Application of a targeted next generation sequencing panel for newborn screening

Hyunjoo Lee

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor Jin-Sung Lee

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This certifies that the Doctoral Dissertation of Hyunjoo Lee is approved.

Thesis Supervisor : Jin-Sung Lee

Thesis Committee Member#1 : Soonmyung Paik

Thesis Committee Member#2 : Kook In Park

Thesis Committee Member#3: Sahng Wook Park

Thesis Committee Member#4: Sung-Chul Jung

The Graduate School
Yonsei University

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<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Application of a targeted next generation sequencing panel for newborn screening

Hyunjoo Lee

Department of Medicine
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Background: Newborn screening (NBS) programs are very important for appropriate management of susceptible neonates to prevent serious clinical problems. However, current biochemical screening programs can provide some rates of false-positive results. After total parenteral nutrition is completely off, repetitive NBS scheduled for neonates admitted in neonatal intensive care unit (NICU) results in delayed diagnosis. Therefore, confirmatory tests are required to precisely identify all affected neonates, and to diagnosis or rule out suspected diseases. Here, we propose a workflow to complement NBS using a targeted next-generation sequencing (TNGS) panel for the early diagnosis of inherited metabolic disorders and for ruling out suspected diseases in high-risk neonates.

Material and Methods: The TNGS panel covered 198 genes associated with actionable genetic and metabolic diseases that are typically included

in a NBS program in Korea, using tandem mass spectrometry. The panel was applied to 48 infants admitted to the NICU of Severance Children's Hospital, Seoul, Korea, between May 2017 and September 2017. The infants were not selected for suspected metabolic disorders.

Results: A total of 13 variants classified as likely pathogenic or pathogenic were detected in 11 (22.9%) neonates, including six genes (*DHCR7*, *PCBD1*, *GAA*, *ALDOB*, *ATP7B* and *GBA*) associated with metabolic diseases not covered in NBS. However, since these metabolic diseases are inherited as autosomal recessive, newborns with one variant have been identified as carriers. One of the 48 infants was diagnosed with an isobutyl-CoA dehydrogenase deficiency, and false positive results of tandem mass screening were confirmed in two infants using the TNGS panel.

Conclusion: The proposed new workflow for the implementation of TNGS in conjunction with conventional NBS can allow for better management of and earlier diagnosis in susceptible infants, thus preventing the development of critical conditions in these infants.

Key words: newborn screening, targeted next-generation sequencing, stressed infants, NBS, false-positive results, inborn errors of metabolism

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I. INTRODUCTION

Newborn screening (NBS) for inborn errors of metabolism is a worldwide public health program implemented to screen asymptomatic newborns for rare inherited diseases for which early treatment results in significant reductions in morbidity and mortality. Thus, the main goal of NBS is to screen all neonates and allow for the early detection of affected infants to prevent serious clinical problems prior to their discharge from the hospital. NBS methods have evolved in recent years, especially with the development of tandem mass spectroscopy (MS/MS), which now facilitates the identification of many disorders in parallel using a single assay.[1,2] In Korea, NBS for over 50 diseases has been supported free of cost by a national program since November 2018.

However, current NBS programs show high rates of false positive results, which is inherent in the design to minimize false negatives. In addition, tests performed on sick, premature, and/or low birth weight infants that require hospitalization in a neonatal intensive care unit (NICU) result in higher

false-positive rates, requiring repeated NBS and additional follow-up tests, which can significantly delay diagnosis and treatment of sick neonates. [3,4]

Thus, there is a need for faster intervention and special planning for the neonate population to make more accurate and timely diagnoses; however, few studies have addressed the best ways to reduce the false positive rate of conventional NBS in the NICU. The Clinical and Laboratory Standards Institute recommended that infants suspected to have a metabolic disorder should undergo a follow-up NBS test to be performed only after ceasing total parenteral nutrition (TPN) or medical therapies initiated owing to the abnormal NBS. However, rapid diagnosis using conventional NBS methods for early intervention remains a challenge in stressed neonates. [5,6]

Recently, next-generation sequencing (NGS) technology has resulted in substantial improvements in diagnosing monogenic disorders in affected neonates with reduced cost, raising the possibility of implementing NGS as part of the NBS program. However, limited data are currently available for the neonatal population due to ethical, regulatory, legal, economic, and technical issues. [4,7-9]

Here, we present a new workflow to screen all infants, including stressed neonates in the NICU at higher risk of false positives, in a timely manner as part of the constitutional NBS program by applying a targeted NGS (TNGS) panel of inborn errors of metabolism. This workflow was applied to complement the previously performed NBS in high-risk neonates admitted to the NICU with the

ultimate goal of achieving early diagnosis in the neonate population (Figure 1).

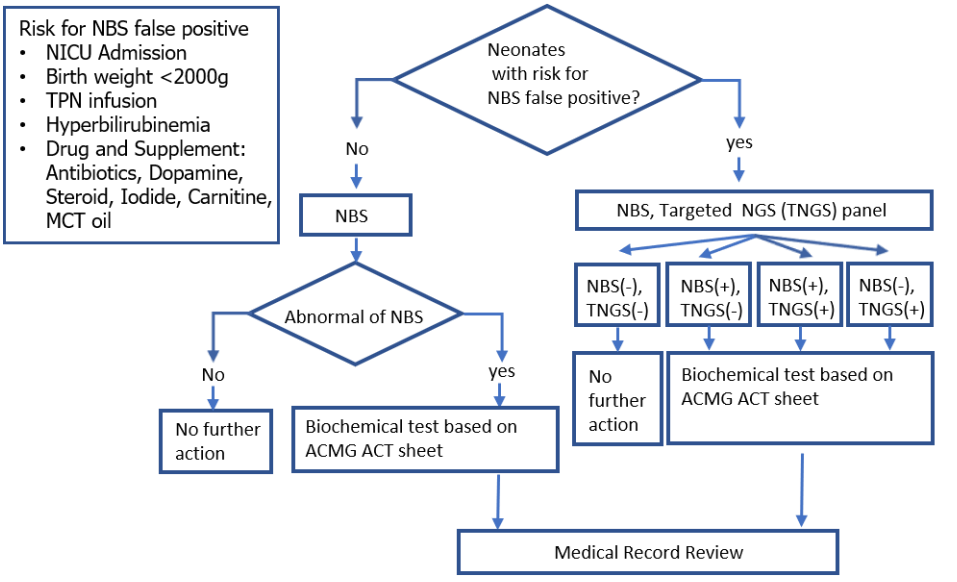

Figure 1. A new algorithm proposed for the diagnosis of inborn errors of metabolism in newborns at risk for false positive results in newborn screening (NBS). TNGS, targeted next-generation sequencing; TNGS (+), detection of variants that are likely pathogenic, pathogenic, or of unknown significance; TNGS (-), detection of benign or like benign variants or no variant detected; NBS (+), abnormal result; NBS (-), no remarkable result.

II. MATERIALS AND METHODS

1. Ethical considerations

The study was approved by the Institutional Review Board of Yonsei

University Health System (IRB, 4-2017-0127). Korean neonates or infants who were admitted to the NICU at Severance Children's Hospital (Seoul, Korea) from May 2017 to September 2017 were included in the study after obtaining written informed consent from the parents/guardians. None of the babies was selected for metabolic disorders in line with the population-based NBS program.

2. Sample preparation, sequencing, and analysis of the TNGS panel

A TNGS panel for neonatal diseases was designed and validated in a previous study [10]. A targeted gene enrichment method was used to construct libraries for subsequent determination of sequences using an NGS method with HiSeq2000 (Illumina, San Diego, CA, USA). In this study, the probe set was designed to capture 1.45 Mb covering the exons and 25 nucleotides at the flanking introns for 198 targeted genes (Appendices Table 1). Probe-library hybridization followed by capture of target genes was performed according to the manufacturer's instructions.

A total of 48 samples were registered for the TNGS panel, which when run together using the developed panel of a 1-Mb region with a 1-Gb output would likely cover ~500× reads. We used blood samples collected on filter paper and DNA was isolated from dried blood spots (DBS) with QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA). The DNA was enzyme-fragmented with NEBNext dsDNA Fragmentase® (New England Biolabs, UK), which was used to construct the library according to the protocol provided by Cellemix (Seoul, Republic of Korea). For library

enrichment, we used a custom probe set synthesized by Cellemix (Seoul, Republic of Korea). The subsequent capturing procedure was performed with the MYbaits® kit and sequencing was performed on a HiSeq 2000 system.

Basespace (Illumina Inc.) was used for data processing, alignment, variant calling, and annotations. The average coverage of target bases was 99.8%, with 98.1% of the regions of interest having at least 10X coverage and 95.4% having at least 20% coverage (Appendices Table 2). The sequenced reads were mapped to the human reference genome (UCSC hg19) with Burrows-Wheeler Aligner (BWA-0.7.7-isis-1.0.0), and variants were identified with the Genome Analysis toolkit (GATK version 1.6.23-gf0210b3). After filtering out variants using an internal database, variants with a minor allele frequency less than 1% in either the 1000 Genomes Project or Exome Aggregation Consortium database were assessed further. The criteria for classification of variants were based on the principle recommended by the American College of Medical Genetics and Genomics (ACMG) standards.[11]

III. RESULTS

1. Participant demographics

To evaluate the usefulness of the TNGS panel in NBS for neonates showing high number of false positive results, we tested 48 clinical samples for newborn diseases associated with 198 genes. The individual participants in the NICU were not selected for metabolic diseases in advance and included both full-term

and preterm infants. In this study, there were 18 (38%) preterm infants and 27 (56%) newborns with a birth weight less than 2000 g (Table 1). The average birth weight was 1998 g (range: 610–3790 g).

Table1. Demographic characteristics of patients

	N	%	Age for additional NBS (Days since birth) Mean ± SD	P value
Gender				0.828
Male	28	(58)	22.7 ± 29.1	
Female	20	(42)	22.1 ± 15.7	
Gestation Age (23~41 weeks)				<0.001
Full-term	30	(62)	7.2 ± 5.5	
Preterm	18	(38)	31.8 ± 26.3	
Birth weight				0.001
<1000 g	11	(23)	40.3 ±13.9	
1000 ~2000 g	16	(33)	31.4 ± 34.8	
2000 ~4000 g	21	(44)	10.4± 9.0	

NBS, newborn screening; SD, standard deviation.

Table2. Details of variants identified

ID	Gender	Gene	Inheritance	Mutation type		Variants	Phenotype NIM	Clinical Comments
Pathogenic, Likely Pathogenic								
D05	Female	*DHCR7*	AR	Frameshift	het	NM_001360.2:c.860delA (p.Asn287ThrfsTer6)	Smith-Lemli-Opitz syndrome (270400)	Edward syndrome, ELBW, gastroschisis,
D10	Female	*PCCB*	AR	Missense	het	NM_001178014.1:c.1364A>G (p.Tyr455Cys)	Propionic acidemia (606054)	VLBW, Hydrocephalus, IVH, s/p ileostomy, s/p EVD, cerebral palsy
D13	Female	*PCBD1*	AR	Missense	het	NM_000281.2:c.263G>A(p.Arg88Gln)	BH4 deficient hyperphenylalaninemia (264070)	ELBW, severe BPD
D17	Male	*GAA*	AR	Missense	het het	NM_000152.3:c.752C>T (p.Ser251Leu) NM_000152.3:c.761C>T (p.Ser254Leu)	Glycogen storage disease II（232300)	ELBW, meconium peritonitis, bowel perforation, severe BPD,
D27	Male	*ALDOB*	AR	Missense	het	NM_000035.3:c.1013C>G (p.Ala338Gly)	Fructose intolerance, hereditary (229600)	LBW
D30	Female	*CYP21A2*	AR	Nonsense	het	NM_000500.7:c.955C>T(p.Gln319Ter)	Congenital Adrenal hyperplasia due to 21-hydroxylase deficiency (201910)	ELBW, ambiguous genitalia, mild BPD
D33	Female	*SLC22A5*	AR	Missense	het	NM_003060.3:c.1400C>G (p.Ser467Cys)	Carnitine transporter deficiency (212140)	IUGR, CMV infection
D37	Female	*GALT*	AR	Missense	het	NM_000155.3:c.998G>A (p.Arg333Gln)	Galactosemia (230400)	IUGR, BPD, NEC, s/p ileostomy,
D39	Male	*ATP7B*	AR	Missense	het	NM_000053.3:c.2333G>T(p.Arg778Leu)	Wilson disease (253200)	Intracranial hemorrhage, Floppy infantile syndrome, Hypoxic ischemic encephalopathy,
D43	Male	*CYP21A2*	AR	Nonsense	het	NM_000500.7:c.955C>T(p.Gln319Ter)	Congenital Adrenal hyperplasia due to 21-hydroxylase deficiency (201910)	Imperforate anus s/p colostomy
D46	Female	*GBA*	AR	Missense	het	NM_001005741.2:c.754T>A(p.Phe252Ile)	Gaucher disease (230800)	VLBW, moderate BPD, neonatal apnea
		GAA	AR	Missense	het	NM_000152.3:c.2015G>A(p.Arg672Gln	Glycogen storage disease II (232300)	

VOUS								
D01	Male	*ACAD8*	AR	Missense Deletion	het het	NM_014384.2:c.557A>G (p.Asn186Ser) NM_014384.2:c.1156_1158delCAG (p.Gln386del)	Isobutyryl-CoA dehydrogenase deficiency (611283)	Newborn sick baby
D02	Female	*CFTR*	AR	Missense	het	NM_000492.3:c.1942G>A (p.Asp648Asn)	Cystic fibrosis (219700)	VLBW, Patau syndrome
D04	Male	*ABCD4*	AR	Missense	het	c.358C>G (p.His120Asp)	Methylmalonic aciduria and homocystinuria (614857)	Newborn sick baby
		MCCC1	AR	Missense	het	NM_020166.3:c.203C>G (p.Ala68Gly)	3-Methylcrotonyl-CoA carboxylase 1 deficiency (210200)	
D08	Male	*DHCR7*	AR	Missense	het	NM_001360.2:c.4G>A (p.Ala2Thr)	Smith-Lemli-Opitz syndrome (270400)	ELBW, moderate BPD, cerebellar hemorrhage
D14	Female	*DMD*	XLR	Missense	het	NM_004006.2:c.10465C>T (p.Arg3489Cys)	Duchenne muscular dystrophy (310200)	ELBW, moderate BPD, cerebellar hemorrhage
		HAL	AR	Missense	het	NM_002108.3:c.995T>C (p.Ile332Thr)	Histidinemia (235800)	VACTERAL, Kabuki syndrome
D32	Female	*HAL*	AR	Missense	het	NM_002108.3:c.1706C>T(p.Pro569Leu)	Histidinemia (235800)	ELBW, severe BPD
D35	Male	*PEX2*	AR	Missense	het	NM_001172086.1:c.206T>C (p.Ile69Thr)	Peroxisome biogenesis disorder 5A (Zellweger) /5B (614866/614867)	Vomiting, Sepsis, Pneumomediastinum
D39	Male	*GALC*	AR	Missense	het	NM_000153.3:c.199A>G (p.Thr67Ala)	Krabbe disease (245200)	Floppy infantile syndrome, Hypoxic ischemic encephalopathy, intracranial hemorrhage,
D42	Male	*ASS1*	AR	Missense	het	NM_000050.4:c.1046T>G (p.Val349Gly)	Citrullinemia (215700)	MRSA sepsis

AR, Autosomal recessive; XLR, X-linked recessive, ELBW, Extremely Low Birth Weight (<1,000g); VLBW, Very Low Birth Weight (<1,500g); LBW, Low Birth Weight (<2,500g); BPD, Bronchopulmonary dysplasia; IUGR, Intrauterine growth retardation; CMV, Cytomegalovirus

2. Variants in the TNGS panel

The clinical characteristics of all newborns included in this pilot study are summarized in Table 2. A total of 25 variants were identified in 19 patients. In accordance with the ACMG guideline, 13 variants classified as "likely pathogenic" or "pathogenic" were detected in 11 (22.9%) of the neonates. In Korea, an extended NBS test using MS/MS is performed to detect approximately 54 metabolic diseases in a conventional NBS program. However, we identified pathogenic or likely pathogenic variants reported in six genes, including those associated with metabolic diseases not covered in the NBS: *DHCR7* (Smith-Lemli-Opitz syndrome), *PCBD1* (BH4-deficient hyperphenylalaninemia), *GAA* (glycogen storage disease II), *ALDOB* (fructose intolerance, hereditary), *ATP7B* (Wilson disease), and *GBA* (Gaucher disease). Two pathogenic *GAA* variants were identified in patient D17; however, those alleles were found in the same strand in the raw data and were negative in the enzyme analysis, suggesting a diagnosis as a carrier of Pompe disease. Of the known pathogenic mutations of the *CYP21A2* gene, p. Gln319Ter is one of the most common variants related to congenital adrenal hyperplasia in Koreans, and the heterozygote mutation was identified in two unrelated patients in this study.[12] One of the patients, D30, a female with ambiguous genitalia, showed normal 17-OHP levels in the NBS screening test, and the electrolyte balance was also normal. The other newborn, D43, was a male who underwent a colostomy operation due to a congenital imperforate anus.

Three of the newborns (D01, D03, D04) showed positive results in a comprehensive NBS. In the case of D01, elevation of C4 using MS/MS resulted in suspicion of an isobutyl-CoA dehydrogenase (IBDH) deficiency and short-chain acyl-CoA dehydrogenase deficiency. According to the ACMG algorithm, ethylmalonic encephalopathy (associated with the *ETHE1* gene) is one of the diseases included in the differential diagnosis NBS.[13] As a result, two mutations in *ACAD8* were identified using the TNGS panel containing genes for all suspected congenital metabolic disorders, and the patient was ultimately diagnosed with IBDH deficiency associated with a novel compound heterozygous variant. For patients D03 and D04, increases in C5-OH acylcarnitine (3-OH isovalerylcarnitine) were reported to result in the same suspected disease of 3-methylcrotonyl-CoA carboxylase deficiency inherited in an autosomal recessive pattern. However, in the former patient, a heterozygote mutation (NM_020166.3: c.1391A>C; p. His464Pro) was identified in the *MCCC1* gene, which is reported to be benign. In the latter case, D04, one unknown variant of the *MCCC1* gene was identified and classified as a variant of unknown significance. In addition, biotinidase deficiency (associated with the *BTD* gene), which is one of the diseases causing elevated C5-OH, is not included in the Korean screening program at present but could be excluded through the TNGS panel. Ultimately, the NBS tests were confirmed to show false positive results in these two patients based on assessment with the TNGS panel.

IV. DISCUSSION and CONCLUSION

In this pilot study, we used a TNGS panel as a supplementary method for the conventional NBS program to determine the cases of false positives and achieve early diagnosis in newborns in the NICU. This expands the applications of few previous studies in which genetic confirmation tests were used only for neonates with abnormal NBS results or symptomatic disorders.[3,7,14,15]

The NBS program is a public health program in Korea aimed at screening every neonate for inherited metabolic disorders, which was introduced to identify conditions that can be critical to the child's health and survival. However, the screening program often produces high number of false positive results, which means that a baby with a suspected diagnosis may not always have health problems. To correctly identify all affected neonates, a confirmatory test, including biochemical analyses, enzyme activity, and genotyping, is required for the positive results determined with NBS. In addition, false positive results are more common for infants in the NICU, or those with low birth weight, that are ill, and on TPN, which require additional follow-up NBS and confirmatory testing. If the blood collection is delayed until the infant is completely off of TPN, it can result in a delayed diagnosis for an infant born with a low birth weight and/or preterm baby.[3-5]

To resolve these issues, we here introduce a new workflow to screen stressed newborns in the NICU undergoing the constitutional NBS program (Figure 1). The DBS samples initially collected from these infants at high risk of false

positive were sent for NBS and the TNGS panel simultaneously. NBS was conducted based on the ACMG ACT sheet and algorithm as the conventional test, followed by interpretation of genetic variants according to ACMG standards. When pathogenic, likely pathogenic, or variants of unknown significance were identified in the infants, the concordance between other confirmatory tests such as biochemical tests and variant classification was evaluated.

This is the first application of the TNGS panel in conjunction with conventional population-based NBS for infants in the NICU to reduce the rate of false positive results and diagnose the precise metabolic disorder. This algorithm might have broad implications for changing the practice in the NICU or cardiac intensive care unit to enable a faster diagnosis and thus allow for more timely intervention. In addition, the repetitive testing without an accurate diagnosis of suspected disease imposes a huge amount of stress on the family because metabolic disorders may cause a fatal progression in some cases. Moreover, individuals with one copy of a recessive allele could be identified with the TNGS panel, allowing for identification of carriers with an asymptomatic condition that might warrant regular follow-up.

A definitive diagnosis was made in only one (D01) of the 48 infants included in this pilot study, which reflects the overall rarity of metabolic disorders. Two asymptomatic infants (D03, D04) yielded false positive results, and the suspected diagnosis from the NBS was ruled out based on the mutation

detected; however, the overall false positive rate of NBS cannot be estimated with this limited sample size. In Korea, although the government-sponsored NBS program using MS/MS can screen over 50 diseases, some critical inherited disorders, such as glycogen storage disease, lysosomal storage disorder, severe combined immune deficiency disorder, adrenoleukodystrophy, and ornithine deficiency are not included. Therefore, rare metabolic disorders and several genetic disorders affecting infants and children could be screened before the onset of clinical signs using the TNGS panel despite the limited sample size in this study.

Integration of the TNGS panel with NBS might help to avoid incidental findings and later-onset diseases rather than conducting whole-genomic sequencing at birth as part of the NBS program and the specific genes included in the panel could be designed considering regional incidences based on ethnic background.[16,17] However, there is still room for improvement in the current TGNS panel, which does not cover deletion/duplication variants and deep intronic or promoter variations sufficiently, potentially resulting in increased false negative results. We also found that compound heterozygote conditions cannot be detected by the TNGS panel alone owing to the technical limitation of distinction between cis or trans variants, except for mutations that are located very close to each other as in infant D17.

Despite these limitations, we demonstrated that screening of ill infants with the TNGS panel could reduce the delayed diagnosis, even for those at high risk

of false positives using the current national NBS program. Moreover, disorders not detected in the current NBS can be diagnosed with the TNGS panel. The proposed practical workflow for conducting the TNGS panel analysis concurrently with initial NBS in stressed babies is expected to substantially reduce the number of unnecessary repetitive NGS tests and allow for the faster detection of rare metabolic disorders in infants.

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APPENDICES

Appendices Table1. The list of 198 genes

Gene		Inheritence	Phenotype MIM
ABCD4	Methylmalonic aciduria and homocystinuria, cblJ type	AR	614857
ACAD8	Isobutyryl-CoA dehydrogenase deficiency	AR	611283
ACADM	Acyl-CoA dehydrogenase, medium chain, deficiency of	AR	201450
ACADS	Acyl-CoA dehydrogenase, short-chain, deficiency of	AR	201470
ACADSB	2-methylbutyrylglycinuria	AR	610006
ACADVL	VLCAD deficiency	AR	201475
ACSF3	Combined malonic and methylmalonic aciduria	AR	614265
ACVRL1	Telangiectasia, hereditary hemorrhagic, type 2	AD	600376
AGL	Glycogen storage disease IIIa, Glycogen storage disease IIIb	AR	232400
AIPL1	Leber congenital amaurosis-4 (LCA4)	AR	604393
ALDH4A1	Hyperprolinemia, type II	AR	239510
ALDOB	Fructose intolerance, hereditary	AR	229600
ALPL	Hypophosphatasia, infantile	AR	241500
ARFGEF2	Periventricular heterotopia with microcephaly	AR	608097
ARSA	Metachromatic leukodystrophy	AR	250100
ARSB	Mucopolysaccharidosis type VI (Maroteaux-Lamy)	AR	253200
ARX	Epileptic encephalopathy, early infantile, 1	XLR	308350
ASL	Argininosuccinic aciduria	AR	207900
ASPA	Canavan disease	AR	271900
ASS1	Citrullinemia	AR	215700
ATM	Ataxia-telangiectasia	AR	208900
ATP7A	Menkes disease	XLR	309400
ATP7B	Wilson disease	AR	253200
ATP8B1	Cholestasis, progressive familial intrahepatic 1	AR	211600
AUH	3-methylglutaconic aciduria, type I	AR	250950
BCAT1	Hyperleucinemia-isoleucinemia or hypervalinemia	NA	NA
BCAT2	Hypervalinemia or hyperleucine-isoleucinemia	NA	NA

BMPR2	Pulmonary hypertension, familial primary, 1, with or without HHT	AD	178600
BRAF	Cardiofaciocutaneous syndrome	AD	115150
BTD	Biotinidase deficiency	AR	253260
BTK	Agammaglobulinemia, X-linked 1	XLR	300755
C2CD3	Orofaciodigital syndrome XIV	AR	615948
C5orf42	Orofaciodigital syndrome VI /Joubert syndrome 17	AR	277170/614615
CACNA1S	Hypokalemic periodic paralysis, type 1	AD	170400
CD320	Methylmalonic aciduria, transient, due to transcobalamin receptor defect	AR	613646
CDKL5	Epileptic encephalopathy, early infantile, 2	XLD	300672
CEP290	Leber congenital amaurosis 10 /Joubert syndrome 5	AR	611755 /610188
CFTR	Cystic fibrosis	AR	219700
CHD7	CHARGE syndrome	AD	214800
CLCN7	Osteopetrosis, autosomal dominant 2/Osteopetrosis, autosomal recessive 4	AD/AR	166600/611490
CLN3	Ceroid lipofuscinosis, neuronal, 3	AR	204200
CLPB	3-methylglutaconic aciduria, type VII, with cataracts, neurologic involvement and neutropenia	AR	616271
CNBP	Myotonic dystrophy 2	AD	602668
COL11A1	Marshall syndrome /Stickler syndrome, type II	AD	154780 /604841
COL11A2	Otospondylomegaepiphyseal dysplasia	AD/AR	184840/215150
COL1A2	Osteogenesis imperfecta, type III /typeIV	AD	259420/166220
COL2A1	Achondrogenesis, type II or hypochondrogenesis	AD	200610
COL4A3	Alport syndrome, autosomal dominant /autosomal recessive	AD/AR	104200/203780
COL4A4	Alport syndrome, autosomal recessive	AR	203780
COL4A5	Alport syndrome	XLD	301050
COL9A1	Epiphyseal dysplasia, multiple, 6/Stickler syndrome, type IV	AD/AR	614135/614134
COMP	Epiphyseal dysplasia, multiple, 1	AD	132400
CPT1A	CPT deficiency, hepatic, type IA	AR	255120
CPT2	CPT II deficiency, infantile	AR	600649

CTNS	Cystinosis, nephropathic	AR	219800
CYBB	Chronic granulomatous disease, X-linked	XLR	306400
CYP21A2	Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency	AR	201910
DDX59	Orofaciodigital syndrome V	AR	174300
DES	Cardiomyopathy, dilated, 1I /Muscular dystrophy, limb-girdle, type 2R	AD/AR	604765 /615325
DHCR7	Smith-Lemli-Opitz syndrome	AR	270400
DLAT	Pyruvate dehydrogenase E2 deficiency	AR	245348
DMD	Duchenne muscular dystrophy	XLR	310200
DNAJC19	3-methylglutaconic aciduria, type V	AR	610198
DUOX2	Thyroid dyshormonogenesis 6	AR	607200
ENG	Telangiectasia, hereditary hemorrhagic, type 1	AD	187300
ETHE1	Ethylmalonic encephalopathy	AR	602473
EYA1	Otofaciocervical syndrome/ Branchiootic syndrome 1	AD	166780/602588
F8	Hemophilia A	XLR	306700
FAH	Tyrosinemia, type I	AR	276700
FBN1	Marfan syndrome	AD	154700
FGFR1	Pfeiffer syndrome	AD	101600
FGFR2	Apert syndrome /Crouzon syndrome	AD	101200/123500
FGFR3	Achondroplasia/Hypochondroplasia	AD	100800/146000
FLNA	Congenital short bowel syndrome/Heterotopia, periventricular	XLR/XLD	300048/300049
G6PC	Glycogen storage disease Ia	AR	232200
GAA	Glycogen storage disease II	AR	232300
GALC	Krabbe disease	AR	245200
GALK1	Galactokinase deficiency with cataracts	AR	230200
GALT	Galactosemia	AR	230400
GBA	Gaucher diseaseI, II, III	AR	230800
GCDH	Glutaricaciduria, type I	AR	231670
GCH1	Dystonia, DOPA-responsive/Hyperphenylalaninemia, BH4-deficient, B,	AD/AR	128230/233910

GLA	Fabry disease	XL	301500
GLB1	GM1-gangliosidosis, type I /Mucopolysaccharidosis type IVB (Morquio)	AR	230500
GLIS3	Diabetes mellitus, neonatal, with congenital hypothyroidism	AR	610199
GNAS	Pseudohypoparathyroidism Ia /Ib/Ic	AD	103580
GUCY2D	Leber congenital amaurosis 1	AR	204000
HADH	3-hydroxyacyl-CoA dehydrogenase deficiency /Hyperinsulinemic hypoglycemia, familial, 4	AR	231530/ 609975
HADHA	long-chain hydroxyacyl-CoA dehydrogenase(LCHAD) deficiency /Trifunctional protein deficiency	AR	609016/609015
HADHB	Trifunctional protein deficiency	AR	609015
HAL	Histidinemia	AD, AR	235800
HBA1	Thalassemias, alpha-	AD	604131
HBA2	Thalassemias, alpha-	AD	604131
HBB	Delta-beta thalassemia /Sickle cell anemia	AD/AR	141749/603903
HLCS	Holocarboxylase synthetase deficiency	AR	253270
HMGCL	HMG-CoA lyase deficiency	AR	246450
HSD17B10	HSD10 mitochondrial disease	XLD	300438
IDS	Mucopolysaccharidosis II	XLR	309900
IDUA	Mucopolysaccharidosis Ih/I hs/Is	AS	607014/607015/607016
IGSF1	Hypothyroidism, central, and testicular enlargement	XLR	300888
IKBKG	Incontinentia pigmenti	XLD	308300
IL2RG	Severe combined immunodeficiency, X-linked	XLR	300400
IVD	Isovaleric acidemia	AR	243500
IYD	Thyroid dyshormonogenesis 4	AR	274800
JAG1	Alagille syndrome 1	AD	118450
KIF1B	Charcot-Marie-Tooth disease, type 2A1	AD	118210
KRAS	Noonan syndrome 3	AD	609942
L1CAM	MASA syndrome,CRASH syndrome	XLR	303350
LMNA	Muscular dystrophy, congenital/ Emery-Dreifuss muscular dystrophy 2, AD /AR/Charcot-Marie-Tooth disease, type 2B1	AD/AD/ AD/AR	613205/181350 /605588
LRP5	Osteopetrosis, autosomal dominant	AD/AR	607634/259770

	1/Osteoporosis-pseudoglioma syndrome		
MCCC1	3-Methylcrotonyl-CoA carboxylase 1 deficiency	AR	210200
MCCC2	3-Methylcrotonyl-CoA carboxylase 2 deficiency	AR	210210
MECP2	Rett syndrome	XLD	312750
MFN2	Charcot-Marie-Tooth disease, axonal, type 2A2A /2A2B	AD/AR	609260 /617087
MLYCD	Malonyl-CoA decarboxylase deficiency	AR	248360
MUT	Methylmalonic aciduria, mut(0) type	AR	251000
NF1	Neurofibromatosis, type 1	AD	162200
NF2	Neurofibromatosis, type 2	AD	101000
NKX2-1	Choreoathetosis, hypothyroidism, and neonatal respiratory distress	AD	610978
NKX2-5	Hypothyroidism, congenital nongoitrous, 5	AD	225250
NRAS	Noonan syndrome 6	AD	613224
OAT	Gyrate atrophy of choroid and retina with or without ornithinemia	AR	258870
OFD1	Orofaciodigital syndrome I /Joubert syndrome 10	XLD/XLR	311200 /300804
OPA3	3-methylglutaconic aciduria, type III/ Optic atrophy 3 with cataract	AR/AD	258501 /165300
OSTM1	Osteopetrosis, autosomal recessive 5	AR	259720
OTC	Ornithine transcarbamylase deficiency	XLR	311250
PAH	Phenylketonuria	AR	261600
PCBD1	Hyperphenylalaninemia, BH4-deficient, D	AR	264070
PCCA	Propionicacidemia	AR	606054
PCCB	Propionicacidemia	AR	606054
PDHA1	Pyruvate dehydrogenase E1-alpha deficiency	XLD	312170
PDHB	Pyruvate dehydrogenase E1-beta deficiency	AR?	614111
PEX1	Peroxisome biogenesis disorder 1A (Zellweger)	AR	214100
PEX10	Peroxisome biogenesis disorder 6A (Zellweger) /6B	AR	614870/614871
PEX12	Peroxisome biogenesis disorder 3A (Zellweger)/3B	AR	614859 /266510
PEX13	Peroxisome biogenesis disorder 11A (Zellweger)	AR	614883/614885
PEX14	Peroxisome biogenesis disorder 13A (Zellweger)	AR	614887
PEX16	Peroxisome biogenesis disorder 8A (Zellweger)/8B	AR	614876 /614877

PEX19	Peroxisome biogenesis disorder 12A (Zellweger)	AR	614886
PEX2	Peroxisome biogenesis disorder 5A (Zellweger) /5B	AR	614866/ 614867
PEX26	Peroxisome biogenesis disorder 7A (Zellweger)/7B	AR	614872 /
PEX3	Peroxisome biogenesis disorder 10A (Zellweger)/10B	AR	614882
PEX5	Peroxisome biogenesis disorder 2A (Zellweger) /2B	AR	214110
PEX6	Peroxisome biogenesis disorder 4A (Zellweger) /4B	AR	614862
PHEX	Hypophosphatemic rickets, X-linked dominant	XLD	307800
PHOX2B	Central hypoventilation syndrome, congenital, with or without Hirschsprung disease	AD	209880
PKD1	Polycystic kidney disease 1	AD	173900
PKD2	Polycystic kidney disease 2	AD	613095
PKHD1	Polycystic kidney disease 4, with or without hepatic disease	AR	263200
PLEKHM1	Osteopetrosis, autosomal recessive 6	AR	611497
PMP22	Charcot-Marie-Tooth disease, type 1A /1E	AD	118220/ 118300
POLR1C	Leukodystrophy, hypomyelinating, 11 /Treacher Collins syndrome 3	AR	616494 /248390
POLR1D	Treacher Collins syndrome 2	AD/AR	613717
PRODH	Hyperprolinemia, type I	AR	239500
PSAP	Krabbe disease, atypical /Gaucher disease, atypical/ Metachromatic leukodystrophy due to SAP-b deficiency	AR	610539 611722/249900
PTPN11	Noonan syndrome 1	AD	163950
PTS	Hyperphenylalaninemia, BH4-deficient, A	AR	261640
QDPR	Hyperphenylalaninemia, BH4-deficient, C	AR	261630
RAF1	Noonan syndrome 5	AD	611553
RBMX	Mental retardation, X-linked, syndromic 11, Shashi type	XLR	300238
RPE65	Leber congenital amaurosis 2	AR	204100
RPGRIP1	Leber congenital amaurosis 6	AR	613826
RPS6KA3	Coffin-Lowry syndrome /Mental retardation, X-linked 19	XLD	303600/ 300844
RS1	Retinoschisis	XLR	312700
SCN1A	Epileptic encephalopathy, early infantile, 6 (Dravet syndrome)	AD	607208

SCN4A	Myotonia congenita, atypical, acetazolamide-responsive/Hypokalemic periodic paralysis, type 2	AD	608390/613345
SERAC1	3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome	AR	614739
SERPINA1	Emphysema due to AAT deficiency	AR	613490
SIX1	Branchiootic syndrome 3	AD	608389
SIX5	Branchiootorenal syndrome 2	AD	610896
SLC22A5	Carnitine deficiency, systemic primary	AR	212140
SLC25A13	Citrullinemia, type II, neonatal-onset	AR	605814
SLC25A15	Hyperornithinemia-hyperammonemia-homocitrullinemia syndrome	AR	238970
SLC37A4	Glycogen storage disease Ib/Ic	AR	232220 / 232240
SLC5A5	Thyroid dyshormonogenesis 1	AR	274400
SMAD4	Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome	AD	175050
SNX10	Osteopetrosis, autosomal recessive 8	AR	615085
SOS1	Noonan syndrome 4	AD	610733
SPATA7	Leber congenital amaurosis 3	AR	604232
STX11	Hemophagocytic lymphohistiocytosis, familial, 4	AR	603552
STX16	Pseudohypoparathyroidism, type IB	AD	603233
TAZ	Barth syndrome	XLR	302060
TCIRG1	Osteopetrosis, autosomal recessive 1	AR	259700
TCOF1	Treacher Collins syndrome 1	AD	154500
TCTN3	Orofaciodigital syndrome IV / Joubert syndrome 18	AR	258860/614815
THRA	Hypothyroidism, congenital, nongoitrous, 6	AD	614450
TNFRSF11A	Osteopetrosis, autosomal recessive 7 / Osteolysis, familial expansile	AR/AD	612301 / 174810
TNFSF11	Osteopetrosis, autosomal recessive 2	AR	259710
TSC1	Tuberous sclerosis-1	AD	191100
TSC2	Tuberous sclerosis-2	AD	613254
TSHB	Hypothyroidism, congenital, nongoitrous 4	AR	275100

TSHR	Hypothyroidism, congenital, nongoitrous, 1 /Hyperthyroidism, nonautoimmune	AR/AD	275200 /609152
UGT1A1	Crigler-Najjar syndrome, type I /II	AR	218800/606785
USH2A	Usher syndrome, type 2A	AR	276901
VHL	von Hippel-Lindau syndrome	AD	193300
VPS33B	Arthrogryposis, renal dysfunction, and cholestasis 1	AR	208085
VWF	von Willebrand disease, type 1/von Willebrand disease, types 2A, 2B, 2M, and 2N	AD/AR	193400
WT1	Wilms tumor, type 1 /Denys-Drash syndrome	AD	194070 / 194080

AD, Autosomal dominant; AR, Autosomal recessive; XLR, X-linked recessive

Appendices Table2. Enrichment summary

Total Length of Targeted Reference	Total Aligned Reads	Percent Aligned Reads	Targeted Aligned Reads	Read Enrichment	Padded Target Aligned Reads	Padded Read Enrichment	Mean Region Coverage Depth	Target Coverage at 1X	Target Coverage at 10X	Target Coverage at 20X
1.46Mb	21.3Mb	99.60%	4.75Mb	22.30%	4.94Mb	23.20%	423.5	99.80%	98.10%	95.40%

ABSTRACT (IN KOREAN)

고위험군 신생아의 선천성 대사이상 선별 검사에 NGS 패널 검사 적용

<지도교수 이 진 성>

연세대학교 대학원 의학과

이 현 주

신생아 선천성 대사이상 선별 검사는 증상이 나타나기 전에 모든 신생아를 대상으로 유전성 대사질환을 진단하여 치명적인 건강상의 문제 발생을 미연에 방지할 수 있도록 적절한 치료 및 관리를 위해 매우 중요한 검사이다. 그러나 현재 시행하는 생화학적 선별 검사는 특히 조산아, 저체중 출생아, 비경구영양법을 유지하고 있는 환아, 그리고 현재 신체 건강 상태의 악화 정도에 의해 위양성의 결과를 보이는 경우가 많기 때문에, 결과 해석에 주의가 필요하며, 정확한 진단을 위해서는 유전자 검사 등의 확진 검사가 추가로 필요했다. 그래서 신생아집중치료실에 입원중인 신생아는 비경구영양을 모두 중단할 정도의 건강의 회복이나 체중이 적정체중으로 증가한 후까지 반복적인 선별검사를 진행하기 때문에 조기선별검사의 목적에 부합되지 못하고 진단이 늦어지는 문제점이 있다. 그러므로 본 연구에서는 고위험군 신생아에서 기존의 신생아 선천성 대사이상 선별 검사에 부가적으로 NGS 패널을 동시에 시행하여, 유전성 대사 질환을 조기에 진단하고 적절한 치료가 더 빨리 이루어질 수 있도록 새로운 알고리즘을 제시하려고 한다.

대한민국에서 현재 텐덤 질량분석기를 이용하여 분석하는

약 50개의 질병과 연관된 신생아 선별 검사의 진단에 필요한 유전자 및 신생아 및 영아기에 영향을 주는 유전자를 포함하여 총 198개의 유전자를 포함한 NGS 패널을 본 연구에 사용했다. 2017년 5월부터 2017년 9월까지 세브란스 병원 신생아과에서 입원치료를 받았던 48명을 대상으로 본 연구의 유용성을 확인하기 위해 파일럿 시험을 진행했다. 대상자는 기존의 신생아 선별검사에서 시행하는 것과 동일하게, 특정 대사 질환이나 유전성 질환이 의심되는 환아에 대해서 선택적인 선별을 진행하지 않았으며, 본 연구의 참여에 부모 중 한 분 이상으로부터 동의서를 받은 환아를 모두 포함 하였다.

총 48명중에서 11명(22.9%)의 신생아에서 13개의 돌연변이가 ACMG (American College of Medical Genetics and Genomics)가이드라인을 기준으로 Pathogenic, Like pathogenic으로 확인되었다. 이 확인된 변이는 기존의 신생아선별검사에서는 포함되어있지 않은 질환과 관련된 6개의 유전자(DHCR7, PCBD1, GAA, ALDOB, ATP7B, GBA)도 포함되었다. 이는 기존의 선천성 대사이상 선별검사에서 진단하지 못했던 질환도 추가적으로 진단이 가능함을 확인한 것이다. 그러나, 이 선천성 대사질환은 상염색체 열성으로 유전되는 질환이므로, 한 개의 돌연변이가 확인된 것은 보인자인 상태를 의미하는 것이다. 대상 환자의 수가 적은 한계가 있는 파이럿 연구이며, 유전성 대사질환이 희귀질환인 것을 고려 할 수 있으나, 본 연구에 포함된 48명의 환아 중에서 1명이 결과적으로 이소부티릴 코에이 탈수소 효소결핍증으로 진단 되었고, 2명의 환아는 기존의 선천성대사 선별검사와 NGS 패널 검사를 동시에 시행 한 결과로 위양성임을 확인하였다.

본 연구에서 제안된 새로운 알고리듬으로 고위험군 신생아에서 현재의 신생아선별검사와 함께 NGS 패널을 동시에 사용하여 유전성 대사질환을 조기에 진단하고, 치명적이고 중대한 질병으로의 진행을 적절한 시기에 중재를 기대할 수 있다. 또한 기존의 신생아 선별검사에서 포함이 되지 않았던 당원병, 암모니아 대사 이상 등의 질환 등을 같이 조기에 진단 할 수 있으며, 위양성일 가능성에 대해서 확인을 위해서 반복적인 선천성 대사 검사를 시행하고 또

확진 검사를 순차적으로 시행하는 동안 그 결과를 기다리는 가족들의 심리적인 부담감의 감소 효과도 기대가 된다.

핵심되는 말 : 신생아 선천성 대사이상 선별 검사, NGS패널