syndrome (SIDS). Nowadays many patients are identified presymptomatically by population newborn screening (NBS) programs. Some patients escape early detection because their symptoms and signs start before NBS test results become available.
Methods: A comprehensive systematic literature review to identify all IEMs associated with RS and SIDS, including their treatability and detectability by NBS technologies.
Results: 42 IEMs were identified in the literature that were associated with either RS or SIDS of which (a) 25 can already present during the neonatal period, (b) treatment is available for at least 32, and (c) 24 can be identified by analysis of acylcarnitines and amino acids in dried bloodspots.
Discussion: We advocate extensive dried blood spot analysis of acylcarnitines and amino acids as a minimal recommended test after both RS and each (unexpected) neonatal death, especially when a dried blood spot for the NBS program has not yet been drawn.

P-052

The use of tandem mass spectrometry (MSMS) based method for determining enzyme activities for six lysosomal storage diseases (LSD) from a single dried blood-spots for use in newborn screening

Ranieri E[1], Stark S[1], Fletcher J M[1], Gelb M H[2]

[1]Genetics & Molecular Path., SAPathology, Adelaide, Australia, [2]Dept of Chemistry, Univ Washington, Washington, United States

Background: The use of an MSMS method for measuring the activity of six LSD enzymes from a single 3mm dried blood spot (DBS) for newborn screening. Included are; galactocerebroside β-galactosidase (GALC, Krabbe disease), acid α-galactosidase A (GLA, Fabry disease), acid sphingomyelinase (ASM, Niemann Pick A/B disease), acid α-glucosidase (GAA, Pompe disease), α-L-iduronidase (IDUA, mucopolysaccharidosis type I and acid β-glucocerebrosidase (ABG, Gaucher disease).
Methods: De-identified DBS were sourced from the SA newborn population (n~1,000) and DBS from positive LSD cases (n=75) obtained from the National Referral Laboratory (NRL); GALC (5), GLA and carriers (14 & 28), ASM A/B (1), IDUA (6), GAA (20) or ABG (20). LSD reagents were obtained from PerkinElmer (Waltham, MA, USA). Enzyme activities were determined against stable isotopes by MRM after a single organic solvent extraction. Analysis by FIA-API5000 with settings: IS voltage 5000, DP 95, CE 55 and CXP 15
Results and discussion: The 1st centile determined from 592 DBS as IU/hr/L wb of ABG 2.2, IDUA 1.3, GAA 4.1, ASM 0.8, GALC 1.8 and GLA 1.3. All LSD cases had activities below the 1st centile, GLA showed some overlap, due to in-source fragmentation, but not seen on API4000/3200 instruments. Each assay showed > 3 orders of magnitude in dynamic range with excellent lower end sensitivity.

P-053

High incidence of heterozygotes of Pompe disease: 2-year experience of newborn screening in Japan

Momosaki K[1], Yoshida S[1 2], Matsumoto S[1], Nakamura K[1], Tasaki R[2], Okumiya T[3], Endo F[1]

[1]Dept Pediat, Kumamoto University, Kumamoto, Japan, [2]Kaketsuken, Research Institute, Kumamoto city, Japan, [3]Analytical Biochemistry, Kumamoto Univ., Kumamoto city, Japan

Background: We started population-based newborn screening (NBS) of Pompe disease in Apr 2013. We also examined the incidence of *GAA* mutation among newborns with low enzyme activities.
Method: We screened 31,274 DBSs from the NBS program in Kumamoto, Japan from April 2013 to January 2015. We assayed acid α-glucosidase (AαGlu) activity in DBS with two different methods; 4-methylumbelliferone (4-MU) method and Ba/Zn method. Definitive diagnosis was done by the measurement of AαGlu activity in fibroblasts and *GAA* mutation analysis using next-generation sequencing technology. Genetic analysis was also performed by Sanger methods.
Results: A total of 91 (0.29%) newborns were recalled for second DBSs with low enzyme activity. AαGlu activity in fibroblasts, carried out for 15 samples with abnormal low enzyme activity (GAA< 4.0), revealed 8 subjects with remarkable low GAA activity at levels seen in patients. 6 of 8 subjects had heterozygous mutations; 4 pathogenic mutations and 2 new mutations.
There were no subjects with 2 independent pathogenic mutations. All 8 screening positive newborns were asymptomatic.
Conclusions: High incidence of heterozygotes of *GAA* mutation among newborns with low enzyme activity was revealed. We need to investigate genotype-phenotype correlation and effect of pseudodeficiency allele on GAA activity.

P-054

Identification of *G6PD* common mutations using a multiplex primer extension-based method

Chiu Y H[1], Liu Y N[1], Chang Y C[2], Liu T T[1 3], Hsiao K J[1 4]

[1]Dept Edu Res, Taipei City Hosp, Taipei, Taiwan, [2]Excelsior Pharmatech Labs, Taipei, Taiwan, [3]VYM Genome Res Center, Natl Yang-Ming, Taipei, Taiwan, [4]Prev Med Found, Taipei, Taiwan

Background: Glucose-6-phosphate dehydrogenase (G6PD, E.C. 1.1.1.49; gene symbol: *G6PD*) deficiency is highly prevalent in Southeast Asians and may lead to extreme hyperbilirubinemia and/or life-threatening bilirubin encephalopathy. Neonatal screening for G6PD deficiency has long been established to provide important information for genetic counseling and to prevent the occurrence of hemolytic crisis in affected populations. However, heterozygous females are difficult to be identified reliably because their enzyme activities may range from deficiency to normal.
Methods: A multiplex polymerase chain reaction coupled with a single-base primer extension method was applied to detect 22 *G6PD* mutations found in Taiwanese population using dried blood spots of 483 male patients.
Results: Four hundred and fifty out of 484 mutant alleles (93.0%) were determined in our assay, among which the c.1376G>T and c.1388G>A mutations of *G6PD* gene were the most frequent ones comprising 47.9% and 21.1% of the mutated alleles, respectively.
Conclusion & Discussion: Our assay is a rapid and precise method for detecting known *G6PD* mutations and can be directly applied to dried blood spot used in newborn screening. This method may be useful to detect female carrier of the border-line G6PD activity with 93% of detection sensitivity in Taiwan.

P-055

Pilot newborn screening for lysosomal disorder by tandem mass spectrometry based method

Polo G[1], Furlan F[1], Kolamunnage T B[1], Del Rizzo M[1], Celato A[1], Giordano L[1], Burlina A P[2], Burlina A B[1]

[1]Div Metab Dis, Dep Ped, Padua Univ Hosp, Padua, Italy, [2]Neurol Unit, St. Bassiano Hospital, Bassano del Grappa, Italy

Background: Several lysosomal storage disorders (LSD) have been recently proposed for inclusion in screening programs because of increasing availability of treatment options and relative prevalence of these conditions. The primary objective of this study was to evaluate the feasibility of a screening population for Pompe disease, Fabry disease, Gaucher disease and MPS I disease using a LC-MS/MS based method.
Methods: Enzyme activities of α-galactosidaseA, α-glucosidase, α-L-iduronidase, β-glucocerebrosidase were determined in dried blood spot (DBS) by stable isotope dilution flow injection analysis MS/MS(FIA-MS/MS) using Perkin Elmer Six-Plex LSD reagents kit.
Results: The performance of each LSD enzyme assay was determined from repeated measurement of 4 levels CDC quality controls (CV intra and inter assay < 15%). Reference ranges for each enzyme were defined from analysis of healthy newborn DBS (n=1000). Enzyme activity in DBS from patients affected by the above mentioned disease were used to have a low enzyme activity in order to test specificity and sensitivity.
Conclusions: The results of this study showed that Perkin Elmer LSD kit perform well on DBS screening allowing simultaneous detection of the 4 enzymes tested. The method was able to distinguish LSD patients from the normal population.

P-056

Free carnitine (FC) levels pattern in a group of very low birth weight newborns identified by a regional screening program

Righi B[1], Bettocchi I[1], Righetti F[1], Baronio F[1], Incorvaia L[1], Vitali A[1], Pession A[1], Cassio A[1]

[1]Dept of Paed, Univ Bologna, Bologna, Italy

Background: There are few and controversial data about the pattern of FC levels measured on dried blood spot (DBS) samples in very low birth weight neonates (VLBW), who were at risk for carnitine depletion compared to full-term neonates.
Methods: In our regional screening program the collection of DBS samples is recommended at 48-72 hours of life. Additional specimens are routinely collected in VLBW (BW < 1800gr) on days 14 and 30. We evaluated 156 VLBW divided into two groups according to FC levels ≥ (Group 1) or < (Group 2) cut-off value on the third sample and 84 neonates with BW > 1800gr (Group 3).
Results: Birth weight (BW) and gestational age (GA) were significantly lower in group 2 than in groups 1 and 3. On day 14, FC levels decreased significantly both in group 1 and 2; on day 30 only in Group 1 there was a significant increase of these levels. In Group 2, newborns with 23-27 weeks GA and BW < 1000gr showed FC levels on day 30 significantly lower than newborns with greater GA and BW.
Conclusions: We identified a subgroup of VLBW particularly at risk for carnitine depletion, whose outcome might be improved by precocious carnitine supplementation.