A novel *MED12* mutation associated with non-specific X-linked intellectual disability

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The mediator complex subunit 12 gene (*MED12*) is responsible for an X-linked recessive intellectual disability syndrome that is characterized by dysmorphic features such as a long, narrow face and blepharophimosis, which is now recognized as an *MED12*-related syndrome. We identified a novel non-synonymous single-nucleotide variant, p.Ile1023Val, in a male patient with non-specific X-linked intellectual disability (XLID). Our results, together with the existence of similar reports, suggest a relationship between *MED12* variants and XLID.

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Obtaining a final diagnosis for a non-specific intellectual disability is challenging. However, the use of massive parallel sequencing with next-generation sequencers has made this task easier. This strategy has also revealed broader genotype–phenotype correlations. It is now possible to determine whether genes that are specific for a distinctive disorder might also be responsible for other clinical characteristics. We report herein a case of a male patient with non-specific intellectual disability who showed a novel mutation in the mediator complex subunit 12 gene (*MED12*), which is known to be responsible for FG syndrome (FGS; Opitz–Kaveggia syndrome, MIM#305450), Lujan–Fryns syndrome (intellectual disability with marfanoid habitus, MIM#309520), and X-linked Ohdo syndrome (Maat–Kievit–Brunner syndrome (MKBS); MIM#300895).

A 12-year-old boy was born to healthy parents at term with a birth weight of 3,700 g. He had two healthy older sisters, and there is no remarkable family clinical history. He has no history of seizures. Because he showed severe developmental delay since infancy, he was admitted to a special education school.

Presently, he is relatively small for his age, with a height of 1.42 cm (10th to 25th percentile), weight of 30.5 kg (3rd to 10th percentile) and occipitofrontal circumference of 52.5 cm (10th percentile). He has distinctive facial features. Finger pads are noted on all fingers. Although he could follow simple verbal commands from his family members, he is aphasic and requires support in his daily life activities. Although it is possible to make eye contact with him, communication is impossible with others. He is often constipated, which is likely because he shows stubbornness in his eating habits. Depending on his mood, he often refuses bathing. Although he does not exhibit aggressive behavior, he often shows panic in circumstances in which he does not get his way. From these findings, we concluded that he has a severe intellectual disability with autistic features.

To make a genetic diagnosis, blood samples were obtained from the patient and his parents after receiving informed consent. This study was performed in accordance with the Declaration of Helsinki Principles, and the ethics committee of Tokyo Women's Medical University gave approval for this study. Genomic DNA was extracted from blood samples and used for further examination. Genomic copy number aberrations were examined using an Agilent SurePrint G3 Human CGH Microarray Kit 8 x 60 K (Agilent Technologies, Santa Clara, CA, USA) as described previously, which demonstrated no aberrations. Subsequently, targeted resequencing was performed using TruSight One v1.0 sequencing panel (Illumina, San Diego, CA, USA), which includes 125,396 probes aimed to capture 11,946,514-bp targeted exon regions consisting of 4,813 genes that are associated with known clinical phenotypes. After constructing the sequence library using 50 ng of genomic DNA, the MiSeq next-generation sequencer (Illumina) was used to sequence 151-bp paired-end reads according to the manufacturer's instructions. The extracted data were mapped to a reference genome (GRCh37/hg19) using BWA Enrichment v1.0 cloud software (Illumina). On an average, 1.093 Gb of targeted aligned sequences and a mean coverage depth of 92 were obtained (the target coverage at 20 × was 95.0%). The extracted variants were annotated and filtered using the Variant Studio software (Illumina; Figure 1a).

A single-nucleotide variant (SNV) in exon 22 of *MED12*, NM_005120.2(MED12_v001):c.3067A>G; NM_005120.2(MED12_i001):p.(Ile1023Val), was identified. The affected amino acid is conserved among mammals (Figure 1d). This variant is not reported in the 1000 Genomes Project (1000G), the Exome Aggregation Consortium (ExAC), or the Human protein polymorphism database (HGV: http://www.genome.med.kyoto-u.ac.jp/SnpDB). The HGVD is the database provided by Kyoto University, and it currently contains genetic variations that have been determined by exome sequencing of 1,208 individuals, and genotyping data of common variations obtained from a cohort of 3,248 individuals, thus indicating a novel variant in the general population. SNP (http://sift.jcvi.org/www/SIFT_help.html) and PolyPhen2 predication (http://genetics.bwh.harvard.edu/pph2/) scores were tolerated (0.73) and benign (0.001); however, the CADD RawScore was 1.386821, and the CADD PHRED score was 15.4 (http://cadd.gs.washington.edu/help.html). Further, MutationTaster

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Figure 1. Sequencing results. (a) Filtering the candidate mutations. Numbers show the results: the top number indicates number of total variants that passed quality control. The second number indicates number of non-synonymous variants. The third number indicates number of the variants with <1% frequency in global population. The fourth number indicates the number of the variants without a dbSNP ID. The fifth number indicates the number of variants after family-based filtering. The variant, which does not appear to have a de novo pattern, an autosomal recessive pattern or an X-linked recessive pattern, was filtered out. The bottom number indicates the number of variants after removal of the simple repeat length changes. (b) IGV presentation of the sequence results from family members. The present patient shows G at the indicated position. The mother is heterozygous for this variant. (c) Sanger sequencing confirmed all of the above results. (d) The affected amino acid is conserved among mammals and chicken. dbSNP, single-nucleotide polymorphism database; HGVD, human genetic variation database; IGV, integrative genomics viewer.

Figure 2. A schematic representation of the primary structure of MED12 and locations of identified mutations. The previously reported mutation identified in the patient with either FG syndrome, Lujan–Fryns syndrome or X-linked Ohdo syndrome is depicted in upper side. The mutation reported in the patient with XLID is in the bottom side. Most of the mutations are located on the narrow region. PQL indicates the domain with a high content of Pro, Gin and Leu residues, as reported by Kim et al. MED12, mediator complex subunit 12; XLID, X-linked intellectual disability.
(c.5898insC) in a patient with non-specific intellectual disability. Subsequently, Tzschach et al.\textsuperscript{15} identified a MED12 variant (p.Arg815Gln) in a familial patient with moderate intellectual disability, short stature, and microcephaly (detailed information is unavailable) in a large cohort study that investigated X-linked intellectual disability (XLID). Most of the reported mutations are located in the narrow region between codon 815 and 1,165 (Figure 2). Although the functional importance of this narrow region is unknown, the localization of p.Ile1023Val, as identified in the present study, in this narrow region would suggest the pathogenesis of this variant. Based on these findings, we deduced that MED12 mutations are related not only to distinctive syndromes but also to non-specific XLID. Determination of the detailed genotype–phenotype correlation of MED12 is required to clarify this relationship.

HGV DATABASE
The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.586.

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COMPETING INTERESTS
The authors declare no conflict of interest.

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