ORIGINAL

Next-generation sequencing for the diagnosis of patients with congenital multiple anomalies and / or intellectual disabilities

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Abstract : Background : In clinical practice, a large proportion of patients with multiple congenital anomalies and/or intellectual disabilities (MCA/ID) lacks a specific diagnosis. Recently, next-generation sequencing (NGS) has become an efficient strategy for genetic diagnosis of patients with MCA/ID. Objective : To review the utility of NGS for the diagnosis of patients with MCA/ID. Method : Patients with MCA/ID were recruited between 2013 and 2017. Molecular diagnosis was performed using NGS-based targeted panel sequencing for 4,813 genes. Promising causative variants underwent confirmation by Sanger sequencing or chromosomal microarray. Results : Eighteen patients with MCA/ID were enrolled in this study. Of them, 8 cases (44%) were diagnosed by targeted panel sequencing. Most of diagnosed patients were able to receive better counseling and more appropriate medical management. Conclusion : NGS-based targeted panel sequencing seems to be an effective testing strategy for diagnosis of patients with MCA/ID. J. Med. Invest. 67 : 246-249, August, 2020

Keywords **:** *next-generation sequencing, targeted panel sequencing, multiple congenital anomalies, intellectual disability*

INTRODUCTION

In neonatal intensive care units (NICUs) and pediatric outpatient clinics, patients with multiple congenital anomalies and/or intellectual disabilities (MCA/ID) of unknown etiology are common. The ability to provide optimal clinical management is dependent on identification of the underlying genetic cause of the disease (1). The strategy for selecting genomic diagnostic tests is highly dependent on the presenting clinical phenotype and differential diagnoses (2). Next-generation sequencing (NGS), particularly whole-exome sequencing (WES) and targeted-panel sequencing (TPS), have been adopted in clinical investigations for patients with undiagnosed MCA/ID (1,3). This is because sequencing costs have become more reasonable, and informatics approaches to NGS reporting have been developed (4). This study reviewed the utility of TPS for molecular diagnosis of patients with MCA/ID of unknown etiology in our hospital.

METHODS

This study was approved by the ethics committee at Tokushima University and all parents provided written informed consent prior to enrolment. Patients with MCA/ID were recruited for this pilot study between 2013 and 2017 from the NICU or pediatric outpatient clinic.

We extracted genomic DNA from lymphocytes according to standard methods. TPS was performed using the TruSight One Sequencing Panel kit (Illumina, San Diego, CA, USA), which includes 4813 genes from the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php) and the Online Mendelian Inheritance in Man database (www.genetests.org), along with other genes identified from commercially available panels, and a MiSeq benchtop sequencer (Illumina), followed by our pipeline for NGS data analysis with a software update specific for a bioinformatics pipeline (5). To identify presumably pathogenic single-nucleotide variants, we excluded sequence variants with low-allele frequencies, that is, > 0.01 included in the 1000 Genomes Project database (http://www.1000genomes.org), National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project (http://evs.gs.washington.edu/EVS), Human Genetic Variation Database (http://www.genome.med.kyoto-u.ac.jp/SnpDB) and integrative Japanese Genome Variation Database (https://ijgvd.megabank.tohoku.ac.jp), and compared candidate variants with the Human Gene Mutation Database Professional (http://www.hgmd.org/) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/). Detection of copy number variants using TPS data with a resolution of a single exon to several exons, depending on exon size, was performed as described previously (5). Validation of single nucleotide variants or small insertions and deletions were performed by Sanger sequencing. Validation and fine mapping of gross deletions were performed by chromosomal microarray using an Affymetrix CytoScan HD chromosome microarray platform (Affymetrix, Santa Clara, CA, USA).

RESULTS

Eighteen patients with MCA/ID were enrolled in the present study. Six patients were enrolled during hospitalization in the

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NICU, whereas 12 patients were enrolled from pediatric outpatient clinic (Table 1). TPS revealed a molecular diagnosis for 8 of 18 (44%) patients (6-10) (Table 2). Clinical benefits from the results of TPS are also shown in Table 2. Case 2 was clinically undiagnosed, but molecularly diagnosed with Lowe syndrome by detecting 1.7 Mb deletion including *OCRL1* at 6 months of age, and this early diagnosis by TPS could lead to better counseling and early initiation of treatment for rickets and renal disorder involving the proximal renal tubules (7). Case 4 presented with cardiac tumors detected on fetal ultrasonography, with tuberous sclerosis suspected from clinical evaluation (Table 1). TPS revealed a 47.8 Kb deletion involving a part of *PKD1* and a part of *TSC2*. This early diagnosis made it possible not only to initiate anti-epileptic pharmacotherapy, but also to detect polycystic kidney disease at preclinical stage. In Case 5, the early diagnosis of achondroplasia allowed us to enact precautions against sudden death due to obstructive respiratory arrest. The established diagnosis by TPS provided beneficial information to care providers regarding the natural history and allowed accurate counseling about reproductive risk. In Case 1 with CHARGE syndrome diagnosed by detecting frameshift deletion in *CHD7*, however, genetic counseling was provided at 147 days of age, but his mother

Table 1. Clinical features and tentative diagnosis before genetic testing.

Case	Tentative clinical diagnosis	Sex	Enrollment	Clinical description
1*(6)	CHARGE syndrome	M	NICU, 3m	cleft lip and palate,choanal stenosis, incomplete atrioventricular septal defect, aortic valve stenosis, dysphagia, growth retardation, cryptorchidism, micropenis, auricular malformation, deafness
2*(7)	Lowe syndrome	M	Outpatient, 6m	cataract, growth retardation, right cryptorchidism, inguinal hernia, hypotonia, nystagmus, delayed development, mild ventriculomegaly, short stature, renal tubular dysfunction
3	Developmental delay	F	Outpatient, 6m	mild developmental delay, imperforate anus, supuravalvular aortic stenosis, peripheral pulmonary artery stenosis
4	Tuberous sclerosis	M	NICU, 1m	cardiac rhabdomyomas, epilepsy, polycystic kidney, subepedymal giant cell astrocytoma, retinal hamartoma
5	Achondroplasia	M	NICU, 2m	macrocephaly, short arms and legs, frontal bossing, mid face hypoplasia
6*(8)	MCA	M	Outpatient, 4m	patent ductus arteriosus, ventricular septal defect, dilated cardiomyopathy, malrotation of intestine
7*(9)	MCA/ID	M	Outpatient, 4y	patent ductus arteriosus, forehead protrusions, flat nose bridge, West syndrome, severe ID
8*(10)	Rett syndrome	M	Outpatient, 3y	hand-wringing, ID, declaration of head growth, loss of acquired hand skill, loss of acquired language skill, impaired gait ability, breathing disturbances, inappropriate laughing/screaming spells
9	Townes Brocks syndrome	F	NICU, 9m	Tetralogy of Fallot, anal atresia, micrognathia, right anotia, left microtia, hypoplasia of the right thumb, hearing loss, vertebral anomalies, right ectopic kidney, epilepsy
10	Rubinstein-Taybi syndrome	M	Outpatient, 5y	myopathy, mental retardation, epilepsy
11	Galloway Mowat syndrome	M	NICU, 4m	congenital nephrotic syndrome, intractable epilepsy, progressive brain atrophy, pulmonary stenosis
12	ID	F	Outpatient, 3y	severe fetal growth restriction, developmental delay, scoliosis, extremely low birth weight infant
13	Blepharophimosis ptosis epicanthus inversus syndrome	F	Outpatient , 5m	blepharophimosis, ptosis, epicanthus inversus
14	MCA/ID	F	Outpatient , 11y	hypertelorism, upslanted palpebral fissure, prominent nasal bridge, mental retardation
15	MCA/ID	M	Outpatient, 9y	hypertelorism, mental retardation, autism spectrum disorder, low set ears
16	MCA/ID	M	Outpatient, 4y	hypertelorism, short columella, frontal bossing, mental retardation, breath-holoding spells, low set ears, craniosynostosis
17	MCA/ID	F	Outpatient , 13y	frontal bossing, mental retardation, migrating partial seizures of infancy, thick lip vermilion, tall chin, repeated hand-rubbing
18	MCA/ID	F	Outpatient, 11y	Severe ID, micrognathia, frontal bossing, auricular deformities, anal atresia, non-compaction of left ventricle, laryngomalacia

* Numbers indicate reference numbers.

Table 2. Details of pathogenic variants identified in molecularly diagnosed patients.

Case	affected gene	mutation type	NCBI Ref Seq	cDNA and protein changes identified	molecular diagnosis (OMIM no)	effects on medical management	genotype/ phenotype correction
1*(6)	CHD7	frameshift deletion	NM_017780.3	c.2966delG: p.Cys989Serfs *3	CHARGE syndrome (214800)	better counseling	Complete
2*(7)	OCRL1	gross deletion	NM_567158	1.7Mb deletion at Xq25-q26.1	Lowe syndrome (309000)	better counseling, early treatment for rickets and renal tubulopathy	Complete
3	LIMK1, ELNPFC2	gross deletion	-	1.4Mb deletion at chr7 : 72, 742, 266-74, 016, 767	Williams-Beuren syndrome (194050)	better counseling and early rehabilitation	Complete
4	TSC2, PKD1	partial deletion	NM_000297.4	47.8 Kb deletion at chr16 : 2121501-2169298	Tuberous sclerosis 2 (613254) Polycystic kidney disease 1 (173900)	better counseling and detection of polycystic kidney at preclinical stage	Complete
5	FGFR3	missense	NM_000142.2	c.1138G>A : p.Gly380Arg	Achondroplasia (10800)	caution for respiratory arrest	Complete
6*(8)	MMP23B, GABRD, SKI, PRDM16	gross deletion	-	2.7Mb deletion at 1p36.33-p36.32	1p36 deletion syndrome (607872)	better counseling	Complete
7*(9)	PHLLP2, WWOXX, HP	gross deletion	-	6.8Mb interstitial deletion at 16q22.2-q23.1	chromosome 16q22 deletion syndrome (614541)	better counseling	Complete
8*(10)	MECP2	duplication	NM_001110792.1	c.23_27dup p.Ser10Argfs*36	Rett syndrome (312750)	better counseling	Complete

* Numbers indicate reference numbers.
NCBI, National Center for Biotechnology Information (US)
cDNA, complementary DNA
OMIM, Online Mendelian Inheritance in Man.

did not accept his diagnosis (6). His parents therefore did not consent to surgery for atrial valve stenosis, and he died of heart failure and arrhythmia.

DISCUSSIONS

Conventional Sanger sequencing has routinely been used to identify pathogenic variants for genetic diseases, but is laborious, expensive and time-consuming for large genes with numerous exons, such as *CHD7* (37 exons covering 8,994 bp of coding sequence), as well as for multiple candidate genes (6, 11) TPS has recently been used for candidate or known disease-associated genes to diagnose patients with various types of disorders including MCA/ID (12). Technological advances have led to the ability to sequence and interpret the entire genome in less than 2 weeks (7).

According to previous reports, NGS revealed the causative gene in 40-59% of patients with MCA in NICU (2). The present study showed a similar frequency of diagnosis (44%) in MCA/ID. Because Case 1 did not present with coloboma, which is seen in 80-90% cases of CHARGE syndrome, it was difficult to obtain the definitive diagnosis due to the wide variance in the phenotype without objective evidence provided by TPS. By TPS, several syndromes were diagnosed before obtaining distinctive features of the face. Case 3 was diagnosed with Williams-Beuren syndrome at 6 months old and Case 6 was diagnosed with 1p36 deletion syndrome at 4 months of age (8). If we waited until the typical clinical features appeared in both cases, we could reach a diagnosis with fluorescence *in situ* hybridization for the specific chromosomal regions once the candidate diseases came to mind due to distinctive clinical features of the face. Because the early diagnosis allows the possibility of better counseling based on accurate outcomes and some degree of relief for family anxiety, early application of TPS may be suitable for analyses of patients with uncertain clinical phenotypes including MCA/ID.

An evaluation of the "best time" to pursue NGS is necessary, to clarify whether this method should be used as the first approach in the NICU or as a final investigation in an outpatient clinic (2). Some have argued that early testing by NGS will save money and time by ending the "diagnostic odyssey" compared with multiple serial testing. An expanded sample of patients, identified consecutively, and investigated prospectively with appropriate controls, will help to answer who and when should be offered NGS.

Several limitations need to be considered for interpreting the present results. First, the rate of genetic diagnosis is affected by the fact that this study includes patients whose clinical diagnoses are almost definitive based on clinical symptoms such as case 4 and case 5. Second, because we used TPS instead of WES, pathogenic variants in the some newly identified disease-causing genes may be missed by genetic testing. In addition, it is possible that variants within promoter and intronic regions, which would affect the transcription and splicing, of disease-causing genes may be missed by TPS. Analysis of transcripts by microarray or RNA sequencing could reveal the underlying pathogenesis.

CONCLUSION

TPS was useful for providing established diagnosis in patients with MCA/ID, although further large study is required.

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