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Molecular Case Studies

RAPID COMMUNICATION

A novel *PAK3* pathogenic variant identified in two siblings from a Japanese family with X-linked intellectual disability: case report and review of the literature

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Abstract Intellectual disability (ID) is a clinically and genetically heterogeneous developmental brain disorder. The present study describes two male siblings, aged 7 and 1 yr old, with severe ID, spastic quadriplegia, nystagmus, and brain atrophy with acquired microcephaly. We used the exome sequencing to identify the causative gene in the patients and identified a hemizygous missense variant, c.1282T>A (p.W428R), in the p21-activated serine/threonine kinase 3 gene (*PAK3*), which is associated with X-linked ID. p.W428R is located within the highly conserved kinase domain and was predicted to induce loss of enzymatic function by three mutation prediction tools (SIFT, PolyPhen-2, and MutationTaster). In addition, this variant has not been reported in public databases (as of the middle of December 2018) or in the data from 3275 individuals of the Japanese general population analyzed using high-depth whole-genome sequencing. To date, only 13 point mutations and deletions in *PAK3* in ID have been reported. The literature review illustrated a phenotypic spectrum of *PAK3* pathogenic variant, and our cases represented the most severe form of the *PAK3*-associated phenotypes. This is the first report of a *PAK3* pathogenic variant in Japanese patients with X-linked ID.

[Supplemental material is available for this article.]

CASE PRESENTATIONS

Patient MR94 II-1 was a 7-yr-old boy born to nonconsanguineous Japanese parents. He was born through normal delivery without asphyxia at 38 wk of gestation. His weight and head circumferences were 2720 g (−0.7 SD) and 31.5 cm (−1.3 SD), respectively. He acquired head control after age 3 yr, sat independently after age 6, but could not form words. He had a febrile convulsion at the age of 2 yr. When he was 5 yr old, his developmental quotient (DQ) was 12. When he was 6 yr 3 mo old, he suffered spastic quadriplegia and horizontal nystagmus. He showed no aggressive, hyperactive, or self-harming behavior but did show autistic stereotype movement. Hypotonia in his trunk, extremities, and facial muscles was noted. His height, weight, and head circumference were 100 cm (−3.0 SD), 14.6 kg (−1.8 SD), and 47 cm (−2.6 SD), respectively. Brain magnetic resonance imaging (MRI) showed cerebral white matter and midbrain atrophy, and a thin corpus callosum (Fig. 1AB; Table 1). His

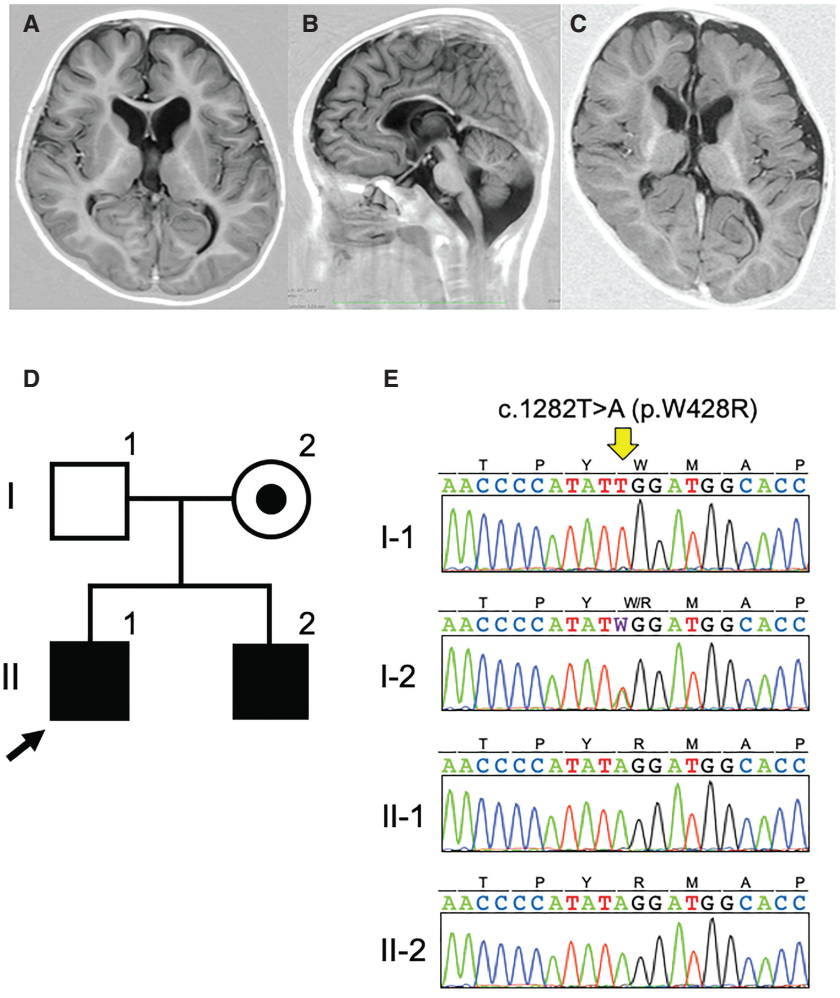

Figure 1. A *PAK3* pathogenic variant in a Japanese family with X-linked intellectual disability (XLID). (*A*,*B*) Coronal and sagittal sections of T1-weighted magnetic resonance images of patient MR94 II-1. (*C*) A coronal section of T1-weighted magnetic resonance images of patient MR94 II-2. Reduced volume of the white matter and midbrain, as well as a thin corpus callosum, were noted. (*D*) Pedigree of family MR94. (*E*) Electrochromatogram of the *PAK3* pathogenic variant, c.1282T>A, identified in two patients in family MR94.

Table 1. Clinical features of patients with c.1282A>T (p.W428R) and reported patients with pathogenic variants in *PAK3*

Phenotypic features	Patient 1	Patient 2	Sample number	Positive rate (%)
Variant	c.1282A>T (p.W428R)			
Intellectual disability	Yes, severe	Yes, severe	34/38	89.5
Epileptic seizures	Yes	No	9/39	23.1
Autistic features	Yes	Yes	6/38	15.8
Aggressive behavior/self-injury	No	No	10/38	26.3
Global developmental delay	Yes	Yes	32/41	78.0
Language impairment	Yes	Yes	36/37	97.3
Spastic quadriplegia	Yes	Yes	4/40[a]	10.0
Nystagmus	Yes	Yes	4/40[b]	10.0
Hypotonia in the trunk and extremities	Yes	Yes	12/35	34.3
Hypotonia in the face	Yes	Yes	12/39	30.8
Microcephaly	Yes	Yes	14/40	35.0
Short stature	Yes	Yes	6/38	15.8
Low set ear	Yes	Yes	6/38	15.8
Long face	Yes	No	10/40[c]	25.0
White matter atrophy	Yes	Yes	2/8	25.0
Midbrain atrophy	Yes	Yes	3/10	30.0
Hypoplasia of the corpus callosum	Yes	Yes	6/9[d]	66.7

[a]Including hemiplegia and lower limb spasticity.
[b]Including abnormality of eye movement.
[c]Including abnormal face shape.
[d]Including dysplastic corpus callosum and agenesis of corpus callosum.

younger brother (MR94 II-2) also had X-linked intellectual disability (XLID). He was delivered by Caesarean section because of the breech presentation without asphyxia at 38 wk of gestation. His weight and head circumferences were 2878 g (−0.3 SD) and 30.5 cm (−2.0 SD), respectively. He rolled over after he was 1 yr old, but at the age of 1 yr and 2 mo, he had no head control, could not form words, and had a DQ < 10. He also showed spastic quadriplegia. His height, weight, and head circumference were 70 cm (−2.7 SD), 6.8 kg (−3.1SD), and 43 cm (−2.3 SD), respectively. No behavioral problems were noted, but autistic features were observed. Hypotonia in his trunk, extremities, and facial muscles was also noted. A brain MRI showed cerebral atrophy, myelination delay, and a thin corpus callosum (Fig. 1C).

VARIANT INTERPRETATION

We identified a hemizygous missense variant, c.1282T>A (p.W428R; GRch37. Chr X. 110439743), in the p21-activated serine/threonine kinase 3 gene (*PAK3:* NM_002578.3; OMIM300142) in MR94 II-1 and MR94 II-2 (Fig. 1D,E; Table 2). The variant was inherited from their mother, who is asymptomatic. The altered amino acid residue (p.W428) is located within the highly conserved kinase domain. The tryptophan residue is conserved from human to zebrafish (Supplemental Fig. 1). Hence, we expected that the variant would abolish the kinase activity of *PAK3*. A c.1282T>A variant in PAK3 has not been deposited in any database, including dbSNP build 151, ESP6500, 1000 Genomes, the Exome Aggregation Consortium, the Human Genetic Variation Database, the Genome Aggregation Database (gnomAD),

Table 2. Genomic findings and variant interpretation

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect (substitution, deletion, etc.)	dbSNP/ dbVar ID	Genotype (heterozygous/ homozygous)	ClinVar ID (optional)	Parent of origin (optional)	Observed effect (if shown to be different from the predicted effect) (optional)	Comments (optional)
PAK3	X	NM_002578.3: c.1282T>A	p.W428R	Missense	Substitution	None	Hemizygous	SCV000914231	X-linked recessive	None	Class 4, likely pathogenic

ClinVar, or the Medical Genomics Japan Variant Database (as of the middle of December, 2018). We also searched the data from 3275 members of the Japanese general population acquired using high-depth whole-genome sequencing (Okada et al. 2018). However, not even a heterozygous carrier was found. Hence, the *PAK3* variant identified in this study is considered to be "an extremely rare variant." In addition, three mutation prediction tools, SIFT, PolyPhen-2, and MutationTaster, predicted the mutation to be "Damaging," with a score of 0, "Probably damaging," with a score 1, and "Disease causing," with a score of 0.99, respectively. Finally, we classified and evaluated this variant using the guidelines for the classification of sequence variants from the American College of Medical Genetics and Genomics (Richards et al. 2015). We concluded that the variant identified in this family is "Class 4 (likely pathogenic)" (Supplemental Table 1). No pathogenic variants in other genes are identified. Array comparative genomic hybridization (aCGH) analyses in both parents and patients also identified no pathogenic copy number variants (Supplemental Table 2).

SUMMARY

Families with a Chromosome X–linked inheritance pattern ID account for 5%–10% of all ID cases, suggesting an important role of the genes mutated on the X chromosome in males (Lubs et al. 2012). X-linked intellectual disability is divided into syndromic XLID (S-XLID) and nonsyndromic XLID (NS-XLID), the latter lacking dysmorphic features or other distinguishing symptoms (Ropers and Hamel 2005). To date, at least 141 genes responsible for S-XLID and NS-XLID have been reported (Neri et al. 2018). Among them, *PAK3* was identified as the causative gene in patients with NS-XLID (OMIM300558; MRX30) (Allen et al. 1998). Currently, 10 types of point mutations, one splicing mutation, one small deletion, and a 90-kb deletion containing exons 6–18 are described in the Human Gene Mutation Database, Professional (Version: 2018.3) as of the middle of December, 2018. These are all found in non-Japanese individuals.

The clinical phenotypes of the patients with *PAK3* mutations are variable, as summarized (Table 1; Supplemental Table 3). We noted several common features, including mild to severe ID (89.5%), language impairment (97.3%), and global developmental delay (78%), as also reported previously (Hertecant et al. 2017; Horvath et al. 2018). Microcephaly (35.0%) and hypotonia (trunk and extremities, 34.3%; face, 30.8%) may represent characteristic features of the patients with *PAK3* mutations. Spastic paraplegia/hemiplegia (10%) and nystagmus (10%) were also found in multiple cases. Less frequent and nonspecific features include epileptic seizures (23.1%), autistic features (15.8%), aggressive behavior/self-injury (26.3%), and short stature (15.8%). Imaging findings were available only in a small number of patients, but hypoplasia of corpus callosum (66.7%), atrophy of midbrain (30.0%), and white matter (25%) were reported.

Our cases showed all features in Table 1 except for aggressive behavior. They also showed more severe ID compared with previously reported patients, suggesting that they had the most severe form of the disease caused by *PAK3* mutations. Spastic quadriplegia

and nystagmus appear to be rare phenotypes, but similar manifestations (i.e., lower limb spasticity and hemiplegia and oculomotor abnormalities) were also reported in other patients, suggesting that these features are possibly associated with *PAK3* mutations. Hertecant et al. (2017) reported a monozygotic twin who presented with ID, behavioral problems, and unique macrocephaly caused by a p.Y427H mutation in PAK3, which is located one amino acid prior to the residue mutated in the present patients, who had acquired microcephaly, as seen in other patients. Reason for this opposite phenotypic expression remains unknown.

In conclusion, we report the first Japanese XLID family with a novel hemizygous *PAK3* mutation. The literature review illustrated the phenotypic spectrum of PAK3-associated XLID and suggested that our cases represent a severe form of the disease.

METHODS

The biobank at the National Center of Neurology and Psychiatry is part of the National BioBank Network in Japan, which is a unique biorepository exclusively dedicated to collecting samples from patients with neuropsychiatric, muscular, and developmental diseases. The biobank contains DNA samples and clinical information from 583 families with neurodevelopmental diseases that were preserved and diagnosed from 2004 to 2016.

We performed exome sequencing to identify the causative gene in the family. Genomic DNAs were extracted from the patients and the parent's bloods by standard protocol. DNAs were processed by SureSelect XT Human All Exon V6 (Agilent Technologies) and BGISEQ DNA library preparation kit (BGI). Captured DNAs were sequenced using MGISEQ2000 (BGI) with 100-bp pair-end reads. Reads were mapped to the human genome reference (GRCh37/hg19) by BWA 0.7.5a-r405. Duplicated reads were removed by Picard 1.99. Variants were identified by Genome Analysis Toolkit v3.5 based on GATK Best Practice Workflow and annotated by ANNOVAR (2018 April 16). In all subjects, at least 98.1% of all coding regions were covered in 10 reads depth (Supplemental Table 4). The variant in *PAK3* was confirmed by Sanger sequencing.

For the aCGH analysis, we utilized SurePrint G3 Human CGH microarray 8 × 60K (Agilent). The experimental procedures were performed according to the manufacturer's protocol (Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis, Version 7.2, Agilent Technologies).

The following Web resources were used:

- dbSNP build 151: https://www.ncbi.nlm.nih.gov/projects/SNP/
- ESP6500: http://evs.gs.washington.edu/EVS/
- 1000 Genomes: http://grch37.ensembl.org/Homo_sapiens/Gene/Variation_Gene/Table?db=core;g=ENSG00000077264;r=X:110187513-110470589
- Exome Aggregation Consortium: http://exac.broadinstitute.org
- Human Genetic Variation Database: http://www.hgvd.genome.med.kyoto-u.ac.jp
- gnomAD: http://gnomad.broadinstitute.org
- ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
- Medical Genomics Japan Variant Database: https://mgend.med.kyoto-u.ac.jp
- OMIM (MRX30): https://www.omim.org/entry/300558?search=x-linked%20intellectual%20disability%20and%20pak3&highlight=disability%20intellectual%20xlinked%20x%20linked%20pak3

ADDITIONAL INFORMATION

Data Deposition and Access

PAK3 variant data was submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) and are available under accession number SCV000914231.

Ethics Statement

Clinical information and samples from patients and their parents were obtained with informed consent. The study was approved by the ethical committee of the National Center of Neurology and Psychiatry, Japan (IRB A2014-081 and XXXX-115 (20-9-6)).

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Author Contributions

E.T., K.I., and Y.G. contributed to the conception and design of the study. A.I. and K.T. contributed to the genome analysis. A.I. contributed to the variant interpretation. E.T., C.A.-H., S.H., Y.I., E.N., K.I., and Y.G. managed the DNA samples and clinical information. S.K., Y.K., Y.M., and M.K. performed the whole genome sequencing and analyzed the data. A.I., E.T., C.A.-H., and K.I. wrote the manuscript and prepared the figures. Y.G. supervised the study.

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Competing Interest Statement

The authors have declared no competing interest.

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