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Clinical Letter

TBCK Encephaloneuropathy With Abnormal Lysosomal Storage: Use of a Structural Variant Bioinformatics Pipeline on Whole-Genome Sequencing Data Unravels a 20-Year-Old Clinical Mystery

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In 1997 we proposed a novel lysosomal disease entity in a 11-year-old girl who had progressive fatal encephaloneuropathy.[1] Hypotonia was present almost from birth, and a neuromuscular disease was suspected. From age two years, global deterioration became evident, with intractable seizures, dementia, muscle weakness with atrophy, and respiratory failure. Autopsy showed normal brain weight despite macroscopic atrophic changes, suggesting that atrophy could be secondary to abnormal storage. Electron microscopy examination of the spianl cord showed zebra body inclusions in the anterior horn neurons (Figure S1). Immunohistochemistry demonstrated that GM2 ganglioside, a sialylated oligosaccharide, bound to a hydrophobic lipid moiety, was a major constituent of the storage material (Figure S2). However, known GM2 gangliosidoses, such as Tay-Sachs and Sandhoff diseases, and other established lysosomal disorders with GM2 ganglioside accumulation, were excluded at the time.

Recently, we reached the molecular diagnosis via whole-genome sequencing (WGS) of the family trio, using a data analysis pipeline with focus on structural variants (details in the Supplemental Data). With this pipeline, we detected two deletions in *TBCK* (NM_001163435.2), which were homozygous in the patient and heterozygous in the parents. Polymerase chain reaction and Sanger sequencing verified the segregation of the deletions with the disease (primer sequences available upon request). A healthy sister was also heterozygous for the variants. The two deletions identified are not reported elsewhere and mapped in a region of autozygosity in the patient, indicating homozygosity by descent. The parents were not aware of consanguinity. The proximal deletion mapped in *TBCK* intron 22 (chr4:107 056 578-107 063 363 bp, hg19), and the distal deletion overlapped introns 22 and 23 resulting in a homozygous loss of *TBCK* exon 23 in the patient (chr4:107 091 826-107 099 220 bp, hg19) (Fig A,B). The distal deletion caused splicing of exon 22 directly to exon 24 in the transcript (Fig C), resulting in loss of the reading frame and generation of a premature stop codon p.Glu687Valfs*8.

Declaration of interest: None.

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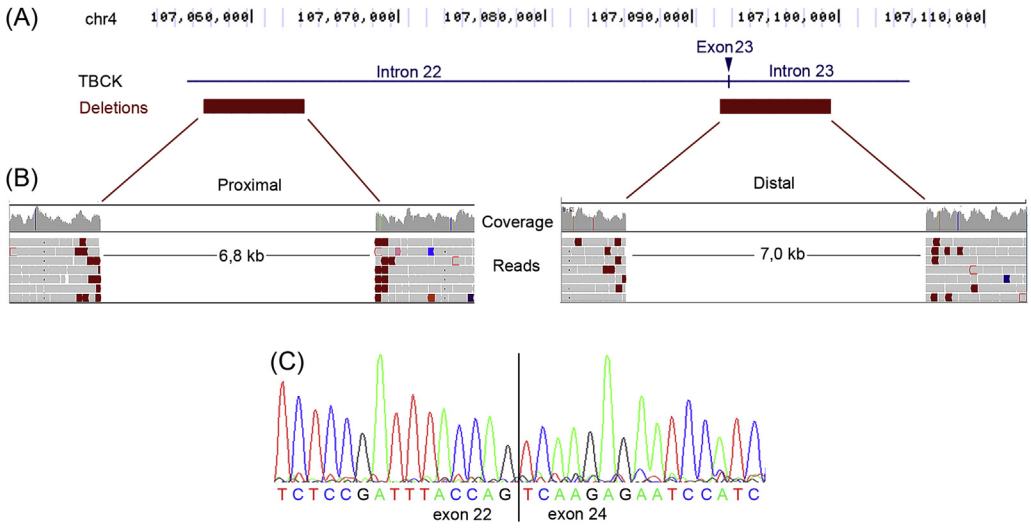

FIGURE. (A) Screenshot from UCSC genome browser (https://genome-euro.ucsc.edu) showing a region in chr4q24 covering *TBCK* (NM_001163435.2) exon 23, which is indicated with the blue arrowhead. The positions of the two deletions within *TBCK* are indicated by the red boxes. The proximal deletion covered a region in *TBCK* intron 22. The distal deletion overlapped part of intron 22, exon 23, and part of intron 23 in *TBCK*. (B) Screenshot from the Integrative Genome Viewer (http://software.broadinstitute.org/software/igv) indicating the two deletions in *TBCK* in the whole-genome sequencing data of the patient. Note the lack of coverage in the deleted regions and the reads with ends spanning the deletions (in red). (C) Sanger sequencing of cDNA from fibroblasts of the patient showing juxtaposition of *TBCK* (NM_001163435.2) exons 22 and 24, and lack of exon 23. The vertical bar indicates the border between exons 22 and 24. The color version of this figure is available in the online edition.

Biallelic loss of function in *TBCK* causes "infantile hypotonia with psychomotor retardation with characteristic facies 3 (IHPRF3)" (OMIM 616900),[2,3] which overlaps the clinical features of the patient.

TBCK inhibits the mTORC1 signaling pathway,[4] causing increased autophagosome flux and inefficient degradation of oligosaccharides, such as GM2 gangliosides.[5] The lamellae inclusions called zebra bodies detected in neural cytoplasm were in line with impaired autophagy typically seen in lysosomal storage diseases, including those causing GM2 ganglioside accumulation.

The field of medical genetics has evolved dramatically in the past 20 years, when laboratory analysis was limited to karyotyping. The implementation of microarrays made an important progress, by detecting deletions and duplications with a resolution of a few tens of kilobases. Whole-exome sequencing (WES) is the current elective method of analysis of Mendelian disease and enables accurate detection of exonic single nucleotide variants and indels (resolution from 4 to 100 bp) and has generated a remarkable amount of knowledge. At present, WES seems to have reached an upper limit in diagnostic yield to approximately 50%,[6] depending on patient cohorts. Decreasing sequencing costs and advances in analytical techniques made WGS accessible. WGS enables additional analytical possibilities and bridges the resolution gap between microarray and WES. In the patient we documented two intragenic deletions below the detection limit of even high-resolution microarrays. Thus we have demonstrated the efficiency of WGS unraveling the complex genomic rearrangement in a 20-year clinical mystery.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pediatrneurol.2019.02.001.

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