COLD SPRING HARBOR
Molecular Case Studies

RESEARCH REPORT

Genetic diagnosis for adult patients at a genetic clinic

Kitiwan Rojnueangnit,[1] Pimjai Anthanont,[2] Thanitchet Khetkham,[3] Sukita Puttamanee,[4] and Chupong Ittiwut[5,6]

[1]Department of Pediatrics, [2]Department of Medicine, Faculty of Medicine, Thammasat University, Pathumthani, 12120 Thailand; [3]Division of Forensic Medicine, Thammasat University Hospital, 12120 Thailand; [4]Faculty of Medicine, Thammasat University, Pathumthani, 12120 Thailand; [5]Center of Excellence for Medical Genomics, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330 Thailand; [6]Excellence Center for Genomics and Precision Medicine, King Chulalongkorn Memorial Hospital, the Thai Red Cross Society, Bangkok, 10330 Thailand

Abstract Clinical utility of genetic testing has rapidly increased in the past decade to identify the definitive diagnosis, etiology, and specific management. The majority of patients receiving testing are children. There are several barriers for genetic tests in adult patients; barriers may arise from either patients or clinicians. Our study aims to realize the detection rate and the benefits of genetic tests in adults. We conducted a prospective study of 10 adult patients who were referred to a genetic clinic. Exome sequencing (ES) was pursued in all cases, and chromosomal microarray (CMA) was performed for six cases. Our result is impressive; six cases (60%) received likely pathogenic and pathogenic variants. Four definitive diagnosis cases had known pathogenic variants in *KCNJ2*, *TGFBR1*, *SCN1A*, and *FBN1*, whereas another two cases revealed novel likely pathogenic and pathogenic variants in *GNB1* and *DNAH9*. Our study demonstrates the success in genetic diagnosis in adult patients: four cases with definitive, two cases with possible, and one case with partial diagnosis. The advantage of diagnosis is beyond obtaining the diagnosis itself, but also relieving any doubt for the patient regarding any previous questionable diagnosis, guide for management, and recurrence risk in their children or family members. Therefore, this supports the value of genetic testing in adult patients.

Corresponding author:
rkitiwan@tu.ac.th

Ontology terms: aortic aneurysm; cleft palate; cleft upper lip; disproportionate tall stature; generalized tonic–clonic seizures on awakening; intellectual disability, moderate; intellectual disability, severe; primary congenital glaucoma; skeletal myopathy

Published by Cold Spring Harbor Laboratory Press

doi:10.1101/mcs.a006235

INTRODUCTION

Currently, the treatment in medicine has changed from the standard guideline treatment (one fit for all) to precision medicine, which is more specific and effective, has the fewest side effects, and avoids unnecessary investigations and therapies for individuals (Ginsburg and Phillips 2018). The goal of precision medicine is to promote better health for everyone. Therefore, getting the right definitive diagnosis by using either clinical recognition and/or specific genetic tests is the starting point. Genetic diseases nowadays encompass at least 7000 diseases (https://www.omim.org/statistics/geneMap), so only doing a physical examination may be challenging for physicians to reach the diagnosis. The advances in technology with genetic tests in clinical settings have been growing rapidly in the past decade for both cytogenetic and molecular genetic tests, such as chromosomal microarray and massive parallel sequencing. Therefore, these genetic tests and genomic medicine are significant tools to help establish the definitive diagnosis. Most genetic studies, ~90%, were requested for pediatric patients, whereas only 11%–12% were performed in adult patients (Yang et al.

2014; Posey et al. 2016). The overall diagnostic yield for molecular genetics was only 12%–22% in adult patients, versus 25%–55% in children (Yang et al. 2014; Posey et al. 2016). In the studies of adult patients with specific symptoms such as neurogenic diseases or cerebellar ataxia, the detection rate was higher (28%–32%) (Bardakjian et al. 2018; Coutelier et al. 2018; Guo et al. 2021; Mergnac et al. 2022; Sainio et al. 2022) than in chronic kidney diseases (12%–17%) (de Haan et al. 2019). Genetic tests for intellectual disability or congenital anomalies have been confirmed to be beneficial in children (Malinowski et al. 2020) and have been given a strong recommendation as first- or second-tier investigations in these pediatric patients by The American College of Medical Genetics and Genomics (Manickam et al. 2021). In comparison, there was only one paper from Canadian Family Physicians that recommended finding the cause of intellectual disability in adults (Sullivan et al. 2018). There have been several barriers for genetic tests in adult patients, including limitation of advanced technology of genetic tests in the past, financial issues due to cost of investigations, lack of interest in finding out the diagnosis, unavailable consent by caregivers who are not their parents, or hesitation to order genetic tests by adult clinicians. However, if a definitive diagnosis has been established, patients may receive more specific monitoring, preventive care, decision-making for management, specific treatment (if available), and recurrence risk evaluation in their families (Baker et al. 2012). Also, a late diagnosis is better than not having a definitive diagnosis, not only for the practical and emotional benefit of patients, but also for family members (Limb et al. 2010). Therefore, our study would like to end the diagnostic journey in adult patients who are suspected of having genetic diseases.

RESULTS

Clinical Presentation

Ten patients (six males and four females) were enrolled in our study; their ages were between 16 and 38 yr (median age 17.5 yr). Our patients were referred from general physicians (four), neurologists (two), a cardiologist (one), an ophthalmologist (one), a cardiovascular surgeon (one), and a general surgeon (one).

Patient 1

A 32-yr-old male presented with recurrent episodes of hypokalemic weakness for >10 yr with a frequency of one to two times per year. His potassium was low (2 mmol/L; normal 3.5–4.5 mmol/L) with alkalosis (HCO_3 37 mmol/L; normal 21–32 mmol/L) when he had symptoms. He also had global developmental delay and intellectual disability. He was able to perform daily living activities, communicate with short words, and do housecleaning. He had epilepsy and bilateral optic disc atrophies. He was the third son of nonconsanguineous parents. His father also had history of episodic weakness and died suddenly at 53 yr old. Physical examination revealed obesity (body mass index [BMI] 37 kg/m^2), head circumference of 59.5 cm (above the 97th percentile), wandering nystagmus, and downslanted palpebral fissure. His karyotype revealed normal male (46,XY). Twenty-four-hour Holter monitor showed no arrythmia. Computed tomography of the brain showed moderate hydrocephalus with few calcified granulomas, 1 cm in size, at the right side of the falx cerebri and right tentorium cerebelli.

Patient 2

A 16-yr-old male presented with intellectual disability, autism, and dysmorphic features. He was born at 7 mo gestational age with a birth weight of 1.9 kg. He had a left unilateral cleft lip and a ventricular septal defect. His developmental quotient was 40. He was the second child of nonconsanguineous parents. Physical examination revealed widely spaced eyes, a high-

arch palate, and postcheiloplasty. His karyotype revealed normal male (46,XY), FISH for 22 q11.2 no deletion, and negative for Fragile X test.

Patient 3

An 18-yr-old female presented with acute visual loss in the left eye due to central retinal artery occlusion. She was born at term with a birth weight of 3 kg, with the complication of heart defect and cleft palate. She had atrial septum defect, secundum type. She was the first child of nonconsanguineous parents. Physical examination revealed a height of 152 cm (at the 10th percentile), ptosis left eye, webbed neck, and low posterior hairline. Her karyotype revealed normal female (46,XX).

Patient 4

A 16-yr-old female presented with episodic weakness since she was 4–5 yr old. The episode lasted for 2–3 d, every 2–4 wk. Her blood test revealed normal potassium (3.8 mmol/L), with mild alkalosis (HCO_3 28 mmol/L), and mild elevated creatinine kinase (315 units/L; normal 27–160 units/L). Her muscle biopsy demonstrated variation of muscle fiber size and increased central nucleus periodic myopathy. She also had cardiac arrhythmia, which was premature ventricular contractions. She was the first child of nonconsanguineous parents; her father and her younger sister also had the same episodic symptoms of weakness and cardiac problem. Her father did not follow up since he was young. Physical examination demonstrated proximal muscle weakness of both upper and lower extremities, and positive Gower's sign.

Patient 5

An 18-yr-old female presented with tall stature. She also had intellectual disability and learning disability, her full intelligent quotient (IQ) was 59. She was the second child of nonconsanguineous parents. Physical examination revealed tall stature (178 cm; more than the 97th percentile), arm span: height was 1.04:1. Her facial features were long face and high-arch palate. Pectus excavatum, scoliosis, and archnodactyly with positive wrist and thumb signs were noted. She had normal karyotype (46,XX) and normal homocysteine level. Her echocardiogram and ophthalmic evaluation were both normal.

Patient 6

A 25-yr-old male presented with jaundice for 2 mo, which was caused by invasive intraductal tubulopapillary neoplasm of the pancreas. He also had dysmorphic features, severe mitral valve regurgitation with left ventricular hypertrophy, aortic root size 3.8 cm (*Z*-score 2.88) (Devereux et al. 2012), and retinal detachment. He had developmental delay and learning disability, which led to him leaving school after grade 5. He had asthma. He was the first child of nonconsanguineous parents. Physical examination revealed bilateral proptosis, micrognathia, high-arch palate with submucosal cleft, pectus carinatum, and scoliosis. His karyotype was normal (46,XY). CT of the chest and abdomen revealed tortuous abdominal aorta with normal diameter.

Patient 7

A 17-yr-old male presented with intellectual disability and dysmorphic features. He was born at term with a birth weight of 2.3 kg. He had developmental delay since he was young. His full intelligent quotient (IQ) was 53. He had a history of recurring respiratory tract infections when he was young. He also had glaucoma. He was the second child of consanguineous parents.

Physical examination revealed coarse hair, coarse face, esotropia, widely spaced eyes, broad nasal bridge, downturned corners of mouth, high-arch palate, and square-shaped fingers. His karyotype revealed normal male (46,XY).

Patient 8

A 17-yr-old male presented with cognitive regression. He was diagnosed with autism, and started treatment at the age of 9 yr. At that time, he was able to walk, run, and talk with several single words. However, for the last 3 years, he lost his ability to walk and to talk and developed a tremor. He was the second child of nonconsanguineous parents. Physical examination revealed hyposthenic build with a BMI of 13.3 kg/m^2, bradykinesia, and tremor.

Patient 9

A 25-yr-old female presented with seizure since she was 4 mo old. She had seizures two to three times per week, 1–2 min each time. She had developmental delay and intellectual disability. She was able to walk and to talk in words, but unable to do her daily activities. Her cognitive function declined for the last 2 years, and she was unable to walk and to talk. She was born at term with no complication. She was the third child of nonconsanguineous parents. Her elder sister also had seizure and intellectual disability, but no investigation was done. The brain MRI demonstrated generalized brain atrophy and architectural change at bilateral hippocampus, compatible with bilateral hippocampal sclerosis. Physical examination revealed normal head size (54 cm; at the 10th–50th percentiles) and contour without any dysmorphic features.

Patient 10

A 38-yr-old male presented with dyspnea for 3 mo, which was caused by aneurysm of the aortic root (9 cm in diameter) with aortic regurgitation. Bentall operation was performed. He was tall and had myopia since he was young. He was the only child of nonconsanguineous parents. There was negative family history of sudden death. Physical examination revealed tall stature (189 cm; above the 97th percentile), arm span: height of 1.05:1, arachnodactyly, pectus excavatum, and pes planus. Ophthalmologic examination demonstrated mild lens subluxation.

All clinical features and HPO terms of 10 patients are summarized in Table 1. Intellectual disability was the most common feature in six out of 10 patients.

Genomic Analysis

ES was performed for all patients, and CMA was performed for only six patients, who had novel or were negative for pathogenic or likely pathogenic variants. The reason for choosing ES first was based on the clinical phenotypes that possibly explain the single gene disorders. Then, for the cases where the known pathogenic variants were not detected by ES, we performed CMA for those cases.

Interestingly, ES revealed four known pathogenic variants in *KCNJ2*, *TGFBR1*, *SCN1A*, and *FBN1*; two novel likely pathogenic and pathogenic variants in *GNB1* and *DNAH9*; and three novel variants (*MID1*, *FOXP1*, and *GLI3*) of uncertain significance (Table 2). Sanger sequencing was performed in patients and six available parents (Fig. 1). The details of two novel likely pathogenic and pathogenic variants, *GNB1*:c.326G > A (p.Gly109Glu) and *DNAH9*:c.5266C > T (p.1756Ter), are presented in Table 2. A total of six pathogenic/likely pathogenic variants were inherited as autosomal dominant (five) and autosomal recessive (one).

CMA revealed variants in two patients; one was a likely pathogenic 3.8-kbp microdeletion on Chromosome 16 (Chr 16:223580–227396; hg 19), which is involved in *HBA1* and *HBA2* genes. The second had multiple large regions of homozygosity on Chromosomes

Table 1. Clinical characteristic and Human Phenotype Ontology terms of each patient

Patient number	Gender	Age at present (years)	Clinical features	Human Phenotype Ontology terms
1 (TU70)	Male	32	Severe ID, recurrent hypokalemic periodic paralysis, positive family history of sudden death, seizure	Intellectual disability HP:0001249, periodic hypokalemic paralysis HP:0008153, seizure HP:0001250, sudden death HP:0001699
2 (TU71)	Male	16	ID (DQ 40), autism, VSD, cleft lip	Cleft upper lip HP:0000204, intellectual disability HP:0001249
3 (TU72)	Female	18	Cleft palate, ASD, central retinal artery occlusion, webbed neck, ptosis	Cleft palate HP:0000175, retinal artery occlusion HP:0025326, webbed neck HP:000046, Noonan syndrome ORPHA:648
4 (TU73)	Female	16	Periodic myopathy, cardiac arrhythmia, positive family history of myopathy in the father and sister	Myopathy HP:0003198
5 (TU74)	Female	18	Tall stature, mild ID (FIQ 59), scoliosis	Tall stature HP:0000098, scoliosis HP:0002650, intellectual disability HP:0001249
6 (TU75)	Male	25	Pancreatic tumor, Pierre–Robin sequence, retinal detachment, severe mitral valve prolapse, pectus carinatum, scoliosis	Mitral valve prolapse HP:0001634, cleft palate HP:0000175
7 (TU77)	Male	17	Mild ID (FIQ 53), short stature, coarse hair, esotropia, widely spaced eyes, glaucoma, history of recurring respiratory tract infections	Intellectual disability HP:0001249, glaucoma HP:0000501, gene list in region of homozygosity (from chromosomal microarray)
8 (TU79)	Male	18	Severe ID, autism, developmental regression, tremor	Developmental regression HP:0002376, progressive intellectual disability HP:0006887, tremor HP:0001337, autistic HP:0000729
9 (TU80)	Female	25	Epilepsy, severe ID	Seizure HP:0001250
10 (TU81)	Male	38	Aortic aneurysm, tall stature, pectus excavatum	Aortic aneurysm HP:0004942

(ASD) Atrial septal defect, (HP) human phenotype, (ID) intellectual disability, (VSD) ventricular septal defect.

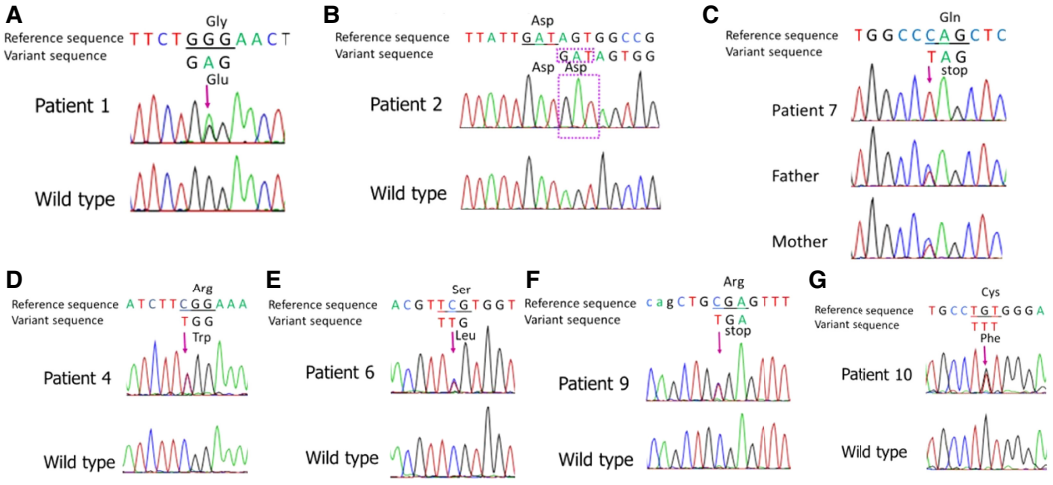

Figure 1. Sanger sequencing for each patient and available parents. (*A*) *GNB1*; c.326G > A (p.Gly109Glu) in Patient 1. (*B*) *MID1* c.1609_1611dup (p.Asp537dup) in Patient 2. (*C*) *DNAH9*; homozygous c.5266C > T (p.Gln1756Ter) in Patient 7 and heterozygous carrier in both parents. (*D*) *KCNJ2;* c.652C > T (p.Arg218Trp) in Patient 4. (*E*) *TGFBR1*; c.722C > T (p.Ser241Leu) in Patient 6. (*F*) *SCN1A;* c.2593C > T (p.Arg865Ter) in Patient 9. (*G*) FBN1; c.5699G > T (p.Cys1900Phe) in Patient 10.

Table 2. Result of genetic tests in each patient

Patient number	Gene	Location (hg19)	Nucleotide changes	Amino acid changes	Zygosity	ACMG classification	gnomAD	Thai exomes	PROVEAN	CADD	M-CAP
1	*GNB1* (NM_001282539.1)	Chr 1:1735962 C>T	c.326G>A	p.Gly109Glu	Het	Likely pathogenic: PM1, PM2, PP2, PP3	Not found	Not found	Deleterious	27.1	Possibly pathogenic
2	*MID1* (NM_000381.3)	Chr X:10422954_10422956 dupATC	c.1609_1611dup	p.Asp537dup	Het	VUS: PM2, PM4, PP3	Not found	Not found	Deleterious	21.5	N/A
3	No clinically significant variants detected	—	—	—	—	—	—	—	—	—	—
4	*KCNJ2* (NM_000891.3)	Chr 17:68171832 C>T	c.652C>T	p.Arg218Trp	Het	Likely pathogenic: PM2, PM5, PP3	Not found	Not found	Deleterious	27	Possibly pathogenic
5	*FOXP1* (NM_001244814.1)	Chr 3:71102888 T>A	c.319A>T	p.Ile107Phe	Het	VUS: BP1, PP3	Not found	2/6412 alleles	Deleterious	28	Possibly pathogenic
6	*TGFBR1* (NM_004612.4)	Chr 9:101900288 C>T	c.722C>T	p.Ser241Leu	Het	Likely pathogenic: PM1, PM2, PP3, PP5	Not found	5/6412 alleles	Deleterious	29.6	Possibly pathogenic
7	*DNAH9* (NM_001372.4)	Chr 17:11607634 C>T	c.5266C>T	p.Gln1756Ter	Hom	Pathogenic: PVS1 PM2, PM3	Not found	Not found	Deleterious	39	N/A
	GLI3 NM_000168.5	Chr 7:42007488 A>G	c.2137T>C	p.Cys713Arg	Het	VUS: BP1, PP3	Not found	Not found	Deleterious	28	Possibly pathogenic
8	No clinically significant variants detected	—	—	—	—	—	—	—	—	—	—
9	*SCN1A* (NM_001165963.4)	Chr 2:166894639 G>A	c.2593C>T	p.Arg865Ter	Het	Pathogenic: PVS1, PM2, PP3, PP5	Not found	Not found	N/A	39	N/A
10	*FBN1* (NM_000138.4)	Chr 15:48738992 C>A	c.5699G>T	p.Cys1900Phe	Het	Likely pathogenic: PM2, PM5, PP3, PP5, BP1	Not found	Not found	Deleterious	32	Possibly pathogenic

(*Continued on next page.*)

Table 2. *(Continued)*

Patient number	Gene	Location (hg19)	Nucleotide changes	Amino acid changes	MutationTaster	Previous report	Inherited	Chromosomal microarray	Diagnosis
1	*GNB1* (NM_001282539.1)	Chr 1: 1735962 C > T	c.326G > A	p.Gly109Glu	Disease causing	—	N/A	arr(1–22)x2, (X,Y)x1	Possible for GNB1 encephalopathy
2	*MID1* (NM_000381.3)	Chr X: 10422954 _10422956 dupATC	c.1609_1611dup	p.Asp537dup	Disease causing	—	N/A	arr(1–22)x2, (X,Y)x1	Possible for Opitz G/BBB syndrome
3	No clinically significant variants detected	—	—	—	—	—	N/A	Chr 16:223580–227396 × 1	Undiagnosed
4	*KCNJ2* (NM_000891.3)	Chr 17: 68171832 C > T	c.652C > T	p.Arg218Trp	Disease causing	Plaster et al. 2001; Tristani-Firouzi et al. 2002; Haruna et al. 2007; Kimura et al. 2012	From affected father	N/A	Andersen-Tawil syndrome
5	*FOXP1* (NM_001244814.1)	Chr 3: 71102888 T > A	c.319A > T	p.Ile107Phe	Disease causing	—	From unaffected father	arr(1–22,X)x2	Undiagnosed
6	*TGFBR1* (NM_004612.4)	Chr 9: 101900288 C > T	c.722C > T	p.Ser241Leu	Disease causing	Adès et al. 2006; Mátyás et al. 2006; Stheneur et al. 2008; Cardoso et al. 2012	N/A	N/A	Loeys–Dietz syndrome
7	*DNAH9* (NM_001372.4)	Chr 17: 11607634 C > T	c.5266C > T	p.Gln1756Ter	Disease causing	—	From both heterozygous parents	Regions of homozygosity of 1q23.2q31.1,6q25.1q26, 8q22.3q24.11,9q31.1q34.13,	Primary ciliary dyskinesia (partial diagnosis)

(*Continued on next page.*)

Table 2. (*Continued*)

Patient number	Gene	Location (hg19)	Nucleotide changes	Amino acid changes	MutationTaster	Previous report	Inherited	Chromosomal microarray	Diagnosis
7	*GLI3* NM_000168.5	Chr 7: 42007488 A > G	c.2137T > C	p.Cys713Arg	Disease causing	—	From unaffected mother	10q11.23q23.31,17p13.1 p11.2, 17q22q25.1	
8	No clinically significant variants detected	—	—	—	—	—	N/A	arr(1–22)x2, (X,Y)x1	Undiagnosed
9	*SCN1A* (NM_001165963.4)	Chr 2: 166894639 G > A	c.2593C > T	p.Arg865Ter	Disease causing	Depienne et al. 2009; Lim et al. 2011; Xu et al. 2015; Kothur et al. 2018	N/A	N/A	Dravet syndrome
10	*FBN1* (NM_000138.4)	Chr 15: 48738992 C > A	c.5699G > T	p.Cys1900Phe	Disease causing	Hung et al. 2009	N/A	N/A	Marfan syndrome

(N/A) Not applicable, (VUS) variant uncertain significance.

1, 6, 8, 9, 10, 11, and 17 with a size between 11 and 39 Mbp with a total size of 167 Mbp (Table 2).

Therefore, impressively, we identified the four definitive genetic diagnoses, two possible genetic diagnoses, and one partial genetic diagnosis in our 10 patients.

DISCUSSION

Our study demonstrates a worthy genetic diagnostic yield for the definitive, possible, and partial diagnosis in adult patients by using singleton ES plus CMA. Although it was clear that trio whole exome gives more yield in diagnosis (Lee et al. 2014; Dragojlovic et al. 2018; Tan et al. 2019), nevertheless, parents of adult patients were less available for testing compared with children patients for several reasons, such as having passed away, living far away, or being reluctant to do tests. The cost of trio ES is another important reason.

Intellectual disability was found in most patients, varying from mild to severe. The yield of intellectual disability diagnosis by using both CMA and ES was 25% (Wang et al. 2020). Our study demonstrated a higher rate of detection in the patients with severe intellectual disability, together with major anomalies or seizure (three out of four, 75%), than those presenting with mild intellectual disability (one out of two, 50%).

In patient 1, a novel variant, p.Gly109Glu in exon 7 of *GNB1*, was identified. This patient's phenotypes of intellectual disability, macrocephaly, and bilateral optic atrophies were compatible with the *GNB1* encephalopathy (OMIM #616973) or intellectual developmental disorder autosomal dominant 42. This has been recently discovered as a new rare disease (Petrovski et al. 2016), given less than 60 clinical case reports related to this gene, and most patients' variants were missense (88%) (Petrovski et al. 2016; Hemati et al. 2018; Da Silva et al. 2021). Nearly 90% of variants were reported in the hotspot location of exon 6 and exon 7 (Hemati et al. 2018; Da Silva et al. 2021), and functional studies of several missense variants in these two exons demonstrated loss of function (Lohmann et al. 2017). Our novel variant has been classified as likely pathogenic as classified by ACMGG, not seen in gnomAD, conserved until *C. elegans* species, and deleterious or possibly pathogenic in all silico predictor programs (CADD, M-CAP, MutationTeaser, and PROVEAN). The *Z*-score for missense variants in *GNB1* is 3.86 (https://gnomad.broadinstitute.org), which above a significant threshold (*Z*-score of 3.09; equal to a *P*-value of 10^{-3}) to distinguish transcripts in a significant depletion of missense variation (Lek et al. 2016; Harrison et al. 2019). Therefore, this patient was a possible diagnosis for *GNB1* encephalopathy.

However, we were unable to identify any genetic cause of the episodic hypokalemic paralysis and history of positive family member with sudden death; nevertheless, all clinical phenotypes of Patient 1 might be caused from more than one etiology. Therefore, further investigation such as genome sequencing may reveal more details in this case.

In Patient 7, there are novel homozygous nonsense variants in *DNAH9*, which correlated with area of loss of heterozygosity identified in chromosomal SNP microarray. This p.Gln1756Ter variant in *DNAH9* is interesting for his condition, given mutation in this gene is associated with primary ciliary dyskinesia (Fassad et al. 2018). This patient had a history of recurrent episodes of pneumonia and recurrent respiratory tract infections when he was young, but we were unable to evaluate his infertility. This novel variant has been pathogenic as classified by ACMGG, and not seen in gnomAD. However, this variant cannot explain his intellectual disability and dysmorphic features. Therefore, we concluded this patient received only partial diagnosis.

Another variant, p.Cys713Arg in *GLI3*, was identified in this patient. He had intellectual disability with widely spaced eyes and broad nasal bridge, but did not have any

macrocephaly or limb anomalies, which present in the classic phenotype of GLI3-related syndrome (Pallister–Hall syndrome or Greig cephalopolysyndactyly). The missense novel variant was classified as uncertain significance by ACMG, plus inherited from an unaffected mother. However, variable phenotype and incomplete penetrance were reported in GLI3-related syndrome (Debeer et al. 2003). Although, we used a combination of genetic testing between chromosomal SNP microarray and ES to analyze this patient, there were no interesting homozygous variants in several regions of homozygosity to explain his intellectual disability and glaucoma.

In Patient 2, a novel in-frame duplication of p.Asp537dup of *MID1* was revealed. Our variant was in exon 8 on the SPRY domain, which is a more common location of mutations (De Falco et al. 2003). MID1 is a microtubule-associated protein. The function of PRY–SPRY domains is critical for looping and ligand binding affinity (Hu et al. 2012). Mutations in these domains disturb the binding function (Woo et al. 2006). There was one previous report for c.1608_1611dupTGAT 4-bp dup (Fontanella et al. 2008), which was the same codon but predicting the effect of frameshift. This novel variant has uncertain significance as classified by ACMGG. Opitz G/BBB syndrome is also a possible diagnosis given this patient's phenotype of intellectual disability, cleft lip, cardiac defect, and widely spaced eyes. *FOXP* pathogenic variants are associated with intellectual disability, minor dysmorphic features, craniosynostosis, and osteochondromas (Tolchin et al. 2020). In Patient 5, the p. Ile107Phe variant in *FOXP* was classified by ACMG as a variant of uncertain significance and was inherited from an unaffected father. Therefore, this patient was less likely to be affected by this variant.

In two cases (Patients 6 and 10) that were clinically suspicious for Loeys–Dietz and Marfan syndrome, the molecular findings were confirmed by identifying known pathogenic variants in *TGFBR1* and *FBN1*, respectively.

TGFBR1, c.722C > T (p.Ser241Leu), was seen in several patients with Loeys–Dietz syndrome (Adès et al. 2006; Mátyás et al. 2006; Stheneur et al. 2008), and a functional study revealed that this variant inhibits the expression of TGF-β signaling by an upstream phosphorylation mechanism (Cardoso et al. 2012). TGF-β signaling involves growth inhibition and suppression of tumor progression; therefore, risk of cancers will increase if TGF-β signal is decreased or absent (MacCarrick et al. 2014). There was a previous report about an increased risk of cancer, including pancreatic cancer in one patient with the *TGFBR1* pathologic variant (Tran-Fadulu et al. 2009), which is the same as in our patient who had pancreatic cancer. TGF-β signal also plays a role in the immune system, which regulates regulatory T cell maturation and immune homeostasis. Therefore, patients with *TGFBR1* are highly likely to develop allergic disease, such as asthma, which was diagnosed in 50% of patients with *TGFBR1* (Frischmeyer-Guerrerio et al. 2013), as presented in our patient.

FBN1, c.5699G > T (p.Cys1900Phe), was reported in a patient with Marfan syndrome (Hung et al. 2009), and although there has not been any functional study analysis for this variant, the same codon variant, p.Cys1900, was also seen in several reports of patients with Marfan syndrome (Arbustini et al. 2005; Stheneur et al. 2009; Franken et al. 2016). Our patient's clinical features were compatible with classic Marfan syndrome.

SCN1A, c.2593C > T (p.Arg865Ter), was identified in patients with epilepsy syndrome (Depienne et al. 2009; Lim et al. 2011; Xu et al. 2015; Kothur et al. 2018), although no previous functional study was performed. This pathogenic variant, p.Arg865Ter, was associated with classic infantile-onset epileptic encephalopathy (Depienne et al. 2009; Lim et al. 2011; Xu et al. 2015; Kothur et al. 2018), which was similar to our Patient 9, who had onset at 4 mo old.

Patient 4 had episodic weakness and cardiac arrhythmia, a known pathogenic variant in *KCNJ2*, c.652C > T (p.Arg218Trp), that has been previously reported in several patients with Anderson syndrome (Tristani-Firouzi et al. 2002; Haruna et al. 2007; Kimura et al. 2012), and a

functional study for this specific variant demonstrated a loss-of-function effect (Plaster et al. 2001).

Our findings strongly present the benefits of genetic testing. The seven patients received a confirmed, possible, or partial diagnosis. The higher detection rate in our study than in previous studies in adult patients (Yang et al. 2014; Posey et al. 2016; Bardakjian et al. 2018; Coutelier et al. 2018; de Haan et al. 2019) might be influenced by the small cohort, which potentially influenced the chances. Clinical phenotypes of Marfan and Loeys–Dietz syndrome were quite directly caused by genetic etiology. Most of the participants did not have any previous genetic tests, except for karyotype in some cases. Severe intellectual disability was more likely be identified in the etiology. The median age of our participants was 17–18 yr, and six cases were younger than 18 yr, which may classify as pediatric patients in other countries; nevertheless, three participants who were undiagnosed were 18 yr. Therefore, the age might not be an issue.

The diagnosis is not only for the patients themselves, but also for Patient 4's father and sister, both of whom had the same symptoms. Although there is no specific treatment, monitoring and surveillance for comorbidity and symptomatic treatment to shorten the episodic duration are applicable. Also, the diagnosis in Patient 10 led to testing of the patient's children, who have a 50% risk of having the same disease. If any child has a mutation, cardiac and ophthalmologic monitoring should be performed, but if there is a negative result, that child does not require any surveillance. The diagnoses led to the patients and their families in our study expressing relief at the outcome.

The limitations of genetic diagnosis in developing countries, such as in Thailand, are due to several reasons. First, the main reason is the cost of genetic tests, which are quite expensive, and most tests are not covered by universal health care or any health insurance. Second, the number of geneticists is limited. Third, the number of patients with rare diseases seems to be small when compared with common health issues, such as infection, hypertension, diabetes mellitus, or malignancy; therefore, the priority of the health policy needs to serve the common diseases. Last, patients and their families generally do not seek a diagnosis because they believe that genetic diseases cannot be cured, and therefore the definitive diagnosis is not important.

In conclusion, genetic etiology was identified for most adult patients who were referred to a genetic clinic, which led to specific management and monitoring for each patient. Our findings encourage clinicians to pursue genetic testing for a definitive diagnosis in adult patients with suspected genetic diseases. Singleton ES should be considered an effective first-tier technique for medical professionals, especially in developing countries. However, our study was limited by having only 10 adult patients, since few parents were available for testing and there was a lack of functional analysis for the novel likely pathogenic variants.

METHODS

This study was approved by the Human Research Ethics Committee of Thammasat University (Medicine), MTU-EC-PE-0-096/63.

All participants were Thai adult patients older than 15 yr of age, who had suspected underlying genetic diseases but did not have any definitive diagnosis and had consulted the genetic clinic at Thammasat University Hospital during August 2020 to February 2021. All recruited patients or their guardians gave written informed consent. Complete patient history, a three-generation pedigree, physical examination data, and all previous tests were collected by a geneticist (K.R.).

Peripheral blood was drawn from the patients and parents, if available. Genomic DNA was isolated from leukocytes by using the Purgene DNA extraction kit. Exome sequencing

(ES) was performed by Illumina HiSeq platform (Macrogen). All variant annotations were analyzed by standard protocol (Burrows-Wheeler Alignment tool [BWA]) (Li and Durbin 2009). Then, the variants were filtered by a minor allele frequency (MAF) of >0.01 in the database for single nucleotide polymorphisms in the whole 1000 genome data (phase 3). Gene target analysis approach was used for their phenotypes based on Human Phenotype Ontology (HPO) terms (Köhler et al. 2014) for each patient. The classification used for genetic variant interpretation was based on recommendations from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology criteria for variant classification (Richards et al. 2015). In silico predictive programs, including M-CAP (http://bejerano.stanford.edu/mcap), CADD (https://cadd.gs.washington.edu/snv), MutationTaster (https://www.mutationtaster.org), and PROVEAN (http://provean.jcvi.org/index.php) were used to determine supposed evidence of pathogenicity of identified variants. Determining the novelty of all variants was done by searching in Clinvar (https://www.ncbi.nlm.nih.gov/clinvar), gnomAD (https://gnomad.broadinstitute.org), HGMD (http://www.biobase-international.com), and our in-house 3206 Thai WES database. Sanger sequencing was performed to confirm the presence of pathogenic and likely pathogenic variants in our patients and available parents.

Chromosome microarray (CMA) was performed by using CytoScan 750K microarray platform (Applied Biosystems), consisting of 750,436 oligonucleotide and 200,436 single nucleotide polymorphism probes across the genome. The analysis was performed by using Chromosome Analysis Suite software version 4.1.0.90 (Applied Biosystems), NCBI build 37.1 (hg19). The Database of Genomic Variants (DGV) and the Thai CNV database (Suktitipat et al. 2014) were used to exclude common structural variations in the Thai population. The classification used for copy number variant interpretation was based on recommendations from the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) (Riggs et al. 2020).

ADDITIONAL INFORMATION

Data Deposition and Access

Additional data are available in the Clinvar database (https://www.ncbi.nlm.nih.gov/clinvar/) with the accession numbers SCV002583251 NM_002074.5(*GNB1*): c.326G > A (p.Gly109Glu); SCV002583252 NM_000381.4(*MID1*): c.1609_1611dup (p.Asp537_Ser538insAsp); SCV002583253 NM_000891.3(*KCNJ2*): c.652C > T (p.Arg218Trp); SCV002583254 NM_004612.4(*TGFBR1*): c.722C > T (p.Ser241Leu); SCV002583255 NM_001372.4(*DNAH9*): c.5266C > T (p.Gln1756Ter); SCV002583256 NM_001165963.4(*SCN1A*): c.2593C > T (p.Arg865Ter); SCV002583257NM_000138.5(*FBN1*): c.5699G > T (p.Cys1900Phe); SCV002583287 NM_001349338.3(*FOXP1*): c.319A > T (p.Ile107Phe); and SCV002583288 NM_000168.6(*GLI3*): c.2137T > C (p.Cys713Arg).

Ethics Statement

Our study was performed in accordance with the Declaration of Helsinki and approved by the Human Research Ethics Committee of Thammasat University (Medicine), MTU-EC-PE-0-096/63. All recruited patients or their guardians gave written informed consent.

Acknowledgments

We thank our patients and their families for participation in this study; the Faculty of Medicine, Thammasat University for grant (no.18/2563); and Ms. Sam Ormond for English language editing.

Competing Interest Statement

The authors have declared no competing interest.

Referees

Jorge Diogo Da Silva
Ann Katherine M. Foreman

Received July 15, 2022; accepted in revised form October 4, 2022.

Author Contributions

K.R. designed the study, performed, and interpreted the genetic tests, and wrote the manuscript. P.A. reviewed the manuscript. T.K. and S.P. performed genetic testing, and C.I. reviewed variant interpretation.

Funding

This research received funding from Faculty of Medicine, Thammasat University (grant no. 18/2563).

REFERENCES

Adès LC, Sullivan K, Biggin A, Haan EA, Brett M, Holman KJ, Dixon J, Robertson S, Holmes AD, Rogers J, et al. 2006. *FBN1, TGFBR1,* and the Marfan–craniosynostosis/mental retardation disorders revisited. *Am J Med Genet A* **140:** 1047–1058. doi:10.1002/ajmg.a.31202

Arbustini E, Grasso M, Ansaldi S, Malattia C, Pilotto A, Porcu E, Disabella E, Marziliano N, Pisani A, Lanzarini L, et al. 2005. Identification of sixty-two novel and twelve known *FBN1* mutations in eighty-one unrelated probands with Marfan syndrome and other fibrillinopathies. *Hum Mutat* **26:** 494. doi:10.1002/humu.9377

Baker K, Raymond FL, Bass N. 2012. Genetic investigation for adults with intellectual disability: opportunities and challenges. *Curr Opin Neurol* **25:** 150–158. doi:10.1097/WCO.0b013e328351820e

Bardakjian TM, Helbig I, Quinn C, Elman LB, McCluskey LF, Scherer SS, Gonzalez-Alegre P. 2018. Genetic test utilization and diagnostic yield in adult patients with neurological disorders. *Neurogenetics* **19:** 105–110. doi:10.1007/s10048-018-0544-x

Cardoso S, Robertson SP, Daniel PB. 2012. *TGFBR1* mutations associated with Loeys–Dietz syndrome are inactivating. *J Recept Signal Transduct Res* **32:** 150–155. doi:10.3109/10799893.2012.664553

Coutelier M, Hammer MB, Stevanin G, Monin ML, Davoine CS, Mochel F, Labauge P, Ewenczyk C, Ding J, Gibbs JR, et al. 2018. Efficacy of exome-targeted capture sequencing to detect mutations in known cerebellar ataxia genes. *JAMA Neurol* **75:** 591–599. doi:10.1001/jamaneurol.2017.5121

Da Silva JD, Costa MD, Almeida B, Lopes F, Maciel P, Teixeira-Castro A. 2021. Case report: a novel *GNB1* mutation causes global developmental delay with intellectual disability and behavioral disorders. *Front Neurol* **12:** 735549. doi:10.3389/fneur.2021.735549

Debeer P, Peeters H, Driess S, De Smet L, Freese K, Matthijs G, Bornholdt D, Devriendt K, Grzeschik KH, Fryns JP, et al. 2003. Variable phenotype in Greig cephalopolysyndactyly syndrome: clinical and radiological findings in 4 independent families and 3 sporadic cases with identified *GLI3* mutations. *Am J Med Genet A* **120a:** 49–58. doi:10.1002/ajmg.a.20018

De Falco F, Cainarca S, Andolfi G, Ferrentino R, Berti C, Rodríguez Criado G, Rittinger O, Dennis N, Odent S, Rastogi A, et al. 2003. X-linked Opitz syndrome: novel mutations in the *MID1* gene and redefinition of the clinical spectrum. *Am J Med Genet A* **120a:** 222–228. doi:10.1002/ajmg.a.10265

de Haan A, Eijgelsheim M, Vogt L, Knoers NVAM, de Borst MH. 2019. Diagnostic yield of next-generation sequencing in patients with chronic kidney disease of unknown etiology. *Front Genet* **10:** 1264. doi:10.3389/fgene.2019.01264

Depienne C, Trouillard O, Saint-Martin C, Gourfinkel-An I, Bouteiller D, Carpentier W, Keren B, Abert B, Gautier A, Baulac S, et al. 2009. Spectrum of *SCN1A* gene mutations associated with Dravet syndrome: analysis of 333 patients. *J Med Genet* **46:** 183–191. doi:10.1136/jmg.2008.062323

Devereux RB, de Simone G, Arnett DK, Best LG, Boerwinkle E, Howard BV, Kitzman D, Lee ET, Mosley TH, Weder A, et al. 2012. Normal limits in relation to age, body size and gender of two-dimensional echocardiographic aortic root dimensions in persons ≥15 years of age. *Am J Cardiol* **110:** 1189–1194. doi:10.1016/j.amjcard.2012.05.063

Dragojlovic N, Elliott AM, Adam S, van Karnebeek C, Lehman A, Mwenifumbo JC, Nelson TN, du Souich C, Friedman JM, Lynd LD. 2018. The cost and diagnostic yield of exome sequencing for children with suspected genetic disorders: a benchmarking study. *Genet Med* **20:** 1–9. doi:10.1038/gim.2017.226

Fassad MR, Shoemark A, Legendre M, Hirst RA, Koll F, le Borgne P, Louis B, Daudvohra F, Patel MP, Thomas L, et al. 2018. Mutations in outer dynein arm heavy chain *DNAH9* cause motile cilia defects and situs inversus. *Am J Hum Genet* **103:** 984–994. doi:10.1016/j.ajhg.2018.10.016

Fontanella B, Russolillo G, Meroni G. 2008. *MID1* mutations in patients with X-linked Opitz G/BBB syndrome. *Hum Mutat* **29:** 584–594. doi:10.1002/humu.20706

Franken R, Groenink M, de Waard V, Feenstra HM, Scholte AJ, van den Berg MP, Pals G, Zwinderman AH, Timmermans J, Mulder BJ. 2016. Genotype impacts survival in Marfan syndrome. *Eur Heart J* **37:** 3285–3290. doi:10.1093/eurheartj/ehv739

Frischmeyer-Guerrerio PA, Guerrerio AL, Oswald G, Chichester K, Myers L, Halushka MK, Oliva-Hemker M, Wood RA, Dietz HC. 2013. TGFβ receptor mutations impose a strong predisposition for human allergic disease. *Sci Transl Med* **5:** 195ra194. doi:10.1126/scitranslmed.3006448

Ginsburg GS, Phillips KA. 2018. Precision medicine: from science to value. *Health Aff (Millwood)* **37:** 694–701. doi:10.1377/hlthaff.2017.1624

Guo MH, Bardakjian TM, Brzozowski MR, Scherer SS, Quinn C, Elman L, Orthmann-Murphy J, Tropea TF, Ellis CA, Gonzalez-Alegre P. 2021. Temporal trends and yield of clinical diagnostic genetic testing in adult neurology. *Am J Med Genet A* **185:** 2922–2928. doi:10.1002/ajmg.a.62372

Harrison SM, Biesecker LG, Rehm HL. 2019. Overview of specifications to the ACMG/AMP variant interpretation guidelines. *Curr Protoc Hum Genet* **103:** e93. doi:10.1002/cphg.93

Haruna Y, Kobori A, Makiyama T, Yoshida H, Akao M, Doi T, Tsuji K, Ono S, Nishio Y, Shimizu W, et al. 2007. Genotype-phenotype correlations of *KCNJ2* mutations in Japanese patients with Andersen-Tawil syndrome. *Hum Mutat* **28:** 208. doi:10.1002/humu.9483

Hemati P, Revah-Politi A, Bassan H, Petrovski S, Bilancia CG, Ramsey K, Griffin NG, Bier L, Cho MT, Rosello M, et al. 2018. Refining the phenotype associated with *GNB1* mutations: clinical data on 18 newly identified patients and review of the literature. *Am J Med Genet A* **176:** 2259–2275. doi:10.1002/ajmg.a.40472

Hu CH, Liu YF, Yu JS, Ng YY, Chen SJ, Su PH, Chen JY. 2012. A *MID1* gene mutation in a patient with Opitz G/BBB syndrome that altered the 3D structure of SPRY domain. *Am J Med Genet A* **158a:** 726–731. doi:10.1002/ajmg.a.35216

Hung CC, Lin SY, Lee CN, Cheng HY, Lin SP, Chen MR, Chen CP, Chang CH, Lin CY, Yu CC, et al. 2009. Mutation spectrum of the fibrillin-1 (*FBN1*) gene in Taiwanese patients with Marfan syndrome. *Ann Hum Genet* **73:** 559–567. doi:10.1111/j.1469-1809.2009.00545.x

Kimura H, Zhou J, Kawamura M, Itoh H, Mizusawa Y, Ding WG, Wu J, Ohno S, Makiyama T, Miyamoto A, et al. 2012. Phenotype variability in patients carrying *KCNJ2* mutations. *Circ Cardiovasc Genet* **5:** 344–353. doi:10.1161/CIRCGENETICS.111.962316

Köhler S, Doelken SC, Mungall CJ, Bauer S, Firth HV, Bailleul-Forestier I, Black GC, Brown DL, Brudno M, Campbell J, et al. 2014. The Human Phenotype Ontology project: linking molecular biology and disease through phenotype data. *Nucl Acids Res* **42:** D966–D974. doi:10.1093/nar/gkt1026

Kothur K, Holman K, Farnsworth E, Ho G, Lorentzos M, Troedson C, Gupta S, Webster R, Procopis PG, Menezes MP, et al. 2018. Diagnostic yield of targeted massively parallel sequencing in children with epileptic encephalopathy. *Seizure* **59:** 132–140. doi:10.1016/j.seizure.2018.05.005

Lee H, Deignan JL, Dorrani N, Strom SP, Kantarci S, Quintero-Rivera F, Das K, Toy T, Harry B, Yourshaw M, et al. 2014. Clinical exome sequencing for genetic identification of rare Mendelian disorders. *J Am Med Assoc* **312:** 1880–1887. doi:10.1001/jama.2014.14604

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, et al. 2016. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536:** 285–291. doi:10.1038/nature19057

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25:** 1754–1760. doi:10.1093/bioinformatics/btp324

Lim BC, Hwang H, Chae JH, Choi JE, Hwang YS, Kang SH, Ki CS, Kim KJ. 2011. *SCN1A* mutational analysis in Korean patients with Dravet syndrome. *Seizure* **20:** 789–794. doi:10.1016/j.seizure.2011.08.002

Limb L, Nutt S, Sen A. 2010. *Experiences of rare diseases: an insight from patients and families*. Rare Disease UK (RDUK), London.

Lohmann K, Masuho I, Patil DN, Baumann H, Hebert E, Steinrücke S, Trujillano D, Skamangas NK, Dobricic V, Hüning I, et al. 2017. Novel *GNB1* mutations disrupt assembly and function of G protein heterotrimers and cause global developmental delay in humans. *Hum Mol Genet* **26:** 1078–1086. doi:10.1093/hmg/ddx018

MacCarrick G, Black JH III, Bowdin S, El-Hamamsy I, Frischmeyer-Guerrerio PA, Guerrerio AL, Sponseller PD, Loeys B, Dietz HC III. 2014. Loeys–Dietz syndrome: a primer for diagnosis and management. *Genet Med* **16:** 576–587. doi:10.1038/gim.2014.11

Malinowski J, Miller DT, Demmer L, Gannon J, Pereira EM, Schroeder MC, Scheuner MT, Tsai AC, Hickey SE, Shen J. 2020. Systematic evidence-based review: outcomes from exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability. *Genet Med* **22:** 986–1004. doi:10.1038/s41436-020-0771-z

Manickam K, McClain MR, Demmer LA, Biswas S, Kearney HM, Malinowski J, Massingham LJ, Miller D, Yu TW, Hisama FM. 2021. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* **23:** 2029–2037. doi:10.1038/s41436-021-01242-6

Mátyás G, Arnold E, Carrel T, Baumgartner D, Boileau C, Berger W, Steinmann B. 2006. Identification and in silico analyses of novel *TGFBR1* and *TGFBR2* mutations in Marfan syndrome-related disorders. *Hum Mutat* **27:** 760–769. doi:10.1002/humu.20353

Mergnac JP, Wiedemann A, Chery C, Ravel JM, Namour F, Guéant JL, Feillet F, Oussalah A. 2022. Diagnostic yield of clinical exome sequencing as a first-tier genetic test for the diagnosis of genetic disorders in pediatric patients: results from a referral center study. *Hum Genet* **141:** 1269–1278. doi:10.1007/s00439-021-02358-0

Petrovski S, Küry S, Myers CT, Anyane-Yeboa K, Cogné B, Bialer M, Xia F, Hemati P, Riviello J, Mehaffey M, et al. 2016. Germline *de novo* mutations in *GNB1* cause severe neurodevelopmental disability, hypotonia, and seizures. *Am J Hum Genet* **98:** 1001–1010. doi:10.1016/j.ajhg.2016.03.011

Plaster NM, Tawil R, Tristani-Firouzi M, Canún S, Bendahhou S, Tsunoda A, Donaldson MR, Iannaccone ST, Brunt E, Barohn R, et al. 2001. Mutations in Kir2.1 cause the developmental and episodic electrical phenotypes of Andersen's syndrome. *Cell* **105:** 511–519. doi:10.1016/S0092-8674(01)00342-7

Posey JE, Rosenfeld JA, James RA, Bainbridge M, Niu Z, Wang X, Dhar S, Wiszniewski W, Akdemir ZH, Gambin T, et al. 2016. Molecular diagnostic experience of whole-exome sequencing in adult patients. *Genet Med* **18:** 678–685. doi:10.1038/gim.2015.142

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, et al. 2015. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* **17:** 405–424. doi:10.1038/gim.2015.30

Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, Raca G, Ritter DI, South ST, Thorland EC, et al. 2020. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet Med* **22:** 245–257. doi:10.1038/s41436-019-0686-8

Sainio MT, Aaltio J, Hyttinen V, Kortelainen M, Ojanen S, Paetau A, Tienari P, Ylikallio E, Auranen M, Tyynismaa H. 2022. Effectiveness of clinical exome sequencing in adult patients with difficult-to-diagnose neurological disorders. *Acta Neurol Scand* **145:** 63–72. doi:10.1111/ane.13522

Stheneur C, Collod-Béroud G, Faivre L, Gouya L, Sultan G, Le Parc JM, Moura B, Attias D, Muti C, Sznajder M, et al. 2008. Identification of 23 *TGFBR2* and 6 *TGFBR1* gene mutations and genotype-phenotype investigations in 457 patients with Marfan syndrome type I and II, Loeys–Dietz syndrome and related disorders. *Hum Mutat* **29:** E284–E295. doi:10.1002/humu.20871

Stheneur C, Collod-Béroud G, Faivre L, Buyck JF, Gouya L, Le Parc JM, Moura B, Muti C, Grandchamp B, Sultan G, et al. 2009. Identification of the minimal combination of clinical features in probands for efficient mutation detection in the *FBN1* gene. *Eur J Hum Genet* **17:** 1121–1128. doi:10.1038/ejhg.2009.36

Suktitipat B, Naktang C, Mhuantong W, Tularak T, Artiwet P, Pasomsap E, Jongjaroenprasert W, Fuchareon S, Mahasirimongkol S, Chantratita W, et al. 2014. Copy number variation in Thai population. *PLoS ONE* **9:** e104355. doi:10.1371/journal.pone.0104355

Sullivan WF, Diepstra H, Heng J, Ally S, Bradley E, Casson I, Hennen B, Kelly M, Korossy M, McNeil K, et al. 2018. Primary care of adults with intellectual and developmental disabilities: 2018 Canadian consensus guidelines. *Can Fam Physician* **64:** 254–279.

Tan TY, Lunke S, Chong B, Phelan D, Fanjul-Fernandez M, Marum JE, Kumar VS, Stark Z, Yeung A, Brown NJ, et al. 2019. A head-to-head evaluation of the diagnostic efficacy and costs of trio versus singleton exome sequencing analysis. *Eur J Hum Genet* **27:** 1791–1799. doi:10.1038/s41431-019-0471-9

Tolchin D, Yeager JP, Prasad P, Dorrani N, Russi AS, Martinez-Agosto JA, Haseeb A, Angelozzi M, Santen GWE, Ruivenkamp C, et al. 2020. *De novo SOX6* variants cause a neurodevelopmental syndrome associated with ADHD, craniosynostosis, and osteochondromas. *Am J Hum Genet* **106:** 830–845. doi:10.1016/j.ajhg.2020.04.015

Tran-Fadulu V, Pannu H, Kim DH, Vick GW, Lonsford CM, Lafont AL, Boccalandro C, Smart S, Peterson KL, Hain JZ, et al. 2009. Analysis of multigenerational families with thoracic aortic aneurysms and dissections due to *TGFBR1* or *TGFBR2* mutations. *J Med Genet* **46:** 607–613. doi:10.1136/jmg.2008.062844

Tristani-Firouzi M, Jensen JL, Donaldson MR, Sansone V, Meola G, Hahn A, Bendahhou S, Kwiecinski H, Fidzianska A, Plaster N, et al. 2002. Functional and clinical characterization of *KCNJ2* mutations associated with LQT7 (Andersen syndrome). *J Clin Invest* **110:** 381–388. doi:10.1172/JCI15183

Wang J, Wang Y, Wang L, Chen WY, Sheng M. 2020. The diagnostic yield of intellectual disability: combined whole genome low-coverage sequencing and medical exome sequencing. *BMC Med Genomics* **13:** 70. doi:10.1186/s12920-020-0726-x

Woo J-S, Imm J-H, Min C-K, Kim K-J, Cha S-S, Oh B-H. 2006. Structural and functional insights into the B30.2/SPRY domain. *EMBO J* **25:** 1353–1363. doi:10.1038/sj.emboj.7600994

Xu X, Yang X, Wu Q, Liu A, Yang X, Ye AY, Huang AY, Li J, Wang M, Yu Z, et al. 2015. Amplicon resequencing identified parental mosaicism for approximately 10% of 'de novo' *SCN1A* mutations in children with Dravet syndrome. *Hum Mutat* **36:** 861–872. doi:10.1002/humu.22819

Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, Ward P, Braxton A, Wang M, Buhay C, et al. 2014. Molecular findings among patients referred for clinical whole-exome sequencing. *J Am Med Assoc* **312:** 1870–1879. doi:10.1001/jama.2014.14601