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Clinical letter

Severe early-onset developmental and epileptic encephalopathy (DEE) associated with novel compound heterozygous pathogenic variants in *SLC25A22*: Case report and literature review

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Dear Sir,

SLC25A22 deficiency is a rare cause of familial and sporadic early onset developmental and epileptic encephalopathy (DEE) and epilepsy of infancy with migrating focal seizures (EIMFS) [1,2]. Patients typically present with refractory seizures, hypotonia, visual inattention with post-retinal dysfunction, microcephaly, neuroimaging abnormalities, burst-suppression pattern on EEG [1,2]. SLC25A22 is an important mitochondrial glutamate transporter in the liver and in the brain, it is highly expressed in astrocytes. However, the exact pathophysiological mechanism in SLC25A22 deficiency is unknown. We report a further case of *SLC25A22*-related DEE due to novel compound heterozygous variants, expanding the phenotypic spectrum of this disorder.

The proband is a 2 years old girl first admitted at our hospital at the age of 3 months for recurrent seizures. She is the second of two children of healthy, nonconsanguineous, caucasians parents. No history of neurological problems has been reported within the family. She was born by uncomplicated delivery at 38 weeks. At birth her weight was 2500 g (5°-10° centile), length was 49 cm (25°-50° centile), head circumference was not reported. The neonatal period was uneventful until the age of 2 months when she presented clusters of left hemiclonic seizures. A few weeks later both focal to bilateral tonic and tonic-clonic seizures appeared accompanied with difficulties in feeding and hypotonia. EEG showed background slowing and rare multifocal bilateral epileptiform abnormalities. Brain MRI revealed mild enlargement of subarachnoid spaces with thin corpus callosum. She was treated with levetiracetam and pyridoxine without response. TORCH screening was negative. When she was admitted to our Department at the age of 3 months, the neurological examination showed seborrheic dermatitis and severe axial hypotonia with a head circumference of 37 cm (3rd centile). The metabolic screening revealed raise of ornithine (111–129 u mol/l, n.v. 21–77), threonine (407-269 umol/l, n.v. 75–203), and citrulline (64-57 umol/l, n.v. 27–43), confirmed in subsequent controls. EEG showed slow, poorly organized and high voltage background activity with multifocal slow sharply contoured waves and high voltage sharp waves (Fig. 1). Valproic acid was started, because clinical and neuroradiological pictures were not indicative of a neurometabolic disorder, with partial response and thereafter phenobarbital was added with

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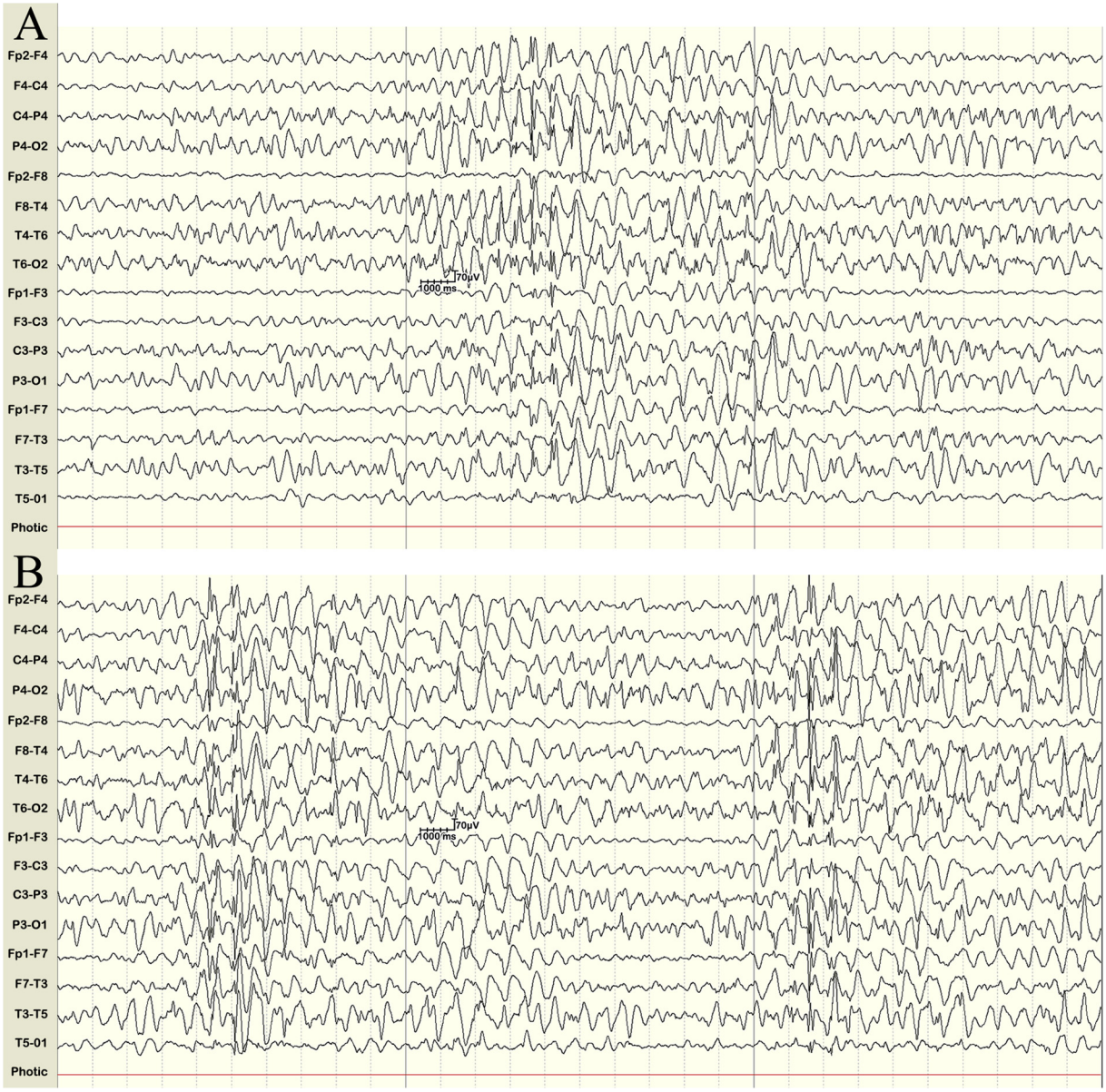

Fig. 1. A–B EEG showing poor organized and high voltage background activity with multifocal epileptiform abnormalities as slow sharply contoured and high voltage sharp waves.

good seizure control. Brain MRI performed at 8 months of age demonstrated marked subarachnoid spaces enlargement, mostly in frontotemporal regions, associated with reduced white matter volume, delayed myelination, callosal and vermis hypoplasia (Fig. 2A–C). Brain MR spectroscopy was normal. Afterwards, a worsening of seizure frequency was controlled with the add-on of topiramate. At last clinical follow-up, at the age of 26 months, she presented hypotonic tetraparesis with no head control, poor eye fixing, and microcephaly (44 cm, < < 3rd centile). A skin biopsy documented an increased lipid content in fibroblasts at OIL RED O staining (Fig. 2D–E). Array-CGH resulted negative. Hence, mutational screening of a panel of 37 genes responsible for Epileptic Encephalopathies was performed by Next Generation Sequencing. Two compound heterozygous variants in *SLC25A22* gene were detected; c.736 T > C [p.Cys246Arg] of paternal origin and c.235 G > C [p.Glu79Gln] of maternal origin. These variants are not listed in ClinVar (www.ncbi.nlm.nih.gov/clinvar/) and HGMD (http://hgmd.cf.ac.uk/) clinical databases and in the database of human polymorphisms gnomAD (https://gnomad.broadinstitute.org/). The pathogenic variants (likely pathogenic according to the ACMG guidelines) involve evolutionary conserved aminoacids and are predicted to have functional consequences in different softwares, with the exception of Mutation Taster for p.Cys246Arg (p.Cys246Arg: Possibly Damaging, score 0.916 [Polyphen2]; Disease Causing, score 180 [Mutation Taster]; Tolerated, score 0.09, [SIFT]. p.Glu79Gln: Probably Damaging, score 0.990 [Polyphen2]; Disease Causing, score 29 [Mutation Taster]; Deleterious, score 0.02 [SIFT]).

Only 16 patients with *SLC25A22* pathogenic variants have been described, all harboring homozygous variants except our and another one case with heterozygous compound variants (see online Table 1). Despite harboring different variants, all patients show common neurological features as severe developmental delay, marked hypotonia, and poor psychomotor acquisitions. Except for one subject starting to show a mild phenotype at 7 years of age [3], all patients have the onset of seizures during the first weeks or months of life. The epileptic phenotype is typically early infantile DEE but may be consistent with any of Ohtahara syndrome, EIMFS or early infantile myoclonic encephalopathy. Focal seizures appear to be the most frequent seizure type. Distinctive EEG features are burst suppression or multifocal epileptic

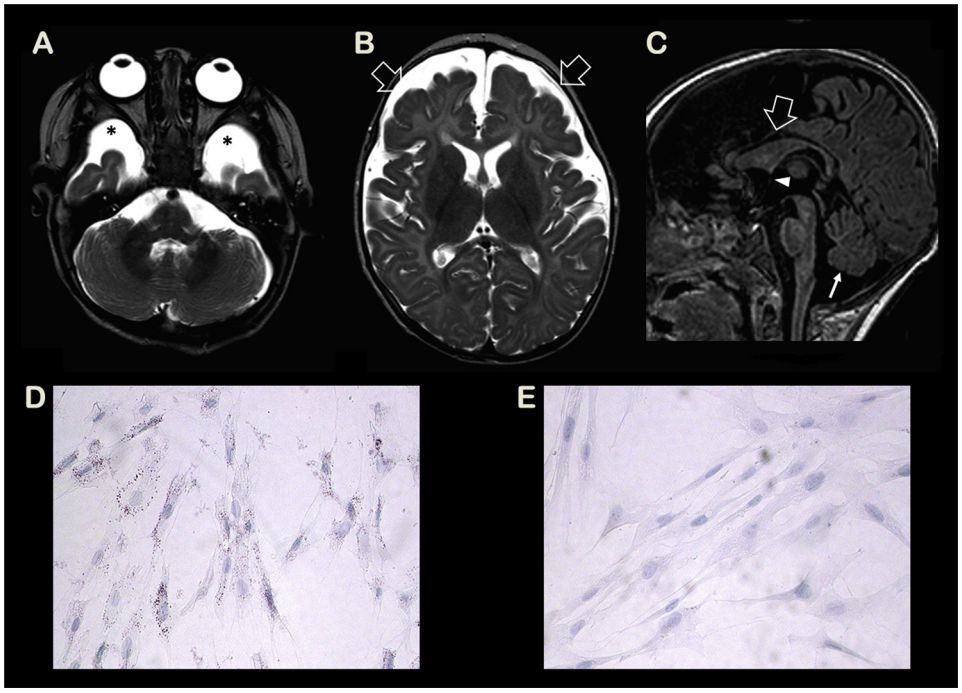

Fig. 2. Brain MRI performed at 8 months of age (A–C) and OIL RED O staining performed in fibroblasts of our patient (D) and a normal control (E). A, B) Axial T2-weighted images reveal reduced white matter volume, delayed myelination, and enlarged subarachnoid spaces in the temporal (asterisks) and frontal lobes (empty arrows). C) Sagittal T1-weighted image shows small corpus callosum (empty arrow), agenesis of the anterior commissure (arrowhead), and small inferior cerebellar vermis (arrow). D, E) OIL RED O staining (magnification 20x) demonstrates an excess of lipid content is evident in fibroblast from patients (red dots, D) in comparison with control fibroblasts (E). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

abnormalities. Most of the patients show drug resistance (69%), while in the present case phenobarbital and topiramate led to a satisfying seizure control. Congenital and secondary microcephaly is frequent (56%). Brain MRI may reveal reduced volume of the frontal and temporal lobes, cerebral and/or cerebellar subarachnoid spaces enlargement, delayed myelination, and callosal hypoplasia.

SLC25A22 pathogenic variants have been related to impaired glutamate synthesis and turnover in the astrocytes and neurons cytoplasm, but also to a malfunction of the mitochondrial respiratory chain or dysfunction in neuronal oxygen handling [4]. Goubert et al demonstrated intracellular glutamate accumulation and lack of glutamate oxidation with lower level of cellular adenosine triphosphate (ATP) in *SLC25A22* silenced astrocytes [4]. Glutamate accumulation in astrocytes is a possible contributory cause of neuronal hyper-excitability, thus explaining the neurological phenotype of these patients.[4]

Reid et al reported several biochemical alterations in patients with *SLC25A22* pathogenic variants, including increased plasmatic levels of proline, ornithine and arginine, and presence of vacuolated fibroblasts in muscular biopsies [3]. Similarly, we detected mild persistent elevation of plasmatic ornithine, threonine, citrulline and lipid deposits within skin fibroblasts. These findings indicate that impaired mitochondrial glutamate transport due to SLC25A22 deficiency may lead to abnormal catabolism and synthesis of several amino acids and lipid accumulation in fibroblasts. These features may be detected at metabolic screenings and skin biopsy. Additionally, these biochemical abnormalities suggest potential roles of SLC25A22 in transportation of other molecules, as proline and pyrroline-5-carboxylate, involved in the amino acids catabolism and synthesis [3].

In conclusion, *SLC25A22* pathogenic variants should be suspected in patients with DEE with polymorphic seizures, severe developmental delay, and microcephaly. Increased amino acids plasmatic levels and vacuolated fibroblasts at skin biopsy might have a role as diagnostic and therapeutic markers in this condition. Animal models are needed to understand the underling mechanisms of this condition thus paving the way for targeted therapies.

Transparency document

The Transparency document associated with this article can be found in the online version.

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