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Research paper

Whole exome sequencing revealed mutations in *FBXL4*, *UNC80*, and *ADK* in Thai patients with severe intellectual disabilities

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ABSTRACT

Intellectual disabilities (ID) are etiologically heterogeneous. Advanced molecular techniques could be helpful in identification of the underlying genetic defects. We aimed to characterize clinical and molecular features of three Thai patients with ID. Patient 1 had ID, hypotonia and lactic acidosis. Patient 2 had ID and growth failure. Patient 3 had ID, seizure, diarrhea and hypoglycemia. Whole exome sequencing found that Patient 1 was homozygous for a nonsense, c.1303C > T (p.Arg435Ter), mutation in *FBXL4*, a gene responsible for encephalomyopathic mitochondrial DNA depletion syndrome-13 (MTDPS13). Patient 2 was compound heterozygous for two novel mutations, c.3226C > T (p.Arg1076Ter) and c.3205C > T (p.Arg1069Ter), in *UNC80*, a known gene of infantile hypotonia with psychomotor retardation and characteristic facies-2 (IHPRF2). Patient 3 was homozygous for a novel missense, c.427T > C (p.Cys143Arg), mutation in *ADK*, a known gene of adenosine kinase deficiency leading to hypermethioninemia. This study expands the mutational spectra of ID genes.

1. Introduction

Rare diseases have been defined as diseases affecting 200,000 people or fewer at any given time in the United States (Rare Disease Act, 2002) or one person out of 2000 or fewer in Europe (de Vrueh et al., 2013). The average estimation is 40 and 50 cases in 100,000 people. Given the rarity of the cases, rare diseases may be underdiagnosed or misdiagnosed. In addition, making diagnosis of rare diseases has been one of the biggest challenges (Thevenon et al., 2016). Whole exome sequencing (WES) is a technique of sequencing the entire coding regions of all genes simultaneously. It has a significant impact on unravelling genetic defects associated with several diseases as well as discovering new rare disease genes.

Intellectual disability (ID) or developmental delay accounts for 1–2% of the general population (Maulik et al., 2011).The etiology of ID could be caused by genetic and non-genetic factors. There are > 900 genetic diseases associated with ID (Chen et al., 2018). WES therefore would be of tremendous benefits in elucidating the genes responsible for ID (Topper et al., 2011). We aimed to use WES to identify the genetic defects in patients with severe ID when intensive investigations could not reveal the underlying causes.

2. Methods

Three undiagnosed patients with severe ID were recruited for WES. Informed consent was obtained from each family. Clinical data were collected. WES was performed and the data were analyzed with bioinformatics tools.

2.1. Clinical information

2.1.1. Patient 1

A 4-month-old female was referred to our hospital because of developmental delay. She was the first child of non-consanguineous

Abbreviations: BWA, Burrows-Wheeler aligner; ID, intellectual disabilities; IHPRF2, infantile hypotonia with psychomotor retardation and characteristic facies-2; mtDNA, mitochondrial DNA; MTDPS13, mitochondrial DNA depletion syndrome-13; WES, whole exome sequencing

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Table 1
Clinical and molecular features of each patient.

	Patient 1	Patient 2		Patient 3
Diagnosis	Encephalomyopathic mitochondrial DNA depletion syndrome-13 (MTDPS13)	Infantile hypotonia with psychomotor retardation and characteristic facies-2 (IHPRF2)		Hypermethioninemia due to adenosine kinase deficiency
OMIM	615471	616801		614300
Symptoms	ID, hypotonia Lactic acidosis	ID, failure to thrive, constipation		ID, epilepsy, hypoglycemia and diarrhea
Age of diagnosis	3 years	1 year		2 years
Gene	*FBXL4*	*UNC80*		*ADK*
Number of reported cases	< 100	< 30		< 30
Year of the first reported case	2013	2016		2011
Zygosity	Homozygous	Compound heterozygous		Homozygous
Nucleotide changes	NM_001278716.1: c.1303C > T	NM_032504.1: c.3226C > T	NM_032504.1:c.3205C > T	NM_001123: c.427T > C
Protein changes	p.Arg435Ter	p.Arg1076Ter	p.Arg1069Ter	p.Cys143Arg
Mutation type	Nonsense	Nonsense	Nonsense	Missense
ExAC	0.00005	No	0.00004	No
De novo mutation	No, inherited from both parents	No, inherited from the father	No, inherited from the mother	No, inherited from both parents
Previous reported mutation	Yes	No	No	No
Polyphen	NA	NA	NA	Probably damaging
SIFT	NA	NA	NA	Damaging
CADD score	44	41	36	23.8

NA, not applicable; ID, intellectual disability.

Table 2
Clinical manifestations of patients with *FBXL4*-related mtDNA maintenance defect.

Symptoms (Frequency > 50%) (El-Hattab et al., 2017)	Frequency (%) (*n* = 87)	Our patient	Symptoms (Frequency < 50%) (El-Hattab et al., 2017)	Frequency (%) (n = 87)	Our patient
Small for gestational age	59	+	Seizure	28	−
Failure to thrive	65	+	Cardiac defect:	20	−
			– Cardiomyopathy or congenital heart disease		
Developmental delay	100	+	Eye:		
			– Strabismus	18	−
			– Nystagmus	16	−
Hypotonia	94	+	Cataracts	7	+
Feeding difficulties	75	−	Elevated liver transaminases	21	−
Lactic acidemia	100	+	Inguinal/umbilical hernia	17	−
Lactate peak in MRS	73	NA	Hyperammonemia	45	+
Abnormal brain MRI:			Distinctive face:		
– White matter abnormalities	70	NA	– Depressed nasal bridge	21	+
– Cerebral atrophy	53	NA	– Low set ears	19	+
			– Prominent forehead	18	−
			Immunology		
			– Recurrent infection	25	−
			– Neutropenia	16	−

NA: not applicable.

parents. Her birth weight was 2190 g (< 3rd centile), head circumference of 34 cm (50th centile) and length of 47 cm (10th centile). Prenatal ultrasound found bilateral dilated ventricles. Postnatally, the patient had developmental delay and hypotonia. She developed posterior bilateral subcapsular cataracts at 7 months of age requiring pars plana lensectomy. Karyotyping and CT of the brain were unremarkable. At 2 years of age, she had generalized tonic clonic seizures without fever and wide anion gap metabolic acidosis. Her head circumference was 43.5 cm, height 79 cm and weight 8.8 cm; all were below the 3rd centile. She remained hypotonia. Laboratory investigations revealed lactate of 7.4 mmol/L (normal < 2), ammonia was 84.3 μmol/L (normal < 40) and venous blood gas with pH 7.35, pCO_2 26 mmHg, pO_2 50 mmHg, and HCO3 14.7 mmol/L. Urine organic analysis revealed a large amount of lactic acid. She was started on thiamine, biotin, carnitine, CoQ10 and Shohl's solution. Her lactate had been in the range of 4–9 mmol/L, ammonia 84–105 μmol/L and HCO3 13–17 mmol/L. At 2 years and 5 months old, she developed Kussmaul breathing and respiratory failure. She required hospitalization for two months and was discharged with respiratory support. She passed away at 3 years of age. Her maximal development was sitting with support at age 2.

2.1.2. Patient 2

A 9-month-old female was brought to hospital because of developmental delay and failure to thrive. She had one sister from the same non-consanguineous parents. Her sister was born without complications but had severe developmental delay and failure to thrive and passed away at the age of 3 years. The patient was born preterm at 36 weeks gestational age with birth weight of 2200 g. At 9 months of age, her weight was 4.3 kg (< 3rd centile), length 65 cm (< 3rd centile) and head circumference 42 cm (25th centile). She still could not hold her head. She had constipation for several months and hypersensitivity to

Table 3
Clinical manifestations of patients with *IHPRF2* mutations.

Clinical feature	Summary of reported patients					
	Perez et al., 2016 (*n* = 7)	Shamseldin et al., 2016 (*n* = 6)	Stray-Pedersen et al., 2016 (*n* = 4)	Valkanas et al., 2016 (*n* = 2)	He et al., 2018 (*n* = 2)	This study (*n* = 1)
Face*						
Downslanting palpebral fissure	3/7	5/6	0/4	2/2	0/2	+
Triangular face	6/7	6/6	2/4	2/2	1/2	+
Frontal bossing	5/7	NA	NA	1/2	2/2	+
Low set/posteriorly rotated ears	7/7	2/5	1/3	0/2	0/2	+
Anteverted nasal tip	7/7	3/6	1/4	0/2	0/2	+
Broad nasal bridge	7/7	2/6	2/4	0/2	0/2	+
Short and smooth philtrum	7/7	1/1	2/3	0/2	1/2	+
Micrognathia	3/7	3/6	2/4	2/2	1/2	+
Thin upper lip	6/7	0/5	0/4	2/2	1/2	+
Tented upper lip	1/2	4/6	3/3	2/2	1/2	+
Hands and feet						
Long thin fingers	7/7	NA	NA	2/2	1/2	−
Tapering of distal phalanx	7/7	NA	NA	2/2	0/2	−
Club feet	4/7	NA	NA	2/2	NA	−
Small hands and feet	NA	NA	3/4	2/2	NA	−
Orthopedic						
Scoliosis	7/7	NA	1/4	2/2	0/2	−
Contractures	7/7	1/1	NA	2/2	NA	−
Ophthalmological findings						
Strabismus	7/7	2/2	NA	2/2	2/2	−
Esotropia	7/7	NA	3/4	1/1	NA	−
Nystagmus	NA	2/2	NA	0/2	NA	−
Gastrointestinal findings						
Constipation	NA	NA	4/4	1/2	½	+
Feeding difficulties	NA	NA	3/4	2/2	0/2	+
Growth findings						
Normal birth parameters	NA	6/6	4/4	2/2	2/2	+
Height < 3rd centile	NA	5/6	4/4	2/2	1/2	+
Weight < 3rd centile	NA	5/6	4/4	2/2	1/2	+
Microcephaly	7/7	3/6	1/4	0/2	1/2	−
Developmental findings						
Severe ID or DD	7/7	6/6	4/4	2/2	2/2	+
Hypotonia	7/7	6/6	4/4	2/2	2/2	+
Global motor delay	7/7	NA	4/4	2/2	2/2	+
Walking achieved	NA	NA	2/4	1/2	1/2	NA
Absent speech or < 5 words	7/7	6/6	4/4	2/2	1/2	NA
Neurological findings						
Dystonic posture of limbs	7/7	NA	NA	2/2	NA	−
Seizures	4/7	1/6	4/4	1/2	1/2	+
Abnormal brain MRI	2/6	1/4	1/4	0/2	0/1	−
Behavioral findings						
Arm flapping	NA	NA	NA	2/2	1/2	−
Hand biting	NA	NA	NA	2/2	1/2	−
Happy disposition	NA	NA	NA	2/2	1/2	+
Self-injury	NA	NA	NA	2/2	1/2	−
Sensory hypersensitivity	NA	NA	3/4	2/2	NA	+

NA: not applicable.

sounds. Physical examination revealed brachycephaly, pointed triangular face, wide forehead, thin and tented upper lip and hypotonia (Suppl. 1). Investigations including ammonia, lactate, thyroid function test, karyotyping, CT and MRI of the brain were unremarkable. Urine organic acid analysis demonstrated moderate excretion of lactic acid and some nonspecific acids. EEG showed intermittent slowing with epileptiform activity over the mid and parasagittal areas. She had myoclonic seizures and was given phenobarbital. She received tube feedings and still could not gain much weight. At 15 months of age, she still had constipation and could not hold her head up. Her weight was 5.5 kg (< 3rd centile), length was 69 cm (< 3rd centile) and head circumference was 41.5 cm (25th centile). She was hypersensitive to sounds. Her sleep had always been interrupted with even little sound.

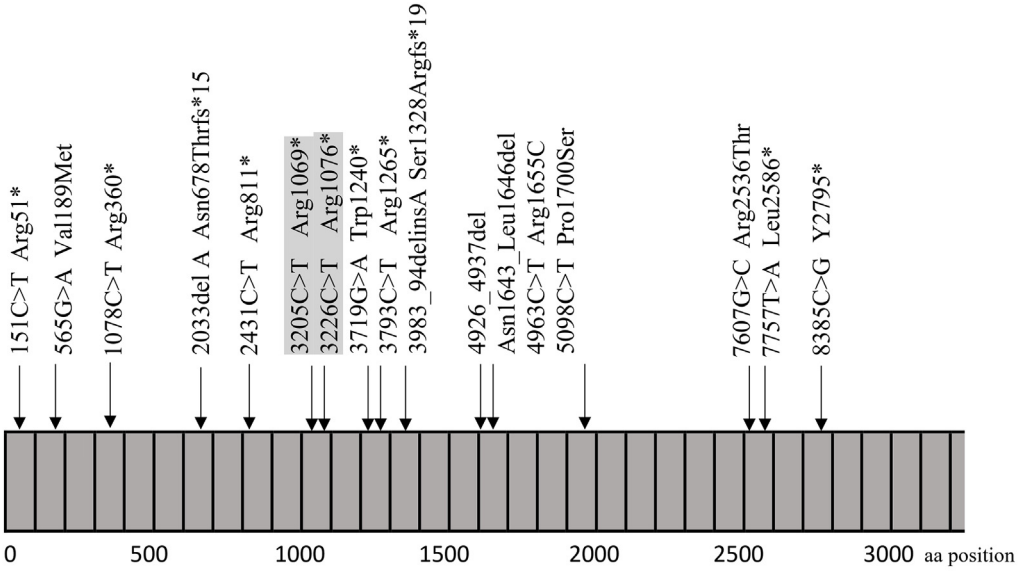
2.1.3. Patient 3

A 5-month-old girl was brought to hospital due to developmental delay and seizure. She was the second child with a birth weight of 3100 g. She was found to have developmental delay at 3 months of age. She had first seizure at 5 months of age which was controlled with phenobarbital. EEG revealed no epileptiform discharges. At age 7 months, she had several seizures and many antiepileptic drugs were given but she continued to seize a few times a day. Repeated EEG revealed no epileptiform discharges. At 10 months of age, she started having diarrhea with loose stool 10 times a day. Her diarrhea was treated with many regimens, total parenteral nutrition, and special formulas (lactose free, soy, pure amino acid formula, rice formula) without improvement. At 16 months of age, she developed alteration of consciousness from hypoglycemia. Laboratory investigations including cortisol, growth hormone, insulin levels were normal. Physical examinations revealed frontal bossing, hypertelorism and sparse hair. Complete blood count revealed only normocytic red blood cells (MCV 90, Hb 11.3 g/dL and Hct 34%). Plasma amino acid and urine organic acid analyses were unremarkable. After final diagnosis through WES, she was started on protein restriction, vitamin B6 and folinic acid. Her diarrhea and hypoglycemia resolved. At 2 years old, her weight was 10.4 kg (25th centile), height 85 cm (50th centile) and head

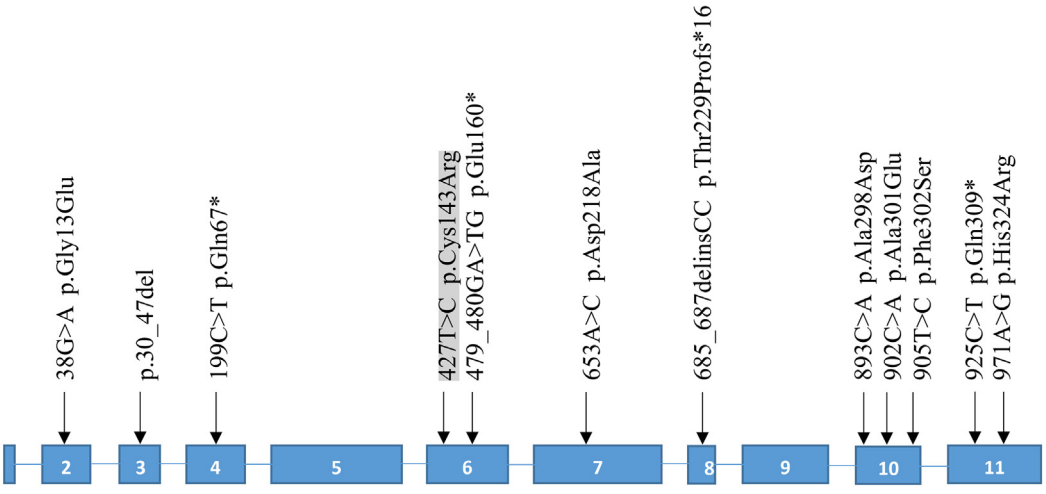
Table 4
Clinical manifestations of patients with ADK deficiency.

	Sex	Methionine (μmol/L)	ALT (U/L)	Age of onset of epilepsy (year)	Age at diagnosis (year)	Cardiac defect	Hypoglycemia	Megaloblastic anemia	Sparse hair	Others
Swedish	M	455	241	1	23	−	NA	NA	NA	−
(Shakiba et al., 2016)	F	886	447	1	10 (deceased)	−	NA	NA	NA	−
Malaysia (Shakiba et al., 2016)	M	800	200	2.11	18	−	NA	NA	NA	−
	M	550	245	10 m	11	PS	NA	NA	NA	−
	M	800	106	1.4	10	ASD	NA	NA	NA	−
	M	600	400	2	7	CoA	NA	NA	NA	−
Turkey (Staufner et al., 2015)	F	56–107	46	5 m	1.5 (deceased)	VSD, PDA, PFO	+	−	−	Strabismus
	M	20–400	469	4 m	4.7	ASD, PDA	+	−	−	Strabismus, Retinal dystrophy
	F	26–867	468	4.8	8.9	PDA, PFO, CoA	+	−	−	Abnormal dentition, Slender hand and feet
Kuwait	F	910	624	8.1	14.6	PFO	+	+	+	Strabismus, Cholelithiasis
(Staufner et al., 2015)	F	162	141	6 m	8.2	−	+	−	−	Discolored teeth, Cholelithiasis
Morocco (Staufner et al., 2015)	M	6–135	160	−	20	−	+	−	+	Slender hand and feet, Cholelithiasis
Italy (Staufner et al., 2015)	M	28–1100	1992	−	3.5	−	+	−	−	Cholelithiasis
Germany (Staufner et al., 2015)	F	30–350	172	−	8	PDA	+	−	+	Slender hands and feet
Iran (Staufner et al., 2015)	F	NA	9	1	29	−	−	+	−	−
	F	NA	13	1	23	−	−	+	−	−
Turkey (Staufner et al., 2015)	M	18–436	113	−	1.9	−	+	−	+	−
Iran (Bjursell et al., 2011)	F	1140	400	−	1	PDA, VSD	−	+	+	Carious teeth
Thailand	F	11–42	47	5 m	1.8	−	+	−	+	−
Frequency of each clinical manifestation (%)			79	74		47	77	30	46	

ASD: Atrial septal defect, CoA: Coarctation of aorta, NA: not applicable, PDA: Patent ductus arteriosus, PFO: Patent foramen ovale, PS: Pulmonic stenosis.



A) Mutations in UNC80 patients. Grey highlight shows novel mutations in this study.



NM_001123.3 (ENST00000372734.3)

B) Mutations in ADK deficiency patients. Grey highlight shows a novel mutation in this study.

Fig. 1. Diagram of the UNC80 (A) and ADK (B) and the position of each mutation. (A) Mutations in UNC80 patients. Grey highlight shows novel mutations identified in this study. (B) Mutations in ADK deficiency patients. Grey highlight shows a novel mutation.

circumference 49 cm (90th centile). She could roll over and had good head control. She continued to seize a few times a day even being treated with two medications.

2.2. DNA isolation and exome sequencing

Genomic DNA was extracted from peripheral blood leukocytes. The DNA samples were prepared as an Illumina sequencing library enriched by TruSeq® Exome Kit (Illumina Inc., Illumina, San Diago, CA) and was sequenced onto NextSeq 500 System (Illumina, San Diago, CA). The Burrows-Wheeler Aligner (BWA) was used to map raw data to the human reference genome version 19. GATK and HaplotypeCaller were used for variant calling. SNVs and Indels were annotated using SnpEff, dbpSNP 142, ClinVar, 1000 Genome, and ESP. The variants were filtered with the 2683 genes reported to be associated with abnormality of the nervous system (HPO, HP:0000707, http://compbio.charite.de/hpoweb/showterm?id=HP:0000118#id=HP:0000707). We then filtered out the variants with frequencies at least 1% in the 1000 Genomes Project and ExAC databases as well as our in-house database of 1084 unrelated Thai exomes.The novel variants were screened in the ClinVar Miner database (https://clinvarminer.genetics.utah.edu/) and the Exome Aggregation Consortium database (exac.broadinstitute.org).

3. Results

WES successfully identified biallelic mutations in all three patients (Table 1). All of the mutations are inherited from parents. A homozygous mutation, c.1303C > T in *FBXL4* was found in Patient 1 (ID with lactic acidosis). This mutation was previously reported to be pathogenic (Bonnen et al., 2013). Heterozygous mutations in *UNC80* were found in Patient 2 (ID with failure to thrive). A homozygous mutation in *ADK* was found in Patient 3 (ID with epilepsy). Patient 1 had symptoms in common with previously reported patients with *FBXL4* mutations (El-Hattab et al., 2017) (Table 2). Clinical manifestations of patients with mutations in *UNC80* (Perez et al., 2016; Shamseldin et al., 2016; Stray-Pedersen et al., 2016; Valkanas et al., 2016; He et al., 2018) and *ADK*

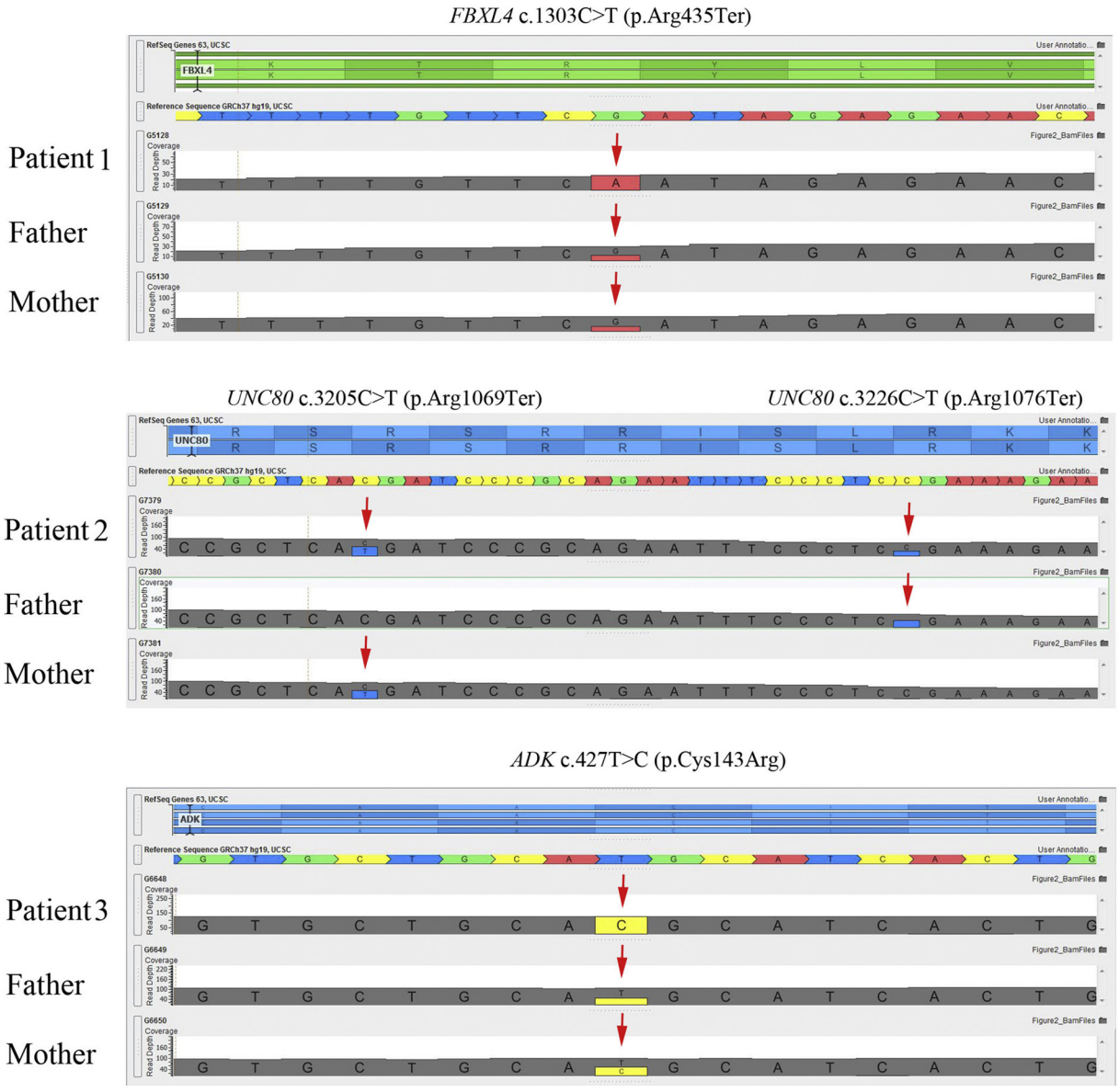


Fig. 2. Nucleotide sequences from BAM files of the patients and their parents. Aligned read coverage of the DNA regions harboring mutations identified in the patients and their parents. Patient 1 is homozygous for the c.1303C > T. The reverse strand shows G > A with all of the 25 reads being A, while her father and mother are heterozygous with the mutant to total reads of 12 to 29 and 15 to 44, respectively. Patient 2 is compound heterozygous for the c.3226C > T (C > T with the mutant to total reads of 48 to 82) and c.3205C > T (C > T with the mutant to total reads of 26 to 65). Her mother and father are heterozygous with the mutant to total reads of 45 to 86 and 40 to 71, respectively. Patient 3 is homozygous for the c.427T > C (T > C with all of the 119 reads being C), while her father and mother are heterozygous with the mutant to total reads of 42 to 103 and 47 to 97, respectively.

(Bjursell et al., 2011; Staufner et al., 2015; Barić et al., 2017) were shown in Tables 3 and 4, respectively. The previously reported mutations in the *UNC80* (Perez et al., 2016; Shamseldin et al., 2016; Stray-Pedersen et al., 2016; Valkanas et al., 2016; He et al., 2018) and the *ADK* (Bjursell et al., 2011; Staufner et al., 2015; Barić et al., 2017) genes were shown in Fig. 1.Nucleotide sequences from BAM files of all three patients with their parents were shown in Fig. 2.

4. Discussion

FBXL4 related mtDNA depletion syndrome has been reported < 100 cases since 2013. It is a multi-system disorder; however the patients primarily manifest as non-specific symptoms: growth failure, feeding difficulty, hypotonia and global developmental delay (Table 2). Other reported symptoms with frequency < 50% (El-Hattab et al., 2017) were not observed in our case. Lactic acidosis was found in this patient when she was 2 years of age. The lactate level should have been tested earlier during investigations of developmental delay even though there was no sign of metabolic acidosis. The cause of primary lactic acidosis can be divided into two groups as defects in the nuclear encoded mitochondria genes and the mitochondrial genes. A specific single gene test for *FBXL4* was not performed in Patient 1 because she had non-specific symptoms. No specific features linking to this gene were observed. Without next generation sequencing or WES, the definite diagnosis is unlikely to be made. For genotype-phenotype correlation, patients with biallelic null variants can survive with a lifespan of four years, significantly lower than those with missense variants (El-Hattab et al., 2017). Our patient had biallelic nonsense variants and survived for three years.

In Patient 2, autosomal recessive condition was predicted because of her previous affected sibling. WES could provide a definite diagnosis when the patient was less than one year of age. She had several features

of UNC80 deficiency causing infantile hypotonia with psychomotor retardation and characteristic facies-2 (IHPRF2) except joint contracture and repetitive movement. Joint contracture was found 100% (10/10) in UNC 80 patients. Repetitive movement such as arm flapping could not be recognized because she was only one year old when diagnosed. Her MRI of the brain was unremarkable. Previous studies have demonstrated some brain abnormalities (Shamseldin et al., 2016; Stray-Pedersen et al., 2016). Her constipation which was described as severe constipation in many other UNC80 cases did not improve after being treated with several regimens. Even tube feeding with high-calorie food, she still had growth retardation as previously described (Valkanas et al., 2016). Her happy manner is similar to that reported in previous studies (Valkanas et al., 2016; He et al., 2018). He et al. (2018) proposed that this behavior resembled that observed in Angelmann syndrome. As shown in Table 3, the findings that > 50% of the mutations (9/16) are nonsense could explain the disease severity.

ADK deficiency was first reported in 2011 by Bjursell et al. (2011) in two Swedish sibs with typical manifestations including severe developmental delay, seizure, liver impairment and elevated plasma methionine. Previous studies (a total of 19 cases in Table 4) reported common clinical features including developmental delay (100%), macrocephaly and frontal bossing in all except one, epilepsy (74%), congenital heart diseases (53%), hypoglycemia (77%) and elevated ALT (79%). Our patient had developmental delay and epilepsy without hypermethioninemia or liver impairment resulting in delay in diagnosis. Diarrhea which was observed in our case has not been previously described. Recurrent hypoglycemia was found in this patient but not from hyperinsulinemia as previously reported (Staufner et al., 2015; Barić et al., 2017). After treatment with folinic acid and protein restriction, diarrhea but not seizure resolved. In addition, recurrent hypoglycemia resolved from therapy without diazoxide, a treatment recommendation of recurrent hypoglycemia caused by hyperinsulinemia in ADK deficiency (Barić et al., 2017). Although the missense variant in the *ADK* gene identified in this patient has never been reported, several lines of evidence suggest it as a disease-causing mutation. Its frequency is very low (minor allele frequencies < 0.01). It was not identified in the ExAC database or 1084 in-house Thai exomes. PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) predicted this variant to be probably damaging with a score of 0.979. In addition, SIFT (http://sift.jcvi.org/) predicted it to be deleterious (Table 1).

We expect the three identified variants to be loss-of-function, as previously shown in other patients with mutations in these three genes (Bonnen et al., 2013; Shamseldin et al., 2016; He et al., 2018; Bjursell et al., 2011).

WES is a useful tool in making diagnosis of severe ID. All of the patients in this study were diagnosed at the age between 1 and 3 years. Early diagnosis could decrease frustration and anxiety in the family and facilitate family planning although specific treatment may not be available. The importance of precise diagnosis is the key step for understanding rare diseases.

We demonstrate the usefulness of WES in identification of rare diseases causing severe ID. In addition, this study expands the mutational spectra of the ID genes.

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Conflict of interest

The authors declare no conflict of interest.

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