Three brothers with a nonsense mutation in \textit{KAT6A} caused by parental germline mosaicism

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Mutations in \textit{KAT6A}, encoding a member of the MYST family of histone acetyl-transferases, were recently reported in patients with a neurodevelopmental disorder (OMIM: \#616268, autosomal dominant mental retardation-32). In this report, we describe three siblings with intellectual disability (ID) or global developmental delay and a \textit{KAT6A} heterozygous nonsense mutation, i.e., c.3070C>T (p.R1024*, ENST00000406337; chr8:41795056G>A on hg19). This mutation was identified by whole-exome sequencing of all three siblings but not in a healthy sibling. The mutation was not detected in the peripheral blood of their parents, suggesting the existence of parental germline mosaicism. The primary symptoms of our patients included severe to profound ID or global developmental delay, including speech delay with craniofacial dysmorphism; these symptoms are consistent with symptoms previously described for patients with \textit{KAT6A} mutations. Although several features are common among patients with \textit{KAT6A} mutations, the features are relatively nonspecific, making it difficult to establish a clinical entity based on clinical findings alone. To the best of our knowledge, this is the first report of cases with a \textit{KAT6A} mutation in an Asian population and these cases represent the first reported instances of germline mosaicism of this disease.

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\textbf{INTRODUCTION}

Intellectual disability (ID) is defined as an intelligence quotient of 70 or below and affects ~1% of children worldwide.\textsuperscript{1} ID is caused by environmental or genetic factors, but the explicit cause is not identified in up to 60% of cases.\textsuperscript{2} Approximately 25–50% of ID cases are thought to have a genetic cause.\textsuperscript{2} A recent large-scale sequencing study identified probable pathogenic mutations in ~40% individuals with ID who underwent whole-exome sequencing (WES) or genome sequencing.\textsuperscript{3}

WES is a powerful tool in identifying genetic alterations in putative genetic disorders, even those that are undiagnosed, providing a molecular diagnosis rate of 25%.\textsuperscript{4} In addition, WES has revealed that ~8% of patients without a definitive causative mutation harbor novel candidate mutations.\textsuperscript{5} Moreover, for some cases of undiagnosed rare diseases, a new entity has been established among patients whose disorder was previously indistinguishable from other diseases expressing similar phenotypes. Recent studies reported patients with \textit{de novo} \textit{KAT6A} mutations among individuals diagnosed with known rare autosomal dominant diseases.\textsuperscript{6–8} Although those patients had similar phenotypes, the features were nonspecific. Thus, it is difficult to distinguish this disease from other diseases based on clinical findings alone.

In this report, we describe three siblings with ID or global developmental delay with a \textit{KAT6A} heterozygous nonsense mutation that was potentially transmitted from one of their parents as a germline mosaicism. This result reinforces ‘germline mosaicism’ in genetic counseling for patients with \textit{de novo} mutation.

\textbf{MATERIALS AND METHODS}

\textbf{Subjects}

Three Japanese brothers from nonconsanguineous parents were analyzed (Figure 1). The brothers exhibited ID and several common features, suggesting that the underlying cause was genetic. The three siblings, their unaffected brother, and their parents were subjected to genetic testing as part of the Initiative on Rare Undiagnosed Diseases in Pediatrics project in Japan. This study protocol has been approved by the Committee for Ethical Issues on Human Genome and Gene Analysis at Nagasaki University. All genetic analyses were performed in the Department of Human Genetics at Nagasaki University.

\textbf{Whole-exome sequencing}

Peripheral blood was obtained with written informed consent and DNA was extracted using the QIAamp DNA Maxi kit (QIAGEN, Hilden, Germany) according to the manufacturer’s protocol. Four subjects, the parents (II-1 and II-7), an affected child (III-1), and an unaffected child (III-2), underwent WES to screen for the candidate causative mutation (Figure 1). Coding exons were captured with the SureSelect XT AUTO HUMAN ALL Exon V5 kit (Agilent Technology, Santa Clara, CA, USA) and sequenced on a HiSeq2500 system (Illumina, San Diego, CA, USA) in rapid mode with 101 bp paired-end reads. Reads were aligned to GRCh37/hg19 with Novoalign (Novocraft Technologies, Selangor, Malaysia) and duplicate reads that were excluded from the following analysis were marked with Novosort software (Novocraft Technologies). Local realignment and variant calling were performed by the Genome Analysis Toolkit.\textsuperscript{9} Generated variant call format files were

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processed in the following two manners. First, trio-based VCS format files were filtered to extract the de novo, homozygous, and X-linked mutations followed by annotation with ANNOVAR. Second, variant call format files were annotated with ANNOVAR then filtered to extract compound heterozygous mutations. This process excluded variants with allele frequencies >0.5% in the Exome Aggregation Consortium (http://exac.broadinstitute.org/), NHLBI GO Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp), or the database of Tohoku Medical Megabank (http://www.dist.megabank.tohoku.ac.jp). Three de novo mutations identified in WES were amplified by PCR in all family members (II-1, II-7, III-1, III-2, III-3, and III-4) followed by direct sequencing to confirm the existence and linkage to phenotype. The primers designed by Primer3Plus (http://www.bioinformatics.nl/primer3plus) were as follows: KAT6A_F, 5′-ATCTCAAACGTGGTCTAA-3′, KAT6A_R, 5′-ATGTGCTAATTCTATTTGGT-3′; CEP89_F, 5′-AACTGGGAACATAGAAAACA-3′, CEP89_R, 5′-CAGTGTCAAGTGTAAGTGA-3′; and CDX4_F, 5′-TCCAATTTCGCTGCGGCACC-3′, CDX4_R, 5′-AGGGCCCAAGTTGCTGTAGTC-3′.

RESULTS

Clinical features

Individual III-1 was a 14-year-old boy who was born at 40 weeks of gestation with a birth weight of 2,914 g. He was intubated for breathing problems soon after birth due to persistent pulmonary hypertension and was hospitalized for 2 months. He sat unassisted at 12 months and walked at 22 months of age. He spoke at the age of 3, but his vocabulary was limited to a few short nursery words. He underwent surgery for undescended testes and inguinal hernia at 10 months of age. At the age of 7, his height was 115.2 cm (−0.63 standard deviation (SD)), his weight was 18.8 kg (−0.89 SD), and his head circumference was 49.6 cm (−1.47 SD). His intelligence quotient was 13 at 13 years of age.

Individual III-3 was a 10-year-old boy who was born at 37 weeks of gestation with a birth weight of 2,910 g. He had meconium aspiration syndrome and stayed in an incubator for 2 weeks. He was repeatedly admitted to hospital with recurrent pulmonary infection during his first year of life. He sat unassisted at 18 months, walked at 30 months old, and spoke at the age of 5. At 2 years of age, his height was 79 cm (−1.8 SD), his weight was 7.63 kg (−3.25 SD), and his head circumference was 49.6 cm (−1.72 SD). His intelligence quotient was 13 at 13 years of age.

Individual III-4 was a 10-month-old boy who was born at 35 weeks of gestation with a birth weight of 2,910 g. He had meconium aspiration syndrome and stayed in an incubator for 2 weeks. He was repeatedly admitted to hospital with recurrent pulmonary infection during his first year of life. He sat unassisted at 18 months, walked at 30 months old, and spoke at the age of 5. At 2 years of age, his height was 79 cm (−1.8 SD), his weight was 7.63 kg (−3.25 SD), and his head circumference was 49.6 cm (−1.72 SD). His intelligence quotient was 26 at 13 years of age.

All three affected siblings have the clinical triad characteristic of the family involving mild to moderate scaphocephaly,
three brothers with a nonsense mutation in KAT6A
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Table 1. Major characteristics of patients with c.3070C>T

<table>
<thead>
<tr>
<th></th>
<th>III-1</th>
<th>III-3</th>
<th>III-4</th>
<th>Arboleda et al.*</th>
<th>Millan et al.*</th>
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<tbody>
<tr>
<td>Age</td>
<td>14 Years</td>
<td>11 Years</td>
<td>1 Years 3 months</td>
<td>5 Years</td>
<td>5 Years</td>
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<tr>
<td>Gender</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
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<tr>
<td>Gastational age</td>
<td>40 Weeks</td>
<td>37 Weeks</td>
<td>35 Weeks</td>
<td>42 weeks</td>
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<tr>
<td>Weight at birth</td>
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<td>2,910 g</td>
<td>2,270 g</td>
<td>1,870 g</td>
<td>—</td>
</tr>
<tr>
<td>Protem at birth</td>
<td>Persistent pulmonary hypertension</td>
<td>Meconium aspiration syndrome</td>
<td>Respiratory problems</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Development</td>
<td>Sit unsuissisted</td>
<td>1 Year</td>
<td>1 Year 6 months</td>
<td>Not yet</td>
<td>1 Year</td>
</tr>
<tr>
<td></td>
<td>Walk</td>
<td>3 Years 10 months</td>
<td>2 Years 6 months</td>
<td>Not yet</td>
<td>4 Years 6 months</td>
</tr>
<tr>
<td></td>
<td>Speech</td>
<td>3 Years (2 words)</td>
<td>5 Years</td>
<td>Not yet</td>
<td>Absent speech</td>
</tr>
<tr>
<td></td>
<td>Intelligence quotient (age at evaluation)</td>
<td>13 (at 13 years)</td>
<td>26 (at 10 years)</td>
<td>Not yet</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>Measures (at 6 years 8 months)</td>
<td>(at 2 years 10 months)</td>
<td>(at 1 year 3 months)</td>
<td>(at 4 years 6 months)</td>
<td>—</td>
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<tr>
<td>Tall (SD)</td>
<td>—1.6</td>
<td>—1.8</td>
<td>0.9</td>
<td>—2.6</td>
<td>&gt;75th Percentile</td>
</tr>
<tr>
<td>Weight (SD)</td>
<td>—0.89</td>
<td>—3.25</td>
<td>—2.0</td>
<td>—0.7</td>
<td>&gt;75th Percentile</td>
</tr>
<tr>
<td>OFC (SD)</td>
<td>—1.47</td>
<td>—1.72</td>
<td>—1.0</td>
<td>—2.52</td>
<td>20–70th Percentile</td>
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<tr>
<td>Physical features</td>
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<td>Head</td>
<td>Scaphocephary</td>
<td>Scaphocephary</td>
<td>Scaphocephary</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Face</td>
<td>Micrognathia, low set ear, malocclusions</td>
<td>Micrognathia, low set ear, malocclusions</td>
<td>Micrognathia, low set ear</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Musculoskeletal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Eye</td>
<td>Axial hypotonia</td>
<td>Axial hypotonia</td>
<td>Axial hypotonia</td>
<td>NE</td>
<td>Strabismus</td>
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<tr>
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<td>Exotropia</td>
<td>NE</td>
<td>Strabismus</td>
<td>Ptosis</td>
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<tr>
<td>Cardiac</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>—</td>
<td>Patent ductus atresia</td>
</tr>
<tr>
<td>Other feature</td>
<td>Feeding difficulty, orthostatic distention, allergic rinitis</td>
<td>Feeding difficulty, mild hearing loss</td>
<td>—</td>
<td>—</td>
<td>Laryngomalacia</td>
</tr>
</tbody>
</table>

Abbreviations: NE, not evaluated; OFC, occipito-frontal circumference.

micronathia, and low-set ears. In addition, feeding difficulties and
axial hypotonia are also observed. Individuals III-1 and III-3 have
strabismus (exotropia) and abnormal teeth (malocclusions). Their
blood cell counts and serum levels of liver enzymes, creatinine,
and electrolytes are within normal ranges. Computed tomography
scanning revealed no evidence of intracranial abnormalities.

WES and Sanger sequencing
The mean depth of coverage for each individual in WES is as
follows: II-1, 74 ×; II-7, 104 ×; II-3, 74 ×; III-2, 77 ×. Trio analysis
revealed that three genes, KAT6A, CEP89, and CDX4, have de novo
heterozygous single nucleotide variations in the affected
individual III-1 but not in unaffected III-2. Sanger sequencing of all
family members (II-1, II-7, III-1, III-2, III-3, and III-4) detected the
KAT6A mutation in affected boys but not in the unaffected boy or
the parents. This de novo nonsense mutation, c.3070C>T (p.R1024*,
ENST00000406337; chr8:41795056G>A on hg19) (Figure 2), was previously reported in two patients.7,8 The
phenotypes of our cases are similar to those cases, and we
concluded that this mutation is causative of disease in the present
family (OMIM: 616268) (Table 1).

DISCUSSION
In this report, we describe three siblings with ID or global
developmental delay caused by a KAT6A mutation. To date, 17
cases with ID or global developmental delay from 16 families with
KAT6A mutations have been reported.6–8 A recent large-scale
study including more than 7,000 individuals with neurodevelopmental
disorder identified 11 patients with potentially damaging
KAT6A de novo mutations.3 This finding may reflect the actual
frequency of the disease.

The main symptoms in our cases involved ID and developmental
delay with very limited verbal development, which is consistent with
previous reports of patients with KAT6A mutations. Craniofacial
abnormalities, especially a small head circumference (some cases met
the criteria of microcephaly), are noted at a high frequency and are
relatively characteristic.6–8 Low-set ears were observed in all our
patients and in 5/7 cases reported by Tham et al.6–8 Feeding difficulties
and axial hypotonia were also observed in most patients, and many
patients had congenital heart disease and strabismus. Scaphocephaly
was common among our patients but has not previously been
observed in other reported cases. Teeth abnormalities and micro-
nathia are common in patients with the
KAT6A mutation, these findings are nonspecific, making the
disorder indistinguishable from similar diseases with ID.

Analysis of parental genotypes in the present study indicated that
the KAT6A mutation of our patients is de novo but that it must
have been transmitted by parental germline mosaicism given its
consecutive occurrence. Germline mosaicism refers to variation in
the genomes of germline cells within an individual.11 Although it
can be beneficial to define the mosaic rate of gametes in such
cases, we did not do so, because it is practically impossible to use
ova for research purposes. Recent deep sequencing or droplet
digital PCR techniques are used to detect a very low prevalence of
somatic mosaicism in the blood;12,13 however, this technology is
not useful to determine the germline mosaic rate. Acuna-Hidalgo
et al.13 reported that the genome-wide analysis of putative de
novo mutations in the proband detected 4/4,081 variants in the
blood of one of the parents. In our exome analysis, the mutation
was not detected within a read depth of 89 in the father (II-1) and
113 in the mother (II-7).

KAT6A, which is also known as MOZ or MYST3 on chromosome
8q11.21, encodes a member of the MYST family of histone acetyl-
transferredes of 2004 amino acids. KAT6A was first described as a fusion protein in patients with acute myeloid leukemia and all members of this family have a histone acetyltransferase domain that acetylates the histone lysine residue and promotes transcription. In zebrafish, the KAT6A ortholog specifies segmental identity in the pharyngeal arches. Kastumoto et al. described the Kato6a homozygous knockout mouse model, in which exon 2 containing the first ATG of Kato6a was replaced with a neo gene cassette, resulting in embryonic lethality and a lack of hematopoietic stem cells. Another homozygous knockout model, in which the neo coding sequence was inserted into exon 16, was also lethal, exhibiting craniofacial and heart abnormalities with normal hematopoietic cells. Although no description exists for heterozygous knockout mouse model defects, the craniofacial and heart abnormalities commonly identified in patients with KAT6A could correspond to this model, except for hematopoietic cell aberrations.

In conclusion, we report three siblings with a KAT6A mutation and ID. This is the first report of KAT6A mutations in an Asian population, and this report also describes the first case of germline mosaicism of the disease. To date, this disease can only be diagnosed after genome-wide testing and the observation of particular craniofacial characteristics. However, the widespread use of WES will lead to an accumulation of patient clinical data that could provide useful information about the diagnosis, prognosis, and future strategies for treating the disease.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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