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This Brief was prepared by Mr Tom Vreugdenburg from the Australian Safety and Efficacy Register of New Intervventional Procedures – Surgical (ASERNIP-S).
TECHNOLOGY BRIEF UPDATE 2014

Technology, Company and Licensing

Register ID WP115

Technology name Neonatal screening for lysosomal storage disorders (LSDs)

Patient indication Detection of LSDs in asymptomatic neonates

Reason for assessment

In 2012, a Technology Brief (see page 13) was completed to investigate the feasibility of lysosomal storage disorder (LSD) screening in newborn populations. In light of developing evidence on the subject, the Brief recommended that the relevant technologies be monitored for 24 months. In line with this recommendation, the purpose of the current Update is to consider the evidence that has emerged since 2012, and determine whether this new evidence may provide additional information to inform policy decisions.

Background

LSDs comprise more than 50 serious, progressive diseases that arise due to an inherited dysfunction in the lysosome. The lysosome is an intracellular organelle with the primary function of degrading substrates through a variety of pathways including endocytosis, phagocytosis and autophagy.1 Lysosomes contain a range of hydrolytic enzymes to undertake degradation. Loss-of-function mutations in one or more of the hydrolytic enzymes, or other integral lysosomal proteins, can lead to substrate accumulation and storage within the lysosome.2 The stored substrate is specific to each disorder, and traditionally the LSDs have been categorised according to the type of substrate stored (for example, mucopolysaccharidoses, oligosaccharidoses, sphingolipidoses, gangliosidoses).2, 3 They may also be classified based on the nature of the defective protein.3 The majority of LSDs are recessively inherited autosomal traits, with several that are recessively inherited X-linked traits. Examples of LSDs include Gaucher’s disease, Fabry’s disease, Tay-Sach’s disease, Pompe’s disease, and Niemann-Pick disease types A and B.4

The accumulation of substrates in LSDs can alter many cellular processes, including lysosomal pH regulation, synaptic release, endocytosis, vesicle maturation, autophagy and exocytosis.1, 3 Progressive lysosomal substrate deposition can occur in cells throughout the body.4 This accumulation can result in the deterioration of cellular and tissue function, and the dysfunction of vital organs, muscles and neurons.1-3 The clinical presentations of LSDs vary, and can be affected by the underlying genetic mutation. The onset of most LSDs occurs in childhood, after the achievement of early developmental milestones; however, some LSDs present in utero or during the newborn period, while others present in late adulthood. Early symptoms can include neurological disorders and slowing of developmental progress, dysmorphic facial appearance, cardiac disease or enlargement of the liver and/or spleen.3
Many disorders affect the central nervous system and most patients have a decreased lifespan and significant morbidity.2

Neonatal screening programs have been in use for decades and are generally intended to differentiate asymptomatic newborns from those without a disease for disorders in which early detection and pre-symptomatic treatment are required to avoid serious clinical harm. A disorder must also have a high enough prevalence to justify inclusion in such programs.4

Multiplex tandem mass spectrometry (MS/MS) is the primary technology used for newborn LSD screening. An older fluorescence assay (4-MU) is used as a comparator to the MS/MS technique in new studies. Details for each technique are outlined in greater detail in the 2012 Brief.

**Stage of development in Australia**

- Yet to emerge
- Experimental
-Investigational
- Nearly established
- Established
- Established but changed indication or modification of technique
- Should be taken out of use

**Australian Therapeutic Goods Administration approval**

- Yes
- No
- Not applicable

Medical device regulations require all in-vitro medical devices (IVDs) introduced onto the Australian market after 1 July 2010 to be included on the Australian Register of Therapeutic Goods (ARTG). Those supplied prior to this date were exempt from ARTG inclusion for a transitional period up to 30 June 2014, recently extended to 30 June 2015.1 As no ARTG entries have been identified for specific LSD IVDs, it is assumed that these assays were available before the 2010 regulations were introduced, and remain exempt from ARTG inclusion until the end of the transition period.

**2014 International utilisation**

<table>
<thead>
<tr>
<th>Country</th>
<th>Trials underway or completed</th>
<th>Limited use</th>
<th>Widely diffused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hungary</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2014 Evidence and Policy

2014 Safety and effectiveness

Since the 2012 Technology Brief was completed, four screening studies have been published that investigate the feasibility of LSD testing in newborn populations (Table 1).1,2-5 Indications for LSD screening in the literature included Pompe disease, Fabry disease, Gaucher disease, Niemann-Pick disease, and Hurler syndrome.

The comparative screening trial conducted by Liao et al (level III-3 screening intervention evidence) screened newborns for four separate indications using a single dried blood spot (DBS) sample. MS/MS was used to measure enzyme activity related to Fabry, Pompe, Gaucher and Hurler disorders simultaneously.2 The results of the MS/MS technique were compared with an historical control cohort of newborns screened with a 4-MU at the same institution. The case series studies by Scott et al,3 Wittmann et al4 and Yang et al5 (level IV screening intervention evidence) screened a single cohort of newborns for multiple LSDs using MS/MS techniques. Safety issues related to the collection of DBS samples were not reported in any of the included studies. The primary efficacy outcomes were the number of LSD cases correctly identified; recall rates for molecular follow-up testing following the initial screen; and positive predictive value (PPV) of the screening assay. A summary of the included studies is provided in Table 1.

Table 1  Newborn screening trials for LSDs published since 2012

<table>
<thead>
<tr>
<th>Study/Design</th>
<th>Study details</th>
<th>Number screened</th>
<th>Number detected</th>
<th>Other findings</th>
</tr>
</thead>
</table>
| Liao et al 2014 Taiwan Non-randomised comparative screening study | • Indications: Fabry, Pompe, Gaucher and Hurler.  
• GAA and GLA enzyme activity measured with the 4-MU assay from January 2008 to January 2010, and MS/MS from February 2010 to January 2013, with confirmatory DNA analysis.  
• GBA enzyme activity measured with MS/MS from September 2012 to January 2013. | 191,786 newborns (GAA)  
191,767 newborns (GLA)  
101,134 newborns (GBA)  
60,473 newborns (IDUA) | 1,394 newborns demonstrated abnormally low levels of GAA (n=874), GBA (N=141) and GLA (n=379) activity in initial DBS sample.  
Repeat DBS testing identified low activity of GAA (n=225), GBA (n=5), GLA (n=79) in 309 newborns.  
Follow-up DNA analysis identified newborns with Pompe (n=16; ~1 in 11,986), Gaucher (n=3; ~1 in 33,711) and Fabry (n=64; ~1 in 2,996) mutations. | The MS/MS method lowered the recall rate from 1.7% to 0.2% for Fabry, and from 1.4% to 0.5% for Pompe compared with the fluorescence method. |
<table>
<thead>
<tr>
<th>Study/Design</th>
<th>Study details</th>
<th>Number screened</th>
<th>Number detected</th>
<th>Other findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yang et al 2014 Taiwan Case series screening study</td>
<td>• Indication: Pompe disease &lt;br&gt;• Enrolment date: January 2008 to May 2012. &lt;br&gt;• GAA enzyme activity measured with 4-MU assay (up to October 2010) and MS/MS (after October 2010), with confirmatory DNA analysis.</td>
<td>402,281 newborns (GAA)</td>
<td>36 newborns demonstrated abnormally low levels of GAA activity, and 4148 had an inconclusive result from the initial DBS sample. &lt;br&gt;Repeat testing of the 4,148 inconclusive results recalled 222 for a third screen due to an inconclusive result, and 158 abnormal samples for molecular testing. &lt;br&gt;The third screening test recalled 127 abnormal results for molecular confirmation. &lt;br&gt;Follow-up DNA analysis of 321 newborns identified six newborns with Pompe disease.</td>
<td>The early identification and treatment of six confirmed cases of infant-onset Pompe disease led to improvement in clinical outcomes in all cases.</td>
</tr>
<tr>
<td>Scott et al 2013 USA Case series screening study</td>
<td>• Indications: Pompe, Fabry and Hurler. &lt;br&gt;• Enrolment date not reported. &lt;br&gt;• GAA, GLA and IDUA enzyme activity measured with MS/MS, with confirmatory DNA analysis.</td>
<td>111,544 newborns (GAA) 108,905 newborns (GLA) 106,526 newborns (IDUA)</td>
<td>39 DBS samples demonstrated abnormally low levels of GLA (n=16), GAA (n=17) and IDUA (n=9) activity. &lt;br&gt;Follow-up DNA analysis identified samples with confirmed Pompe (n=4; ~1 in 27,886), Fabry (n=7; ~1 in 15,558) and Hurler (n=3; ~1 in 35,509).</td>
<td>PPV (Fabry) = 0.43 False positive rate (Fabry): 1 in 12,100 PPV (Pompe) = 0.24 False positive rate (Pompe): 1 in 8600 PPV (Hurler) = 0.33 False positive rate (Hurler): 1 in 17,750</td>
</tr>
<tr>
<td>Wittmann et al 2012 Hungary Case series screening study</td>
<td>• Indications Pompe, Gaucher, Fabry and Niemann-Pick. &lt;br&gt;• Enrolment date not reported. &lt;br&gt;• GAA, GBA, GLA and ASM enzyme activity measured from DBS via MS/MS with confirmatory DNA analysis.</td>
<td>44,024 newborns (GAA, GBA, GLA and ASM)</td>
<td>663 newborns (1.7%) demonstrated abnormally low levels of GBA (n=141), ASM (n=114), GAA (n=163), and GLA (n=224) activity in initial DBS sample. &lt;br&gt;Repeat DBS testing identified low activity of GBA (n=17), ASM (n=5), GAA (n=64), GLA (n=34) in 120 newborns. &lt;br&gt;Follow-up DNA analysis identified newborns with confirmed Gaucher (n=3; ~1 in 14,675), Niemann-Pick (n=2; ~1 in 22,012), Pompe (n=9; ~1 in 4,892) and Fabry (n=3; ~1 in 14,675).</td>
<td>24 DBS samples with poor integrity were excluded from further analysis. DNA analysis was indeterminate for two newborns with suspected Fabry, and three with suspected Pompe.</td>
</tr>
</tbody>
</table>

Table notes: ASM = acid sphingomyelinase (deficient in Niemann-Pick A/B); DBS = dried blood spot; GAA = acid α-glucosidase (deficient in Pompe); GBA = acid β-glucosidase (deficient in Gaucher); GLA = acid α-galactosidase (deficient in Fabry); IDUA = α-L-iduronidase (deficient in Hurler); MS/MS = tandem mass spectrometry; 4-MU = fluorescence assay; PPV = positive predictive value.
The primary aim of this non-randomised historically controlled trial (level III-3 screening intervention evidence) was to determine the efficacy of the MS/MS technique for measuring enzyme activity related to four LSDs. A total of 191,786 newborns were enrolled in the study between February 2010 and January 2013. Low activity levels of acid α-glucosidase (GAA; deficient in Pompe), acid β-glucosidase (GBA; deficient in Gaucher) and acid α-galactosidase (GLA; deficient in Fabry) were determined via MS/MS using a DBS sample collected up to three days after birth. The high-throughput multiplex MS/MS technique allowed all indications to be tested simultaneously from a single DBS sample. Screening results from the MS/MS technique were compared with the 4-MU assay in the Pompe and Fabry populations. An additional 60,473 samples were screened for α-L-iduronidase deficiency (IDUA; deficient in Hurler) via MS/MS without informed consent, and were not subject to recall DBS screening or molecular confirmation. The screening results from this population are not reported in this Brief due to the lack of sample verification in positive test cases.

Healthy control samples from the Centre for Disease Control in the United States were used to determine the intra-assay and inter-assay reliability of the MS/MS test. Newborns were initially screened using the MS/MS technique, and enzyme-deficient samples were subject to repeat testing using the same DBS sample. Samples that displayed low enzyme activity on both MS/MS assays were referred for confirmatory genetic analysis. The 4-MU assay was used to identify patients with suspected Fabry and Pompe from January 2008 to January 2010, prior to the availability of the MS/MS technique. Recall rates, confirmed diagnoses and PPVs for both tests were compared.

There may be some overlap between the cohort of newborns screened by Liao et al. and Yang et al. The extent to which this may have occurred is unclear. Both trials were conducted at the Chinese Foundation of Health; however, Yang et al also enrolled newborns screened at the Taipei Veterans General Hospital. The proportion of newborns from each screening centre was not reported.

Efficacy

Direct evidence of the effectiveness of LSD screening (i.e. health outcomes following earlier diagnosis) was not reported. Surrogate outcomes included the number of LSD cases detected through screening, recall rates and PPV. Fewer newborns screened with the MS/MS technique were recalled for repeat testing due to low GLA (0.2% vs. 1.7%) and GAA (0.5% vs. 1.3%) activity compared with the 4-MU assay. Fewer newborns were referred for confirmatory molecular analysis for suspected Fabry disease following the MS/MS assay compared with 4-MU (0.04% vs. 0.1% respectively); however, refer rates for Pompe were equivalent for the MS/MS and 4-MU assays (0.12% vs. 0.11% respectively). The MS/MS technique demonstrated a higher PPV than 4-MU for both Fabry (0.96 vs. 0.61) and Pompe (0.07 vs. 0.04) diseases, and this was reflected in the reduced number of false positive test
The diagnostic yield of the MS/MS assay in patients screened for Gaucher disease is presented in Table 1.

**Yang et al 2014**

This single arm screening study (level IV screening evidence) reported results from a population-based newborn screening programme in Taiwan, and aimed to develop a diagnostic protocol for infantile Pompe disease. Newborns were consecutively enrolled in the study between January 2008 and May 2012. GAA activity (deficient in Pompe disease) in DBS samples was measured using the 4-MU assay between January 2008 and October 2010, and the MS/MS technique between November 2010 and May 2012. Newborns that had an inconclusive test result (0.51 to 2.0 μmol/L/h) were recalled for a second screening assay. Newborns with inconclusive results on the second assay (0.51 to 1.0 μmol/L/h) were referred for a third and final screening assay. All newborns with abnormally low GAA activity (≤0.50 μmol/L/h) at any stage in the screening process were referred directly for molecular confirmation.

As stated previously, there may be some overlap between the cohort of newborns screened by Liao et al and Yang et al. The newborns screened by Yang et al and Liao et al may also overlap with those screened in the study by Lin et al, which appeared in the original Technology Brief. Both studies screened a sample of newborns at the Chinese Foundation of Health between January 2008 and January 2009. There does not appear to be any overlap between the other recently published studies and those identified in the 2012 Technology Brief.

**Efficacy**

From 402,281 newborns screened, 4,148 (1.0%) were recalled for a second screening test and 222 (0.06%) were recalled for a third screening test. Overall, 321 (0.01%) newborns were referred for molecular confirmation and six were diagnosed with Pompe disease. In addition to screening outcomes, patient outcomes were also reported. Enzyme replacement therapy (ERT) was commenced in five newborns by 20 days of age and in one newborn at 79 days of age. These patients received follow-up echocardiography and biochemistry testing monthly for the first six months post-diagnosis, and then every three to six months. All patients treated with ERT demonstrated an improvement in motor skills measured using validated tools, and did not require a wheelchair or walking device at the most recent follow-up.

**Scott et al 2013**

Scott et al aimed to evaluate the feasibility of using multiplex MS/MS assay to screen newborns for Fabry disease, Pompe disease and Hurler syndrome simultaneously (level IV screening evidence). The study utilised 111,544 anonymous DBS samples collected through a state-based newborn screening programme in Washington, United States. Samples that
demonstrated abnormal GAA (deficient in Pompe), GLA (deficient in Fabry) and α-L-iduronidase (IDUA; deficient in Hurler) enzyme activity were further evaluated with genotype sequencing. Due to the use of anonymous DBS samples, infants with abnormal nucleotide changes indicative of each disease were not able to be followed-up to receive appropriate treatment.

**Efficacy**

A total of 108,905 samples were tested for GLA activity, of which 16 (0.01%) demonstrated abnormally low enzyme activity (<1.91 μmol/L/h) and were referred for genotype sequencing. Due to the use of anonymous DBS samples, infants with abnormal nucleotide changes indicative of each disease were not able to be followed-up to receive appropriate treatment. The estimated PPV for detecting Fabry disease was 0.43 (95% confidence interval [CI] 0.21, 0.70). GAA activity was measured in 111,544 samples, of which 17 (0.02%) demonstrated low enzyme activity (≤2.60 μmol/L/h). Four samples had nucleotide changes that may lead to the development of Pompe disease later in life. The estimated PPV for detecting Pompe disease was 0.24 (95% CI [0.08, 0.50]). Finally, IDUA activity was tested in 106,526 samples, of which three reported nucleotide changes that correlated with Hurler syndrome. It was not clear how many samples returned an abnormal result on the initial screening test. The estimated PPV for detecting Hurler syndrome was 0.33 (95% CI [0.08, 0.65]).

Wittmann et al 2012

The study by Wittmann et al screened a cohort of 40,424 newborns for Pompe, Fabry, Gaucher, and Niemann-Pick disease types A and B using the MS/MS technique (level IV screening evidence). DBS samples were obtained through a population-based newborn screening programme in Hungary. Samples that demonstrated abnormally low GAA (deficient in Pompe) GLA (deficient in Fabry) GBA (deficient in Gaucher) and acid sphingomyelinase (ASM; deficient in Niemann-Pick) enzyme activity were recalled for two repeat screening tests conducted simultaneously. Persistently low samples across all three screening tests were further analysed using molecular testing to confirm the diagnosis. Cut-off values for the initial screen were based on the distribution of enzyme activity in the first 1000 samples (Table 3). Cut-offs between the 0.25th and 0.5th percentiles were adopted.

**Efficacy**

A total of 141 samples (0.35%) were recalled for repeat screening due to low GBA activity, of which 17 (0.04%) were considered abnormal following repeat screening, and three were diagnosed with Gaucher disease following molecular testing. A total of 114 samples (0.29%) were recalled for repeat screening due to low ASM activity, of which five (0.01%) were further investigated with molecular testing, and two were diagnosed with Niemann-Pick type A or B. GAA activity was abnormal in 163 newborns (0.41%) on the initial screen, of which 64 (0.16%) were persistently low on repeat testing, and nine were diagnosed with Pompe disease on molecular analysis. Two cases of suspected Pompe could not be confirmed on molecular analysis. Finally, 224 newborns (0.56%) demonstrated abnormal
GLA activity on the initial screen, 34 (0.09%) were persistently abnormal on the repeat screening, and three were confirmed to have Fabry disease. Two cases of suspected Fabry disease could not be confirmed on molecular analysis. The overall recall rate for secondary, duplicate screening was 0.35 per cent.

2014 Economic evaluation

No cost-effectiveness studies of neonatal screening for LSDs were identified.

2014 Ongoing research

There are two ongoing trials investigating neonatal screening or diagnosis of LSDs (Table 2).

<table>
<thead>
<tr>
<th>Trial identifier/ location</th>
<th>Trial status</th>
<th>N</th>
<th>Design</th>
<th>Indication</th>
<th>Study start date</th>
<th>Estimated completion date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00787865 USA</td>
<td>Recruiting</td>
<td>100</td>
<td>Case control</td>
<td>Krabbe disease</td>
<td>April 2008</td>
<td>April 2014</td>
</tr>
<tr>
<td>NCT01409486 Israel</td>
<td>Unverified</td>
<td>10,000</td>
<td>Prospective case series</td>
<td>Pompe disease</td>
<td>September 2011</td>
<td>September 2013</td>
</tr>
</tbody>
</table>

Trial NCT00787865 is investigating the use of diffusion tensor imaging (DTI) to differentiate newborns with rapid infantile Krabbe disease from those with slower juvenile or adult forms of the disease. It is proposed that DTI be used in newborns with a positive DBS screening test to better inform treatment options. The primary outcome measure of this study is DTI of corticospinal tracts at birth, with follow-up imaging at one and two years of age; the secondary outcome is motor development over the same period.

Trial NCT01409486 aims to investigate a screening assay for Pompe disease in newborns. This study was last verified in August 2011 and is assumed to have been terminated.

2014 Other issues

An Australian-based pilot screening study of neonatal screening for LSDs was identified in the 2012 Technology Brief. Conducted at the South Australian Neonatal Screening Centre (SANS), the study has now concluded (personal communication, SANS), but is yet to be reported. While the research team from SANS has been involved with a similar trial conducted by the Mayo Clinic in the United States, no ongoing trials of neonatal LSD screening in Australia have been identified.

Neonatal screening for LSDs was discussed by the Human Genetics Society of Australasia’s Newborn Screening Committee in July 2014. The committee supported the inclusion of Hurler syndrome and Pompe disease into state-based newborn screening programmes; however, this information is not publicly available (personal communication, SANS). As newborn screening policy is currently determined by individual states, the addition of these indications to newborn screening programmes is dependent on jurisdictional funding.
and service availability. Testing services for Hurler syndrome and Pompe disease have only been identified in South Australia through SA Pathology laboratories.\textsuperscript{11}

In 2014, a working group was established with the aim to develop a national policy framework for newborn bloodspot screening programs in Australia.\textsuperscript{12} The policy framework will include criteria and a pathway to guide the assessment of conditions for inclusion in newborn bloodspot screening, such as LSDs. The development of the policy framework is being led by the Newborn Bloodspot Screening Working Group (NBSWG), under the remit of the Standing Committee on Screening. Future policy decisions on the expansion of newborn screening in Australia will be informed by the national policy framework.

Screening for Krabbe disease is no longer recommended by the Secretary’s Advisory Committee on Heritable Disorders in Newborns and Children in the Unites States, as trial evidence has demonstrated limited improvement in patient outcomes from early intervention.\textsuperscript{13, 14} The benefits and harms of early treatment for Krabbe disease are not well understood, and early treatment may have limited value in improving patients’ long-term prognosis.\textsuperscript{13}

In addition to the screening trials, two technical studies were identified that investigated ways to optimise LSD screening methods.\textsuperscript{15, 16} One study by Aldemir et al described differences in enzyme activity relating to gestational age and gender, while Chiang et al sought to determine optimal cut-offs for enzyme activity.\textsuperscript{15, 16}

Technical Studies

\textbf{Aldemir et al 2013}\textsuperscript{15}

As demonstrated in Table 3, enzyme activity cut-off values used in LSD screening vary between the included studies. They were largely based on the observed distribution of enzyme activity in control populations.\textsuperscript{2-4}

**Table 3  Cut-off values used to determine a positive screening test result in screening trials**

<table>
<thead>
<tr>
<th>Study</th>
<th>ASM (μmol/h/L)</th>
<th>GAA (μmol/h/L)</th>
<th>GBA (μmol/h/L)</th>
<th>GLA (μmol/h/L)</th>
<th>IDUA (μmol/h/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liao et al 2014</td>
<td>N/A</td>
<td>1.6</td>
<td>7.5</td>
<td>1.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Scott et al 2013</td>
<td>N/A</td>
<td>2.6</td>
<td>N/A</td>
<td>1.9</td>
<td>3.6</td>
</tr>
<tr>
<td>Yang et al 2013</td>
<td>N/A</td>
<td>1.0</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Wittmann et al 2012</td>
<td>2.0</td>
<td>3.0</td>
<td>3.5</td>
<td>2.5</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table notes: ASM = acid sphingomyelinase (deficient in Niemann-Pick A/B); GAA = acid α-glucosidase (deficient in Pompe); GBA = acid β-glucosidase (deficient in Gaucher); GLA = acid α-galactosidase (deficient in Fabry); IDUA = α-L-iduronidase (deficient in Hurler); N/A = not applicable.

Aldemir et al aimed to identify reference intervals for enzyme activity related to GAA, GBA and GLA in a population of Turkish newborns, and to investigate enzyme activity related to gestational age and sex.\textsuperscript{15} A total of 130 healthy newborns between 33 and 40 weeks of gestational age were enrolled in the study. Core blood samples and DBS samples were
obtained after informed consent was given by the primary caregivers. GLA, GBA and GAA activities were measured using the 4-MU technique.

There was no significant difference in enzyme levels between newborns delivered via caesarean and those delivered vaginally. Newborn girls had higher GLA activity than boys, but there was no significant difference observed for GAA or GBA. Newborns delivered before 38 weeks demonstrated significantly lower levels of GLA and GBA compared with those delivered at 39 weeks or later. There was no observed difference in GAA activity by gestational age. GBA activity was shown to increase significantly with gestational age, while GLA activity decreased. These results may have implications for the timing of newborn screening for LSDs; however, it was suggested that further research was needed to investigate appropriate cut-off values for newborn screening.

Chiang et al 2012

Chiang et al investigated the optimal cut-off values for enzyme activity related to Pompe disease in a Taiwanese population. The trial used DBS samples collected through the national newborn screening programme described earlier by Liao et al. Between October 2005 and December 2011, a total of 473,738 samples were collected and analysed for GAA activity using the 4-MU method. From trial initiation until October 2007, a cut-off value for the ratio of neutral α-glucosidase (NAG) to acid α-glucosidase (GAA) of at least 25 was used to select newborns for repeat testing. From October 2007, an increased cut-off ratio of at least 30 was implemented to reduce the high recall rate. Data from samples collected in the screening programme were retrospectively analysed to determine the optimal cut-off values for identifying newborns with Pompe disease.

A total of 2,210 newborns were recalled for repeat testing from 2005 to 2011, of which 28 were confirmed to have the disorder. The initial NAG:GAA cut-off ratio of at least 25 returned 0.82 per cent of newborns for repeat testing. The increased ratio of at least 30, implemented from October 2007, led to a recall rate of 0.26 per cent. Analysis of the screening results indicated that an NAG:GAA ratio of at least 60 would have resulted in a recall rate of 0.01%, with a PPV of 0.63 (95% CI [0.47, 0.78]) and a false positive rate of 0.003 per cent. In this scenario, only 41 newborns would have been recalled for repeat testing; however, two late-onset Pompe cases would have been missed and the false negative rate would have been 7.1 per cent. It was suggested that the screening algorithm could be further improved by the addition of acarbose inhibition as a second-tier test for newborns with an inconclusive result on the first DBS test. This would lead to a PPV of 0.93 (95% CI not reported).

2014 Summary of findings

Additional screening trials published since the 2012 Technology Brief have demonstrated the feasibility of screening for Pompe disease, Fabry disease, Gaucher disease and Hurler syndrome. The use of the MS/MS technique, compared with fluorescence assays, allows for
more efficient testing of multiple indications simultaneously. Use of this technique was also associated with a reduction in the number of cases requiring additional testing following an equivocal test result. Although additional studies investigated the role of gestational timing and optimal cut-offs for enzyme activity to differentiate healthy and affected newborns, primary research in an Australian population is required to determine the optimal screening algorithm for Australia.

The addition of Hurler syndrome and Pompe disease to existing neonatal DBS screening programmes was recently supported by the Human Genetics Society of Australasia. The technology to conduct neonatal screening for LSDs is widely available across Australia; however, only one laboratory actively conducts LSD testing for Hurler and Pompe. The expansion of state-based screening programmes to include these indications is yet to occur, and will be influenced by a range of factors beyond the evidence for efficacy presented in this Technology Brief.

2014 HealthPACT assessment

Based on the current level of evidence and lack of ongoing registered clinical trials, the uptake and diffusion of neonatal screening for LSDs in Australia is unlikely to be influenced by additional evidence in the near future. Further, policy decisions on the expansion of newborn DBS screening in future will be informed by the newborn bloodspot screening policy framework currently under development. This Brief should be disseminated to the jurisdictions and to the Secretariat of the Blood Spot Screening Policy Working Group and no further research on behalf of HealthPACT is warranted at this time.

2014 Number of studies included

All evidence included for assessment in this Technology Brief has been assessed according to the revised NHMRC levels of evidence. A document summarising these levels may be accessed via the HealthPACT website.

| Total number of studies | 4 |
| Total number of Level III-3 studies | 1 |
| Total number of Level IV studies | 3 |

2014 References


2012 TECHNOLOGY BRIEF

Register ID WP115

Name of technology Neonatal screening for lysosomal storage disorders

Purpose and target group Detection of lysosomal storage disorders in the neonate population

Stage of development in Australia

☐ Yet to emerge
☐ Experimental
☒ Investigational
☐ Nearly established

Australian Therapeutic Goods Administration approval

☐ Yes
☐ No
☒ Not applicable

International utilisation

<table>
<thead>
<tr>
<th>Country</th>
<th>Trials underway or completed</th>
<th>Limited use</th>
<th>Widely diffused*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
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<td></td>
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</tr>
<tr>
<td>Austria</td>
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<td></td>
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</tr>
<tr>
<td>Italy</td>
<td>✓</td>
<td></td>
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</tr>
<tr>
<td>Taiwan</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>✓</td>
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</tbody>
</table>

* While the required technology is widely diffused, widespread population screening has only been conducted in the context of trials

Impact summary

Laboratories that perform routine analysis of heel prick tests may have the necessary equipment to provide additional screening for lysosomal storage disorders (LSDs) in neonates; however should a national neonatal screening program be introduced in Australia and New Zealand, additional investment in both technology and staffing would be required. In addition to these economic considerations, there are ethical considerations that also surround the uptake of this technology. Newborn screening for selected genetic disorders using dried blood spots (DBS) is currently occurring in Australia at a population level, but
does not routinely include LSD screening. A small technical feasibility study for newborn LSD screening has been published using DBS cards collected from the South Australian Screening Centre; however, the feasibility of including LSD screening as part of a national neonatal screening program has not been assessed in Australia.5

Background

LSDs comprise more than 50 serious, progressive diseases that arise due to an inherited dysfunction in the lysosome. The lysosome is an intracellular organelle with the primary function of degrading substrates through a variety of pathways including endocytosis, phagocytosis and autophagy.1 Lysosomes contain a range of hydrolytic enzymes to undertake degradation. Loss-of-function mutations in one or more of the hydrolytic enzymes, or other integral lysosomal proteins, can lead to substrate accumulation and storage within the lysosome.2 The stored substrate is specific to each disorder, and traditionally the LSDs have been categorised according to the type of substrate stored (for example, mucopolysaccharidoses, oligosaccharidoses, sphingolipidoses, gangliosidoses).2, 3 They may also be classified based on the nature of the defective protein.3 The majority of LSDs are recessively inherited autosomal traits, with several that are recessively inherited X-linked traits. Examples of LSDs include Gaucher’s disease, Fabry’s disease, Tay-Sach’s disease, Pompe’s disease, and Niemann-Pick disease types A and B.4

The accumulation of substrates in LSDs can alter many cellular processes, including lysosomal pH regulation, synaptic release, endocytosis, vesicle maturation, autophagy and exocytosis.1, 3 Progressive lysosomal substrate deposition can occur in cells throughout the body.4 This accumulation can result in the deterioration of cellular and tissue function, and the dysfunction of vital organs, muscles and neurons.1-3 The clinical presentations of LSDs vary, and can be affected by the underlying genetic mutation. The onset of most LSDs occurs in childhood, after the achievement of early developmental milestones; however, some LSDs present in utero or during the newborn period, while others present in late adulthood. Early symptoms can include neurological disorders and slowing of developmental progress, dysmorphic facial appearance, cardiac disease or enlargement of the liver and/or spleen.3 Many disorders affect the central nervous system and most patients have a decreased lifespan and significant morbidity.2

Treatment is generally directed towards symptomatic care of secondary complications; however, for some LSDs, haematopoietic stem-cell transplantation (from bone marrow or cord blood) and enzyme replacement therapy are emerging as promising treatments.2, 4 Due to the progressive nature of these disorders, the effectiveness of these therapies relies heavily on early detection and treatment. Haematopoietic stem-cell transplantations may be more successful in neonates due to the natural immaturity of the immune system.5 Neonatal screening programs have been in use for decades and are generally intended to differentiate asymptomatic newborns from those without a disease for disorders in which
early detection and presymptomatic treatment are required to avoid serious clinical harm. A disorder must also have a high enough prevalence to justify inclusion in such programs. Several studies have been published that implemented pilot screening for LSDs to assess the practicality and appropriateness of including these disorders in neonatal screening panels. In addition, several states in the USA have passed legislation to include some LSDs in their neonatal screening programs, while regional neonatal screening programs are commencing in parts of Europe.

Screening for LSDs typically involves the collection of DBS, with subsequent enzyme activity analysis, using such technology as electrospray ionisation tandem mass spectrometry (ESI-MS). First introduced in 1993, ESI-MS has been shown in multiple studies to be technically feasible. ESI-MS enables the simultaneous screening of several enzyme activities related to LSDs from DBS samples and high-throughput, multiplex assays have been developed to simplify and expedite workflow. More advanced DNA-based genetic mutation analysis is generally used to confirm a suspected positive result from an enzyme activity screen, however, one study by Chien et al (2012) has indicated that mutation analysis as a primary screening method may be more sensitive than the enzyme-based technique. Numerous genetic and biomarker studies for the detection of LSDs are currently being conducted.

Clinical need and burden of disease

There are more than 50 different forms of LSDs. While each individual disorder is rare, when considered as a group, the prevalence in Australia has been estimated at 1 in 7,700 births. More recent estimates are available from other countries, such as a recent screening study in Austria which put the combined incidence of LSDs at approximately 1 in 2,315 births. Due to the rarity of LSDs, there are limited data available on the burden of disease in Australia. Most LSDs can be classified by ICD-10-AM code as either ‘E74 Other disorders of carbohydrate metabolism’ or ‘E75 Disorders of sphingolipid metabolism and other lipid storage disorders’. In Australia in 2009 – 2010, there were 490 hospital separations for the disease classification ‘Other disorders of carbohydrate metabolism’ and 1,864 hospital separations for the disease classification ‘Disorders of sphingolipid metabolism and other lipid storage disorders’. More specific LSD data are not available.

Diffusion of technology in Australia

Newborn screening for selected genetic disorders using DBS is currently occurring in Australia at a population level, but does not routinely include LSD screening. A small technical feasibility study for newborn LSD screening has been published using DBS cards collected from the South Australian Neonatal Screening Centre. Since 1998, an LSD pilot project has been in operation at the South Australian Neonatal Screening Centre to determine the effectiveness of the test for identifying neonates at risk of having an LSD.
Comparators

The comparator for the introduction of widespread neonatal LSD screening is diagnosis once clinical symptoms have emerged. Issues around widespread screening implementation versus the comparator of no widespread screening are predominantly economic and ethical, rather than technical.\(^2,18\)

Safety and effectiveness

Study description

An Austrian study provided the first evidence for the introduction of a screen for multiple LSDs into a nation-wide neonatal screening program.\(^4\) Additional population-wide screening studies for single selected LSDs have been conducted in several countries (Table 1).\(^14,19-25\)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Population-wide screening studies in single selected lysosomal storage disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>Study details</td>
</tr>
<tr>
<td><strong>Pompe’s disease</strong></td>
<td></td>
</tr>
<tr>
<td>Chien et al (2008)(^19), Chien et al (2009)(^20), Labrousse et al (2010)(^22).</td>
<td>• Pilot screening program (~45% of all newborns in Taiwan).</td>
</tr>
<tr>
<td>Taiwan</td>
<td>• GAA measured from DBS via fluorescence assay, with confirmatory DNA analysis.</td>
</tr>
<tr>
<td><strong>Fabry’s disease</strong></td>
<td></td>
</tr>
<tr>
<td>Spada et al (2006)(^25).</td>
<td>• Pilot screening program of consecutive newborn males in a region in Italy.</td>
</tr>
<tr>
<td>Italy</td>
<td>• GLA measured from DBS via a fully automated system, with confirmatory DNA analysis.</td>
</tr>
<tr>
<td>Hwu et al (2009)(^21).</td>
<td>• Pilot screening program of consecutive newborns (~40% of all newborns in Taiwan)</td>
</tr>
<tr>
<td>Taiwan</td>
<td>• GLA measured from DBS, with further confirmatory analysis.</td>
</tr>
<tr>
<td>Study</td>
<td>Study details</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------------------------------------------------</td>
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<tr>
<td>Lin et al (2009)[23]</td>
<td>Pilot screening program of consecutive newborns (~55% of all newborns in Taiwan)</td>
</tr>
<tr>
<td>Lin et al (2009)</td>
<td>GLA measured from DBS, with further confirmatory analysis.</td>
</tr>
<tr>
<td>Chien et al (2012)[4]</td>
<td>Pilot screening program of consecutive newborns in Taiwan</td>
</tr>
<tr>
<td>Chien et al (2012)</td>
<td>DNA-based mutation analysis as primary screen, for specific GLA mutation linked to late-onset disease.</td>
</tr>
<tr>
<td>Krabbe’s disease</td>
<td>Orsini et al (2009)[24]</td>
</tr>
<tr>
<td>Orsini et al (2009)</td>
<td>GALC measured from DBS via a high-throughput system.</td>
</tr>
</tbody>
</table>

DBS: dried blood spot; GAA: α-glucosidase; GLA: α-galactosidase A; GALC: galactocerebrosidase

Mechtler et al (2012) implemented a multiplex high-throughput screening assay for Gaucher’s disease, Pompe’s disease, Fabry’s disease, and Niemann-Pick disease types A and B in an anonymous prospective nation-wide screening study in Austria. The aim of the study was to assess the practicality and appropriateness of including these disorders in neonatal screening panels; these issues could be considered the main effectiveness measures for the introduction of LSD screening.⁴

DBS samples were collected consecutively from 34,736 newborns from January to July 2010, as part of the routine national Austrian newborn screening program which covers more than 99% of births. All samples successfully screened with the regular screening panel for endocrine and metabolic disorders were additionally analysed for four different lysosomal enzyme activities: acid β-glucocerebrosidase (GBA; deficient in Gaucher’s disease), α-galactosidase A (GLA; deficient in Fabry’s disease), α-glucosidase (GAA; deficient in Pompe’s disease), and acid sphingomyelinase (ASM; deficient in Niemann-Pick disease type A and B). The DBS screen for LSDs was performed using ESI-MS. The authors adapted a direct multiplex assay to allow for use of automated sample preparation and pipetting steps with liquid handling stations to enable high-throughput screening. Quality control measures were...
implemented in line with neonatal screening procedures for other metabolic disorders, and 32 blood samples from affected individuals served as controls. DBS from potentially enzyme-deficient infants were retested in duplicates, and positive results were diagnostically confirmed by subsequent mutational analysis of genomic DNA.\footnote{4}

**Safety**

Safety aspects of screening for LSDs were not specifically reported. The study made use of DBS samples that are routinely collected from neonates for screening for other disorders, and presumably no additional safety issues applied to the LSD screen.\footnote{4}

**Effectiveness**

The adapted biochemical multiplex screening assay for the selected LSDs was successful for all 34,736 samples. The 32 control samples from known affected patients had low enzyme activities that could be clearly distinguished from healthy controls. The first line ESI-MS screening identified 124 samples with low enzyme activity. When the samples were retested in duplicates from the same DBS card, 38 neonates were found to have low enzyme activity (4 had low GBA, 5 had low GAA, 28 had low GLA, and 1 had low ASM).

Mutation analysis was subsequently performed in the 38 suspected cases, and 15 were positive by genetic testing and classed as confirmed cases. The confirmed cases included two for Gaucher’s disease (low GBA), four for Pompe’s disease (low GAA) and nine for Fabry’s disease (low GLA), with no confirmed cases of Niemann-Pick types A/B disease (low ASM). This gave a combined incidence of 1 per 2,315 births (95\% confidence interval (CI) of 1 per 1,403 to 1 per 4,136 births), and individual disorder incidence rates of 1 per 17,368 births for Gaucher’s disease, 1 per 8,684 births for Pompe’s disease, and 1 per 3,859 births for Fabry’s disease, with Niemann-Pick types A/B disease not found in the tested population.

Most mutations (75\%) were associated with a mild phenotype related to later onset and slow disease progression. The positive predictive value (PPV) for the ESI-MS screening test was 40\% (95\% CI 24 – 57\%) overall for the LSDs screened, 50\% (95\% CI 7 – 93\%) for Gaucher’s disease, 80\% (95\% CI 28 – 99\%) for Pompe’s disease, 32\% (16 – 95\%) for Fabry’s disease, and 0\% (95\% CI 0 – 95\%) for Niemann-Pick types A/B disease. The false positive rate per million was 660 (95\% CI 420 – 990) overall for the LSDs screened, 60 (95\% CI 10 – 210) for Gaucher’s disease, 30 (95\% CI 1 – 160) for Pompe’s disease, 550 (95\% CI 330 – 850) for Fabry’s disease, and 30 (95\% CI 1 – 160) for Niemann-Pick types A/B disease.\footnote{4}

**Cost impact**

Mechtler et al (2012) reported that to add the detection of several LSDs to routine neonatal screening, an additional tandem mass spectrometry system and at least one extra laboratory worker for sample preparation were needed. The total analysis time for 100 samples was approximately three hours. The reagent costs were about €1 per sample for all
four multiplexed LSDs, which the authors stated was much the same as for other screening assays.\textsuperscript{4} Other costs associated with the test include labour, equipment maintenance (for both sample preparation and screening) and writedown.

Downstream costs that also need to be considered include those to confirm the diagnosis, input from specialist clinical consultants, genetic counselling services; as well as participation in paediatric metabolic programs following a positive diagnosis.

LSD treatment costs are also relevant, as the detection of more LSDs at birth may result in earlier uptake of available treatments. While the majority of treatments are directed towards symptomatic care, primary therapies such as haematopoietic stem cell transplantation and recombinant enzyme replacement therapies could enable early treatment before irreversible damage occurs.\textsuperscript{2, 18} For many countries, expense is a large consideration in the treatment of LSDs as most new therapies have a high cost, require considerable expertise, and often lack sufficient evidence of effectiveness.\textsuperscript{2}

**Ethical, cultural or religious considerations**

As with any screening program, there are many ethical considerations around neonatal screening for LSDs.\textsuperscript{2} Such ethical considerations include how best to treat presymptomatic individuals with positive screen results, and how best to inform parents of the likely outcomes for the affected individual and also of future reproductive risks. Many countries have adopted a conservative approach to neonatal screening, possibly due to the scarcity of formal evidence of benefit and the fear of harm of false positive or negative screening results.\textsuperscript{18}

For some LSDs, there is some limited evidence that presymptomatic diagnosis and early treatment can favourably alter the natural history of the disease.\textsuperscript{24} For example, in the Taiwanese screening study for Pompe’s disease, the screening program was shown to improve clinical outcomes in infants identified by screening, compared with infants identified by clinical signs and symptoms.\textsuperscript{20}

For many LSDs, effective primary therapies have not yet been found, and a presymptomatic diagnosis may have little effect on disease progression. Detection of a known mutation can provide guidance on the likelihood of a classic or late-onset disease phenotype; however, precise genotype-phenotype correlations are not possible for many LSDs, particularly when novel mutations are detected.\textsuperscript{18} Uncertainty regarding how a disease will manifest translates into uncertainty regarding the appropriate age to commence treatment.\textsuperscript{18} Identification of adult-onset variants and variants of uncertain significance could potentially be identified in greater numbers than the early infantile forms of LSDs, and some patients with these variants may never develop symptoms or require therapy.\textsuperscript{2}

The manner in which trials to display the feasibility of neonatal screening are conducted also raises ethical issues. Anonymous study designs, such as those performed by Mechtler et al
(2012)\(^4\) and Orsini et al (2009)\(^{24}\) where routine DBS samples were de-identified for the additional LSD screen, preclude clinical assessment of neonates identified as having a disorder by the screening process. Future studies without informed consent and clinical assessment could be deemed unethical.\(^{18}\)

**Other issues**

Fletcher & Wilcken (2012)\(^{18}\) noted that assessing the benefit of neonatal screening for LSDs is difficult due to the rarity of the disorders. Frameworks are being developed to systematically assess the merits of screening for additional disorders.\(^{26}\)

Many of the studies included were sponsored by the manufacturer or distributor of enzyme(s) used in the treatment of LSDs, with employees of these companies included as study authors.

**Summary of findings**

From the primary study by Mechtler et al (2012)\(^4\), nation-wide neonatal screening for several LSDs using ESI-MS was technically feasible. More accurate confirmation of an LSD could be obtained after repeated biochemical screening and genetic testing. Mechtler et al (2012)\(^4\) stated that the combined incidence of the four LSDs screened was higher than expected (1 per 2,315 births). This was in agreement with some of the additional screening studies for single LSDs that also noted that neonatal screening produced higher incidence rates than previously recorded clinical diagnosis rates.\(^{14, 21, 23, 25}\) The mutation analyses in the studies by Mechtler et al (2012)\(^4\), Chien et al (2012)\(^{14}\), Hwu et al (2009)\(^{21}\), Lin et al (2009)\(^{23}\), and Spada et al (2006)\(^{25}\) detected a high proportion of mutations linked with late-onset disease symptoms. Individuals with such mutations are likely to appear asymptomatic in early years, and without neonatal screening would only be identified later in life or not at all.

Whilst LSD screening is technically effective, and holds no more safety risk than current screening programs using DBS, the introduction of an expanded nation-wide neonatal screening program for LSDs is associated with both economic and ethical issues. The value of detecting an LSD at birth is clear when favourable outcomes are dependent on early initiation of treatments. More ethical issues surround the detection of LSDs where treatments are less effective, or where the likelihood and timing of symptom development is unknown.\(^2\) Economic considerations include the downstream costs associated with confirmatory diagnosis, specialist clinical consultant input, genetic counselling services and participation in paediatric metabolic programs following a positive diagnosis.
HealthPACT assessment:

Based on the increasing availability of evidence for population-wide neonatal LSD screening, and the economic and ethical issues surrounding this technology, HealthPACT recommended that the technology be monitored for 24 months.

Number of studies included

All evidence included for assessment in this Technology Brief has been assessed according to the revised NHMRC levels of evidence. A document summarising these levels may be accessed via the HealthPACT web site: http://tinyurl.com/99kkraa.

Total number of studies: 9
Total number of level IV studies: 9

References


Search criteria to be used (MeSH terms)

Search terms:

(lysosomal storage disorders OR lysosomal storage disease OR Gaucher’s disease OR Gaucher disease OR Pompe’s disease OR Pompe disease OR Fabry’s disease OR Fabry disease OR Niemann-Pick disease)

AND

(screening OR diagnosis)

AND

(neonatal OR neonate* OR baby OR babies OR infant* OR newborn*)

AND

(population OR nationwide)