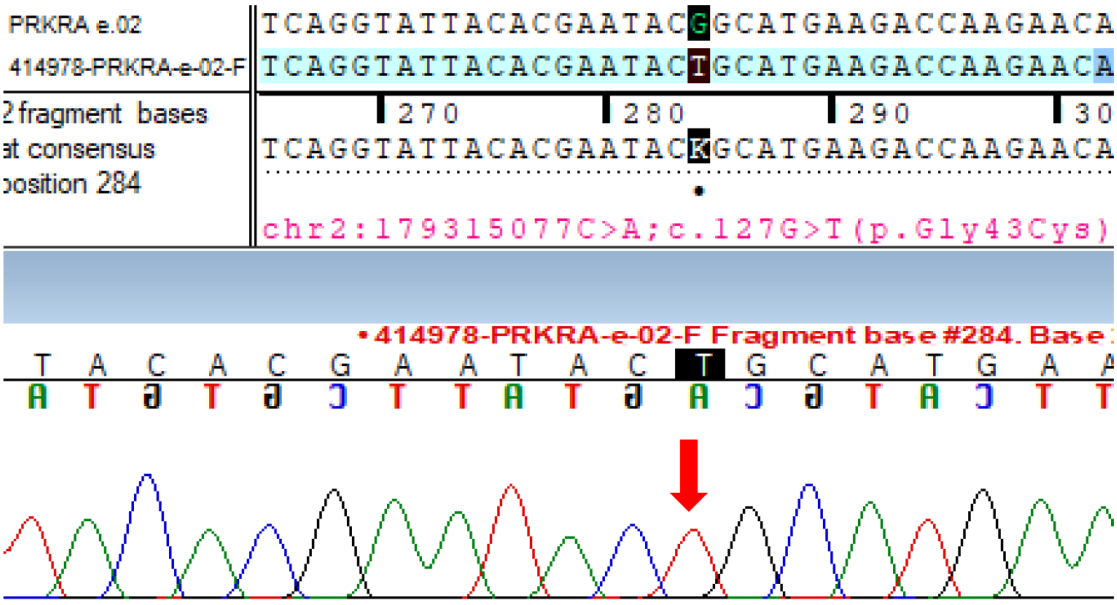
Laboratory investigations

Older child: Complete blood count, glucose, and thyroid, liver, and kidney function tests were normal. Uric acid, ceruloplasmin, and lactate levels were within range. Tandem mass spectrometry and urine organic acid analysis yielded no abnormality. Cerebrospinal fluid study (cell count, glucose, protein, and lactate) was normal. Clinical exome sequencing detected a homozygous variant in the *PRKRA* gene (c.127G>T; G 43C; exon 2). The *in silico* predictions of the variant are possibly damaging by PolyPhen-2 and damaging by LRT and MutationTaster2. The reference codon is conserved across species.

Younger child: Exon 2 of the PRKRA gene was amplified by polymerase chain reaction and the product was sequenced using Sanger sequencing. The sequence was aligned to the *PRKRA* reference sequence ENST00000325748.4.[1] The same variant was found in homozygous state (eFigure), as in the older child.



eFigure 1. Sequence chromatogram and alignment to the reference sequence shows variation in the exon 2 of the *PRKRA* gene (c.127G>T).

DYT16

DYT16 is attributed to biallelic pathogenic variants in the *PRKRA* gene which encodes PACT, a stress-response protein. Affected children manifest dystonia that commonly has onset in a limb and gradual progression to other body parts; the cranial (oromandibular, laryngeal) region is strikingly affected. Patients may have mild parkinsonism, pyramidal signs, and delayed speech developement.[2] Some of them have abrupt presentation or exacerbation with febrile illness. Till date, 10 pathogenic variants (T34S, G43S, V72F, C77S, H89fs, C213F, C213R, P222L, S235T, S265R) have been reported across the world.[3] PACT-dependent activation of protein kinase R (PKR) leads to phosphorylation of the translation initiation factor eIF2α and cellular apoptosis. It has been shown that the P222L mutation results in sustained activation of PKR, making the cells markedly sensitive to endoplasmic reticulum stressors and vulnerable

to apoptosis.[4] The G43S variant is at the same location as the one detected in our patients. Conceivably, the G43C PACT triggered abnormal activation of PKR and intensified neuronal apoptosis under the stressful cellular environment during febrile illness.

References

1. ENSEMBL: https://www.ensembl.org
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4. Vaughn LS, Bragg DC, Sharma N, Camargos S, Cardoso F, Patel RC. Altered activation of protein kinase PKR and enhanced apoptosis in dystonia cells carrying a mutation in PKR activator protein PACT. J Biol Chem. 2015 Sep 11;290(37):22543-57.