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RESEARCH ARTICLE

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A novel frameshift variant in *SON* causes Zhu-Tokita-Takenouchi-Kim Syndrome

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Abstract

Background: Zhu-Tokita-Takenouchi-Kim syndrome is a severe multisystem developmental disorder characterized by intellectual disability, developmental delay, malformations of the cerebral cortex, epilepsy, vision problems, musculoskeletal abnormalities, and congenital malformations. This syndrome is caused by heterozygous pathogenic variants in the *SON* gene at chromosome 21q22.1.

Objectives: The aim of this study was to investigate the pathogenesis of a 4-year-old Chinese child who displayed severe intellectual disability, delayed psychomotor development, and facial dysmorphism.

Methods: A sequential detection including chromosome karyotyping, chromosome microarray analysis (CMA), and whole-exome sequencing (WES) was performed on this child. The familial verification of WES result was conducted by Sanger sequencing.

Results: A de novo frameshift variant *SON*: c.5230delC (p.Arg1744ValfsTer29) was identified in the proband. The identical variant was not found in his family members. The frequencies of this variant in gnomAD/gnomAD_EAS databases were both none.

Conclusions: This study substantiates that *SON*: c.5230delC (p.Arg1744ValfsTer29) is a pathogenic variant of Zhu-Tokita-Takenouchi-Kim syndrome and it is the first time to report Zhu-Tokita-Takenouchi-Kim syndrome in China.

KEYWORDS

intellectual disability, psychomotor development, *SON*, Zhu-Tokita-Takenouchi-Kim syndrome

1 | INTRODUCTION

Zhu-Tokita-Takenouchi-Kim (ZTTK) syndrome (MIM#617140) is a severe multisystem developmental disorder characterized by intellectual disability and delayed psychomotor development. ZTTK syndrome is an autosomal dominant disorder. Affected individuals had mild to moderate facial dysmorphisms, including facial asymmetry, low-set ears, horizontal eyebrows, short philtrum, a broad and/or depressed nasal bridge, epicanthal folds, and prominent forehead.[1] Most patients also have hypotonia, poor feeding, musculoskeletal abnormalities, eye and/or vision abnormality, heart defects, and urogenital malformation. Brain magnetic resonance imaging (MRI) usually shows developmental abnormalities including cortical and/or cerebellar atrophy, gyral changes, and thin corpus callosum.[1] In 2015, Zhu et al[2] described a 5-year-old girl with developmental delay, seizures, minor dysmorphisms, leukodystrophy, macrocephaly, intestinal atresia, and ventriculoseptal defect. This patient had a frameshift variant in the *SON* gene, which is a ubiquitously expressed

and phylogenetically conserved gene that encodes a DNA-binding protein. Afterward, Tokita et al,[3] Takenouchi et al,[4] and Kim et al[1] reported 28 unrelated patients in 2016, including a patient previously reported by Zhu et al, with a complex neurodevelopmental disorder associated with mild to severe intellectual disability. All of the patients carried variants in the *SON* gene which detected by whole-exome sequencing. Thus, ZTTK syndrome was named after the initials of these authors and was determined to be caused by pathogenic variants in the *SON* gene.

SON gene located at 21q22.1 which spans 34,478 base-pair (bp) and contains 12 exons. SON is a nuclear speckle protein able to bind to both DNA and RNA, and it shares homology with pre-mRNA splicing accessory factors.[5-7] A growing number of research demonstrated that *SON* maintains accurate splicing of human mRNAs and pointed toward an important role for *SON* in regulating the expression of genes involved in cell cycle progression and pluripotency.[8-10] So far, a total of 23 variants in *SON* have been reported in 28 patients, including 17 frameshift, 2 missense, 2 nonsense, and 2 in-frame-deletion variants. There are 7 unrelated individuals carrying the *SON*: c.5753_5756del (p.Val1918Glufs*87) variant. Twenty of these 23 variants occurred in exon 3, 2 in exon 2, and 1 in exon 4.[2-4] The vast majority of the patients were from Europe and the United States, with only one case from Japan, while no one has been reported in China.

Here, we reported a de novo heterozygous frameshift variant, *SON*: c.5230delC (p.Arg1744ValfsTer29), identified in a 4-year-old Chinese child with severe intellectual disability and delayed psychomotor development.

2 | MATERIALS AND METHODS

2.1 | Subjects

The proband was a 4-year-old boy, and he is the first child of his healthy non-consanguineous parents (Figure 1A). The mother was 28 years old at delivery with mild thalassemia. Prenatal ultrasound suggested the fetus had a small gall bladder and a confluent kidney. The proband was prematurely delivered at 33^{+5} weeks with 1690 g weight. During the infant period, he had severe hypotonia and poor feeding. He showed significant delay in psychomotor development and severe intellectual disability. At the age of 4 months, the psychomotor development index (PDI) was <50 (average index: 100) and the mental development index (MDI) was 57 (average index: 100). At the age of 6 months, the psychomotor development index (PDI) was also <50 (average index: 100) and the mental development index (MDI) was 61 (average index: 100). He can only help standing and unable to walking when he was 15 months old. At the age two, he developed seizure and it lasted up

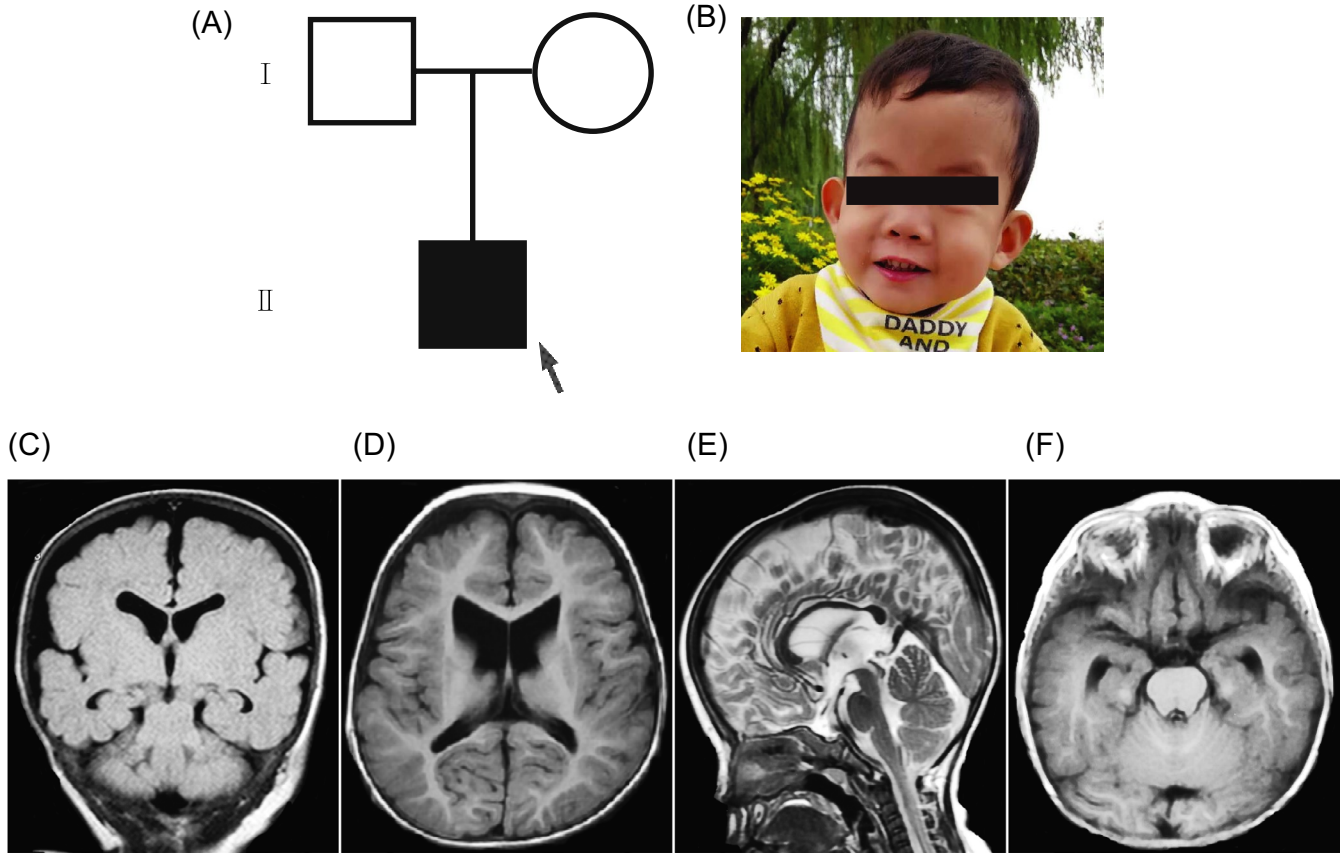

FIGURE 1 Pedigree structure, photograph, and brain MRI of proband. A, Family pedigree of the patient. The proband is indicated with a black arrow. B, The face characteristic of the proband. C-F, The brain MRI of proband which showed parallel bilateral lateral ventricles, ventricular enlargement, and thin corpus callosum

to 30 minutes. He showed distinctive facial features with a low-set ears, a short philtrum, a broad and depressed nasal bridge, epicanthal folds, and prominent forehead (Figure 1B). The brain MRI results of the proband at 1-year-old showed ventricular enlargement, parallel bilateral lateral ventricles, and thin corpus callosum (Figure 1C-F).

2.2 | Chromosome karyotyping

Conventional chromosome karyotyping by G-banding was performed on the proband to detect overall chromosomal anomalies in accordance with routine operation procedure.[11] The karyotype result was analyzed in accordance with ISCN 2016.[12]

2.3 | DNA extraction

Peripheral blood samples were obtained from the proband and his parents. Genomic DNA from 200 μL peripheral blood was extracted using a TIANamp Genomic DNA Kit according to manufacturer's protocol.

2.4 | Chromosome microarray analysis

The Affymetrix CytoScan 750K gene chip was used for DNA digestion, amplification, purification, fragmentation, labeling, hybridization, rinsing, staining, and scanning. ChAS software was used to analyze the scanned data. The reporting threshold of copy number variant was missing or repeated ≥400 Kb, and loss of heterozygosity (LOH) ≥10 Mb.

2.5 | Whole-exome sequencing

DNA fragments were hybridized and captured by IDT's xGenExome Research Panel (Integrated DNA Technologies). The Novaseq6000 platform (Illumina), along with 150 bp pair-end reads, was used for sequencing genomic DNA. The sequencing reads were aligned to the human reference genome (hg19/GRCh37) using the Burrows-Wheeler Aligner. Base quality score recalibration, indel realignment, duplicate removal, insertions/deletions (indels), and single-nucleotide variants' (SNVs) detection were performed using Genome Analysis Toolkit (GATK) HaplotypeCaller. Variants were annotated using the ANNOVAR software and then filtered according to their predicted effects and allele frequencies in the available public databases gnomAD (http://gnomad.broadinstitute.org/). The results were verified by Sanger sequencing.

3 | RESULTS

3.1 | Clinical data analysis

The proband had typical clinical features of ZTTK syndrome including intellectual disability, brain malformation, neurological abnormalities, kidney malformation, and facial dysmorphism. The clinical characteristics of the proband and the 28 reported patients were summarized in Table 1.

3.2 | Genetic testing

The karyotype of the proband was normal. The CMA result showed no chromosome numerical abnormality or pathogenic copy number variant. To further clarify the pathogenesis of the disease, whole-exome sequencing was performed to the proband. A de novo heterozygous frameshift variant *SON*: c.5230delC (p.Arg1744ValfsTer29) was found in the patient and confirmed by Sanger sequencing (Figure 2). Furthermore, the proband's parents did not carry this variant.

3.3 | Bioinformatic analysis

The gnomAD database population frequency was none, gnomAD_EAS population database east Asian population frequency was

TABLE 1 Clinical features of subjects with *SON* variants

Characteristics	Proband	Number of affected individuals*	Percentage
Intellectual disability	yes	28/28	100%
Facial dysmorphism	yes	28/28	100%
Brain malformation	yes	22/28	79%
Musculoskeletal abnormalities	no	23/28	82%
Short stature	yes	18/28	64%
Hypotonia	yes	21/28	75%
Seizures	yes	13/28	46%
Eye and/or vision abnormality	no	20/28	71%
Urogenital malformation	yes	9/28	32%
Heart defect	no	5/28	18%

*The number of affected individuals previously reported.

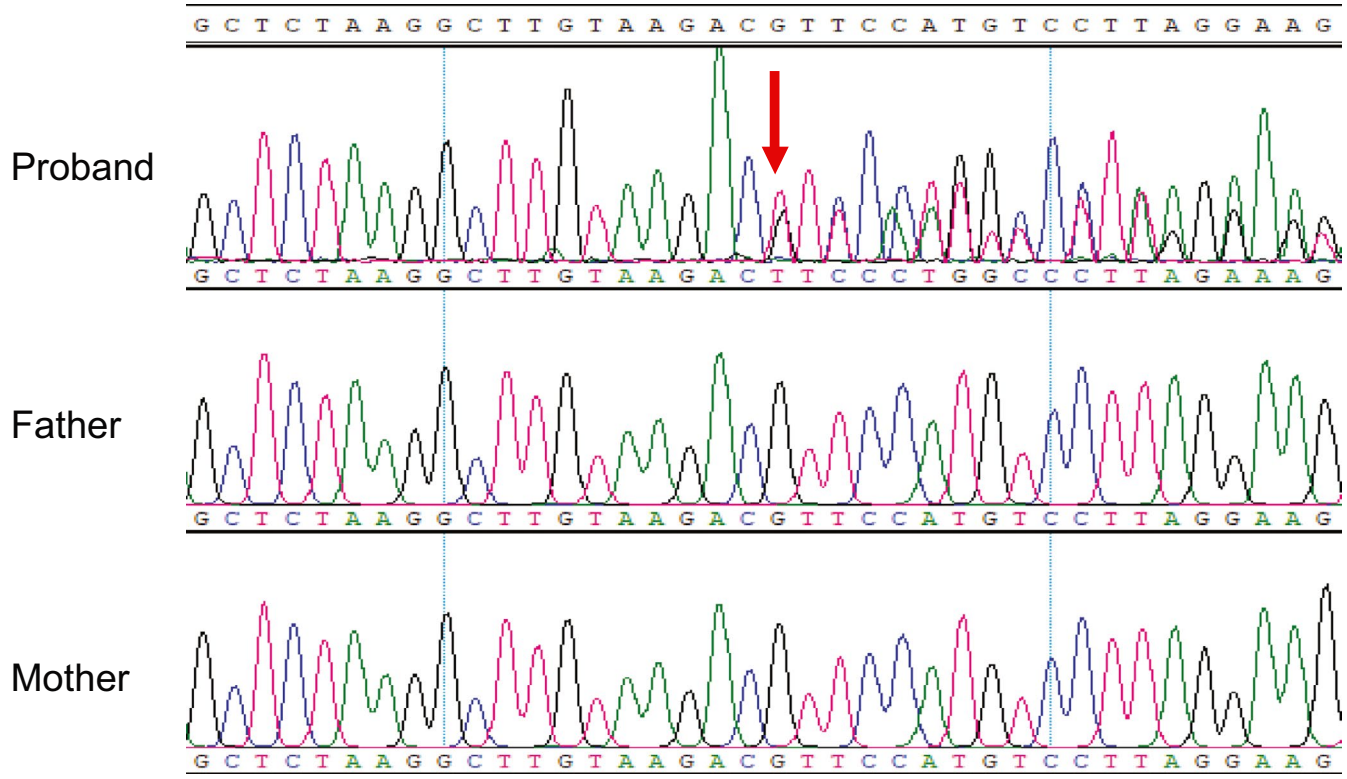

FIGURE 2 Sanger sequencing analysis of the family. Upper panel: segments of genomic DNA sequences of the proband showing a variation (SON: c.5230delC) which the region indicated by the red arrow. Middle panel: segments of genomic DNA sequences of the proband's father. Lower panel: segments of genomic DNA sequences of the proband's mother

none, and the population frequency was not seen in the internal database. According to the American College of Medical Genetics and Genomics (ACMG) criteria, this novel variant was classified as pathogenic variant (PVS1 + PS2+PM2 + PP4).

4 | DISCUSSION

Discovery of the *SON* gene came in 1988 when a partial clone called *SON3* was isolated from a human embryonic cDNA library and noted as having unusual sequence structure. *SON3* region codes for protein with the presence of a cluster of short tandem repeats from 7 to 19 amino acid long. Also, *SON3* showed homology to the DNA-binding structure proteins as well as the human MYC and MOS proto-oncogene.[13] The full length human *SON* was independently identified by Wynn et al which shares 84.2% amino acid identity with its mouse homolog.[5] SON was initially considered to be a DNA-binding protein.[14-16] Moreover, SON contains a RS domain that is enriched in serine(S)/arginine(R) dipeptides which shares homology with proteins involved in mRNA processing.[5,17] Furthermore, SON co-localized with pre-mRNA processing factors in nuclear speckles[5-7] and the presence of double-stranded RNA-binding domain and G-patch domain[18] all hinted at pre-mRNA splicing functions for SON.

Pre-mRNA splicing regulation in eukaryotes is critical for maintaining proper gene expression as well as protein biodiversity. Mis-regulation of pre-mRNA splicing can produce aberrant proteins disrupting biochemical pathways and/or cell growth controls which contribute to human disease.[19] Several independent studies demonstrated that SON plays a critical role in pre-mRNA splicing. Depletion of *SON* in human embryonic stem cells results in splicing defects of transcripts encoding for pluripotency regulators such as *OCT4*, *PRDM14*, *E4F1*, and *MED24*.[10] Knockdown of *SON* leads to exon skipping in pre-mRNAs for chromatin-modifying enzymes, including *ADA*, *HDAC6*, and *SetD8*.[8] In 2016, 28 individuals with pathogenic variants in *SON* were reported who were mainly characterized by intellectual disability and delayed psychomotor development.[2-4] To identify molecular mechanisms underlying the clinical features of individuals with *SON* haploinsufficiency, Kim et al examined global expression patterns upon *SON* knockdown in cellular systems. They noticed that a group of genes playing pivotal roles in neuronal cell migration, embryonic survival, metabolism, and mitochondrial function showed significantly decreased expression upon *SON* knockdown.[4] Moreover, *SON* knockdown in kidney cell lines leads to abnormal pre-mRNA splicing, resulting in decreased expression of several established congenital abnormalities of the kidney and urinary tract (CAKUT) genes.[20] These might partially explain why the individuals with *SON* heterozygous variants showed such severe clinical manifestations.

In this study, we identified a novel variant, namely *SON*: c.5230delC (p.Arg1744ValfsTer29), in a Chinese boy by whole-exome sequencing. This frameshift variant occurred in exon 3 which results in the deficiency of G-patch domain and double-stranded

RNA-binding motif (DSRM) domain. G-patch domain was found in a number of RNA-binding proteins and also in proteins containing RNA-binding domains.[21] DSRM domain is highly specific for double-stranded RNA which was found in a variety of proteins including dsRNA dependent protein kinase, RNA helicases, and dsRNA dependent adenosine deaminases.[21] Accordingly, the proband who showed such severe symptoms was possibly because of the defective RNA splicing of multiple genes critical for brain development, neuron migration, and metabolism.

In conclusion, we confirmed that *SON*: c.5230delC (p.Arg1744ValfsTer29) is a pathogenic variant of ZTTK syndrome which enriched the *SON* gene variant spectrum. And it is the first time to report the *SON* variant which leads to ZTTK syndrome after its naming in 2016. Also, it is the first time to report ZTTK syndrome in Chinese population.

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