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Report of two children with global developmental delay in association with *de novo TLK2* variant and literature review

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Abstract

Objective: We describe clinical details, including novel findings, of two further children with the newly defined *TLK2* related disorder.

Method: One patient was recruited to the Deciphering Developmental Delay (DDD) Study to identify underlying aetiology of global developmental delay. The other was detected on whole exome sequencing as part of second line investigations following normal microarray.

Results: Both patients were found to have *de novo* heterozygous pathogenic *TLK2* variants. A novel c.6del p.(Glu3Lysfs*) loss-of-function frameshift variant was found in Patient 1. A c.1121+1G>A splice- donor variant was detected in Patient 2.

Discussion: *TLK2* related neurodevelopmental disorder is a specific syndrome that has been recently described. Global developmental delay, behavioural problems, gastrointestinal disorders and typical facial dysmorphism are common features. Neuropsychiatric disorders, ophthalmic, musculoskeletal and cranial abnormalities, as well as short stature, have also all been described. The novel findings we describe include sleep disturbance, non-differentiation of lateral semi-circular canals (where asymmetric semi-circular canals were a feature in the previous cohort), vesico-ureteric reflux and bilateral periauricular skin tags.

Conclusion: Here we report a novel *TLK2* variant and previously undescribed features of *TLK2* related disorder, to expand the clinical phenotype and provide further genotype-phenotype correlation.

Introduction

Intellectual disability, and its precursor diagnosis global developmental delay, describe lack of achieved expected skills across more than one area of development, with diagnosis based on limitations to adaptive, cognitive and social behaviour that require increased support for the individual. Prevalence is around 1-3% (Moeschler et al., 2014). Aetiology can be a combination of genetic and environmental factors, ranging from chromosomal disorders or syndromes, single gene disorders, peri or post-natal hypoxic events, or cerebral malformations (Tikaria A et al., 2020). Other causes include metabolic, endocrine, trauma, infections, exposure to toxins and neuromuscular conditions. Despite this, the majority of causes of global developmental delay are idiopathic and require further investigation (Khan et al., 2021).

Studies have shown that genetic aetiology of global developmental delay can be found in up to 47% of all cases (Belanger et al., 2018). Chromosome microarray is the first line genetic test in children with global developmental delay with unclear cause, with diagnostic yield of up to 20% (Belanger et al., 2018). Further targeted gene panels, whole exome or whole genome sequencing can be carried out with diagnostic yield of up to 40% (Belanger et al., 2008). The importance of achieving an underlying genetic diagnosis is to help predict a patient's individual clinical course and prognosis. Other benefits in reaching a diagnosis include knowledge of gene mechanisms and risk of inheritance, potential for patient research opportunities and future treatment options, provision for family support such as genetic counselling and ceases the need for further unwarranted investigations (Moeschler et al., 2014).

Recently identified in a large meta-analysis, Tousled-like kinase 2 (*TLK2)* gene variants have been speculated to be a candidate gene for neurodevelopmental problems and intellectual disability (Lelieveld S H et al., 2016). *TLK2* is a protein coding gene involved in maintaining genome stability; involved in chromatin assembly and cellular repair (Mortuza et al., 2018). *De novo* heterozygous *TLK2* variant associated phenotype has been subsequently described as a distinct syndrome in 40 individuals in the literature thus far (Reijnders et al., 2018). Here we describe two further children with global developmental delay with known *TLK2* gene variants.

Clinical reports

Patient 1:

This patient was the second male child to non-consanguineous parents, born at term in good condition, weighing 3.2kg. Pregnancy was normal and detailed family history was non-contributary.

Concerns about speech and language development began at 18 months when the few single words he had acquired since the age of 12 months had started to regress. At 24 months, he had no words, only vocalisation of sounds and noises. Assessment of hearing and vision were normal. Monthly speech and language therapy subsequently helped him regain initial lost words, and more, and helped him develop Makaton signing as an additional method of communication. By 3 years of age, he was able to speak in two-to-three-word sentences and ask questions.

Concurrent development of social communication and understanding were also delayed. His first social smile was at 10 weeks and he was not able to follow simple instructions at 24

months of age. At 2 years 9 months he had paucity of eye contact, showed little reciprocity, had sensory intolerances and obsessive behavioural traits in line with autism spectrum disorder. At 3 ½ years, he would refer to himself as a baby and he was not able to indicate wet nappies; his learning at this age was significantly delayed with a developmental age consistent with a child of 12 to 18 months.

There were early concerns from parents with regards to excessive crying up to 14 hours a day since the neonatal period, and subsequent explosive temper tantrums and prolonged crying associated with self-injurious behaviour as a toddler. He would poke his eyes or hit himself in the face and head-bang when excited or angry. Parents describe him as having a high pain threshold. An orthotics helmet was worn during early childhood for protection of self-injurious behaviour.

In terms of early motor milestones, he first sat at 8 months and walked at 16 months. Subsequent motor milestones were reached appropriately by 3 years. As a toddler, he had a tendency to drool (Figure 1a). Despite fussy eating, he had been described to generally eat well and had no bladder or bowel concerns, although he had been on iron supplementation during early childhood due to iron deficiency anaemia. Sleep initiation remained an ongoing issue, with difficulty predominantly in getting to sleep, typically around the early hours of the morning. He has never been medicated for sleep issues.

Clinical examination revealed dysmorphic facial features of prominent nasal bridge with square nose and broad nasal tip, overhanging columella, blepharophimosis, telecanthus, narrow mouth, pointed chin and posteriorly rotated ears (Figures 1a, 1c, Table 2). Examination also revealed broad fingers and toes (Figure 1b). Spine and palate were normal.

Sporadic weight, height and head circumference measurements remained around the 50th centile from birth to most recent measurements.

Cytogenetics showed normal male karyotype (46, XY), testing for Fragile X was negative and microarray analysis detected no clinically significant imbalance. This patient was presented at a regional dysmorphology meeting where a possible diagnosis of SBBYS variant Ohdo Syndrome was considered. He was enrolled in the Deciphering Developmental Delay (DDD) study which identified a *de novo TLK2*, NM_006852.3: c.6del p.(Glu3Lysfs*), likely pathogenic loss of function frameshift variant. This variant is very close to the start codon, and may be subject to start-proximal NMD insensitivity (Lindeboom et al., 2016). However, use of the next in-frame start codon would result in truncation of 22% of the protein, so this variant is likely to cause a substantial, if not complete, loss-of-function effect. It is not reported in gnomAD database.

Aged 10 years, he is educated with 1:1 support in a mainstream school with an Education Health Care Plan in place. Issues with behaviour now supersede concerns with learning. Behaviour continues to be destructive and disruptive at school, although reportedly well-behaved with his parents. Poor sleep initiation remains but there are no other health issues of concern. There remains continued input from child psychology with regards to his behaviour. He continues to be followed up in community paediatrics.

Patient 2:

This female patient was the first child of non-consanguineous parents, born at term by emergency c-section due to foetal bradycardia, weighing 2.6kg. Pregnancy was complicated by intrauterine growth restriction and polyhydramnios, requiring monitoring scans but no specific intervention. She was born in poor condition with APGAR scores of 1 and 7 at 1 and 5 minutes respectively, requiring inflation breaths at birth, but did not require admission to the Neonatal Unit. At birth, weight and head circumference were both <0.4th centile and she required an incubator to maintain body temperature. She was discharged from hospital after 4 days having received support with establishing feeding.

The difficulties with feeding resulted in initial poor growth; at 2 months of age weight was 0.4th centile whilst height and weight had increased to 2nd – 9th centiles. Lansoprazole and ranitidine were started for presumed gastro-oesophageal reflux with no improvement. At 5 months of age, she was referred to dieticians for further input and was commenced on a high calorie milk. She subsequently developed a food aversion which resulted in gastroenterology involvement with fundoplication and percutaneous gastrostomy insertion at the age of 2 years and 10 months. Now, she drinks water orally from a bottle but is otherwise entirely fed via percutaneous gastrostomy with a blended diet. At the age of 3 years and 6 months, her weight is 50th centile with height 0.4th to 2nd centile.

Dysmorphic features included multiple bilateral preauricular skin tags, prominent and low set ears, up-slanting palpebral fissures, overhanging columella, and bilateral hallux valgus (Table 2). MRI head and spine revealed no focal parenchymal abnormality but an incidental finding of non-differentiation of the lateral semi-circular canals bilaterally which was subsequently confirmed on HR-CT (Figures 2a,b,c,d). Hearing tests had always been normal. At 3 months

of age, parents had concerns that she was not fixing or following. Ophthalmology assessment revealed delayed visual maturation and slightly alternating divergent squint, although no specific concerns about sight.

In terms of motor skills, she first sat with support at 6 months, crawled at 12 months and walked at 26 months. Other features of developmental delay became apparent over time including tip-toe walking, delayed social communication and poor verbal skills. At age 3 years she had only monosyllabic babble with no words, was not responding to her name or pointing. Developmental skills at 3 years were in keeping with an infant of 9 – 12 months of age. Now at age 3 years and 6 months, she has started to say some single words but primarily has non-verbal communication. Recent concerns from parents and speech and language therapy have emerged with regards to a possible diagnosis of autism spectrum disorder. She pulls parents by the hand to attend her needs, rarely responds to her name, is frequently rocking, and has repetitive hand and eye movements. She mainly engages in self-directed play, has reduced eye contact and sensory behaviours.

Co-existing medical problems included severely disturbed sleep with snoring, where she would take a long time to settle to sleep and would wake 20 to 30 minutes later, remaining awake for the rest of the night. Sleep clinic diagnosed rhythmic movement sleep disorder and restless leg syndrome, for which she was initially commenced on Melatonin, with little effect. This was changed to Clonazepam with much improvement; subsequent sleep study showed good sleep efficiency where she would sleep through the night, waking only once or twice.

She was also found to have bilateral Grade 4 vesicoureteric reflux at the age of 2 months when admitted to hospital for investigation into feeding difficulties. She was started on prophylactic antibiotics at this time.

Cytogenetics showed normal female karyotype (46, XX). Microarray analysis detected no clinically significant imbalance. Further trio exome sequencing detected a *de novo* heterozygous splice site variant NM_006852.3: c.1121+1G>A, which abolishes the intron 12 splice donor site. The least severe predicted consequence is exon 12 skipping, preserving the reading frame and loss of 7% of the protein. Alternately, the variant may lead to incorporation of a premature termination codon and nonsense-mediated decay, resulting in no protein being produced. It is not reported in gnomAD database. This variant had been previously identified in one other patient with neurodevelopmental disorder (Table 1) (Reijnders et al., 2018).

Materials and Methods

Patient 1

The first child was recruited to the Deciphering Developmental Disorders (DDD) study. Trio-based exome sequencing was performed on the individual and his parents. This was carried out at the Wellcome Trust Sanger Institute using Agilent 2x1M for array-based comparative genomic hybridization (aCGH), Illumina 800K SNP genotyping to identify copy number variants, and Agilent SureSelect 55MB Exome Plus with Illumina HiSeq for exome sequencing (Wright et al., 2015). Putative *de novo* mutations were identified from exome data using DeNovoGear software, interpreted for their clinical relevance based on ACMG/AMP and ACGS guidelines (Richards et al., 2015; Ellard et al., 2020) and were validated using

targeted Sanger sequencing. Genomic variants were filtered by DDD on the basis of six factors, of which the first five were automated and the last done manually: (1) frequency, prevalence <1% of the variant in the general population; (2) function, most severe predicted functional consequence; (3) location, genomic location compared with Developmental Disorders Genotype-to-Phenotype database (DDG2P) of published genes; (4) variant type, genotype (heterozygous or homozygous) and loss or gain for small copy number variants; (5) inheritance, aspects of the pipeline that are dependent on inheritance information derived from parental data are shaded; and (6) phenotype, patient phenotype was manually compared against published phenotypes for a particular gene (Wright et al., 2015).

No other variants were detected in this individual. Analysis and validation of the *TLK2* variant was performed locally.

Patient 2

The second child was referred for trio exome analysis. Sequencing was performed at Exeter Genetics Laboratory at Royal Devon and Exeter Trust. Trio whole exome sequencing analysis of the coding region and conserved splice sites of 23,244 genes by next generation sequencing was carried out using Twist Core Human Exome/ Illumina NextSeq. The lab selected in de novo, compound heterozygous or homozygous rare variants with a population frequency of < 0.01% in Exome Aggregation Consortium (ExAC http://exac.broadinstitute.org/) or the Exome variant server (EVS http://evs.gs.washington.edu/EVS/) using their inhouse bioinformatics pipeline. Variants were interpreted for their clinical relevance based on ACMG/AMP and ACGS guidelines (Richards et al., 2015; Ellard et al., 2020). No other variants were reported by the Exeter Laboratory.

Discussion

TLK2 is part of a family of serine/threonine kinases**.** The *TLK2* gene (OMIM * 608439) has been mapped to chromosome 17q23.2 using fluorescent in situ hybridisation and is expressed in almost all human tissues (Yamakawa et al., 1997). Tousled-like kinases were first discovered to be involved in chromatin assembly (Sillje et al., 1999). They are regulated by cell-cycle dependent phosphorylation and are more active in the S phase of the cell cycle (Sillje et al., 1999). It is now understood that Tousled-like kinases are involved in the DNA damage checkpoint, as well as DNA replication and transcription (Groth et al., 2003; Mortuza et al., 2018). TLK2 can be activated externally as well as by autophosphorylation; the proposed mechanism is a two-stage enzyme activation whereby initial phosphorylation triggers a cascade of conformational protein changes, which leads to new sites which are subsequently targeted, eventually leading to full activation (Mortuza et al., 2018). Loss of function pathogenic variants in *TLK2* that have been identified in the new *TLK2* related disorder showed impaired TLK2 activity with reduced autophosphorylation (Lelieveld S H et al., 2016). It is understood that intellectual disability and neurodevelopmental disorders are often a result of *de novo* gene mutations in protein coding genes (Lelieveld S H et al., 2016).

There have been several proposed mechanisms for *TLK2* causing intellectual disability. Through mice studies, it had been proposed that the developmental delay/ intellectual disability seen with *TLK2* variants could arise as a result of placental defects, as embryonic fatality was seen in mice with no *TLK2* expression (Segura-Bayona et al., 2017). However, there were no clear placental phenotypes in heterozygous mice, so this is unlikely causative (Segura-Bayona et al., 2019). Of note, neural progenitor cells are very sensitive to DNA damage, therefore it is possible that reduced functioning of *TLK2* can result in structural central nervous system problems and therefore developmental delay, as is seen in many other

neurodevelopmental disorders (McKinnon, 2017). Another possibility is that micro-exon splicing is regulated by *TLK2*; micro-splicing defects have been suggested as a possible mechanism in idiopathic autism, which could possibly account for some of the social communications difficulties as part of global developmental delay seen in these patients with *TLK2* variant (Segura-Bayona et al., 2019). It has been suggested that some *TLK2* missense mutations result in a mild dominant negative effect, as seen in four different mutations where kinase activity levels in vitro were significantly reduced, however, the likely mechanism of pathology being haploinsufficiency (Segura-Bayona et al., 2019). More research into protein regulation and pathogenesis of described phenotype is required to further understand disease process.

De novo and heterozygous dominant *TLK2* pathogenic variants have been found to display a consistent phenotype of neurodevelopmental delay, behavioural problems, gastrointestinal disorders and dysmorphic features (Reijnders et al., 2018). RNA analysis detected that these identified missense and C-terminal truncating variants resulted in loss of function and therefore likely mechanism of pathology being haploinsufficiency. Thus far, 40 individuals (38 children and 2 of their mothers) from 7 different countries have been identified using whole exome or whole genome sequencing methods - the first 5 cases were identified by Lelieveld S H et al (2016) and later analysed with the remainder by Reijnders et al (2018). The majority of variants were found to be *de novo*. Of all of these, the majority had motor and language delay and 72% were diagnosed with intellectual disability, of which the majority were classified as mild (Reijnders et al., 2018).

A single case report of homozygous missense *TLK2* gene variant described a child with significantly more severe global developmental delay and gastrointestinal symptoms, in

addition with cerebellar vermis hypoplasia, spastic tetraparesis, hydronephrosis and West syndrome (Töpf et al., 2019). Clinical features were more severe than those previously described in the *TLK2* syndrome. This missense variant (hg19 chr17: g.60599574 A>G; c.163 A>G; p.(Lys55Glu)) affected a highly conserved amino acid, which proposes a different mechanism of disease than previously described in *de novo* or dominant mutations (Töpf et al., 2019). Dysmorphic facial features were consistent with those most commonly seen in the previously described phenotype: telecanthus, up-slanting palpebral fissures, broad nasal bridge and thin vermillion of the upper lip. Both parents of the homozygous proband were clinically unaffected; both were heterozygous carriers of the missense mutation. This is in contrast to the two mothers described by Reijnders et al (2018) who, through the process of parental testing for inheritance status in the heterozygous dominant probands, were found to have heterozygous dominant variants themselves, both of whom had a milder phenotype that had not been previously investigated. This shows that population prevalence of both heterozygous recessive or dominant *TLK2* gene mutations may be higher than currently recognised. A similar phenomenon can be seen in other known inherited heterozygous gene variants, where heterozygous parents of a proband are either clinically unaffected or have very mild disease, such as *ASXL3* (Schirwani et al., 2021) and in several copy number variants such as 22q11.2 microdeletion syndrome (Kylat, 2018).

The features of the two individuals we describe fit the newly described *TLK2* syndrome (Table 2). In addition to the features previously described, both our patients had sleep disturbance; Patient 1 had difficulties with sleep initiation and Patient 2 was diagnosed with a rhythmic sleep disorder requiring Clonazepam. The prevalence of sleep disorders has not been previously quantified by Reijnders et al., 2018, although may be seen commonly due to its association with autism spectrum disorder and ADHD. Although both of our patients have

features of autism spectrum disorder, neither have a formal diagnosis thus far. Patient 2 also has a diagnosis of restless leg syndrome, which has been described in one other patient with *TLK2* variant (Table 3) (Reijnders et al., 2018). Another significant finding previously described was a feature found on MRI head in Patient 2: non-differentiation of lateral semi-circular canals, suggestive of a developmental anomaly. This is in conjunction with normal audiology tests. In contrast, two patients with hearing loss in conjunction with asymmetric semi-circular canals have been previously reported (Reijnders et al., 2018). Patient 2 also has vesicoureteric reflux, whereas urinary tract abnormalities have not been previously described. Both individuals fit the facial phenotype for *TLK2* variant. Of note, an additional facial dysmorphism seen in Patient 2 was multiple bilateral preauricular skin tags, which has not been previously described.

Conclusion

Intellectual disability describes reduced or slower than normal cognitive and intellectual development than what would normally be expected for age. Its common precursor, global developmental delay, also encompasses delay in cognition along with motor, speech and language and social communication difficulties. Causes of these are multiple and varied. Protein coding pathogenic gene variants are often a cause of such neurodevelopmental disorders.

Here we describe two children with *de novo TLK2* variant associated with global developmental delay. Other features include behavioural difficulties, poor acquisition of language, delayed communication, motor difficulties and typical facial dysmorphism. Both patients have a phenotype in keeping with the distinct *TLK2,* neurodevelopmental syndrome.

We also describe one novel variant not previously reported in the literature. Additional findings we report that may be an extension of the evolving phenotype include: sleep disturbance, non-differentiation of lateral semi-circular canals, vesico-ureteric reflux and bilateral periauricular skin tags.

Further case reports will continue to expand the described genotype-phenotype and provide information on other associated features in order to understand the variable expressivity of this condition.

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) or http://www.ddduk.org/access.html (http://www.ddduk.org/access.html) for full acknowledgement.

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Conflicts of interest

None to declare

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