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SHORT COMMUNICATION

A case of autism spectrum disorder arising from a *de novo* missense mutation in *POGZ*

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Autism spectrum disorder (ASD) is a clinically heterogeneous psychiatric disorder with various genetic backgrounds. Here, we report a novel mutation in the pogo transposable element-derived protein with zinc finger domain gene (*POGZ*) identified by trio-based whole exome sequencing. To date, a total of seven *de novo POGZ* mutations in ASD have been reported. POGZ contains a total of five functional domains, and this study reports the first *de novo* missense mutation in the centromere protein B-like DNA-binding domain. *POGZ* is highly expressed in the human fetal brain and is involved in mitosis and the regulation of neuronal proliferation. Therefore its loss-of-function or pathogenic missense mutations are likely to be causative of ASD.
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INTRODUCTION

Autism spectrum disorder (ASD) is characterized by deficits in social communication and interactions, stereotyped or repetitive behaviors, and restricted interests.[1] The prevalence of ASD is estimated to be 62 in 10 000 and the diagnosed population has increased during the last decade.[2] The clinical heterogeneity of ASD may be reflected by its heterogeneous genetic complexity.[3] More than 100 autism-related genes, copy number variations and 158 linked regions have been reported.[1,4] To date, at least six large-scale trio- or quads-based ASD whole exome sequencing (WES) studies have discovered *de novo* mutations in various genes that are causative of ASD.[3,5–9] Mutant genes in ASD can be also associated with intellectual disability (ID) and epilepsy.[3,5,10] Moreover, *de novo* loss-of-function (LoF) single nucleotide variants and insertions/deletions are observed at higher frequencies in ASD individuals than in controls.[5–9,11] Genes showing LoF mutations in schizophrenia are also found as *de novo* mutations in ID and ASD, including the sodium channel, voltage-gated, type II, alpha subunit gene.[12] Recently, the pogo transposable element-derived protein with zinc finger domain gene (*POGZ*) has become a plausible candidate gene for ASD as *de novo* mutations were identified in at least seven independent ASD patients by WES studies.[7,10–12] Here, we report a Japanese individual with ASD possessing a novel *de novo* missense mutation in *POGZ* detected by trio-based WES.

MATERIALS AND METHODS

Case report

The patient was born at 39 weeks of gestation to healthy, non-consanguineous Japanese parents as the first child after a normal pregnancy. His birth weight was 3715 g (+1.79 s.d.), height 48.6 cm (−0.24 s.d.) and occipital–frontal circumference 34.0 cm (+0.44 s.d.). On examination at 5 years of age, his height was 106.2 cm (−0.21 s.d.), weight 17.9 kg (+0.26 s.d.), and occipital–frontal circumference 46.5 cm (−2.69 s.d.). He spoke no meaningful words, had a severe ID, limited social interactions, communication difficulties and repetitive behavior, and demonstrated an interest in water, and shiny or spinning objects. He was diagnosed with ASD showing level-3 social communications and level-3 restricted interests and repetitive behaviors both requiring very substantial support, and global developmental delay according to the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). Based on the Enjoji developmental assessment,[13] his developmental quotient was 16 (at age 5, motor development was judged to be at the 15-month-old level, daily living activities at 11.5 months and communication at 6.5 months). Dysmorphic facial features were noted including strabismus, a broad, low nasal bridge, anteverted nares, a prominent cupid bow and upturned corner of the mouth, and edematous hands (Figure 1). G-banded chromosomal analysis showed 46,XY,inv(9)(p12q13), which was derived from his mother with the normal phenotype. Brain magnetic resonance imaging at the age of 18 months and electroencephalography at 3 years were both normal.

Whole exome sequencing

Genomic DNA was extracted from the peripheral blood of the patient and his parents. Approximately 3 μg was sheared and coding regions captured using a SureSelect Human All Exon V4 (51 Mb) library (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Captured DNA was sequenced on the HiSeq2000 (Illumina, Inc., San Diego, CA, USA) with 101 bp paired-end reads. Image analysis and base calling were performed by sequence control software real-time analysis and CASAVA software v1.8 (Illumina, Inc.). The quality controlled (Path Filter) reads were mapped to the human reference genome (UCSC hg19, NCBI build 37), using Novoalign 2.08.02 (http://www.novocraft.com/). After the removal of PCR duplication by

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Picard 1.55 (http://broadinstitute.github.io/picard/), single nucleotide variants and short insertions and deletions (Indels) were called using Genome Analysis Toolkit (GATK) 1.6–5 (http://www.broadinstitute.org/gatk/). Called single nucleotide variants and Indels were annotated using ANNOVAR (http://www.openbioinformatics.org/annovar/). Through this flow, common variants registered in dbSNP137 (minor allele frequency $\geqslant 0.01$) were removed. Of all variants within exons or ± 30 bp from exon–intron boundaries, those registered in dbSNP137, the National Heart Lung and Blood Institute Exome Sequencing Project Exome Variant Server (NHLBI-ESP 6500, http://evs.gs.washington.edu/EVS/) and our in-house (exome data from 575 Japanese individuals) databases were removed. The variants were confirmed by Sanger sequencing using an ABI PRISM 3500xl autosequencer (Life Technologies, Carlsbad, CA, USA).

RESULTS AND DISCUSSION

To explore the genetic basis of ASD, we performed trio-based WES involving the patient and both parents. The mean depth of the RefSeq coding sequence was 125.11–164.12 reads, with 91.3–93.1% being covered by $\geqslant 20$ reads. One *de novo* missense mutation was detected and predicted as pathogenic by SIFT,[14] PolyPhen2,[15] and MutationTaster[16] (Table 1): *POGZ* NM_015100.3: c.3118G>A (p.Glu1040Lys). Sanger sequencing confirmed the mutation to be *de novo*.

During the last five years, *de novo* mutations in various genes have been implicated as causative of ASD.[17] Interpretation of the pathogenicity of these mutations in neuropsychiatric diseases was performed by protein–protein interaction analysis and expression studies, which indicated that *POGZ* is a transcriptional regulator gene in neuronal networks.[9,18] It encodes a heterochromatin protein 1 α-binding protein containing a cluster of multiple C2H2-type zinc fingers that may regulate gene expression,[19] a centromere protein (CENP) B-like DNA-binding domain, and a DDE domain (Figure 2a).[20] POGZ is involved in mitosis and neuronal proliferation,[20] so mutations in this gene may cause dysregulation of the neuronal proliferation associated with ASD.

To date, seven *de novo POGZ* mutations have been reported: three frameshifts, two stop–gain and two missense mutations have been detected on the screening for ASD, ID or schizophrenia, though clinical information was limited.[7,10–12] One of the missense mutations (p.Tyr597Cys) within a zinc finger domain showed a highly pathogenic predicted score, while the other was predicted to be a benign amino acid change and occurred outside the functional domains (Table 1).[11] Here, we report the first *de novo* missense mutation in the CENP-B-like domain, which is predicted to be highly pathogenic (Figure 2).

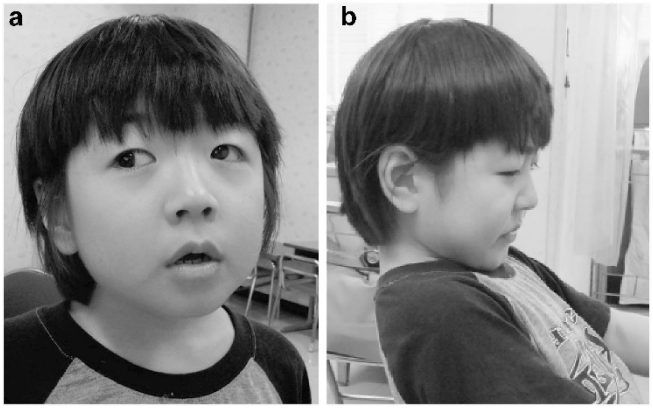

Figure 1 Craniofacial features of the patient at 5 years of age ((**a**) frontal view and (**b**) lateral view) demonstrating broad and low nasal bridge, anteverted nares, prominent cupid bow and upturned corner of the mouth. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

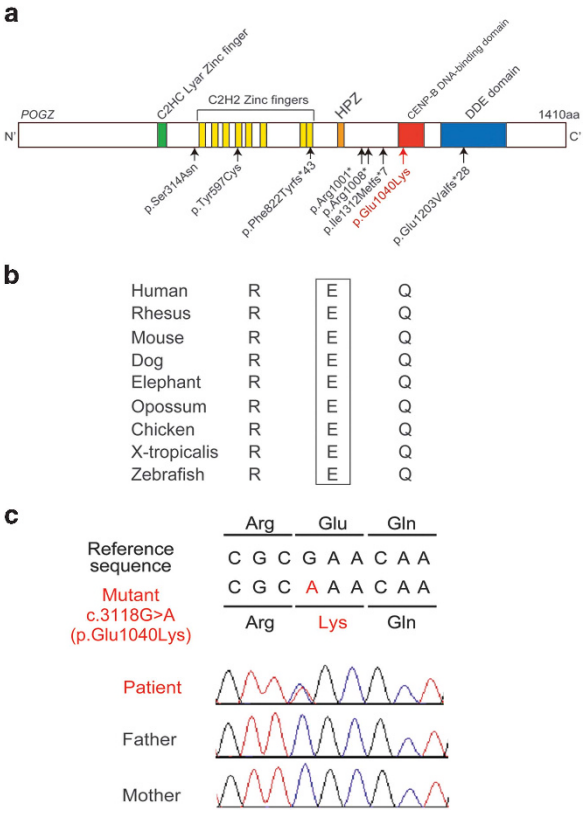

Figure 2 Schematic presentation of the POGZ protein and location of its mutations. (**a**) Schematic of POGZ and its five functional domains. The novel mutation identified in our patient (red) and the seven known mutations are shown below the protein. (**b**) Electropherograms of the patient and his parents showing the presence of the *de novo* mutation. (**c**) Interspecies evolutionary conservation of the original amino acid residue altered by the *de novo* mutation. POGZ, pogo transposable element-derived protein with zinc finger domain gene.

Table 1 Functional predictions of three *de novo* missense mutations in *POGZ*

Gene	*Accession no.*	*Mutation*	*Amino-acid change*	*SIFT*	*PolyPhen2*	*MutationTaster*	*Patient*
POGZ	NM_015100.3	c.3118G>A	Glu1040Lys	0.01 (damaging)	0.998 (probably damaging)	0.99999 (disease causing)	Present case
POGZ	NM_015100.3	c.1790A>G	Tyr597Cys	0 (damaging)	0.999 (probably damaging)	0.997 (disease causing)	Iossifov *et al.*,[11]
POGZ	NM_015100.3	c.941G>A	Ser314Asn	0.211 (tolerated)	0.09 (benign)	0.853 (polymorphism)	Iossifov *et al.*,[11]

Abbreviation: POGZ, pogo transposable element-derived protein with zinc finger domain gene.
P.Glu1040Lys was found in the present patient and the other two were reported previously.

In conclusion, we identified a novel *de novo POGZ* mutation that appears to be causative of ASD. Future studies should accumulate more ASD patients harboring *POGZ* mutations to delineate phenotypic features by *POGZ* abnormality.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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