Novel Causative Variants in *DYRK1A*, *KARS*, and *KAT6A* Associated with Intellectual Disability and Additional Phenotypic Features

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Abstract

Keywords

- ▶ ataxia
- ▶ hearing loss
- ▶ intellectual disability
- ▶ microcephaly
- ▶ seizures

Patients with unclear patterns of developmental and cognitive delay may go years without a definitive diagnosis despite extensive testing due to overlapping phenotypes of many genetic disorders. In this study, we identified causative variants in *DYRK1A*, *KARS*, or *KAT6A* in four individuals with global developmental delay and various findings including microcephaly and sensorineural hearing loss using whole exome sequencing. We present the cognitive, neurologic, and physical findings of four individuals to expand the clinical knowledge of possible features of the phenotypes of three rare genetic disorders. Through this process, we provide support for the use of whole exome sequencing in the setting of severe, intellectual disability or in those in whom a genetic disorder is suspected despite initial negative testing.

Introduction

Identifying a definitive diagnosis in individuals with developmental and cognitive delay is a challenging aspect of clinical genetics. Although chromosomal microarray analysis is the first line in genetic screening for such patients, it only has a 12 to 19% rate of genetic diagnosis.^{1,2} For the undiagnosed individuals with unclear phenotypic patterns, whole exome sequencing (WES) has emerged as the most effective laboratory testing in identifying a definitive diagnosis.^{3,4} In the current study, we report on four patients of various ages in three unrelated families who presented with a wide array of fractures, including undiagnosed global developmental delay, microcephaly, sensorineural hearing loss, ataxia, and additional signs and symptoms. Chromosomal microarray was nondiagnostic in these four patients. WES revealed causative variants in *DYRK1A* (MIM 600855), *KARS* (MIM 601421), and *KAT6A* (MIM 601408). All these variants were confirmed by Sanger sequencing.

Clinical Report

A summary of phenotypic features of all three families is shown in ▶Table 1 and specific features are exhibited in ▶Fig. 1. WES was performed as previously reported.^{5,6} The institutional review board of the University of Miami approved the study, and a signed informed consent form was obtained from each participant or, in the case of a minor, from parents.

Family 1

The proband is an 8-year-old Caucasian/Latin American girl (birth weight 2.41 kg, 3rd percentile; birth length 44.5 cm, < 3rd percentile) born at term via vaginal delivery whose neonatal period was complicated by a 5-day stay in the neonatal intensive care unit (NICU) due to feeding difficulties and apnea. She was immediately noted to have distinctive facial features, a high-arched palate, and aplasia cutis congenita (▶Fig. 1A). An echocardiogram done at the time

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Table 1 Comparison of phenotypic features of present and prior cases

Gene	Present case	Summary—54 cases	Present case—case 1	Present case—case 2	Summary—8 cases	Present case	Summary—17 cases
<i>Neurologic features</i>	<i>DYRK1A</i>	<i>DYRK1A</i>	<i>KARS</i>	<i>KARS</i>	<i>KARS</i>	<i>KAT6A</i>	<i>KAT6A</i>
Global developmental delay	+	53 (98%)	+	+	4 (50%)	+	17 (100%)
Speech delay	+	51 (94%)	+	+	5 (63%)	+	17 (100%)
Abnormal gait/ataxia	+	26 (48%)	—	+	—	—	—
Hypotonia	—	—	+	+	1 (13%)	+	13 (76%)
Seizures	+	28 (52%)	+	+	2 (25%)	—	1 (6%)
Febrile convulsions	—	31 (57%)	—	—	—	—	—
Hearing impairment	—	—	+	+	5 (63%)	—	—
Elevated CPK	+	—	—	—	—	—	—
Other neurologic features	Autism	16 (Autism) (30%)	—	—	4 (50%)	—	2 (12%)
<i>Craniofacial features</i>							
Facial gestalt	+	53 (98%)	—	—	—	+	16 (94%)
Microcephaly	+	44 (81%)	+	+	1 (13%)	+	14 (82%)
Bitemporal narrowing	—	Common	—	—	—	+	7 (41%)
Broad nasal tip	—	—	—	—	—	+	14 (82%)
Large/low set/posteriorly rotated ears	Small/ — /+	Common/ — /—	—	—	—	—/ + /+	4/5/7 (24/29/41%)
Thin upper lip	—	—	—	—	—	—	9 (53%)
Epicanthal folds	—	—	—	—	—	—	4 (24%)
Preauricular pits/sinus	—	—	—	—	—	+	3 (18%)
Downturn corners of mouth	—	—	—	—	—	—	5 (29%)
Other craniofacial features	Downslanting palpebral fissures	53 (98%)	—	—	—	Protruding tongue, full lower lip	5 (29%)
<i>Other features</i>							
Cardiac defect	Subvalvular aortic membrane	8 (15%)	—	—	—	ASD	11 (65%)
Brain abnormalities	—	32 (59%)	—	—	—	—	2 (12%)
Feeding problems	+	44 (81%)	—	—	—	+	11 (65%)
Eye problems	Ptosis, strabismus, Duane's anomaly	25 (46%)	—	—	3 (38%)	Ptosis, esotropia, myopia, astigmatism	11 (65%)
Other features	Aplasia cutis congenita, broad thumb, great toe, and terminal phalanges, VUR		Wheelchair-bound, osteoporosis, β -thalassemia minor	Torticollis		Single palmar crease	1 (6%)

Abbreviations: ASD, atrial septal defect; CPK, creatine phosphokinase; EEG, electroencephalogram; VUR, vesicoureteral reflux.
Note: Comparison of the phenotypic features of the present case(s) for *DYRK1A*, *KARS*, and *KAT6A* with a summary of the most common phenotypic features described in previously published cases. Numbers in the summary columns indicate the total number of patients described as having a given phenotypic trait out of the total number of cases noted at the top of each summary column. Percentages in the summary columns indicate the percentage of the total number of patients described as having a given phenotypic trait out of the total number of cases.
A (+) symbol in a column indicates that the given phenotypic feature was reported in the case.
A (—) symbol in a column indicates that the given phenotypic feature was not reported in the case.

Fig. 1 Images depicting key phenotypic features of present cases. Family 1: *DYRK1A*—(A) deep set eyes with down-slanting palpebral fissures, ptosis of the left eyelid and strabismus of the left eye, synophrys, high nasal bridge, prominent nose with columella extending beyond the nasal tip; (B) broad first digits on the upper extremity; (C) posteriorly rotated ears with small earlobes. Family 3: *KAT6A*—(D) coarse facial appearance, full lower lip, protruding tongue, bilateral ptosis (right greater than left), microcephaly; (E) single palmar crease; (F) bilateral preauricular pits and low-set ears.

showed an obstructive subvalvular aortic membrane and patent ductus arteriosus (PDA) for which she underwent surgical repair. On ultrasound, the patient was shown to have left vesicoureteral reflux complicated by multiple urinary tract infections, but this subsequently resolved. She has global developmental delay and intellectual disability. She walked at age 2 years and 9 months, and she currently has a broad, unsteady gait. Her speech is limited to a few words including colors and numbers. She developed slowly without regression. She is currently able to swim and feed herself. At age 3, she had significantly elevated creatine phosphokinase (CPK) levels. CPK electrophoresis revealed macro-CK type 1, which does not have a demonstrated pathologic significance.⁷ CPK levels at ages 4 and 6 were 3849 IU/L and 8076 IU/L, respectively. A subsequent muscle biopsy showed congenital fiber-type disproportion (CFTD). At 6 years of age, she was diagnosed with autism spectrum disorder and developed a seizure disorder now managed by Levetiracetam (Keppra) (►Table 1). Family history is remarkable for a previous miscarriage in the early first trimester and an abortion of a fetus with partial chromosomal duplication (22q13.1). The elder sibling has attention-deficit/hyperactivity disorder (ADHD) and dyslexia. There is no history of birth defects.

On physical examination she has deep set eyes with down-slanting palpebral fissures, ptosis of the left eyelid, and strabismus of the left eye (►Fig. 1A). Duane's anomaly is present on the right eye, and this eye does not move beyond midline to the lateral position. She has broad first digits on the upper and lower extremities and broad terminal phalanges of the upper extremities (►Fig. 1B). Additional facial features include synophrys, high nasal bridge, a prominent nose with columella extending beyond the nasal tip, and posteriorly rotated ears with small earlobes bilaterally (►Fig. 1C) (►Table 1). Her gait is wobbly, wide-based, and uncoordinated; she is unable to run. Biometry at her last evaluation revealed a weight of 22.7 kg (8th percentile), height of 121.5 cm (4th percentile), and head circumference of 50.5 cm (< 25th percentile) with a body mass index (BMI) of 15.36 kg/m² (33rd percentile).

Prior negative genetic testing includes a karyotype and single-nucleotide polymorphism (SNP) microarray analysis, Fragile X, 7-dehydrocholesterol testing, *PTPN11* (MIM 176876) mutation analysis, and transferrin isoelectric focusing. Metabolic testing with plasma amino acids, urine organic acids, plasma acylcarnitine profile, and total and free carnitines was also within normal range. Mutation analyses for

Table 2 Characteristics of the identified variants

Gene	Transcript ID	cDNA	Protein	SIFT	Polyphen2	PROVEAN	CADD	ExAC	ACMG
DYRK1A	NM_130436.2	c.638-1G > T	n.a.	n.a.	n.a.	n.a.	25	0	P
KARS	NM_001130089.1	c.1577C > T	p.Ala526Val	DM	PDM	DM	32	0	VUS
KARS	NM_001130089.1	c.1466T > G	p.Phe489Cys	DM	PDM	DM	29.2	0	VUS
KAT6A	NM_001099412.1	c.3850_3851dup	p.Gln1285Glyfs*10	n.a.	n.a.	n.a.	32	0	P

Abbreviations: ACMG, American College of Medical Genetics (Richards et al, 2015); CADD, combined annotation-dependent depletion; cDNA, complementary DNA; DM, damaging; ExAC, Exome Aggregation Consortium allele frequency; n.a., not available; P, pathogenic; PDM, probably damaging; PROVEAN, Protein Variation Effect Analyzer; SIFT, sorting intolerant from tolerant; VUS, variant unknown significance.
Note: Summary of the characteristics of the novel variants found in the present cases that were used to determine pathogenicity.

three genes associated with CFTD, *TPM3* (MIM 191030), *ACTA1* (MIM 102610), and *SEPN1* (MIM 606210) were negative. In addition, sequencing of the mitochondrial genome and electron transport chain complexes in muscle specimens were both negative.

WES of the parents-patient trio revealed a heterozygous de novo variant (NM_130436.2:c.638-1G > T) in *DYRK1A* in the proband. The variant has not been previously reported and is defined as pathogenic according to the American College of Medical Genetics (ACMG) 2015 guidelines (►Table 2).⁶ Although the patient's clinical phenotype resembled that of Rubinstein-Taybi syndrome, mutations in the two known causative genes, *CREBBP* (MIM 600140) and *EP300* (MIM 602700) were not found.

Family 2

Family 2 consists of two Caucasian sisters born to nonconsanguineous parents. Individual 1 is an 18-year-old woman (birth weight 3.37 kg, 48th percentile) born at term via cesarean section with an uncomplicated neonatal period. Her mother was age 32 years and her father was age 30 years at the time of her birth. She was first noted to have microcephaly < 5th percentile at 9 months of age and had a normal computed tomographic (CT) scan at that time. She sat at 9 month and she was thought to have mild cerebral palsy at 13 months. Her developmental milestones improved with occupational and physical therapies. At 15 months, she developed coordination problems; she crawled at 17 months. She has always been with walker or wheelchair-bound. The patient's development is notable for significantly delayed speech with an inability to communicate without utilizing sign language. At 16 months, she was diagnosed with hearing loss, and a unilateral cochlear implant was placed at 22 months. At 2.5 years, she had a fall complicated by epidural hematoma and a seizure. CT of the brain showed calcification of the left occipitoparietal junction. Although she did not continue to have seizures, further EEG studies were consistent with symptomatic generalized epilepsy, and she was diagnosed with absence seizures. The patient was diagnosed with osteoporosis at age 18 with a history of two prior hip fractures (►Table 1). Family history is significant for dyslexia in a paternal uncle and grandmother and for delayed walking in a paternal first cousin.

Her physical examination showed spasticity with increased deep tendon reflexes. No dysmorphic facial

features were seen and external ears were normal. Biometry at her last evaluation revealed a weight of 35.56 kg (< 1st percentile), height of 146.5 cm (1st percentile), and head circumference of 51 cm (< 2nd percentile) with a BMI of 16.57 kg/m² (1st percentile).

Individual 2 is a 15-year-old girl (birth weight 2.61 kg, 6th percentile; birth length 49.5 cm, 53rd percentile) born full term via normal spontaneous vaginal delivery (NSVD) with an uncomplicated neonatal period except for torticollis until 17 months. Her mother was age 35 years and her father was age 33 years at the time of her birth. She was found to have profound, bilateral sensorineural hearing loss at age 6 months. She was also noted to be hypotonic with global developmental delay and microcephaly, and she currently studies at a second grade level. She received her first cochlear implant at 15 months and a second one subsequently. Though she benefitted from these, her speech remained significantly delayed. She also demonstrated delayed fine and gross motor skills; she first walked at 4 years of age. An EEG performed at age 14 due to a history of seizures demonstrated a focal area of epileptogenicity in the left occipital region (►Table 1).

On physical examination, she had low muscle bulk and a wide-based stance with an uncoordinated gait. There were no dysmorphic facial features and external ears were normal. Biometry at her last evaluation revealed a weight of 37.9 kg (< 1st percentile), height of 155.5 cm (16th percentile), and head circumference of 52.7 cm (between 2nd and 50th percentile), with a BMI of 15.68 kg/m² (2nd percentile).

Prior testing including karyotype, SNP array, mitochondrial electron transfer chain enzymes in a muscle biopsy, mitochondrial genome sequencing, metabolic workup with plasma amino acids, urine organic acids, acylcarnitines, total and free carnitines, and *GJB2* (MIM 121011) sequencing were negative for both cases.

Filtering of the variants obtained from WES shared by both siblings revealed two novel (NM_001130089.1:c.1577C > T (p.Ala526Val) and NM_001130089.1:c.1466T > G (p.Phe489Cys)) heterozygous variants in the *KARS* gene. Each parent was found to be heterozygous for one variant. Neither of these two variants is present in Exome Aggregation Consortium (ExAC), 1000 Genome, ClinVar, or NHLBI GO Exome Sequencing Project (ESP) databases. Based on the ACMG 2015 guidelines, these two variants were interpreted as variants of uncertain significance (►Table 2).⁶ However,

their co-segregation with the phenotype as an autosomal recessive trait makes them a very likely cause of the observed phenotype.

Family 3

The patient is a 3-year-old Hispanic boy born at 39 weeks (birth weight 3.26 kg, 31st percentile; birth length 48.3 cm, 26th percentile) via cesarean section due to nuchal cord. The neonatal period was significant for poor feeding and vomiting that required a 9-day hospital admission. His mother's age was 35 years and his father 39 years at the time of the patient's birth. The pregnancy was significant for gestational diabetes treated with dietary modification. He exhibited hypotonia and delayed gross motor developmental history that included lifting his head at 3 months, sitting at 12 months, pulling to stand at 20 months, and crawling and kneeling at 21 months. His speech was also delayed as he started babbling at 22 months. His fine motor skills have improved with therapy to grabbing objects and playing with both hands. He has had a normal audiology evaluation as well as a normal EEG and magnetic resonance imaging (MRI) of the brain. At age 2 years, an echocardiogram showed a 7-mm-small atrial septal defect (ASD), secundum type. The patient's family history is significant for a paternal second cousin with autism and speech delay (►Table 1).

On physical examination, the patient was found to have bilateral ptosis (right greater than left), microcephaly, bilateral preauricular pits, and low-set ears (►Fig. 1D, F). He has a coarse facial appearance and protruding tongue that lead to an open mouth expression; he also has difficulty swallowing (►Fig. 1D). Additionally the patient has a full lower lip, a single palmar crease, and generalized hypotonia (►Fig. 1D–F). There are no stigmata of neurocutaneous conditions. Ocular abnormalities include esotropia, astigmatism, and myopia. He has torticollis. Biometry at his last evaluation revealed a weight of 15.2 kg (49th percentile), height of 100 cm (62nd percentile), and head circumference of 46.5 cm (< 2nd percentile) with a BMI of 15.2 kg/m².

A chromosomal microarray and testing for fragile X, Angelman's syndrome, Smith-Lemli-Opitz syndrome, mucopolysaccharidoses, and congenital disorders of glycosylation were negative. Testing for inborn errors of metabolism with plasma amino acids, acylcarnitine profile, total and free carnitines, and urine organic acid was also negative. WES in the parents-patient trio revealed a heterozygous de novo (NM_001099412.1:c.3850_3851dup [p.Gln1285Glyfs*10]) variant in *KAT6A* in the proband. Based on the ACMG 2015 guidelines, the variant is interpreted as pathogenic (►Table 2).⁶

Discussion

This report serves to expand the clinical knowledge of possible phenotypic features of three rare genetic variants as well as to further support the use of WES in undiagnosed patients believed to have a genetic cause for their condition. All presented cases demonstrated cognitive delay, microcephaly, and various distinctive features.

There have been at least 54 previously reported cases of variants in *DYRK1A*^{8–10} (►Table 1). Here we report a novel de novo splice-site variant that is predicted to cause abnormal gene splicing and is interpreted as pathogenic.⁶ There were many common features among the previously reported cases that were shared with the present case. These features include global developmental delay, speech delay, abnormal gait, seizures, microcephaly, and feeding problems. Dysmorphic facial features were also found in a majority of prior cases, including deep set eyes with abnormal palpebral fissures, a prominent nasal root, bitemporal narrowing, and prominent and dysplastic ears. Our case shared downsloping palpebral fissures, a high nasal bridge, and dysplastic ears, but she did not exhibit bitemporal narrowing and had distinctly small ear lobes in contrast to the notably enlarged ears of prior cases (►Fig. 1A, C). Hand and foot abnormalities were also noted in both our cases and previous cases. Prior cases reported 25 patients with hand and foot abnormalities, including long, tapered fingers, high arches, and polydactyly, whereas our case exhibited broad first digits of the upper and lower extremities and broad terminal phalanges.

Our case also exhibited features that have not been previously reported in association with *DYRK1A* variants or have only been previously reported in limited cases. Cardiac abnormalities including ventricular septal defect (VSD), PDA, and aortic valve stenosis have been observed in only eight prior cases whereas our case presented with subvalvular aortic membrane and PDA. Of note, our case had aplasia cutis congenita and vesicoureteral reflux that have not been reported previously with *DYRK1A* mutations, although other renal abnormalities have been described, including cryptorchidism, unilateral renal agenesis, pelvic kidney, renal cysts, hypospadias, micropenis, and bilateral hydronephrosis.¹⁰ Interestingly, our case had significantly elevated CPK and CFTD on muscle biopsy in addition to an ataxic gait. Although an abnormal gait has been noted in 26 previously published cases, there was no mention of specific muscular disorders.

There have been at least eight previously published cases with variants in *KARS*^{11–14} (►Table 1). We report two novel missense variants, which co-segregate with the phenotype as an autosomal recessive trait. These variants are not present in databases, and *in silico* analyses predict that they disrupt the protein function (►Table 2). Common neurologic features among the present patients and prior cases include global developmental and speech delay, hypotonia, seizures, and hearing impairment. However, one of the two present cases had abnormal gait, ataxia, and limited coordination, which have not been previously described. Other neurologic features were seen in half of prior cases including decreased nerve conduction velocities and thinning of the corpus callosum. Ocular abnormalities were also noted in prior cases but not in the present cases including strabismus, ophthalmoplegia, and pendular nystagmus. In addition, microcephaly was present in both the current cases (one resolved subsequently) and was only noted in one prior case presented by McMillan et al.¹³ Further medical complications seen in the present cases included β -thalassemia minor and osteoporosis. The diagnosis of β -thalassemia minor was made

using hemoglobin electrophoresis; WES did not reveal a pathogenic variant in the *HBB* gene. The osteoporosis may be related to the immobility, and wheelchair-bound status of the patient which is a result of the *KARS* mutation, but it is possible that a separate, unrelated cause for this condition could also exist as well. Although there have been no previous descriptions of developmental delays in carriers of *KARS* variants, McLaughlin et al reported a heterozygous *KARS* variant that they believed to be causative in a patient clinically diagnosed with Charcot-Marie-Tooth disease.¹² However, to our knowledge, a clinically significant phenotype in carriers has not been identified in other *KARS* variants. Though the paternal family history of dyslexia and delayed walking is interesting to note, WES was not performed on extended family members and we do not have evidence to state whether or not these traits are related to *KARS* variants. The parents of the present cases were asymptomatic and did not exhibit any neurologic symptoms.

There are at least 17 previously reported cases of individuals with variants in *KAT6A*.^{15–17} We report a novel, heterozygous de novo duplication causing a frameshift in protein structure that is interpreted as pathogenic.⁶ Common neurologic features shared in all previously reported cases with the present case include global developmental delay and speech delay, whereas hypotonia was shared among our case and thirteen others. Other neurologic features seen in two previously reported cases but not in the present case included hyperintense signaling in the posterior periventricular white matter and a missing olfactory bulb. Similar craniofacial features between the present case and previously published cases include microcephaly, a broad nasal tip, bitemporal narrowing, and low-set and posteriorly rotated ears. A unique facial characteristic of our present case is the presence of a protruding tongue, which was shared with only one prior case. In addition, preauricular pits and a single palmar crease were noted in our case and only three prior cases but were lacking in the majority of other cases. However, craniofacial features commonly seen in previous cases but not in our current case included large ears, a thin upper lip, epicanthal folds, and dental/oral anomalies. Ocular problems were present in a majority of prior cases and the present case with ptosis and strabismus being most common. Eleven of 17 previously reported cases had a cardiac defect, including PDA, ASD, patent foramen ovale, and VSD. The present case had an ASD. Feeding difficulties were exhibited in the majority of previous cases but not in the present case.

The value of WES in patients with undiagnosed disease has been illustrated in several studies.^{18–22} The overall diagnostic yield of WES is estimated to be between 27 and 28.8% with higher yields of up to 55% for subgroups of patients.^{19,23} Because of the falling costs of sequencing and its high diagnostic yield, WES is rapidly becoming a clinical tool for the evaluation of intellectual disability.²⁴ O'Roak et al successfully utilized WES to identify new autism spectrum disorder candidate genes among 60 individuals and shortly thereafter sequenced > 2,000 probands at low cost to identify recurrently disrupted candidate genes.²⁵ Based on our experience and the literary evidence, we recommend com-

prehensive genetic testing such as WES in patients who present with intellectual disability and remain undiagnosed after first-tier genetic evaluation.

Web Resources

ClinVar: <http://www.ncbi.nlm.nih.gov/clinvar/>
Exome Aggregation Consortium (ExAC): <http://exac.broadinstitute.org/>
1000 Genomes: <http://www.1000genomes.org/>
NHLBI GO Exome Sequencing Project (ESP): <http://evs.gs.washington.edu/EVS/>
Polyphen2: <http://genetics.bwh.harvard.edu/pph2/>
SIFT: <http://sift.jcvi.org/>
Combined Annotation Dependent Depletion (CADD): <http://cadd.gs.washington.edu/>
Protein Variation Effect Analyzer (PROVEAN): <http://provean.jcvi.org/index.php>

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