A 2nd tier (reflex-test) blood-spot test for MMA was added to the South Australian newborn screening programme in 2007 as a specific marker for a group of IEM collectively known as methylmalonic acidemias. The MMA reflex test was introduced due to the low predictive value of C3-carnitine for identifying disorders of cobalamin metabolism and to the false positives due to prematurity and hyperbilirubinaemia in neonates.

A simple non-derivatised blood-spot LC-MS/MS MMA method using a 5 × 100 mm Phenomenex C6-phenyl column with acetonitrile:water:formic acid at a flow rate of 150 µL/min directly into an API4000 MS/MS (SCIEX) operated in negative ion mode. MMA was eluded from a 3 mm blood-spot and determined against (d3)-MMA using MRM pairs of 117.1/73.1 & 120.1/76.1 in a 5 min isocratic LC run. A MMA level of 3.3 µmol/L whole blood equivalent to the 99th centile is used as an action limit.

Since 2007 over 1135 dried blood-spot MMA determinations have been performed as a result of a primary elevation in C3-carnitine, in addition to the related ratios C3/C2, C3/C16 & C3/methionine. From the screened population this represented <0.01%. To date, of the 1135 MMA determinations 104 babies required further follow-up either for a repeat blood-spot collection or recalled for plasma & urine MMA and B12 determination at an average age of 21 days. We have identified 26 neonates with significant B12 deficiencies and who have been treated representing ~25% of the recalled neonates. A case of cobalamin A deficiency (cblA; p.R145X/p.D292V)) was identified with a presenting MMA level of 18.3 µmol/L whole blood. In addition we have identified maternal B12 deficiency, as a result of an elevated blood-spot MMA in her baby.

Inclusion of a 2nd tier MMA identifies B12 deficiencies as well as disorders of cobalamin metabolism while significantly reducing the false positive rate associated with the measurement of C3-carnitine.

O13. A Novel Method for Inclusion of Urea Cycle Disorders into Newborn Screening

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The inclusion of urea cycle disorders (UCD) detection into newborn screening (NBS) is highly desirable; however it is hampered by the lack of a specific marker for most of these disorders. So far, the common feature of UCDs, hyperammonemia, is not directly detectable in dried blood spots (DBS). The detection of secondary elevations of glutamine seemed so far not feasible based on the assumption of the instability of glutamine in DBS. We describe here a reliable method for the simultaneous detection of lysine and glutamine from DBS in multiple reaction monitoring (MRM) with a second-tier ultra-high performance liquid chromatography-(UPLC)-method for the separation and specific quantitation of glutamine. We combined this newly developed method with the measurement of all specific amino acids (arginine, arginino succinic acid, citrulline, ornithine, and proline), N-acetyl-glutamate, and orotic acid. This combination proofed to be a reliable and sensitive method for the detection of all UCDs by tandem-mass spectrometry NBS. The next step will be a prospective study with dried blood samples from patients with hyperammonemia, for further testing of the method.

O14. Long-Term Outcome of Newborn G6pd Screening Program in Taiwan

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Severe neonatal jaundice (NJ) triggered by environmental factors and/or medications is the major health impact of Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency in newborns. If not prevented or treated properly, it may lead to kernicterus and cause death or permanent neurological damages. The incidence of G6PD deficiency in Taiwan is about 2%. It has been found that 30% of NJ admitted to hospital was G6PD deficient with 16% mortality and 32% developed kernicterus in 1970s.

The nationwide newborn G6PD screening program in Taiwan was started in 1987 and the coverage rate has reached >99% since 1996. The effectiveness of this screening program to prevent mortality and sequela of NJ is studied.

The patient data of hospital admission with NJ after discharged from the birthing facility between 2000 and 2010 were retrieved from the National Health Insurance Research Database, which covered >98% population of Taiwan. There were 8,635 NJ (0.38%) admissions from 2,297,867 live births and 14 of them treated with exchange transfusion. Only 2 of the NJ cases dead within 1 month of age and 8 of them developed kernicterus not due to isoimmunization. The average immediately severe morbidity and mortality were about 1 (0–2) case per year nationwide. Long-term follow up those NJ cases born between 2000 and 2004 up to 6 years old have found a higher risk of developmental delay, hearing loss, speech disorders, attention deficit hyperactivity disorder (ADHD), and mental retardation comparing to the control cohort.

The results indicated that the newborn G6PD screening program in Taiwan almost eliminated severe morbidity and mortality caused by NJ with G6PD deficiency after discharge from birth facilities. However, close follow-up of those cases with NJ admission are still needed for early intervention of developmental delay, mental disorders, hearing loss, and speech problems.

O15. Pseudo-Deficiency of Alpha-L-Iduronidase: A Challenge Faced in the Newborn Screening for Mucopolyssacharidosis I

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Mucopolysaccharidosis I is one of the new targets for NBS. Babies identified with the severe form of MPS I should be considered candidates for HSCT, and the ones with the attenuated form are considered for ERT, with firm evidences on benefits of early treatment. There are several platforms available for MPS I screening, and we ran a pilot project using the digital microfluidics technology (Baebies, Inc.). The α-L-iduronidase (IDUA) activity was determined in 8550 babies, randomly selected among the ones referred to CTN for routine NBS. One sample showed low IDUA activity (0.8 μmol/L/h, with cut off = 5.0). The IDUA activity in this sample was tested also by manual fluorimetry (undetectable) and by TMS (undetectable). The baby was retrieved and urine and blood samples were collected. Urinary GAGs were normal by standard DMB method (197 μg/mg creat, normal range = 133 to 460 μg/mg creat) and GAG electrophoresis showed a normal pattern. IDUA activity was normal in plasma (11 nmol/h/mL, normal range = 6.6 to 34 nmol/h/mL) but slightly reduced in leucocytes (11 nmol/h/mg prot, normal range = 27–171). Molecular analysis of the IDUA gene allowed the identification of the mutation {c.251G>C [p.(G84A)} in one allele, predicted as possibly pathogenic by Poly-Phen 2, SIFT and Provean. In the same codon where already described two pathogenic mutations (p.G84E and p.G84S). A genetic variation previously associated with pseudo-deficiency was found in the other allele {c.246C>G [p.(H82Q)]}.This comprehensive evaluation allowed us to predict a clinically normal child, as the residual activity provided by the p.(H82Q) allele allows a normal degradation of GAGs. This case illustrates the challenges faced by NBS of LSDs, with the need of an comprehensive algorithm to properly manage the cases when a screening test results positive.

O16. Full Population Screening for Pompe, Fabry, Gaucher, Mucopolysaccharidosis Type I and Krabbe Disorders in the State of Missouri

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