Title:

Severe neurodevelopmental disease caused by a homozygous *TLK2* mutation

Running title: Severe recessive *TLK2* case

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Abstract

A distinct neurodevelopmental phenotype characterised mainly by mild motor and language delay and facial dysmorphism, caused by heterozygous *de novo* or dominant mutations in the *TLK2* gene has recently been described. All cases reported carried either truncating variants located throughout the gene, or missense changes principally located at the C-terminal end of the protein mostly resulting in haploinsufficiency of *TLK2*. Through whole exome sequencing we identified a homozygous missense variant in *TLK2* in a patient showing more severe symptoms than those previously described, including cerebellar vermis hypoplasia and West syndrome. Both parents are heterozygous for the pathogenic variant and clinically unaffected, highlighting that recessive variants in *TLK2* can also be disease-causing and may act through a different pathomechanism.

Keywords: TLK2, autosomal recessive, neurodevelopmental disease, dysmorphism

Introduction

The *TLK2* gene encodes the Tousled-like kinase 2, a nuclear serine/threonine kinase known to be involved in DNA replication and chromatin assembly by phosphorylating chromatin assembly factors, such as ASF1, and regulating histone usage.[1] *TLK2* was suggested as a candidate gene for intellectual disability (ID) in a meta-analysis of *de novo* mutations in 2,104 exome trios[2], and recently Reijnders et al.[3] associated it with ID within a broader phenotype in a cohort of 40 individuals from 38 families. The cases presented with a distinct neurodevelopmental phenotype characterised by mild motor and language delay, behavioural problems, facial dysmorphism and gastro-intestinal symptoms caused by heterozygous *de novo* or dominant mutations in *TLK2*. Truncating (nonsense, frameshift and splice site) variants located throughout the gene, or missense changes principally located at the C-terminal end of the protein were identified amongst the cohort. RNA analysis from patient derived cell lines suggested that the mutations resulted in a loss-of-function variant and that the most likely disease mechanism was haploinsufficiency of *TLK2*. Here, we report a child carrying a homozygous missense variant in *TLK2* showing more severe symptoms than those previously described.

Subject and methods

Patient was recruited at the Department of Paediatric Neurology, Izmir, Turkey. Written informed consent was obtained from the parents.

Whole exome sequencing (WES) was performed using Illumina exome capture (38 Mb target) at the Broad Institute of MIT and Harvard, Cambridge, USA. Data analysis was carried out on the RD-Connect Genome-Phenome Analysis Platform

(https://platform.rd-connect.eu/). Standard filtering criteria with MAF<1% and high/moderate variant effect predictor (VEP) were used.

Clinical description

The patient is a 6-year-old girl and the second child of unaffected consanguineous parents of Turkish origin; her 9-year-old brother is healthy (Figure 1). She was born at term but had intrauterine growth retardation (birth weight 2,400 gr). Intrauterine foetal movements were reduced and she had hypotonia and hip dislocation at birth. She presented with ~~intractable generalised seizures and~~ epileptic spasms at the age of 6 months. Her EEG showed hypsarrhythmia suggesting West syndrome. She has been seizure-free since 3 years of age.

On clinical examination at 6 years of age her psychomotor and speech development were severely delayed, she could sit without support but was unable to walk or speak. She had microcephaly, coarse facial appearance and dysmorphic facial features including telecanthus, upslanting palpebral fissures, thin vermilion upper lip, big mouth and broad nasal bridge (Figure 2a and 2b), and a hemangioma on the left parietal scalp. In addition, she had feeding difficulties, dysphagia, peripapillary atrophy on fundoscopic examination and conductive hearing loss in the left ear. Neurological examination detected spastic tetraparesis with increased deep tendon reflexes, head titubation, trunk ataxia and dysmetria. She also presented with constipation and behavioural problems including aggression and irritability. Her brain MRI at age 2 years showed cerebellar vermis hypoplasia (Dandy-Walker variant) and dilatation of lateral ventricles (Figure 2c and 2d). Abdominal MRI detected medullary nephrocalcinosis and hydronephrosis.

Results

The proband carries a homozygous missense variant (hg19 chr17: g.60599574 A>G; c.163A>G; p.Lys55Glu), which affects a highly conserved amino acid, predicted to be damaging by all tested *in-silico* tools (Combined Annotation Dependent Depletion CADD 27; top 0.2% most deleterious variants). This variant has been detected only once, in a heterozygous European, out of 122,547 individuals in gnomAD (http://gnomad.broadinstitute.org) and is absent from a cohort of 1,182 ethnically-matched Turkish control individuals (TUBITAK MAM-GMBE dataset).

Sanger sequencing showed that the variant is not present in the healthy brother, and that both parents are heterozygous carriers. On examination, they were clinically unaffected and show no signs of facial dysmorphy or ID (Figure 1).

Discussion

We report a homozygous missense variant in *TLK2* in a patient with a neurodevelopmental disorder with severe motor and language delay, behavioural problems, facial dysmorphism and gastro-intestinal symptoms. Most of the symptoms overlap with those reported by Reijnders in patients carrying heterozygous mutations in *TLK2*, however the presentation of our index case is much more severe with profound ID, intractable epilepsy during early childhood, spastic tetraparesis and structural brain anomaly. The dysmorphic features were remarkably similar. Conductive hearing loss, microcephaly and non-specific brain anomalies were present in a minority of reported cases (<25%), however we are not aware of cerebellar vermis hypoplasia, which we observed in the homozygous individual. Although the majority of the patients reported by Reijnders had language and cognitive delay (92% and 74%) this was mainly mild ID (IQ 50-70). In contrast, our patient presents severe neurodevelopmental delay with no acquired speech (although non-verbal IQ was not

formally assessed). Constipation and feeding difficulties were similar to other individuals with heterozygous mutations in *TLK2*.

The clinical presentation fits the one described by Reijnders et al. for heterozygous *TLK2* patients, however our index case present more severe symptoms. All nine missense variants identified in the 38 families described by Reijnders et al. were located either in the catalytic domain or in the coiled-coil motifs of the protein. Our variant is located at the N-terminus in a region where no functional domains are known (Figure 3). This region however is expected to contain a nuclear localization signal (NLS) as mutants lacking the first 160 amino acids fail to localise to the nucleus[4]. In addition, more than 20 phosphorylation sites, including p.Thr52 and p.Tyr70, have been identified in the N-terminal domain of TLK2, suggesting that this is potentially an important regulatory domain in vivo (https://www.phosphosite.org). *In silico* tools (cNLS Mapper, http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi) predict a monopartite NLS (^{61}RNRKRKAEPY^{70}) starting at position p.Arg61. NLSs consist of stretches of basic amino acids, primarily lysine and arginine, which bind to negative charges in the binding grooves of the transport receptor. Amino acid substitutions of lysine residues within NLSs can completely abolish their cargo nuclear import[5]. Although speculative, it may be hypothesised that the change from a positive (Lys) to a negative (Glu) side chain amino acid at the proximal position p.Lys55 may result in a change in conformation partially occluding the binding between the NLS-cargo and the import receptor. This would lead to impaired nuclear import and in turn, diminished *TLK2* activity.

As nephrocalcinosis had not been seen in any of the reported *TLK2* patients so far we looked for variants in other disease-causing genes which might explain this symptom in our patient. The proband carries a heterozygous variant in the *ABCC6* gene, associated

with Pseudoxanthoma elasticum (PXE; OMIM #177850), a connective tissue disorder. This missense variant (p.Arg391Gly) has been previously reported as pathogenic in a recessive case of PXE[6]. Interestingly, a number of PXE patients presenting with nephrocalcinosis have been reported in the literature (~~see~~ [7-9]) highlighting that PXE should be considered as a differential diagnosis. Although PXE is mainly a recessive disorder, heterozygous individuals with symptoms suggestive of PXE have been described[10], suggesting that the ABCC6 variant may be responsible for the renal phenotype in our patient.

In summary, here we describe a patient with a homozygous *TLK2* mutation leading to an autosomal recessive severe neurodevelopmental disorder, highlighting that certain *TLK2* mutations can cause a recessive disease, as the heterozygous parents were healthy. The clinical presentation of our patient shows similarities with the previously reported patients carrying heterozygous *TLK2* mutations, however the symptoms are more severe and complicated with additional features, such as cerebellar vermis hypoplasia and West syndrome. A reduction of available nuclear TLK2 would be the proposed mechanism, although this requires further investigation. We also highlight the importance of searching for not only *de novo* and dominant, but also recessive variants in *TLK2* in patients with neurodevelopmental disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

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References

1. Sillje HH, Nigg EA: Identification of human Asf1 chromatin assembly factors as substrates of Tousled-like kinases. *Current biology : CB* 2001; **11:** 1068-1073.

2. Lelieveld SH, Reijnders MR, Pfundt R *et al*: Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. *Nature neuroscience* 2016; **19:** 1194-1196.

3. Reijnders MRF, Miller KA, Alvi M *et al*: De Novo and Inherited Loss-of-Function Variants in TLK2: Clinical and Genotype-Phenotype Evaluation of a Distinct Neurodevelopmental Disorder. *American journal of human genetics* 2018; **102:** 1195-1203.

4. Mortuza GB, Hermida D, Pedersen AK *et al*: Molecular basis of Tousled-Like Kinase 2 activation. *Nature communications* 2018; **9:** 2535.

5. Kalderon D, Richardson WD, Markham AF, Smith AE: Sequence requirements for nuclear location of simian virus 40 large-T antigen. *Nature* 1984; **311:** 33-38.

6. Chassaing N, Martin L, Mazereeuw J *et al*: Novel ABCC6 mutations in pseudoxanthoma elasticum. *The Journal of investigative dermatology* 2004; **122:** 608-613.

7. Crespi G, Derchi LE, Saffioti S: Sonographic detection of renal changes in pseudoxanthoma elasticum. *Urologic radiology* 1992; **13:** 223-225.

8. Gayen T, Das A, Roy S, Biswas S, Shome K, Chowdhury SN: Pseudoxanthoma elasticum and nephrocalcinosis: Incidental finding or an infrequent manifestation? *Indian dermatology online journal* 2014; **5:** 176-178.

9. Seeger H, Mohebbi N: Pseudoxanthoma elasticum and nephrocalcinosis. *Kidney international* 2016; **89:** 1407.

10. Martin L, Maitre F, Bonicel P *et al*: Heterozygosity for a single mutation in the ABCC6 gene may closely mimic PXE: consequences of this phenotype overlap for the definition of PXE. *Archives of dermatology* 2008; **144:** 301-306.

Titles and legends to figures

Figure 1

Pedigree and Sanger segregation of the *TLK2* variant (hg19 chr17:60599574 A>G). The index case is homozygous for the alternate allele (G/G) whereas her unaffected brother and parents are homozygous wild type (A/A) and heterozygous (A/G), respectively (highlighted in yellow). No dysmorphic features are observed in the healthy family members, including those who are carriers for the *TLK2* variant.

Figure 2

Clinical findings. (A & B) Patient photographs at 6 years of age showing facial dysmorphism: telecanthus and upslanting palpebral fissures, thin vermilion upper lip, big mouth and broad nasal bridge; (B) Sagittal T2-weighted cranial MRI showing cerebellar vermis hypoplasia (Dandy-Walker variant); (C) Axial T2-weighted cranial MRI showing dilatation of lateral ventricules.

Figure 3

Schematic representation of the *TLK2* missense variants identified. In black, the p.Lys55Glu reported here; in grey, the nine mutations previously reported by Reijnders et al.