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RESEARCH LETTER

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SLC35F1 as a candidate gene for neurodevelopmental disorders resembling Rett syndrome

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To the Editor,

Rett syndrome (RTT, OMIM #312750) is a neurodevelopmental disorder with an incidence of 1 in 10,000 live female births. In classic RTT, affected girls present with psychomotor regression around age 6–18 months after initial normal development, and progressively develop a severe condition associated with motor, cognitive, and behavioral impairment. Most cases of classic RTT are related to pathogenic variants in the Methyl CpG-binding protein 2 gene (*MECP2*). Patients with atypical or variant forms of RTT exhibit many of the clinical signs of RTT, but do not necessarily show all the classic characteristics of the disorder (Neul et al., 2010). Among the atypical forms, individuals with the early seizure onset RTT variant (Scala et al., 2005), who manifest epilepsy before regression, have mutations in the cyclin-dependent kinase-like 5 gene (*CDKL5*), and patients with congenital RTT, who show early developmental delay, have molecular defects in the forkhead box G1 gene (*FOXG1*) (Ariani et al., 2008). However, no pathogenic variants in the aforementioned genes are identified in around 10% of patients clinically diagnosed as RTT.

Next generation sequencing (NGS) and especially exome sequencing have emerged as powerful tools for the identification of additional new genes involved in rare genetic diseases (Zhu et al., 2015) and for the diagnosis of patients without a known genetic cause or with uncertain clinical manifestations (Negri et al., 2019). Indeed, several uncommon causative genes for classic/variant RTT or similar phenotypes (RTT-like) have been discovered in the last few years (Vidal et al., 2019).

We report on a 27-year-old woman recruited among the patients attending the RTT Clinic at the Child and Adolescent Neuro-Psychiatry Unit of ASST Santi Paolo Carlo Hospital (University of Milan, Italy). She exhibited an RTT-like phenotype but was negative after classic molecular analyses, and was found to carry a novel heterozygous missense variant in *SLC35F1*.

The patient was the third child of unrelated parents, born by caesarean section with 9/10 Apgar score.

Global developmental delay was noted since the age of 3 months; she sat unsupported at the age of 36 months.

At the age of 3 months, she experienced generalized tonic and tonic–clonic seizures, which became drug resistant, except for a seizure-free period between the age of 4 and 9 years. At the age of 9 years, tonic seizures with perioral cyanosis and clonic components in the face and upper limbs reappeared, presenting in clusters, with

monthly frequency. Several antiepileptic drugs as well as vagal nerve stimulation did not control her seizures.

She never acquired independent walking and developed spastic tetraplegia in adulthood. Although not formally tested, she had severe intellectual disability (ID). Speech was limited to few intentional vocalizations. Intermittent stereotypies involving both hands, namely hand washing and mouthing, were present since the age of 2 years. Bruxism during wakefulness occurred almost daily.

Background electroencephalography (EEG) activity was diffusely slow with sharp-waves on both temporal regions. Brain magnetic resonance imaging (MRI) showed nonspecific abnormalities in the white matter on both hemispheres.

She experienced several episodes of pneumonia with severe respiratory deficit and received percutaneous endoscopic gastrostomy at the age of 24 years to prevent ab ingestis pneumonia. The patient recently passed away at the age of 27 years due to cardiac arrest during sleep.

Metabolic tests (plasma and urine amino acids, urine organic acids), genetic analyses (karyotype, chromosomal microarray, *MECP2*, *CDKL5*, and *STXBP1* sequencing and del/dup analyses), and transferrin isoelectrofocusing were normal.

DNA was then extracted from the patient's (blood and saliva) and parents' (blood) samples with the Wizard Genomic DNA Purification Kit (Promega, Madison, WI) and Quick-DNA Miniprep Plus Kit (Zymo Research, Freiburg im Breisgau, DE). Genomic DNA was enriched for the targeted exome with the Agilent SureSelect AllExon v7 kit according to the manufacturer's protocol and sequenced on the Illumina HiSeq3000 platform at CRS4 NGS Core facility. Data analysis was carried out as previously described in Di Fede et al. (2020). Exome sequencing identified a heterozygous missense variant in *SLC35F1*: c.1037T>C; p.(I346T) in exon 8 (RefSeq NC_000006.12, NM_001029858.4) in both the saliva and blood proband's samples. The variant was confirmed by Sanger sequencing in the trio, is *de novo*, and absent from population databases (now registered in the LOVD website as individual ID #00324959). This variant is predicted as damaging by several prediction tools (BayesDel_addAF, DANN, EIGEN, FATHMM-MKL, LIST-S2, MutationTaster, PrimateAI, and REVEL), and is classified as likely pathogenetic (PS2, PM2, and PP3) according to the ACMG guidelines (Richards et al., 2015).

Little is known about *SLC35F1*, which is mainly expressed in the brain, and its protein product, which is thought to be a nucleotide sugar transporter (Song, 2013). However, in 2015, Szafranski and colleagues (Szafranski et al., 2015) showed that deletions in a chromosomal region including regulatory sequences of *SLC35F1* (6q22.1q22.31) are associated with pediatric epilepsy, thus suggesting a neurodevelopmental role for this gene. In addition, a recent study demonstrated that Slc35f1 co-localizes in mouse with Rab11, a protein fundamental for dendritic spine formation and mutations in which in humans have been associated with developmental and epileptic encephalopathy (Farenholtz et al., 2019). These data support both the pathogenicity of the variant found in our patient and the presumed role of SLC35F1 in synaptic plasticity.

Most of the previously described patients (Szafranski et al., 2015) showed variable severity of ID, stereotyped behaviors, and mild neurological signs. Epilepsy and EEG abnormalities were reported in half of the affected individuals, who frequently had drug-resistant seizures. Our patient exhibited a more severe phenotype characterized by spastic tetraplegia, absent speech, hand stereotypies, bruxism, drug-resistant seizures, and recurrent respiratory infections. We cannot exclude that these characteristics may appear or worsen with age, as our patient is the oldest reported thus far, or that different mechanisms in the same gene may cause slightly different phenotypes.

Although further work needs to be done, these premises together with our findings suggest *SLC35F1* as an excellent candidate gene for developmental and epileptic encephalopathies resembling Rett syndrome.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Aglaia Vignoli and Angela Peron critically recruited and clinically evaluated the patient; Elisabetta Di Fede, Elisa Adele Colombo, and Cristina Gervasini performed the variants analysis. All the authors wrote the text.

DATA AVAILABILITY STATEMENT

The variant is available in the LOVD website as individual ID #00324959. The raw data are available from the corresponding author upon reasonable request.

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