distribution of saposins in tissue samples from LSD patients and control individuals also found that saposin D is more abundant in unaffected tissue than the other saposins (22). This observation together with our findings suggests that saposin D may have a longer half-life in cells. The higher concentrations of saposin B in plasma compared with whole blood may suggest a functional difference in extracellular and intracellular saposins and could result from either an increased half-life of this saposin in plasma or its preferential release from cells. However, the mechanism by which saposins are released into circulation is unclear.

Three hypotheses previously have been suggested for the accumulation of saposins in tissues of LSD patients, which may also apply to their increase in plasma: (a) The synthesis of saposins may be stimulated by the accumulation of either the defective enzyme or lipid substrates as a compensatory mechanism. (b) Saposins may be codelocalized with the accumulated substrates. (c) Saposins may not be able to interact with deficient enzymes and therefore cannot undergo normal metabolism (23). However, other studies have shown that saposin accumulation may be nonspecific in that it is unrelated to the catabolic defect (15). Because saposin B is known to stimulate the hydrolysis of cerebroside sulfate, the storage product of MLD, we expected that it would be increased in plasma from MLD-affected individuals; however, this was not the case. This observation would suggest that saposin B does not accumulate with its substrate in MLD, although this may be a tissue-specific phenomenon that is not reflected in plasma concentrations of saposin B.

We also observed that there was a general correlation between the LSD groups that store sphingolipids or sphingolipid derivatives and those that showed an increase in saposin concentrations. For example, Niemann-Pick disease (type A/B), in which sphingomyelin is stored, showed increases in all saposins, whereas of the MPS groups studied, only MPS II showed an increase in saposin C (Table 2). In addition, saposins were not increased to the same extent in demyelinating disorders such as MLD and Krabbe disease, compared with other lipidoses. This has also been noted in previous tissue studies (22). The observed increase of saposins in the various LSD groups may therefore relate to both the tissues involved in storage and the type of substrate stored. Moreover, the degree of saposin increase may also reflect the severity of the disorder.

The results presented in this study are based on the analysis of plasma samples from clinically affected LSD patients varying in age from newborns to adults. Although LSDs are progressive disorders that often take years to present clinically, there is considerable evidence from both human (24, 25) and animal models (11, 26) that biochemical storage commences early in gestation and is well advanced at birth. The presence of increased saposin concentrations in 11 of the 13 newborns in the LSD group would also suggest that, at least in these individuals, the biochemistry is well advanced at birth. Although these results support the usefulness of saposins as screening markers for LSD, further studies on the newborn population will be required to confirm the usefulness of saposins as markers for the early detection of LSD.

As with the LAMP-1 and LAMP-2 studies (13, 14), we have used the 95th percentile as a cutoff for the control population; however, a 5% recall would clearly be unacceptable in a newborn-screening program. We propose to develop a two-tier screening program in which the top 1–5% of the population (based on the primary screening markers) would then be examined further. Using the same Guthrie card, a panel of second-tier diagnostic
assays designed to detect the storage product for the particular disorder involved would be performed. This would effectively identify the affected individuals from the false positives identified in the first-tier screen. We currently are developing the second-tier assays for this procedure, utilizing tandem mass spectrometry.

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References


