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SHORT COMMUNICATION

# A novel *de novo POGZ* mutation in a patient with intellectual disability

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***POGZ*, the gene encoding pogo transposable element-derived protein with zinc-finger domain, has been implicated in autism spectrum disorder and it is widely expressed in the human tissues, including the brain. Intellectual disability (ID) is highly heterogeneous neurodevelopment disorder and affects ~2–3% of the general population. Here we report the identification of a novel frameshift mutation in the coding region of the *POGZ* gene (c.1277_1278insC), which occurred *de novo* in a Chinese patient with ID. *In silico* analysis and western blotting revealed this frameshift mutation generating truncated protein in peripheral blood lymphocytes, and this may disrupt several important domains of *POGZ* gene. Our finding broadens the spectrum of *POGZ* mutations and may help to understand the molecular basis of ID and aid genetic counseling.**
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## INTRODUCTION

Intellectual disability (ID) is a heterogeneous group of neuro-developmental disorders and may be caused by genetic and environmental factors. About estimate 2–3% of the general population is affected in ID.[1] The etiology of ID is genetically heterogeneous, and *de novo* point mutations may account for 22% in severe ID cases.[2] Recently, hundreds of candidate ID genes were revealed by large-scale trio-based next-generation sequencing studies,[2,3] suggesting *de novo* mutations are strongly enriched in ID patients.

*POGZ* is a gene that codes for the pogo transposable element-derived protein with zinc finger domain, which plays a role in mitotic cell cycle progression, and is involved in kinetochore assembly and mitotic sister chromatid cohesion.[4] Recently researches highlight the gene as a chromatin modifier that has significant role in psychiatric disorders and autism spectrum disorder (ASD).[5–7] Here we report a novel *de novo* frameshift mutation in *POGZ* gene in Chinese patient with ID.

## MATERIALS AND METHODS

### Case report

A female patient aged 5 years, the first child of healthy non-consanguineous parents, was born at 39 weeks of gestation after a normal pregnancy. Prenatal period was normal. Her birth weight was 3013 g (+3.39 s.d.). Family history was negative for ID and other neurological disorder. She could raise her head at 4 months and walked alone at 18 months. Later her speech and physical development was delayed. She could only count numbers within 20, and her developmental quotient was 54 at 3.5 years of age.

On examination at 5 years of age, her weight was 18 kg (+0.04 s.d.), height was 108 cm (+0.24 s.d.) and occipital frontal circumference was 47cm (+0.08 s.d.). Patient was assessed by using Wechsler Preschool and Primary Scale of Intelligence-4th edn, autism behavior checklist and Achenbach system of empirically based assessment. Patient could only speak simple sentences, had a mild ID (intelligent quotient = 63). Patient had a low frustration tolerance and displayed intermittent tantrum behaviors. The craniofacial features consisting of high-arched eyebrows, a broad, low nasal bridge, anteverted nares and a thin upper lip (Figure 1). Single-nucleotide polymorphism array analysis (Illumina Human660 BeadsChip, Illumina Inc., San Diego, CA, USA) confirmed no pathogenic copy-number variation change in the patient.

### Next-generation sequencing

In this study, we used molecular inversion probes to sequence in 764 patients (ID 729, epilepsy 27 and ASD 8) and 772 of parents' samples. Informed consent from all the parents and approval from the local institutional review board were obtained for the molecular studies. DNA was extracted from peripheral blood samples.

Molecular inversion probes were designed to cover coding region of *POGZ* gene. The libraries were amplified during 22 cycles of PCR, during which an 8-bp sample barcode was introduced. After the barcode library was purified and quantified, it was sequenced via paired-end 100-bp reads with an 8-bp barcode read on Illumina HiSeq2000 sequencer. The reads were performed arm trimming (MIPGen v.1.0), alignment (BWA v.0.7.8), multi-sample genotype calling (GATK Unified Genotyper v.3.2-2) and variants were annotated with SeattleSeq138. Variants with an alternative allele frequency $<0.005$ in the NHLBI Exome Sequencing Project Exome Variant Server (ESP6500), or not present in our exome database of ~500 individuals and the 1000 Genomes Browser were included before the analysis as previously described.[8] The variants were validated by Sanger sequencing.

### Western blot analysis

In the patient, the c.1277_1278insC frameshift mutation in the exon 9 disrupts the zinc-finger domain. To validate the functionality of *de novo* mutation in

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*POGZ*, we detected the *POGZ* encode protein by western blot analysis in peripheral blood lymphocytes. The protein lysates were electrophoresed on 8% SDS–polyacrylamide gel electrophoresis gel and transferred onto a polyvinylidene difluride membrane. The membranes were incubated with anti-human *POGZ* polyclonal antibody (rabbit polyclonal; Abcam, Cambridge, MA, USA), followed by detection with secondary horseradish peroxidase-conjugated goat anti-rabbit IgG antibody. Proteins were visualized using the enhanced chemiluminescence system. Densitometry was analyzed by Quality One Image software (Biorad, Hercules, CA, USA).

## RESULTS AND DISCUSSION

In this study, we found one novel *de novo* mutation of *POGZ* in patient with mild ID, leading to the cause of a premature truncated protein in peripheral blood lymphocytes (Figure 2a). The antibody detected wild-type *POGZ* at the correct size (predicted molecular weight ~155 kD), and also detected the truncated protein in patient (Figure 3). The expression of wild-type *POGZ* protein in the patient was reduced respectively ($n=3$, $P<0.05$, Student's *t*-test).

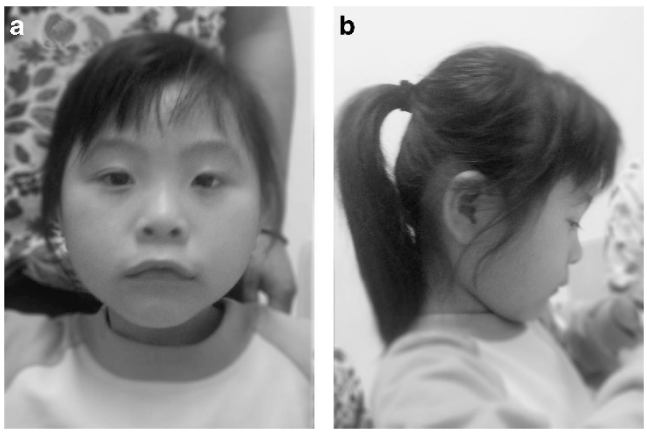


**Figure 1** Clinical photographs of patient harboring a *de novo* mutation (c.1277_1278insC) in POGZ. (**a**) Frontal view and (**b**) lateral view demonstrate high-arched eyebrows, broad and flat nasal bridge and thin upper lip. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

ID is a multifarious neurodevelopmental disorder and may exhibit overlapping phenotypes with autism and epilepsy.[9] The next-generation sequencing provides us a great opportunity to study the genetic cause of psychiatric disorder. Recently studying the *de novo* variations offered more insights into genetic-determining genes in ID.[10] In previous studies, loss of function mutations in *POGZ* gene were found significantly enriched in patients with ASD and ID by large-scale next-generation sequencing.[11,12] Our case provides evidence for the important role of *POGZ* gene in the development of ID.

The *POGZ* gene is located on chromosome 1q21.3, which encodes several domains including multiple C2H2-type zinc fingers, a centromere protein (CENP) B-like DNA-binding domain, and a DDE domain, which may have an important role in mitosis and neuronal proliferation.[4,13] The DDE domain is characterized by a catalytic site composed of two or three aspartic acid, which allows DNA-modifying reactions such as strand cleaving, nicking and ligation.[14] Recent research suggests those DNA-binding domain protein may function in the role of human neurodevelopmental disorders.[15]

For now, eight *de novo POGZ* mutations have been identified through next-generation sequencing, including missense, frameshift and stop-gain mutations, which have been associated with ID and ASD.[16,17] Here we report the first *de novo* frameshift mutation in the zinc-fingers domain, yielding a premature truncated protein that may disrupt DNA and protein binding activation (Figure 2b).

Moreover, a recent report described a Japanese patient with a *de novo* missense mutation identified by trio-based exome sequencing. The mutation was located in the CENP B-like DNA-binding domain and the patient was described having ASD, severe ID and global

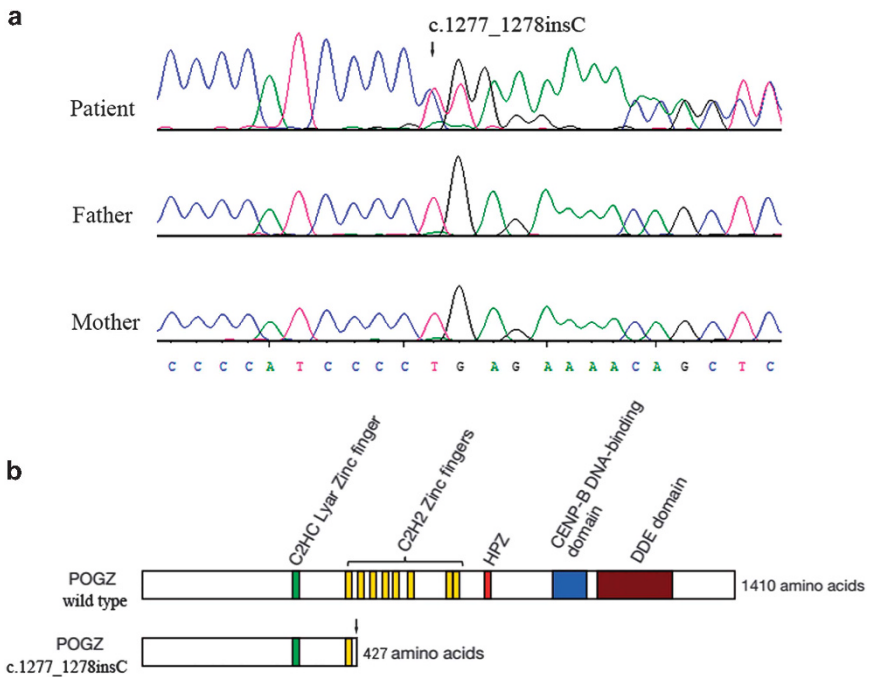


**Figure 2** (**a**) Electropherograms of the patient and her parents showing the presence of the *de novo* mutation in *POGZ* gene. (**b**) Schematic overview of the *POGZ* full-length and truncating protein, the frameshift variation was marked by black arrow. *In silico* analysis the frameshift mutation (c.1277_1278insC) was predicted causing a premature truncated protein in *POGZ* with a short N-terminal domain.

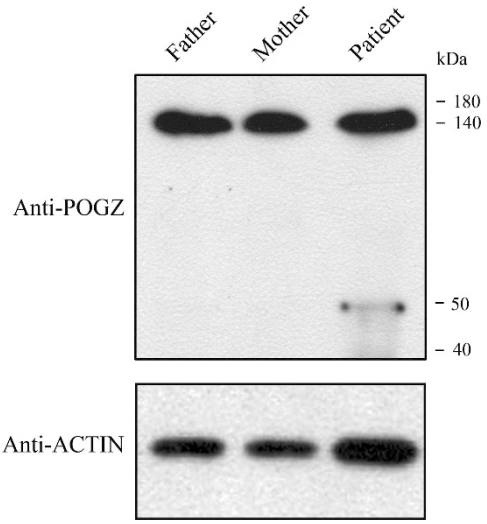


**Figure 3** Western blot analysis of POGZ protein variation from patient and his parents in peripheral blood lymphocytes. Anti-POGZ antibodies were used to recognize the wild type and novel POGZ mutant. Densitometric results shows wild-type POGZ level was significantly reduced in the patient ($n=3$; $P<0.05$, Student's *t*-test).

developmental delay. After the identification of this *de novo* mutation in *POGZ*, we tried to collect all available patient's clinical data and images to reevaluate the diagnosis of psychiatric disorder. Psychiatrist evaluated the patient and found mild ID with stereotyped behaviors in this patient; however, ASD was absent (autism behavior checklist total score 19; sensory 3, relating 0, body and object use 7, language 3, social and self-help skills 6). Photos of the patient at the age of 5 years clearly showed such as broad and flat nasal bridge and thin upper lip consistent with previously described in the Japanese patient.[17] Those evidence were found in our patient suggest phenotypic heterogeneity may exist among patients with mutation in *POGZ* gene.

In summary, the patient features are consistent with mild ID caused by a *de novo* frameshift mutation in *POGZ* gene. Our research supports the hypothesis that *de novo* variants represent significant causal factors in ID and extend the phenotype of patient with *POGZ* haploinsufficiency.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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