ANNALS OF LABORATORY MEDICINE

# A Population-Based Genomic Study of Inherited Metabolic Diseases Detected Through Newborn Screening

## SUPPLEMENTAL INFORMATION

### Supplemental Method 1

#### Current newborn screening pipeline

Newborn screening (NBS) tests were performed in Green Cross Laboratories, previously certified by the College of American Pathologists. Nationwide tests are conducted for congenital hypothyroidism (CH), galactosemia, maple syrup urine disease (MSUD), phenylketonuria (PKU), congenital adrenal hyperplasia (CAH), and homocystinuria, while expanded tests are performed to identify other amino acid diseases, fatty acid oxidation diseases, and organic acid diseases by MS/MS screening in Korea. The levels of thyroid hormone, 17-hydroxyprogesterone (17-OHP), and total galactose were measured in 120,700, 120,494, and 120,504 newborns, respectively. Among the study population, expanded newborn screening (NBS) tests based on tandem mass spectrometry (MS/MS) were conducted in 93,165 newborns. Levels of thyrotropin (TSH), free T4 (FT4), galactose, and 17-OHP are measured using Quantase N-TSH Screening kits (Bio-rad, UK), Microplate Neonatal FT4 (Daiichikishimoto Clinical Lab, Japan), Quantase Neonatal T-Galactose (Bio-rad, UK), and Microplate Neonatal 17-OHP Kit (Bio-rad, UK), respectively. Other metabolite levels, including those for acylcarnitines and amino acids, are determined by LC-MS/MS on API 3000 (AB SCIEX, USA). Cutoffs for FT4 and TSH are determined by values below the 99th percentile and four SDs above the mean of the reference population, respectively. Cutoffs for the other metabolites are set at 5-6 SD above the mean of the reference population. The cases with metabolite levels higher than the cutoff were retested using second samples. "Presumptive positive" was defined as the individuals with abnormal levels of a metabolite in the two separate samples.

#### DNA preparation and targeted sequencing

Several discs (3-5/sample) were collected from DBS and were incubated overnight at 37°C in nuclei lysis buffer (500 μL) and proteinase K (15 μL). After protein precipitation, the tube was centrifuged at 13,000 rpm for 10 min. The supernatant was transferred to a new tube and isopropanol (500 μL) was added. After centrifugation at 13,000 rpm for 2 min, the supernatant was discarded and 70% ethanol (500 μL) was added. After additional centrifugation at 13,000 rpm for 2 min, the pellet was air-dried for 15 min. The concentration of extracted DNA was measured by the Pico-Green dsDNA assay (Quanti-iTTMPicoGreen dsDNA kit from Invitrogen).

Briefly, 10 ng of DNA (per pool) was amplified using customized AmpliSeq primers (in two primer pools consisting of 1,034 and 1,020 primer pairs) and an Ion AmpliSeq kit 2.0. The amplicons were clonally amplified by emulsion PCR, using the IT OneTouch Template Kit 2.0 on an IT OneTouch system (Life Technologies) following the manufacturer's instructions. Twenty barcoded samples on an Ion 318 chip and 32 barcoded samples on a Proton 1 chip were sequenced. In addition to sequencing 37 control samples and 269 newborn samples, we conducted additional sequencing in duplicate using DNA from both WB and DBS from one healthy control to validate the use of DBS for NGS.

#### Sanger sequencing

The mutated DNA was amplified by PCR using primer pairs designed with Primer3 software. Relevant regions were sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems Foster City, CA, USA).

## Supplemental Method 2

Selection criteria for haplotype analysis

We limited the target to medically important diseases and chose cases with identical mutations in more than two samples. For genotyping, we applied the following criteria: SNPs located in the genomic region surrounding recurrent mutations; SNPs with AF higher than 5% in the Korean HapMap; intergenic regions extended by about 10 fold of the candidate gene size; a distance of 20-200 kb between intergenic SNPs; a distance of 1-20 kb between intragenic SNPs.

## SUPPLEMENTAL TABLES