Novel De Novo Heterozygous Variants in the SON Gene Causing ZTTK Syndrome: A Case Report of Two Patients and Review of Neurological Findings

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Abstract

Zhu-Tokita-Takenouchi-Kim (ZTTK) syndrome is a newly described autosomal dominant multisystem developmental disorder resulting from a mutation of the SON gene located on chromosome region 21q22.11. It is characterized by heterogeneous features such as intellectual disability, facial dysmorphisms, poor feeding, vision abnormalities, musculoskeletal anomalies, congenital heart and genitourinary system defects, as well as several unique neurological findings including seizures, tone abnormalities, autism spectrum disorder and variable brain abnormalities noted on neuroimaging. Unfortunately, we lack adequate information regarding the spectrum of these neurological symptoms. In this study, we report 2 new unrelated cases of ZTTK syndrome, and identify new pathogenic variants in the SON gene through microarray analysis and whole-exome sequencing. We also emphasize the neurological manifestations of the syndrome in our patients and discuss the significance of gathering more data regarding neurological presentation, particularly seizure characteristics and long-term developmental progression. This information will be crucial to help understand long-term neurodevelopmental prognosis in these patients.

Keywords
developmental delay, hypotonia, novel variants, SON gene, Zhu-Tokita-Takenouchi-Kim (ZTTK) syndrome

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Introduction

The Zhu-Tokita-Takenouchi-Kim (ZTTK) syndrome, Online Mendelian Inheritance in Man (OMIM) 617144, is a relatively newly described autosomal dominant multisystem developmental disorder characterized by heterogeneous features, including intellectual disability, facial dysmorphisms, poor feeding, musculoskeletal anomalies, and congenital heart and genitourinary system defects.¹⁻¹¹ It results from a genetic mutation affecting the SON gene, a ubiquitously expressed and evolutionarily conserved gene in vertebrates located on the human chromosome region 21q22.11. SON haploinsufficiency leads to defective RNA splicing of multiple genes critical for neural and multi-organ development, causing multi-organ defects, including of the nervous system.¹⁻¹¹

ZTTK has been characterized by several neurological findings. Tone abnormalities, epilepsy or other electroencephalographic (EEG) abnormalities, and autism spectrum disorder occur in about 62% of the subjects.⁴⁷ These neurological findings are paralleled by variable brain malformations identified on magnetic resonance imaging (MRI).¹⁻¹¹

With only 60 reported cases since its first description in 2015 which has expanded our understanding of this condition, some aspects remain elusive, particularly in terms of genotype-phenotype correlation and progression of neurological symptoms. In this paper, we present two unrelated patients with ZTTK syndrome carrying novel de novo heterozygous variants of the SON gene, with an aim to highlight their neurological findings.

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Clinical Findings

Case 1. 11-month-old girl born at 41 weeks of pregnancy (Figure 1A and B). Detailed history and physical examination findings are presented in Table 1. The patient had several neurological findings, including hypotonia, psychomotor retardation and developmental delay. Head ultrasound revealed callosal hypoplasia with decreased periventricular white matter. These findings were confirmed with brain MRI showing diffuse loss of cerebral white matter with thinned corpus callosum, and ventriculomegaly (Figure 1C).

Case 2. 2-year-old girl born at 41 weeks of gestation. Detailed history and physical examination findings are presented in Table 1. In terms of neurological findings, patient developed febrile seizures at one year of age, however EEG was unremarkable. She had appropriate cognitive but delayed language function for her age. Brain MRI showed polymicrogyria (Figure 1D). Neurological examination revealed hypotonia, head lag and a positive Babinski sign.

Genetic Findings. Patient 1: microarray-based chromosome analysis was performed using the I Scan System with the Global Diversity Array-8 v1.0 Array BeadChip. This microarray consists of >1 000 000 genetic markers. The markers provide information on the copy number status of the entire genome and provide single nucleotide genotyping that allows for detection of mosaicism, uniparental disomy, loss of heterozygosity and identity by descent. Patient hybridization data was compared to a compilation of information obtained from multiple controls including the HapMap set of 270 controls. Criteria for designating a reportable aberration include deletion larger than 200 kb with a minimum of 20 consecutive markers disrupted and duplication larger than 500 kb.

Findings: chromosome microarray analysis: arr[GRCh37] 21q22.11 (34816414_34941521)x1;125kb deletion of 21q22.11 including the majority of the TMEM50B gene, all of the GART gene, and exons 1–8 of the SON gene, including exon 3 which includes the RS and RNA binding domains (chr 21:34, 816, 414-34, 941, 521).

Patient 2: Whole-exome sequencing: The Agilent SurSelect Clinical Research Exome kit was used to target known disease-associated exonic regions of the genome. The targeted regions were

Figure 1. Facial characteristics and brain MRI findings in patients with ZTTK syndrome. (A) and (B) facial features of patient 2 showing hypertelorism, smooth philtrum and epicanthal fold (obtained with parental consent). (C) MRI findings of ventriculomegaly (yellow arrow), loss of cerebral white matter, thinned corpus callosum and (D) polymicrogyria (blue arrow).
Table 1. Clinical Features of Patients 1 and 2 with ZTTK in Comparison to Findings Reported in the Literature.

| Clinical characteristics | Patient 1 | Patient 2 | Literature review |
|--------------------------|-----------|-----------|------------------|
| Current age              | 11 months | 2 years   |                  |
| Sex                      | Female    | Female    |                  |
| Weight                   | 20lb 10oz (70.61%) at 11 months | 25lb 9.2oz (33.93%) at 2 years |
| Length                   | 71.3 cm (25.81%) at 11 months | 84.5 cm (40.69%) at 2 years |
| Head circumference       | 45.7 cm (77.9%) at 11 months | Not available |
| Pregnancy                | Peripartum cardiomyopathy in mother | Uncomplicated |
| Gestational age          | Full term | Full term |                  |
| Mode of delivery         | Spontaneous vaginal | Spontaneous vaginal | |
| Post-natal course        | Feeding difficulties, failure to thrive | Feeding difficulties | |
| Therapeutic interventions| Physical therapy, occupational therapy, speech therapy | Physical therapy, occupational therapy, speech therapy | |
| Neurological findings    | Hypotonia, developmental delay | Hypotonia, febrile seizures, developmental delay | Hypotonia, developmental delay/intellectual disability,1–7,9–12 seizures,1–3,6,7,10–12 autism spectrum disorder,2,3,10,11 speech delay11 |
| EEG findings             | N/A       | Normal    | Changes in the left mid-temporal area9 |
| Imaging findings         | Diffuse loss of cerebral white matter with fully formed but uniformly thinned corpus callosum, polymicrogyria, ventriculomegaly | Polymicrogyria, loss of cerebral white matter, ventriculomegaly | Ventrilocorony,1,2,5,9–11 corpus callosum thinning/agenesis,2,4,7,10,11 loss of white matter,1,2,5,9–11 cortex/ cerebellar abnormalities,2,10,11 plagiocephaly, thin septum pellucidum, incomplete hippocampal inversion, delayed myelination,11 |
| Dysmorphic features      | Low set ears, down slanting palpebral fissures, flat nasal bridge, wide set eyes and frontal bossing | Down slanting palpebral fissures, hypertelorism, flat philtrum, thin upper lip | Short and smooth philtrum, thin lips, epicanthal fold, frontal bossing, down slanting palpebral fissures, depressed nasal tip, low hanging columella, sparse eyebrows, low set ears, inverted nipples, wide space front teeth, sandal gap, prominent chin, deep set eyes, tubular nose, hypoplastic nails, ptosis, prognathism, high palate, sparse hair, macrocephaly1,2,2–9,12 |
| Ophthalmological findings| Hyperopia and anisocoria | None | Exotropia, myopia, nystagmus, strabismus, hypermetropia, astigmatism, convergent squint, cortical visual impairment1,2,3,6,10,11 |
| Gastrointestinal abnormalities | Gastroesophageal reflux disease | Gastroesophageal reflux disease | Gastroesophageal reflux disease, intestinal atresia, intestinal malrotation, failure to thrive, gastrostomy -tube feeding, diarrhea, reflux, gastric dysmotility, dysphagia1,2,6,9–11 |
| Cardiovascular abnormalities | None | None | Hypoplastic left heart syndrome, Patent ductus arteriosus/atrial/ventricular septal defect1,2,3,6,10,11 |
| Genitourinary abnormalities | None | None | horseshoe/dysplastic kidneys, unilateral renal agenesis, vesicoureteral reflux, recurrent urinary tract infections, pyelectasis, nephrocalcinosis2,3,6,10,11 |
| Musculoskeletal abnormalities | None | None | Joint hypermobility, scoliosis/kyphosis, hemi vertebral, arachnodactyly, contractures, pectus excavatum, syndactyly, clinodactyly, pes planovalgus, talipes equinovarus, pes planus, genu varus, short toes, hip dysplasia2,7–9,12 |
| Hematological/            | None | None | Thrombocytopenia,7 immunoglobulin |

(continued)
sequenced using the Illumina NextSeq 500/550 or NovaSeq 6000 system with 150 bp paired-end reads. Illumina DRAGEN Bio-IT Platform software was used was used for sequence alignment and comparison to the human genome build 19. The emedgene software was used to filter and analyze sequence variants identified in the patient and compare them to the sequences of the parents.

Findings: heterozygous 5 base-pair duplication starting at nucleotide 1186 of the SON gene (NM_032195.2):c.1186_1192dup, p.(Pro398ArgfsTer31)), with reading frame shift and generation of premature termination codon with loss of the full-length 2426 amino acids protein due to truncation. Both identified variants have a null frequency in tested database (gnomAD https://gnomad.broadinstitute.org/).

Discussion

ZTTK is a newly discovered syndrome with a collection of symptoms involving most organ systems. To date, 60 cases have been reported. Our patients presented with several symptoms similar to those described, including dysmorphic features, congenital anomalies and gastroesophageal reflux.1–7,9–11

Our patients also presented with neurological findings and imaging abnormalities reported in the literature. Both manifested hypotonia and developmental delays. Indeed, neurological findings predominate in ZTTK; all examined children presented with varying degrees of intellectual disability.1–11 Hyper or hypotonia occurred in 75% of patients, and autism spectrum disorder occurred in about 62% of cases.7 Patient 2 developed febrile seizures, albeit with unremarkable EEG, consistent with previous reports where epilepsy or other EEG abnormalities occurred in about 46%7,60%10 and 50%11 of cases. In a more recent study, clinical seizures were reported in patients from 6 months to 8 years; five of nine individuals were noted to have seizures associated with febrile illness, and three were noted to have at least one episode of status epilepticus. Seizures were successfully managed with medication including levetiracetam, carbamazepine, or clobazam.11 However, despite seizures being a defining feature of ZTTK syndrome, information is lacking regarding medical management and long-term prognosis. Moreover, EEG studies have been under-reported with results only available for three patients thus far.5 Of these, one study confirmed changes in the left mid-temporal area on EEG in a patient with febrile seizures.6

Brain MRI showed diffuse loss of cerebral white matter, ventriculomegaly and polymicrogyria, consistent with literature where brain malformations were observed in 85.9% of the subjects.1–11 Interestingly, patient 1 had more pronounced developmental delay compared to patient 2, and whether that correlates with MRI findings and/or specific SON gene mutation remains to be determined.

Among the SON variants reported so far, 69% were frameshift variants, 13% were nonsense variants, 1% were a splice site variant, 13% were missense variants, and 4% were in-frame deletions. Five variants were reported in multiple individuals: a 4-bp deletion, NC000021.8:g.34927290_34927293del) was found in 13 individuals; three other small deletions, NC000021.8:g.34923418_34923419del (p.(Val629Alafs*56)), NC000021.8:g.34925389_34925393del (p.(Met1284Ilefs*2)), and NC000021.8:g.34927547del (p.(Val2004Trpfs*2)), as well as one substitution, NC000021.8:g.34924871C>T (p.(Arg1112*)), were each identified in two individuals. Variants were de novo in all cases in which both parents were available for testing, except for one maternally inherited case reported.11 A comparison of clinical features where cases with a missense or in-frame variant (n = 6) to those with loss-of-function variants (n = 54) showed increased incidence of multisystem involvement including abnormal brain imaging, genitourinary anomalies, and growth deficiency in the latter group. Our report adds two new cases and genetic findings to the existing body of literature.

In Patient 1, we identified a novel 125kb deletion of 21q22.11, and exons 1–8 of the SON gene, including exon 3 which includes the important RS and RNA binding domains. The deletion also included the majority of the TEM50B gene, all of the GART gene, however, TMEM50B and GART have not been associated with abnormal phenotypes. In Patient 2, we found a new 5-base-pair duplication heterozygous frameshift variant c.1186_1192dup in the SON gene resulting in premature termination codon, causing loss of the full-length amino acids due to truncation protein. Interestingly, similar genetic findings were reported for one other patient with c.3597_3598dup and p.(Pro1200Argfs*17); both patients had reported seizure activity with no EEG abnormalities.10 Both variants reported here are novel and have not been previously described. Moreover, although one other patient has been reported to have whole gene deletion,10 this is the first report of deletion of

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**Table 1.** Continued.

| Clinical characteristics | Patient 1 | Patient 2 | Literature review |
|--------------------------|-----------|-----------|------------------|
| Immunological abnormalities | Chronic otitis media with tympanostomy tubes | | deficiency and recurrent infections,1,3,4,10–12 |
| Other congenital anomalies | Cleft palate and laryngomalacia | None | deep vein thrombosis,8 transient ischemic attacks,7 IgA and IgG deficits11 |
| Neurodevelopment status to date | Sits independently, rolling, pulling up and walking with support, language grossly delayed | Improving fine motor skills such as pincer grasper and gross motor skills, walking, language delayed | Laryngomalacia,11 cleft palate5,14 and craniosynostosis2 |
exons 1–8 of SON gene, where previously all of the causative SON variants that have been reported involved exon 3 with the exception of one case involving exon 4 and one involving exon 10.10

In conclusion,1 we report two additional cases of ZTTK syndrome and describe two de novo novel disease-causing variants in SON, with a first report of loss-of-function mutation in SON, enriching clinical and genetic data of the condition. It is unclear thus far if seizures in the ZTTK syndrome result from primary genetic abnormalities or secondary to well-defined structural abnormalities, of which some may also have genetic causes. Given that there has been no correlation so far between variants in the SON gene and epilepsy in those patients, a more expansive library of cases would be helpful in understanding if the development of seizures depends on the variation locus in this population. Moreover, as we continue to study this syndrome, it would be crucial to continue to collect information on seizure management and appropriate control. Indeed, because of the heterogeneous nature of this syndrome, particularly in terms of neurological findings, it remains difficult to find clinical and prognostic information for affected individuals. Particularly, we still lack information regarding descriptive EEG findings, progression of seizure activity with age, and appropriate treatment options in this population. We also lack information regarding long term intellectual/cognitive development and school performance, as well as neuropsychiatric comorbidities. Having patients follow up regularly with a neurologist and keeping track of this information can help drive evidence-based management of neurological symptoms as well as understand the long-term neurodevelopmental prognosis in future patients.

Author Contributions
Maya Eid: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data. Sonal Bhatia: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data.

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