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Inherited and *de novo* variants extend the etiology of *TAOK1*-associated neurodevelopmental disorder

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SHORT RUNNING TITLE

Four patients with *TAOK1*-associated syndrome

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ABSTRACT

Alterations in the *TAOK1* gene have recently emerged as the cause of developmental delay with or without intellectual impairment or behavioral abnormalities (MIM #619575). The thirty-two cases currently described in the literature have predominantly *de novo* alterations in *TAOK1* and a wide spectrum of neurodevelopmental abnormalities. Here, we report four patients with novel pathogenic *TAOK1* variants identified by research genome sequencing, clinical exome sequencing, and international matchmaking. The overlapping clinical features of our patients are consistent with the emerging core phenotype of *TAOK1*-associated syndrome: facial dysmorphism, feeding difficulties, global developmental delay, joint laxity, and hypotonia. However, behavioral abnormalities and gastrointestinal issues are more common in our cohort than previously reported. Two patients have *de novo* *TAOK1* variants (one missense, one splice site) consistent with most known alterations in this gene. However, we also report the first sibling pair who both inherited a *TAOK1* frameshift variant from a mildly affected mother. Our findings suggest that incomplete penetrance and variable expressivity are relatively common in *TAOK1*-associated syndrome, which holds important implications for clinical genetic testing.

INTRODUCTION

The *TAOK1* gene gets its name from the number of amino acids for which it encodes; a “thousand and one” amino acids, thus the name “thousand and one” kinase. *TAOK1* is located at cytogenetic band 17p11.2 and is a MAP3K serine/threonine kinase, with the kinase domain located at the N-terminus of the gene. It contains a serine rich domain as well as two coiled-coil domains. *TAOK1* is expressed ubiquitously but has highest expression in testes and brain (Fang, Lai et al. 2020, van Woerden, Bos et al. 2021). This gene is constrained for both missense variants (234 observed, 553 expected, $Z = 4.82$) and for loss of function variants (11 observed, 67 expected, $pLI = 1$) according to the gnomAD database (Karczewski, Francioli et al. 2020) v2.1.1, which lists only two nonsynonymous variants with minor allele frequency >0.0004 in human population cohorts. *TAOK1* has been reported to have a variety of functions including regulation of p38 MAPK, JNK, and Hippo signaling pathways (Plouffe, Meng et al. 2016, Fang, Lai et al. 2020). It is also thought to play a role in cytoskeleton regulation, neuron development, inflammation and other pathways (Li, Liu et al. 2019, Zhu, Yu et al. 2020, van Woerden, Bos et al. 2021). Loss of *TAOK1* has been shown to result in mitotic abnormalities, suggesting a role for the gene in proper chromosome transmission during cell division (Liskovych, Goncharov et al. 2019, Fang, Lai et al. 2020, Kouprina, Liskovych et al. 2020, Shi, Zhang et al. 2021).

Alterations in *TAOK1* are associated with developmental delay with or without intellectual impairment or behavioral abnormalities (MIM #619575). A microdeletion of *TAOK1* was reported in a patient with neurodevelopmental abnormalities in 2016 (Xie, Fan et al. 2016). In 2019, Dulovic-Mahlow et al. reported eight individuals with *de novo* alterations in *TAOK1*. Five

of these patients were among 6,504 patients referred to Centogene AG for diagnostic exome sequencing, while the other three were identified by international matchmaking (Sobreira, Schiettecatte et al. 2015). All eight patients presented with nonspecific developmental delay, with some individuals sharing additional clinical features (muscular hypotonia and facial dysmorphism). In 2021, van Woerden et al reported 23 additional patients with *TAOK1* variants (van Woerden, Bos et al. 2021) identified by matchmaking efforts. Like the previous study, most of the variants identified were *de novo* alterations (16/23) with predicted loss-of-function effects (18/23) supporting haploinsufficiency as the mechanism underlying disease (Dulovic-Mahlow, Trinh et al. 2019, van Woerden, Bos et al. 2021). Functional assessment of missense variants, however, revealed that some reduced TAOK1 protein levels consistent with a loss-of-function effect, but others (notably p.Leu548Pro) exhibited increased protein expression suggestive of a dominant-negative effect. Even overexpression of wild-type TAOK1 disrupted neuronal morphology and migration, suggesting that tight regulation of this gene during development is required for normal neuronal function (van Woerden, Bos et al. 2021).

The clinical presentation of *TAOK1*-associated neurodevelopmental disorder from the two cohort studies remains variable. The most common features include developmental delay and intellectual disability, macrocephaly, neonatal feeding difficulties, behavioral problems, hypotonia, joint laxity, and some mild facial dysmorphisms (frontal bossing, downslanting palpebral fissures, long philtrum, and a bulbous nasal tip). Given this broad range of reported clinical features, more studies of *TAOK1* patients are needed to understand the genotypic and phenotypic spectrum of this disorder. Here, we describe four patients from three families with *TAOK1*-associated neurodevelopmental disorders, including the first report of an affected sibling

pair. Our results extend the clinical manifestations observed in *TAOK1* patients and emphasize that both inherited and *de novo* variants can cause disease.

RESULTS

Clinical presentation and family history

Patient 1 (Family 1)

Patient 1 prenatal and perinatal complications included polyhydramnios, maternal uterine rupture at delivery following a failed vaginal birth after caesarian (VBAC) attempt, and intermittent hypoglycemia for approximately two days. At 6 months of age this male infant was being treated for feeding issues and gastroesophageal reflux (GERD), but at his last evaluation, his eating behavior had improved and he was noted to be eating a normal diet. At 18 months of age he was first seen by genetics for macrocephaly, delay, and failure to thrive. He was 3yr. 4mo. at last evaluation at which time he was noted to have global developmental delay including speech delay with only a few words. He had decreased strength, decreased central tone, joint laxity, and was reported to be clumsy. Gross motor skills were delayed (crawling and sitting 2yrs, walked ~2.5yrs). He was noted to have a tremor when stressed. He was referred to the Child Development Center at our hospital due to disruptive behavior, but was not quantitatively evaluated for autism. He was macrocephalic (53.5 cm; >>99%ile) with frontal prominence of the skull but was overall reported to be non-dysmorphic. His linear growth was reported to be normal but his height was 91.4 cm (5 %ile; Z= -1.68) and his body mass index was 97.75 %ile (Weight: 61 %ile). Brain magnetic resonance imaging (MRI) on two occasions revealed

ventriculomegaly and mild bilateral parietal white matter volume loss and cerebellar tonsillar ectopia not meeting Chiari I malformation criteria. Skin findings included livedo reticularis (Table 1).

Clinical microarray testing revealed a 1.27Mb gain at 8p22 that was classified as a VUS, but considered non-contributory to the patient's phenotype. Prader-Willi / Angelman syndrome methylation studies and myotonic dystrophy testing were normal.

Patient 2 (Family 1)

Patient 2 is the older sibling of patient 1. Polyhydramnios was noted prenatally. At her latest evaluation at age 4yr. 10mo. of age, this female patient was noted to have global developmental delay, gross motor delay (rolled 13 mo., sat 14 mo., crawled 18 mo., walked at 2 y.). Her speech was significantly delayed and although she has many words, her language is difficult to understand. She communicates by motions and signals in combination with speaking. She was noted to have hypotonia and hyperflexibility. She had a history of GERD and pica, but was eating normal foods at her last evaluation with a body mass index (BMI) of 80.86 %ile (Weight: 46 %ile) a height 102.5 cm (19 %ile; Z= -0.87). She had stereotypic repetitive behaviors and was referred to the Child Development Center but not quantitatively evaluated for autism. Her growth was slow but steady. She is macrocephalic (fronto-occipital circumference = 52 cm; 90.38% %ile) but was not otherwise noted for dysmorphic features. Brain MRI on two occasions revealed mild dilation of the lateral and third ventricles as well as a small arachnoid cyst. In addition, she had congenital heart defects including tetralogy of Fallot, ventriculoseptal defect,

and a bicuspid aortic valve; phenotype characteristics not previously reported in other *TAOK1* patients (Table 1).

Clinical microarray revealed the same 1.27Mb gain at 8p22 originally identified in her brother. Clinical exome sequencing (WES) performed at 23 mo. of age uncovered a heterozygous likely pathogenic frameshift alteration in *NOTCH1* (NM_017617.5: c.4758_4759insCA: p.Asn1587GlnfsTer30) that was reported as the probable cause of her cardiac abnormalities (Garg, Muth et al. 2005, McBride, Riley et al. 2008). This variant was inherited from an asymptomatic father, consistent with reduced penetrance reported for *NOTCH1* associated congenital heart defects (Zahavich, Bowdin et al. 2017). Her neurodevelopmental features, however, remained unexplained.

Patients 1 and 2 were referred along with their parents for research whole-genome sequencing (WGS) under an Institutional Review Board (IRB)-approved translational research study at the Institute for Genomic Medicine at Nationwide Children's Hospital. The most plausible candidate variant shared by both siblings was a heterozygous frameshift deletion in *TAOK1*, NM_020791.4:c.2203delA, p.(Arg735fs). The variant is absent from gnomAD and other public databases and predicted to result in nonsense mediated decay of the transcript. Retrospective review of the clinical WES data for patient 2 indicated that this variant was observed, but not assessed due to the lack of an OMIM disease association for *TAOK1* at the time (late 2018; OMIM did not add the gene-disease association until September 2021). This demonstrates the necessity of re-evaluating exome data periodically for negative patients.

Interestingly, the *TAOK1* frameshift variant (p.Arg735fs) in patients 1 and 2 was maternally inherited (Figure 1A). The mother was considered unaffected, was macrocephalic (FOC = 62.6 cm (>>99%ile)), and anecdotally has tremor with stress. She also reported minor learning difficulties and lax ankle joints as a child. The father was noted to be macrocephalic but was otherwise unaffected. The parents report European ancestry. No other family members have reported phenotypes consistent with *TAOK1*-related disease, though some maternal relatives were noted to have severe congenital heart defects (Figure 1A).

Several other rare nonsynonymous variants in disease-associated genes were shared by the proband and sibling; these are detailed in Supplemental Table 2. Two rare, maternally-inherited missense variants were identified in genes associated with autosomal dominant conditions that did not match the proband or sibling: *FNI*, associated with glomerulopathy with fibronectin deposits 2 (MIM #601894) and Spondylometaphyseal dysplasia (MIM #184255), and *NODAL*, associated with autosomal dominant visceral heterotaxy 5 (MIM #270100; not evident in either proband or sibling). The siblings also shared a paternally-inherited missense variant in *MYH9* (MIM #160775) which is associated with two types of autosomal dominant hearing loss; both they and their father had normal hearing. The proband only was heterozygous for a missense variant in *MAGEL2*, associated with autosomal dominant Schaaf-Yang syndrome (MIM #615547), but in which only loss-of-function changes have been reported to cause disease.

Finally, we evaluated a rare missense variant in *NFI* (NM_000267.3: c.4363C>T, p.Arg1455Cys) that was heterozygous in both children. This variant has been observed in human populations, albeit at extremely low allele frequency (4 heterozygotes in gnomAD; population

MAF=0.0000174). It affects a highly conserved residue and is predicted to be damaging by most *in silico* tools (24/26, REVEL score=0.7239). Of more than 2,000 pathogenic / likely pathogenic variants in *NFI* reported by clinical laboratories (ClinVar), the majority (90%) have loss-of-function effects. The p.Arg1455Cys variant has been reported by two clinical laboratories as a variant of uncertain significance (VUS). Although the children and their father (who also carries this variant) have macrocephaly, a feature sometimes reported in Neurofibromatosis syndrome (Clementi, Milani et al. 1999), none of them were reported to have café au lait spots or other skin findings characteristic of the disease (reviewed in (Williams, Lucas et al. 2009)).

Patient 3 (Family 2)

Patient 3 is a 3-year-old male of European ancestry. Evaluation at 2.5 years of age revealed intellectual disability and global developmental delay. He was ambulatory but had frequent falls. Brain imaging by MRI revealed ventriculomegaly but no other abnormalities. He was reported to have autism, poor eye contact, staring spells, and restless sleep. He was macrocephalic (FOC = 54 cm; >99 %ile; Z= 2.99) with some relatively minor facial dysmorphisms including prominent cheeks, slightly prominent forehead, and tenting of the upper lip. Prenatal polyhydramnios was reported and feeding difficulties were noted at birth. Gastrointestinal issues included nausea, reflux, diarrhea, and constipation and he was eventually found to have tracheoesophageal fistula, annular pancreas, duodenal atresia, mild right caliectasis, and penile adhesions. Despite feeding problems, his BMI was 85.50%ile (Weight: 90%ile) and his height was 96.6 cm (76%ile) and was thought to possibly have overgrowth issues and relatively large size. He was noted to have hypotonia, including facial hypotonia, and increased joint mobility along with slightly short appearing digits. Strabismus and ptosis were noted (Table 1). At 2 years of age he was referred

to the Child Development Center at our hospital and diagnosed with autism after a clinical interview and psychological evaluations (ADI-R, AVABO, DP-3). The family history was not particularly suggestive of other similarly affected individuals in the family. Both parents were noted to have some degree of macrocephaly, a relative with learning disabilities was reported, and two paternal half siblings were noted to have attention deficit disorder (ADHD) (Figure 1B).

Clinical whole exome sequencing was performed on the proband and both biological parents. A heterozygous 4bp intronic deletion near the 3' splice donor site of exon 2 of the *TAOK1* gene was detected (NM_020791.4: c.132+3_132+6delAAGT) in the proband but not present in either parent. This alteration was confirmed *de novo* by Sanger sequencing of the proband and parents, and parentage was confirmed. The variant is absent from gnomAD and other public databases and is predicted to result in complete loss of splicing by multiple algorithms (ESEfinder (Cartegni, Wang et al. 2003) and SpliceAI (Smith, Zhang et al. 2006), see Table 2). We classify the variant as likely pathogenic (Table 2). Two additional *de novo* alterations were identified in this patient (Supplemental Table 2). Both are missense changes classified as variants of uncertain significance (VUS); their contributions to patient 3's clinical features are unclear. In addition to exome sequencing, Fragile X testing and microarray were performed for patient 3 and reported as normal.

Patient 4 (Family 3)

Patient 4 is of Dominican and Puerto Rican descent and originally presented with hypotonia and developmental delay (first words 18 months, pulled to stand 13-14mo. walking 2.5 years, not toilet trained at 5yrs). Pregnancy was normal but delivery was induced due to maternal

hypertension. She was 6lbs 14oz. at birth. She has a history of GERD as an infant and is reported to have chronic constipation. Her last evaluation at 5 years of age determined that her height was 40th%ile, BMI was 98%ile (Weight: 96%ile), and head circumference was 20%ile. Her language was delayed, but she is currently talking more and speaking in full sentences. Adaptive skills evaluation revealed an FSIQ of 58 (subscores 50-60 range except for processing speed score of 77) and a Vineland 3 adaptive behavioral assessment score of 65 (communication 58, daily living skills 60, socialization 76, motor skills 70). Behavioral concerns included selective mutism, inattention at school, and anxiety. She does not have autism. Medical records also indicate a complex febrile seizure lasting greater than 10 minutes in the setting of a urinary tract infection. Brain MRI showed multifocal subcortical gliosis thought to be consistent with chronic manifestation of perinatal infection or ischemia. Facial features reported were a prominent forehead, flat nasal bridge with short columella, prominent lips, and a short philtrum. She was noted to have tapered fingers with squared fingertips and persistent fetal finger pads. She also had 2/3 toe syndactyly, sandal gap, and pes planus. She is noted to have astigmatism and wears glasses. Skin findings included a strawberry hemangioma on her chest and a café au lait spot on her neck. Imaging by ultrasound revealed a small right kidney with a simple cortical cyst. Also of note, is a history of thrombocytopenia, bruising without trauma, and poor response to vaccinations. She was seen by endocrinology for premature adrenarche (Table 1). Family history did not reveal any relatives with particularly striking phenotype correlation (Figure 1C).

Patient four's diagnostic evaluation prior to exome sequencing included normal microarray testing (Affimetrix Cytoscan HD Array), normal Prader Willi methylation analysis (SNRPN Southern blot), and metabolic screening that was non diagnostic showing a mildly elevated

ammonia level of 59 and mild nonspecific elevation of serum amino acids. Clinical whole exome sequencing through GeneDx identified a *de novo* NM_020791.4(*TAOK1*): c.1324C>T: p.Arg442Trp missense variant that was classified by GeneDX as a VUS but classified as likely pathogenic herein (Table 2). A paternally inherited missense variant in *TNFRSF13B* (NM_012452.3:c.310T>C:, p.(Cys104Arg)) was classified as pathogenic but not thought to play a major role in the phenotype of patient 4 (Supplemental Table 2). No other variants of interest were included in the clinical exome report.

Genomic Analyses

The three *TAOK1* variants in this study have not been previously reported in databases or the literature (Figure 3A). The variant in Family 1 is a frameshift change located in a C-terminal region of the gene in which many loss-of-function variants have been reported by Dulovic-Mahlow et al. (Figure 3B) (Dulovic-Mahlow, Trinh et al. 2019), van Woerden et al. (Figure 3C) (van Woerden, Bos et al. 2021), or to the ClinVar database (Figure 3D) including several that are more C-terminal than the variant reported here. This variant is in exon 18 of 20 (NM_020791.4) and is expected to result in nonsense mediated decay. The c.132+3_132+6delAAGT variant reported in patient 3 appears to be the most N-terminal splice variant reported to date and likely results in nonsense mediated decay. The region in which the missense alteration in patient 4 (p.Arg442Trp) is found does not belong to a known domain or recognition motif, but UniProt identifies nearby residue 445 as a phosphorylation site (Dephoure, Zhou et al. 2008, Zhou, Di Palma et al. 2013). Similar numbers of missense and loss of function variants have been

reported. There does not appear to be any regions of the gene with greater prevalence of reported disease-causing variants, but rather disease-causing variants of all types are distributed somewhat uniformly across the gene (Figure 3). As discussed above, loss of function appears to be a genetic mechanism of disease, but the mechanism of disease associated with missense variants is not yet clear.

Phenotypic Analyses

Overall, the 4 patients exhibited the most common features of developmental delay with or without intellectual impairment or behavioral abnormalities listed in OMIM (MIM #619575) including frontal bossing, developmental delay, hypotonia, and neonatal feeding difficulties (Table 1). In addition, consistent features across our patients would add to or provide more definition to the phenotype described in OMIM including joint hypermobility, gross motor delay, speech and language delay, increased BMI, autistic features, and GERD. Patients presented herein were not reported to have ADHD, but patient 4 was reported to have inattentiveness. Patients all had behavioral abnormalities overlapping with autism spectrum disorder. Despite feeding issues, all patients had elevated BMI, a feature also not listed in OMIM. Although our patients were not reported to be particularly dysmorphic, patient 3 had some similarities to the mild facial gestalt reported in other *TAOKI* patients, while patient 4 had some facial features that differed from previous reports (Dulovic-Mahlow, Trinh et al. 2019, van Woerden, Bos et al. 2021). In addition to the overlapping phenotypes listed in table 1, detailed evaluation of each patient revealed additional unique phenotype characteristics, some of which could be an

expansion of the *TAOK1* disease phenotype or phenotypes associated with additional independent or modifier variants (Supplemental Table 2 and 3).

DISCUSSION

Although a patient with a microdeletion of *TAOK1* was reported in 2016 (Xie, Fan et al. 2016), most patients with *TAOK1* alterations described in the literature (31 of 32) were part of two recent studies (Dulovic-Mahlow, Trinh et al. 2019, van Woerden, Bos et al. 2021). The neurodevelopmental syndrome associated with variants in *TAOK1* was recognized by OMIM in September 2021 (MIM #619575), but our understanding of the genotypic and phenotypic spectrum of disease remains incomplete. Here, we describe four new patients whose overlapping clinical features further solidify the central phenotype *TAOK1*-associated syndrome: neonatal feeding difficulties, global developmental delay, intellectual disability, behavioral abnormalities, hypotonia, joint hypermobility, and craniofacial dysmorphism (van Woerden, Bos et al. 2021). Some variable phenotypes from previous patient cohorts are more consistent in ours, notably gastrointestinal reflux (4/4), increased BMI (4/4), macrocephaly (3/4), and ventriculomegaly (4/4). Also, kidney abnormalities – present in patients 3 and 4 in this cohort – have only been reported in one other patient to our knowledge.

The *TAOK1* variants identified in our patients are novel and have not been previously reported to our knowledge. The variants identified in patients 3 and 4, like the majority of reported *TAOK1* alterations, were demonstrated to be *de novo* and were absent from confirmed parental samples (Dulovic-Mahlow, Trinh et al. 2019, van Woerden, Bos et al. 2021). The variant identified in patients 1 and 2, however, was inherited from a mother who was initially considered asymptomatic. Although multiple affected siblings with *TAOK1*-associated syndrome have never been described (to our knowledge), vertical transmission of *TAOK1* variants has been reported in

at least three other families (van Woerden, Bos et al. 2021). Two of those families had a family history of neurodevelopmental disease, but one did not. Taken together, these observations suggest that inheritance of *TAOK1* disease-causing variants from a mildly affected parent may be a relatively common phenomenon. If true, this has important implications for clinical testing; inheritance of a *TAOK1* variant from a reportedly asymptomatic parent should no longer be considered evidence against pathogenicity.

Patient 2 in our study had congenital heart defects -- including tetralogy of Fallot, ventriculoseptal defect, and a bicuspid aortic valve -- which have not been reported in any *TAOK1* patients to our knowledge. Instead, we attribute these features to her likely pathogenic variant in *NOTCH1*, a gene associated with a wide spectrum of developmental heart defects (Garg, Muth et al. 2005, McBride, Riley et al. 2008, Zahavich, Bowdin et al. 2017). These findings are consistent with the increasingly recognized phenomenon of patients with blended clinical phenotypes caused by multiple independent variants (Smith, Blanco et al. 2019). These cases highlight the value of comprehensive exome or genome sequencing as a first-line test for diagnosis of genetic disorders.

The emerging picture of *TAOK1*-associated syndrome, with a wide range of neurodevelopmental and behavioral manifestations as well as *de novo* and transmitted genetic etiologies, is more reminiscent of autism spectrum disorder than a severe monogenic neurodevelopmental disease. Indeed, *TAOK1* first surfaced as a potential disease-associated gene in a single patient in an autism cohort paper (De Rubeis, He et al. 2014). With the identification and phenotype information of additional patients (Dulovic-Mahlow, Trinh et al. 2019, Satterstrom, Kosmicki et

al. 2020, van Woerden, Bos et al. 2021), *TAOK1* is currently recognized as a high-confidence autism susceptibility gene in the SFARI database (SFARI score=1.0, EAGLE score=8.25). As additional *TAOK1* variants are identified, the phenotype spectrum will become further solidified.

METHODS

Family 1. Genomic DNA was extracted from blood for the proband, his affected sister, and both parents. Paired-end sequencing (2x151bp) libraries were created with NEBNext Ultra II (New England Biolabs, Ipswich, MA, USA) and sequencing was performed using the NovaSeq6000 instrument (Illumina Inc., San Diego, CA, USA) according to the manufacturer protocols. Reads were mapped to the GRCh38/UCSC hg38 reference sequence. Secondary data analysis was performed using Churchill (Kelly, Fitch et al. 2015), which implements the Genome Analysis Toolkit (GATK) “best practices” workflow to allow for a computationally efficient analysis of whole-genome sequencing (WGS) data. We generated 179-238 Gbp of uniquely mapped reads per individual, achieving ~45.8x haploid coverage on average. Sequencing metrics are provided in Supplemental Table 1. VarHouse, an in-house annotation framework, was used to annotate variants with predicted gene/transcript effect (snpeff/ANNOVAR), the damaging scores of various *in silico* tools, and population allele frequencies from gnomAD and other databases. Our general approach to genomic analysis for rare diseases has been described (Koboldt, Kastury et al. 2018, Koboldt, Mihalic Mosher et al. 2018, Mihalic Mosher, Zygmunt et al. 2019). Briefly, we prioritized rare, likely-damaging variants that segregate with disease under a plausible inheritance model. Due to the family history, multiple inheritance modes – including recessive, X-linked, and dominant inheritance with incomplete penetrance – were considered.

Family 2. Proband and parental peripheral blood samples were submitted for clinical exome sequencing to The Steve and Cindy Rasmussen Institute for Genomic Medicine at Nationwide Children's Hospital, Columbus, Ohio. Genomic DNA extraction from peripheral blood and

genotyping assay using a custom Agena MassArray panel (Agena) to ensure sample provenance and verify familial relationships was performed as described by (Miller, Lee et al. 2020). Libraries underwent target capture using SureSelect Human All Exon V6 (Agilent) followed by paired-end 151-bp sequencing to at least 100× mean depth on a HiSeq 4000 (Illumina), with 95.7% of targeted bases at 20× or greater. Similar to the approach described for Family1, secondary analysis was performed using Churchill 3.0 and variant annotation was performed by VarHouse. Human Phenotype Ontology (HPO) terms (Köhler et al. 2017) inferred from clinician-provided phenotypes by Mr. Phene (an in-house tool) were used to prioritize variants in phenotypically-relevant genes for clinical variant interpretation. Reported variants were confirmed by PCR and Sanger sequencing in the proband and both parents as previously described (Miller, Lee et al. 2020).

Family 3. Proband and parental peripheral blood samples were submitted for clinical exome sequencing to GeneDx (Gaithersburg, MD). Genomic DNA was enriched for the complete coding regions and splice site junctions for most genes of the human genome using a proprietary capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets were simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads were assembled and aligned to the reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. Using a custom-developed analysis tool (XomeAnalyzer), data were filtered and analyzed to identify sequence variants and most deletions and duplications involving three or more coding exons (Retterer, Scuffins et al. 2015). The reported variants were converted to GRCh38 coordinates using VarSome (Kopanos, Tsiolkas et al. 2019).

ADDITIONAL INFORMATION

Data Deposition and Access

Data deposition is not permitted under the current consents, but these variants and our interpretations have been submitted to the ClinVar database (accession numbers VCV001333717.1, VCV001328968.2, and VCV001307834.1).

Ethics Statement

Written informed consent was obtained for all patients reported in this study. Families 1 and 2 consented for clinical exome sequencing as well as research studies under a research protocol approved by the Institutional Review Board at Nationwide Children's Hospital (IRB18-00662, Gene Discovery in Clinical Genomic Patients).

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Author Contributions

J.M.H. and D.C.K.: analysis and manuscript preparation. K.M., D.B., A.S., E.d.l.R.: clinical evaluations of families 1 and 2. L.J.M. and J.L.S.: clinical evaluation and analysis of family 3. R.W.: study coordinator (family 1). M.M.: Variant analysis. S.C.R. and C.E.C.: clinical laboratory protocols (families 1 and 2). R.K.W.: research study funding and leadership.

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FIGURE LEGENDS

Figure 1. Pedigrees for families 1-3. Arrows indicate the index proband in each family; colored shading indicates variant carrier status and reported clinical features. (A) Pedigree for family 1. (B) Pedigree for family 2. (C) Pedigree for family 3.

Figure 2. Genomic analysis uncovers rare deletions in *TAOK1* in two families. Both panels show sequencing data aligned to the GRCh38 reference sequence as visualized in the Integrative Genomics Viewer (IGV) v2.8.0. (A) Quad whole-genome sequencing data for family 1, in which patients 1 and 2 are heterozygous for a single-base deletion inherited from their mother. (B) Trio exome sequencing data for family 2, in which the proband is heterozygous for a de novo 4-base splice site deletion.

Figure 3. Protein structure of *TAOK1* and location of reported variants (amino acid position) by source. The first plot shows the variants in this study; the second and third show variants reported in two previous studies. The bottom plot shows ClinVar pathogenic / likely pathogenic variants retrieved 27 September 2021. Variant diagrams were generated using lollipops v1.5.3 using information from UniProt (ID #Q7L7X3).

FIGURES

FIGURE 1

Figure 1. Pedigrees for families 1-3. Arrows indicate the index proband in each family; colored shading indicates variant carrier status and reported clinical features. (A) Pedigree for family 1. (B) Pedigree for family 2. (C) Pedigree for family 3.

A.

- *TAOK1*(NM_020791.4): c.2203delA: p.Arg735fs
- *NOTCH1*(NM_017617.5):c.4758_4759insCA: p.As1587GlnfsTer30
- macrocephaly
- autism
- learning disability / developmental delay
- seizures
- tremor when stressed
- severe congenital heart defects
- atrial septal defect (self-resolved)

B.

Family 2

- *TAOK1*(NM_020791.4): c.132+3_132+6delAAGT: p.?
- macrocephaly
- ADHD
- learning disability / developmental delay

C.

Family 3

- *TAOK1*(NM_020791.4):c.1324C>T: p.Arg442Trp
- bicuspid aortic valve, aortic stenosis
- autism
- SIDS
- delayed milestones (resolved)

FIGURE 2

Figure 2. Genomic analysis uncovers rare deletions in *TAOK1* in two families. Both panels show sequencing data aligned to the GRCh38 reference sequence as visualized in the Integrative Genomics Viewer (IGV) v2.8.0. (A) Quad whole-genome sequencing data for family 1, in which patients 1 and 2 are heterozygous for a single-base deletion inherited from their mother. (B) Trio exome sequencing data for family 2, in which the proband is heterozygous for a de novo 4-base splice site deletion.

A.

B.

FIGURE 3

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TABLES

Table 1. Demographics and phenotype characteristics of the four patients reported in this study. Only phenotypes observed in at least two individuals are shown (see Supp. Table 3 for full details).

Demographics and Variant Information					
Family:	Family 1	Family 1	Family 2	Family 3	
Patient:	Patient 1	Patient 2	Patient 3	Patient 4	
Gender:	Male	Female	Male	Female	
Age at last evaluation:	3yr. 4 mo.	4yr. 10 mo.	2yr. 10 mo.	5yr	
TAOK1 Variant NM_020791.4 (GRCh38):	c.2203delA p.Arg735fs	c.2203delA p.Arg735fs	c.132+3_132+6 delAAGT p.?	c.1324C>T p.Arg442Trp	
Inheritance:	Het./Maternal	Het./Maternal	Het/de novo	Het/de novo	
Growth and Development					
HPO ID	Term Description	Patient 1	Patient 2	Patient 3	Patient 4
HP:0001263	Global developmental delay	+	+	+	+
HP:0002194	Delayed gross motor development	+	+	+	+
HP:0007015	Poor gross motor coordination	+		+	
HP:0000750	Delayed speech and language development	+	+	+	+
Brain imaging					
HP:0002119	Ventriculomegaly	+	+	+	
Behavior					
HP:0000708	Behavioral abnormality	+	+	+	+
HP:0000729	Autistic behavior		+	+	+
HP:0100852 ; HP:0000739	Abnormal fear/anxiety-related behavior	+	+		+

	(Stress induced tremor) ; Anxiety				
<i>Neuromuscular</i>					
HP:0001252	Hypotonia	+	+	+	+
HP:0001382	Joint hypermobility	+	+	+	+/-
<i>Feeding / Gastrointestinal</i>					
HP:0008872	Feeding difficulties in infancy	+	+	+	
HP:0002020	Gastroesophageal reflux	+	+	+	+
HP:0004324	Increased BMI	+	+	+	+
HP:0002019	Constipation			+	+
<i>Genitourinary</i>					
HP:0000077	Abnormality of the kidney			+	+
<i>Craniofacial Dysmorphisms</i>					
HP:0000256	Macrocephaly	+	+	+	
HP:0011220	Prominent forehead	+		+	+
HP:0001999	Abnormal facial shape			+	+
<i>Hands and Feet</i>					
HP:0011297	Abnormal digit morphology		+	+	+
<i>Prenatal</i>					
HP:0001561	Polyhydramnios	+	+	+	

Table 2. Genomic findings and variant interpretation for the TAOK1 variants identified in our patients. Genomic coordinates reflect build GRCh38.

Patient	Genomic Location	HGVS cDNA	HGVS Protein	Zygosity /Origin	Interpretation
1	chr17: 29530460	NM_020791.4: c.2203delA	TAOK1: p.(Arg735AspfsTer6)	Het / maternal	LP (PVS1, PM2, PP1)
2	chr17: 29530460	NM_020791.4: c.2203delA	TAOK1: p.(Arg735AspfsTer6)	Het / maternal	LP (PVS1, PM2, PP1)
3	chr17: 29451682	NM_020791.4:c.132+3_132+6del	TAOK1: p.(Phe44+3) splice	Het / <i>de novo</i>	LP (PS2, PM2, PP3*)
4	chr17: 29502709	NM_020791.4:c.1324C>T	TAOK1: p.(Arg442Trp)	Het / <i>de novo</i>	LP (PS2, PM2, PP3**)

*PP3 was applied due to complete loss of donor splicing predicted by **BDGP: Splice Site Prediction by Neural Network** (fruitfly.org)(native score = 0.95, altered score = not found (threshold 0.1) and **ESEFinder** (Cartegni, Wang et al. 2003) 3.0 (cshl.edu)(native score = 5.41210, altered score = not found (threshold 1.0).

**For patient 4, PP3 was applied because the majority of computational algorithms (18 of 25, including SIFT, MutationTaster, PROVEAN, Polyphen 2, CADD, and others) reported by VarSome (Kopanios, Tsiolkas et al. 2019) predict pathogenicity.

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