Title: De novo mutation in SLC25A22 gene: expansion of the clinical and electroencephalographic phenotype.

Running Head: De novo mutation in SLC25A22 gene

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Abstract

The SLC25A22 (Solute Carrier Family 25, Member 22) gene encodes for a mitochondrial glutamate/H+ symporter and is involved in the mitochondrial transport of metabolites across the mitochondrial membrane. We hereby report a 12-year-old girl presenting with early-onset epileptic encephalopathy, hypotonia, and global developmental delay. Whole exome sequencing identified a novel homozygous missense mutation in SLC25A22 gene (c.97A>G; p.Lys33Glu), as the likely cause of the disease. The phenotype of our patient and EEG recordings do not completely overlap with the phenotypes previously described, leading to a new and more complex form of disease associated with SLC25A22 variants, characterized by dyskinetic movements and oculogyric crisis.

Keywords: dyskinetic movements; epileptic encephalopathy; glutamate; oculogyric crisis; SLC25A22 gene

Introduction

The SLC25A22 (Solute Carrier Family 25, Member 22, OMIM *609302) gene, or GC1 (Glutamate Carrier 1), encodes for a mitochondrial glutamate/H+ symporter and is located on the short arm of chromosome 11 (Chr 11p15.5) (Palmieri et al., 2004).

SLC25A22 is a member of the SLC25 gene family which is involved in mitochondrial transport of metabolites across the mitochondrial membrane. SLC25A22 is similar for structure and function to SLC25A18 (Solute Carrier Family 25, Member 18, OMIM * 609303 (609303), one out of the two known isoforms of mitochondrial glutamate/H+ symporters, also known as GC2 (Glutamate Carrier 2) (Palmieri et al., 2004; Molinari et al., 2009). The SLC25 gene family encodes mitochondrial carriers that transport a variety of metabolites across the inner mitochondrial membrane. Although SLC25A22 is present ubiquitously, it is mainly expressed
in astrocytes, liver, and pancreas; it plays a role in carrying glutamate into and out the mitochondria across their inner membrane, regulating the accumulation of glutamate into these organelles. The variants in SLC25A22 have been associated with a different form of epileptic encephalopathies, such as Ohtahara Syndrome (OS), Early Myoclonic Encephalopathy (EME), and Malignant Migrating Partial Seizures of Infancy (MMPSI), all of them characterized by early-onset seizures (Poduri et al., 2013). To date, to the best of our knowledge, one patient with a variant in SLC25A22 has been so far described with a phenotype of late-onset seizures (Giacomini et al., 2019).

OS is a rare and severe disease affecting infants within the first 3 months of life. Tonic spasms are the most common seizures and can occur as a cluster or single episodes and be refractory to treatment. Focal and myoclonic seizures can also occur. The electroencephalogram (EEG) is characterized by a burst-suppression pattern both in waking and sleeping states (Mastrangelo and Leuzzi, 2012). OS phenotype caused by SLC25A22 mutations mostly overlaps with other OS forms associated with other genes’ mutations (i.e.: CDKL5 or ARX) (Sartori et al., 2011).

It is featured by myoclonic seizures within the first month of life and late-onset tonic spasms, hypotonia, microcephaly, abnormal visual evoked potential (VEP), and electroretinogram (Mastrangelo and Leuzzi, 2012). EME is a rare epileptic encephalopathy characterized by erratic myoclonus and refractory partial seizures with neonatal or early infantile-onset and suppression-burst EEG pattern during sleep. EME may be caused by metabolic disorders, such as non-ketotic hyperglycinemia and organic acidemias, or genetic disorders due to mutations in ERBB4, SIK1, SLC25A22, KCNQ2 and GABRB2 genes (Mastrangelo and Leuzzi, 2012; Giacomini et al., 2019). MMPSI is a severe and rare epileptic encephalopathy. EEG abnormalities involve different regions of the brain, migrating from one region to another one and leading to focal seizures, which are typically multifocal, independent, and drug-resistant.
To date, *KCNT1*, *PLCBI*, *SCN1A*, *SCN8A*, *TBC1D24* and *SLC25A22* are the genes associated with MMPSI (Poduri et al., 2013; Striano et al. 2014).

Herein, we describe a patient with a novel homozygous mutation in *SLC25A22* gene associated with a complex epileptic and neurodevelopmental phenotype not overlapping with the previously described cases.

**Case study**

The patient is a Caucasian 12-years-old girl born on the 36th weeks of gestational age from a complicated pregnancy with intrauterine growth restriction and fetal bradycardia which required the caesarean section. The parents of the patient are not consanguineous, but they originated from a small village in Sicily, Italy. Severe generalized hypotonia, poor feeding, and early psychomotor impairment with low reactivity to visual stimuli were detected from the first weeks. Since the 15th day of life, she presented epileptic seizures with hypertonia, staring, facial rush, and clonic movements of the lower left limb that occurred several times daily and lasted about one minute.

The EEG during sleep showed pseudoperiodic high-voltage slow theta-delta activity, interspersed with sporadic brief sequences of rapid activity with spikes and sharp waves in temporal and occipital regions. Valproic acid treatment was set and Vigabatrin was successively added to the therapy, with partial benefit. At the age of 18 months, seizures occurred during sleep and were characterized by sudden awakening, left lateral head deviation with clonic movements, oral automatism, drooling and dyskinetic movements of the left lower and upper limb. Furthermore, she presented significant oculogyric crisis with horizontal rhythmic and/or multidirectional eyes movement. EEG recording revealed diffuse and irregular high-voltage spike-wave and slow delta activities discharges, interspersed with brief tracts of diffuse background slowing according to a fragmented hypsarrhythmia pattern.
Over the following years occurred minor changes, and although antiepileptic polytherapy was modified with levetiracetam, phenobarbital, ethosuximide, clobazam, and ACTH added in turn, always with partial benefit on seizure control. An EEG recording showed a progressively better organization of background activity during awakening with the persistence of diffuse and irregular rapid high-voltage discharges and pseudoperiodic pattern arising during sleep (Video 1). When she was 11 years old, a new EEG showed a pattern similar to the previous ones. Mild dysmorphic facial features of patient including microcephaly and facial asymmetry with a triangular shape, macrognatia, wide mouth with dental diastema, and crowding (Fig. 1). The patient never reached any motor and language skills, presenting distal spasticity at the four limbs and severe intellectual disability. Extensive routine and metabolic investigations assessed according to previously described methods were unremarkable (Di Rosa et al., 2006). Moreover, muscle biopsy, electromyography, auditory brain response, and ophthalmologic evaluation, were all reported to be normal. Although, at the age of 3 months, amino acid testing showed a slight raise of glycine (blood levels 528 micromol/L, and urine levels 1134 micromol/L), not confirmed in subsequent controls. Visual evoked potentials showed no response. Brain magnetic resonance imaging (MRI), performed at 3 months, 1 year, 3 years, and 5 years of age, showed a progressive enlargement of the subarachnoid space, mainly in the frontal lobe, a volumetric reduction of supratentorial and subtentorial structures with marked enlargement of liquor spaces, symmetrical alterations of the frontal and occipital periventricular white matter, atrophy of the cerebellar vermis and lobes (Fig. 2).

Genetic analyses

Karyotype, mitochondrial DNA evaluation and array comparative genome hybridization (array-CGH) were reported as normal. Whole-exome sequencing (WES) was performed both in the proband and her unaffected parents. The Nextera Rapid Capture Enrichment kit (Illumina) was used according to the manufacturer instructions. Libraries were sequenced in
an Illumina HiSeq3000 using a 100-bp paired-end reads protocol. Sequence alignment to the human reference genome (UCSC hg19), and variants call and annotation were performed as described elsewhere (Salpietro et al., 2019). We removed all synonymous changes and variants not shared by the patient and the two parents. The raw list of single nucleotide variants (SNVs) and indels was then filtered. Only exonic and donor/acceptor splicing variants were considered. In accordance with the pedigree and phenotype, priority was given to rare variants [<1% in public databases, including 1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium (ExAC v0.2)] that were fitting a recessive model. No novel/rare plausible segregating compound heterozygous variants were identified in the family’s WES data. A homozygous missense mutation in SLC25A22 (c.97A>G; p.Lys33Glu), not described before and absent from publicly available databases, was identified in the proband, and in the heterozygous state in the unaffected parents. This mutation was predicted as deleterious by Sorting Intolerant From Tolerant (SIFT) and damaging by Polyphen-2. The missense mutation affects a conserved residue (GERP++ 3.25) of the first repeat of the protein encoded by SLC25A22. Segregation analysis at the DNA level performed by traditional Sanger sequencing confirmed the mutation as homozygous in the proband and heterozygous in the parents (Fig. 3).

**Discussion**

We hereby reported a patient affected by EIEE with early-onset drug-resistant seizures characterized by clonic jerks and dyskinetic movements at face and limbs, oculogyric crisis, axial hypotonia, distal spasticity, and severe cognitive impairment, carrying an inherited novel homozygous mutation in SLC25A22 gene (c.97A>G; p.Lys33Glu), detected by WES trioanalysis. The homozygous mutation in SLC25A22 has been considered the most likely explanation for the disease pathogenesis in our child, as supported by the published
association of this gene with different epileptic encephalopathy phenotypes, even if not completely overlapping with our patient one.

To date, 19 patients with SLC25A22 variants mutations have been described, all of them affected by hypotonia and epileptic seizures. Early-onset refractory seizures were described in all the patients; just one 7-years-old subject showed a mild phenotype. An abnormal EEG was described in quite all the patients, with a burst-suppression pattern in five out of them, hypsarrhythmia in one patient, multifocal discharges in four subjects, and multifocal discharges with focal spikes in three patients. Brain MRI has been described as normal in four children. Hypoplastic corpus callosum and/or hypoplastic splenium has been described in eight patients, hypoplastic cerebellum in six patients, delayed myelination in four patients, frontotemporal hypoplasia in one patient, brain atrophy in five subjects, subarachnoid enlargement in four patients, and abnormalities of the insular cortex in one patient.

Hyperprolinaemia was detected in five patients and it could contribute to neuropsychiatric phenotype as previously reported. Dystonic movements have been described in two patients, with dyskinetic movements in one of them (Table 1). Significantly none of the patients presented an epileptic phenotype completely overlapping with our patient, particularly regarding oculogyric crisis with horizontal rhythmic or multidirectional eye movements, which have not been previously reported in association with SLC25A22 mutation. Also, EEG discharges are not classifiable as suppression-burst and do not show migrating features.

Differently from the previous reported cases of early-onset epileptic encephalopathy, our patient’s phenotype does not fulfil the clinical and EEG “criteria” for the diagnosis of the EIEE forms associated to SLC25A22 mutations, such as EIEE3, EME, and MMPI (Molinari et al., 2005; Raux et al., 2006; Molinari et al., 2009; Guilmatre et al., 2010; Cohen et al., 2014; Poduri et al., 2014; Reid et al., 2017; Lemattre et al., 2019; Giacomini et al., 2019.)
Moreover, the reported mutation is located in exon 3; precisely, in a helical transmembrane domain. To date, there are not patients reporting mutation in this specific region. Lemattre et al. suggested that mutations involving other helical transmembrane domains present a more severe phenotype; accordingly with the proposed classification and clinical presentation of our patient, we could match her phenotype with a severe clinical picture (Lemattre et al., 2019). It could be thought that pathophysiological mechanisms underlying SLC25A22-related neurodevelopmental/epileptic features implicate abnormal glutamate transport into neuronal cells. In particular, Goubert et al. described that SLC25A22 inhibition determining an accumulation of glutamate in rat cortical astrocytes; thus, they hypothesized that the inactivation of enzymes/proteins involved in astrocytic glutamate metabolism is a key-mechanism causing EEEs and/or MPSI (Goubert et al., 2017). It is presumable that this mechanism link between the oculogyric crisis too.

However, although other authors yet described encephalopathies caused by mutations in genes involved in glutamate metabolism (GRIN1, GRIN2A, GRIN2B, and GRIN2D) and epilepsy/movement disorders, to date, there are few evidences of association between SLC25A22 gene and movement disorder, whereas no patients with oculogyric crisis are reported (Nicotera et al., 2019; Fernández-Marmiesse et al., 2018; Salpietro b et al., 2019).

The involvement of glutamate in ocular movements’ regulation has been reported in Rapid eye movement sleep behavior disorder, which is a known prodromal sign or precursor of Parkinson disease, moreover, an increase in glutamatergic activity of the pedunculopontine nucleus (PPN) of the brain stem is seen in experimental models of Parkinsonism in rats (Pourmirbabaei et al., 2019).

Further studies are needed to confirm and understand how dyskinetic movement and oculogyric crisis with horizontal rhythmic or multidirectional eye movements can also occur in patients with SLC25A22 mutations.
Conflict of interest: The authors declare no conflict of interest.

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Figure Legends:

**Figure 1.** Mild dysmorphic facial features of patient including microcephaly and facial asymmetry with a triangular shape, macrognatia, wide mouth with dental diastema, and crowding.

**Figure 2.** Brain Magnetic Resonance Imaging showing an enlargement of the subarachnoid space, mainly in the frontal lobe, a volumetric reduction of supratentorial and subtentorial structures with marked enlargement of liquor spaces, symmetrical alterations of the frontal and occipital periventricular white matter, atrophy of the cerebellar vermis and lobes.

**Figure 3.** Pedigree, Sanger sequencing, multiple-sequence alignment of SLC25A22 of the patient. (A) The pedigree diagram of the family with an SLC25A22 mutation. (B) Individual results of Sanger sequencing showing the proband (II.1) carrying the homozygous SLC25A22 mutation (c.97A>G; p.Lys33Glu), while being heterozygous in the two parents I.1 and I.2. (C) Inter-species alignment performed with Clustal Omega shows the complete conservation down to invertebrates of the amino acid residue affected by the substitution. UniProt references: Human: Q9H936; Mouse: Q9D6M3; Rat: A0A0G2K5L2; Bovin: Q08DK4; Danre: F8W3S7; Cattle: A0A4W2DTA2; Drosophila: Q9VGF7; C. elegans: Q93540.

**Video Legend:**

**Video 1:** EEG recorded at the age of 10 years shows awakening with the persistence of diffuse and irregular rapid high-voltage discharges and pseudoperiodic pattern arising during sleep. Video shows the oculogyric crisis with horizontal rhythmic and/or multidirectional eyes movement, lateral head deviation with clonic movements, oral automatism, and dyskinetic movements of the lower and upper limb (left>right).
**Table Legend:**

**Tab. 1:** Clinical features of the patients with variants in SLC25A22.