LETTER TO THE EDITOR

Progressive cerebellar atrophy caused by heterozygous TECPR2 mutations

To the Editor,

Biallelic mutations in TECPR2 (OMIM #615000) are known to cause neurodevelopmental and progressive neurodegenerative disorders. TECPR2, an autophagy protein, regulates the lysosomal targeting of autophagosomes through interaction with ATG8 family proteins. A single TECPR2 homozygous mutation was first identified as the cause of a novel subtype of hereditary spastic paraplegias (previously known as SPG49; OMIM #615031) in five individuals from three Jewish Bukharian families (Oz-Levi et al., 2012). Through a recent international collaboration, Neuser et al. (2021) described the genotype and phenotype of an additional 17 individuals with bi-allelic TECPR2-variants, bringing the total count of affected individuals described in the literature up to 26 and expanding the phenotype to hereditary sensory and autonomic neuropathy type IX with developmental delay (HSAN9; OMIM #615031; Anazi et al., 2017; Heimer et al., 2016; Palma et al., 2021; Patwari et al., 2020; Zhu et al., 2015). The phenotype includes global developmental delay, intellectual disability, axial and appendicular hypotonia, dysarthria, spasticity, and ataxia. Peripheral neuropathy, areflexia and/or hyporeflexia, and autonomic dysfunction characterized by central hypoventilation and abnormal gastrointestinal motility are additional features. Here we describe for the first time a female patient diagnosed with progressive cerebellar atrophy with global developmental delay, hypotonia, areflexia, and sleep apnea with a stop-gained mutation and a novel frameshift mutation in the TECPR2 gene that resulted in loss of protein expression.

The proband was a Caucasian female adopted at birth. As a newborn, she vomited frequently, had multiple bouts of pneumonia, and was diagnosed with failure to thrive. An MRI at five months showed a Chiari I malformation and tonsillar ectopia. At 17 months she underwent G-tube insertion, Nissen fundoplication, and posterior fossa decompression. Over time her gait became more ataxic. She was jittery upon waking, wobbly, and had a tremor even upon standing. An MRI at this time showed atrophy of cerebellar vermis, olive, and pons while her myelination had improved (Figure 1a). A muscle biopsy at 3.5 years of age did not show specific histopathological features. Biochemical studies of the respiratory chain revealed normal enzyme activity. A repeat assay showed borderline complex I activity but was not sufficiently low on a consistent basis to be diagnostic itself. Between 3 and 5 years of age, she suffered progressive hearing loss, increased fatigue, worsening vision, and deterioration in speech with slurring. She still followed directions, crawled, and cruised. Clinical features included hypotonia, areflexia, facial weakness, a myopathic shape to her mouth, and midface hypoplasia. A sleep study revealed central sleep apnea, and she underwent a tonsillectomy and adenoidectomy. Over the last years of her life she became combative, aggressive, hurting herself and others, and with head banging when upset. She experienced episodes of posturing and left arm stiffness. An MRI of the brain at four years showed cerebellar vermis atrophy, flattening of the pons, and diffuse T2/FLAIR hyperintensity in the white matter (Figure 1a). She required supplemental oxygen. She passed away at age 5 years 10 months of hypoventilation due to central apnea.

The patient was deceased at the time of enrollment and the parents provided informed written consent for participation in the Western Institutional Review Board Protocol #20120789. Previously acquired DNA from blood and frozen skeletal muscle biopsy tissue from the vastus lateralis muscle was obtained.

Research whole-exome sequencing analysis was performed on peripheral blood and revealed two heterozygous mutations in the TECPR2 gene: (a) a novel dinucleotide deletion NM_014844.5:c.1318_1319del resulting in a frameshift mutation in exon 8, truncating the protein after 13 amino acids (p.Leu440fs) and (b) non-sense mutation NM_014844.5:c.3072G>A resulting in a stop codon (p.Trp1024Ter). The p.Trp1024Ter variant is listed in ClinVar (# 957432) as pathogenic by one commercial testing company and previously seen in an individual with autosomal recessive SPG49 (Landrum et al., 2018).
Because biological parental samples were unavailable it is unclear whether the two variants are in cis or trans.

Truncated proteins due to loss of function (LoF) mutations, like the two described in our patient, are reported as being targeted for proteasome degradation (Oz-Levi et al., 2012). We performed immunoblot analysis of skeletal muscle tissue lysate from the proband and a control sample to validate the functional consequences of both mutations. A TECPR2 band (140 kDa) was detected in the control human skeletal muscle sample but not in the patient skeletal muscle lysate (Figure 1b) suggesting that the truncated TECPR2 proteins are degraded. This finding supports our contention that the two TECPR2 variants are in trans.

Mutations in TECPR2 have been described in cases of autosomal recessive spastic paraplegia (SPG49) and classified under congenital disorders of autophagy, part of an emerging group of inborn errors of metabolism (Ebrahimi-Fakhari et al., 2016). Research shows that TECPR2 is a human ATG8 binding protein and positive regulator of autophagy. Recent studies in fibroblasts of patients with TECPR2 mutations have shown that the targeting of autophagosomes to lysosomes is performed through the TECPR2 carboxy-terminal TECPR domain which interacts with VAMP8 (OMIM #603177), a lysosomal SNARE protein, as well as Atg8-family proteins and the HOPS complex on the autophagosomal membrane through the TECPR2 LIR motif (Fraiberg et al., 2021). Tamim-Yecheskel et al. (2021) created a tecpr2 knockout mouse model demonstrating an age-dependent accumulation of autophagosomes in the brain and spinal cord that suggest a defect in the targeting of these vesicles to the lysosomes. Stadel et al. (2015) described a role of TECPR2 in mediating COPII-dependent endoplasmic reticulum export in cooperation with LC3C (OMIM #609605), a protein required for autophagosome formation. Depletion of TECPR2 reduced the numbers of ER exit sites (ERES) and substantially delayed ER export. HSP patient fibroblast cell lines with TECPR2 mutations showed decreased levels of SEC24D (OMIM #607186), a COPII coat protein, and delayed ER export. These studies demonstrated that TECPR2 regulates autophagy, possibly through maintaining functional ERES.

Our patient showed the common features associated with TECPR2-related disorder, including global developmental delay, intellectual disability, hypotonia, areflexia, dysarthria, and ataxia as well as central hypoventilation and delayed gastrointestinal motility. Her MRIs demonstrated progressive cerebellar atrophy, flattening of the pons, and early-onset delayed myelination. As described in other individuals with TECPR2 mutations (Patwari et al., 2020), our patient demonstrated progressive central apnea due to respiratory cycle dysregulation and eventually relied on supplemental oxygen and passed away from respiratory failure. Similar to other reported cases of HSAN9 our patient had two LoF mutations resulting in suspected proteasome degradation as demonstrated by western immunoblot analysis of her skeletal muscle tissue showing undetectable TECPR2 protein.

In conclusion, we report a child with a progressive, multisystem disorder who underwent exome sequencing using samples acquired post-mortem. We suggest that she...
had a very rare disorder of autophagy caused by two truncating pathogenic variants in TECPR2. These findings further support the recent publication by Neuser et al. and demonstrate the severity of this form of neuropathy.

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**CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

**REFERENCES**

Anazi, S., Maddirevula, S., Salpietro, V., asi, Y. T., Alhasli, S., Alhashem, A., Shamseldin, H. E., AlZahrani, F., Patel, N., Ibrahim, N., Abdulwahab, F. M., Hashem, M., Alhashmi, N., Al MurshedI, F., Al Kindy, A., Aishaer, A., Rumayyan, A., Al Tala, S., Kurdi, W., ... Alkuraya, F. S. (2017). Expanding the genetic heterogeneity of intellectual disability. *Human Genetics*, 136(11-12), 1419–1429. https://doi.org/10.1007/s00439-017-1843-2

Ebrahimi-Fakhari, D., Safarri, A., Wahlster, L., Lu, J., Byrne, S., Hoffmann, G. F., Jungbluth, H., & Sahin, M. (2016). Congenital disorders of autophagy: an emerging novel class of inborn errors of neuro-metabolism. *Brain*, 139(2), 317–337. https://doi.org/10.1093/brain/awv371

Fraibel, M., Tamim-Yecheskel, B.-C., Kokabi, K., Subic, N., Heimer, G., Eck, F., Nalbach, K., Behrends, C., Ben-Zeev, B., Shatz, O., & Elazar, Z. (2021). Lysosomal targeting of autophagosomes by the TECPR domain of TECPR2. *Autophagy*, 17(10), 3096–3108. https://doi.org/10.1080/15548672020.1852727

Heimer, G., Oz-Levi, D., Eyal, E., Edvardson, S., Nissenkorn, A., Ruzzo, E. K., Szeinberg, A., Maayan, C., Mai-Zahav, M., Efrati, O., Pras, E., Reznik-Wolf, H., Lancet, D., Goldstein, D. B., Anikster, Y., Shalev, S. A., Elpeleg, O., & Ben, Z. B. (2016). TECPR2 mutations cause a new subtype of familial dysautonomia like hereditary sensory autonomic neuropathy with intellectual disability. *European Journal of Paediatric Neurology*, 20(1), 69–79. https://doi.org/10.1016/j.ejpn.2015.10.003

Landrum, M. J., Lee, J. M., Benson, M., Brown, G. R., Chao, C., Chitipiralla, S., Gu, B., Hart, J., Hoffman, D., Jang, W., Karapetyan, K., Katz, K., Liu, C., Maddipatla, Z., Malheiro, A., McDaniel, K., Ovetsky, M., Riley, G., Zhou, G., ... Maglott, D. R. (2018). ClinVar: Improving access to variant interpretations and supporting evidence. *Nucleic Acids Research*, 46(D1):D1062–D1067. https://doi.org/10.1093/nar/gkx1153

Neuser, S., Brechmann, B., Heimer, G., Brössle, I., Schubert, S., O’Grady, L., Zech, M., Srivastava, S., Sweetser, D. A., Dincer, Y., Mall, V., Winkelmann, J., Behrends, C., Darras, B. T., Graham, R. J., Jayakar, P., Byrne, B., Bar-Aluma, B. E., Haberman, Y., ... Ebrahimi-Fakhari, D. (2021). Clinical, neuroimaging, and molecular spectrum of TECPR2-associated hereditary sensory and autonomic neuropathy with intellectual disability. *Human Mutation*, 42(6), 762–776. https://doi.org/10.1002/humu.24206

Oz-Levi, D., Ben-Zeev, B., Ruzzo, E. K., Hitomi, Y., Gelman, A., Pelak, K. et al (2012). Mutation in TECPR2 reveals a role for autophagy in hereditary spastic paraparesis. *American Journal of Human Genetics*, 91, 1065–1072. https://doi.org/10.1016/j.ajhg.2012.09.015

Palma, J.-A., Yadav, R., Gao, D., Norcliffe-Kaufmann, L., Slagenhaupt, S., & Kaufmann, H. (2021). Expanding the genotypic spectrum of congenital sensory and autonomic neuropathies using whole-exome sequencing. *Neurology Genetics*, 7(2), e568. https://doi.org/10.1212/NXG.0000000000000568
Patwari, P. P., Wolfe, L. F., Sharma, G. D., & Berry-Kravis, E. (2020). TECPR2 mutation–associated respiratory dysregulation: More than central apnea. *Journal of Clinical Sleep Medicine, 16*(6), 977–982.

Stadel, D., Millarte, V., Tillmann, K. D., Huber, J., Tamin-Yecheskel, B. C., Akutsu, M., Demishtein, A., Ben-Zeev, B., Anikster, Y., Perez, F., Dötsch, V., Elazar, Z., Rogov, V., Farhan, H., & Behrends, C. (2015). TECPR2 cooperates with LC3C to regulate COPII-dependent ER export. *Molecular Cell, 60*(1), 89–104. https://doi.org/10.1016/j.molcel.2015.09.010

Tamim-Yecheskel, B.-C., Fraiberg, M., Kokabi, K., Freud, S., Shatz, O., Marvaldi, L., Subic, N., Brenner, O., Tsoory, M., Eliam-Altstadter, R., Biton, I., Savidor, A., Dezorella, N., Heimer, G., Behrends, C., Ben-Zeev, B., & Elazar, Z. (2021). A tecpr2 knockout mouse exhibits age-dependent neuroaxonal dystrophy associated with autophagosome accumulation. *Autophagy, 17*(10), 3082–3095. https://doi.org/10.1080/15548627.2020.1852724

Zhu, X., Petrovski, S., Xie, P., Ruzzo, E. K., Lu, Y.-F., McSweeney, K. M., Ben-Zeev, B., Nissenkorn, A., Anikster, Y., Oz-Levi, D., Dhindsa, R. S., Hitomi, Y., Schoch, K., Spillmann, R. C., Heimer, G., Marek-Yagel, D., Tzadok, M., Han, Y., Worley, G., ... Goldstein, D. B. (2015). Whole-exome sequencing in undiagnosed genetic diseases: Interpreting 119 trios. *Genetics in Medicine, 17*(10), 774–781. https://doi.org/10.1038/gim.2014.191