Short Report

Mutations in the mitochondrial glutamate carrier SLC25A22 in neonatal epileptic encephalopathy with suppression bursts


Neonatal epileptic encephalopathies with suppression bursts (SBs) are very severe and relatively rare diseases characterized by neonatal onset of seizures, interictal electroencephalogram (EEG) with SB pattern and very poor neurological outcome or death. Their etiology remains elusive but they are occasionally caused by metabolic diseases or malformations. Studying an Arab Muslim Israeli consanguineous family, with four affected children presenting a severe neonatal epileptic encephalopathy, we have previously identified a mutation in the SLC25A22 gene encoding a mitochondrial glutamate transporter. In this report, we describe a novel SLC25A22 mutation in an unrelated patient born from first cousin Algerian parents and presenting severe epileptic encephalopathy characterized by an EEG with SB, hypotonia, microcephaly and abnormal electroretinogram. We showed that this patient carried a homozygous p.G236W SLC25A22 mutation which alters a highly conserved amino acid and completely abolishes the glutamate carrier’s activity in vitro. Comparison of the clinical features of patients from both families suggests that SLC25A22 mutations are responsible for a novel clinically recognizable epileptic encephalopathy with SB.

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Neonatal epileptic encephalopathy with suppression bursts (NEESBs) is a rare condition characterized by the onset of seizures in the first months of life and interictal ‘suppression burst’ (SB) electroencephalogram (EEG) pattern. This specific pattern is described as generalized and multifocal, high-voltage, spikes and sharp wave complexes alternating with periods of suppression
of the electrical activity (1, 2). In this group of pathology, two diseases have been described: early myoclonic encephalopathy (EME) and early infantile epileptic encephalopathy (EIEE). However, it may be difficult to distinguish these two entities for given patients, especially in the first months of life and since not all patients fulfill the criteria of either EIEE or EME (3–5).

In 1976, Ohtahara and co-workers described the EIEE syndrome (6–8) and proposed that this syndrome represented one of three so-called ‘age-dependent epilepsy encephalopathy syndromes’ which also include West and Lennox-Gastaut syndromes (9, 10). The characteristics of EIEE syndrome are: onset in early infancy (neonatal period through the first few months of life); tonic spasms (brief tonic seizures) as the predominant seizure type; SB EEG pattern; medically intractable seizures; severe psychomotor retardation; poor prognosis; evolution to West syndrome and then to Lennox-Gastaut syndrome (8).

A number of etiological factors have been associated to EIEE syndrome. In a series of 16 patients, Ohtahara and colleagues reported 4 cryptogenic and 12 symptomatic cases. Among the symptomatic patients, four were thought to have suffered neonatal anoxia, two presented Aicardi syndrome and the remainder had cerebral malformations, i.e. porencephaly or cerebral atrophy or dygenesis (11). None had specific metabolic disorders. Recently, mutations in ARX (one polyalanine expansion) and Munc18-1 (one deletion, four missense mutations, MIM #612164) were reported in patients with EIEE (12, 13). Treatments with hormonal therapy (typically ACTH) or antiepileptic drugs are not effective (8). The outcome of this condition is poor; the majority of patients either die within the first few years of life or survive in a vegetative state.

Recently, genetic analyses of a multiplex consanguineous family allowed us to identify a homozygous mutation in the SLC25A22 gene (also named ‘GC1’, MIM #609302, NM_024698), encoding a mitochondrial glutamate carrier, responsible for an autosomal recessive form of neonatal epileptic encephalopathy (14). To estimate the prevalence of SLC25A22 mutations in epileptic encephalopathy, 30 patients (25 previously described (14) and 5 additional presented in Table S1, supporting information online) were screened. We found two recurrent variations (c.234 C>T and c.448 C>G) and identified a second SLC25A22 mutation. This report presents the detailed clinical characteristics, genetic analyses and biochemical findings of this new patient. On the basis of the present and previous data, we suggest that SLC25A22 mutations may be responsible for a clinically recognizable epileptic encephalopathy with SB.

Methods

Mutation screening of the SLC25A22 gene

In addition to the 25 patients previously analyzed for the SLC25A22 gene (14), 5 new patients were screened. Their clinical features are presented in Table S1 (supporting information online). Blood samples were obtained from siblings and their parents after informed consent, and genomic DNA was isolated from blood leukocytes using the Nucleon kit (Amersham-GE Health Care Europe-Saclay-France) according to the manufacturer’s instructions. Polymerase chain reaction (PCR) amplification of each SLC25A22 exon and splicing junctions was performed as previously described (14). PCR products were purified with Exo-SAP (Amersham-GE Health Care Europe-Saclay-France) and directly sequenced on an automated sequencer (ABI 3130xl, Applied Biosystems Courtaboeuf-France) using the Dye Terminator method according to the manufacturer’s instructions.

Construction of the expression plasmid for the human SLC25A22 mutant

The human SLC25A22 expression construct has been reported previously (15). The mutations Gly236Trp or Gly236Ala were introduced into the wild-type human SLC25A22 cDNA by overlap–extension PCR (16). Subsequently, the wild-type and mutant proteins were overexpressed in Escherichia coli BL21(DE3) (17). Transport activities of the recombinant purified proteins were assayed as described previously (18). The amount of protein incorporated into liposomes was estimated as described by Fiermonte et al. (19). Approximately 20% of wild-type or SLC25A22 mutant proteins were reconstituted.

Results

Clinical evaluation

The patient (IV.3) was the third child of first Algerian cousin parents who had two healthy daughters (IV.1 and IV.2) aged 7 and 3 years old, respectively (Fig. 1a). His father (III.3), 29 years old at birth of IV.3, presented cerebral palsy due
Fig. 1. Genetic analysis of Family 2. (a) Pedigree of the family and haplotypes at the SLC25A22 locus. The black symbol indicates the affected individual. Haplotypes are shown under each genotyped individual. Markers are from telomere to centromere and their positions, based on the UCSC Genome browser, are indicated in base-pairs (bp). Genotypes are based on the CEPH human diversity panel with respect to a control DNA provided by the CEPH. (b) Electropherograms of SLC25A22 exon 8 in a control (left) and the affected patient (right). DNA sequencing identified a homozygous substitution c.706G>T. (c) SLC25A22 mutation and phylogenetic analysis of known mitochondrial glutamate carriers and aspartate/glutamate carriers in various species. The underlined amino acid indicates the substitution caused by the mutation.
SLC25A22 mutations: a new case

to neonatal anoxic encephalopathy and his mother (III.4), 29 years old, was healthy. The patient was born at 39 weeks of gestation (Apgar score: 10; birth weight: 2850 g; and cranial circumference: 34 cm) after a normal pregnancy.

During the first month of life, physical examination showed axial hypotonia, weak suction and incomplete archic reflexes. Seizures were observed from day 5, consisting of epileptic spasms followed by focal, secondary generalized seizures with eye deviation associated with limb and facial clonic movements. The first interictal EEG performed at day 8 showed SBs (Fig. 2a). Biochemical, imaging and neurophysiological explorations are listed in Table S2 (supporting information online). Diverse antiepileptic drugs (vigabatrin, carbamazepine, stiripentol and phenobarbital) were tested without any effect.

At 6 months of age, the patient presented clusters of epileptic spasms and interictal EEG showed hypsarrhythmia with a diagnosis of West syndrome (Fig. 2b). Electromyogram performed at 7 months, nerve conduction velocity at 2 and 7 months, and high-resolution karyotype performed at 7 months were normal (Table S2, supporting information online). At 1 year of age, tonic seizures appeared with persistent focal secondary generalized seizures occurring in clusters several times a day (Fig. 2c). At 5 years, seizures progressively decreased without any antiepileptic drugs change but relapsed at 7 years.

A brain magnetic resonance imaging (MRI) (7 years) showed cerebellar hypoplasia without brainstem anomalies, dysmorphism of the corpus callosum with hypoplasia and abnormal splenium, abnormal gyration of both tempo-parietal regions, and abnormal myelination of temporal poles with lack of differentiation of white and gray matters (Fig. S1, supporting information online). Electroretinogram (ERG), performed at 7 months of age and repeated at 8 years, showed progressive alteration and abolition of macular and peripheral responses (Table S2, supporting information online).

At 10 years of age, the patient still exhibited tonic seizures, no psychomotor acquisition, vegetative state, microcephaly (cranial circumference: 50 cm, −2 SD), severe hypotonia and pyramidal syndrome without spasticity. EEG has no organization, consisting of diffuse slow waves and spike waves (Fig. 2d).

Genotyping and sequencing analysis

Genotyping analysis showed homozygosity at the SLC25A22 locus, from telomere to marker

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**Fig. 2.** Electroencephalogram evolution. (a) One month of age, wakefulness: no physiological background activity, suppression-burst pattern characterized by multifocal or generalized, high-voltage, spikes and slow wave complexes. (b) Six months of age, wakefulness: typical hypsarrhythmia. (c) One year of age, sleep: tonic seizure characterized by generalized fast rhythms during 4 s. (d) Seven years of age, wakefulness: no occipital physiological activity, generalized slowing of the tracing with multifocal spikes.
We identified a homozygous substitution, c.706G>T, which leads to the change of a highly conserved amino acid p.Gly236Trp (Fig. 1b,c). This substitution segregated in the family and was not found in 200 unrelated control individuals (100 of Arab origin and 100 of various ethnic origins). This mutation identification led to a prenatal diagnosis and the birth of a healthy girl (IV.4, Fig. 1a) who is heterozygous for the mutation.

Glutamate exchange and transport in vitro

The altered glycine residue is highly conserved across species in all glutamate and aspartate/glutamate carriers (SLC25A12/AGC1 [MIM #603667] and SLC25A13/AGC2 [MIM #603859]; Fig. 1c) (20), suggesting an important role for this residue in protein function. To test the functional consequences of this substitution, wild-type and SLC25A22 (G236W) mutant proteins were overexpressed in E. coli and purified. Equivalent amounts of each protein were used for in vitro reconstitution of the transporter into liposomes (18). Observation of the time course of [14C]glutamate uniport and the [14C]glutamate/glutamate exchange in reconstituted liposomes showed that the wild-type SLC25A22 protein efficiently catalyzed glutamate exchange and uniport, whereas neither uniport nor exchange was observed in the mutated G236W protein, despite normal insertion of the SLC25A22 mutant protein into the liposomal membrane (Fig. 3).

To confirm the role of the G236 residue, we introduced a different mutation at this position (G236A). Indeed, in some proteins, glycine can be replaced by an alanine without any significant loss of activity (21). By contrast, the G236A mutant protein exhibited a significant decrease in exchange activity (about 10% of the wild-type protein) and nearly no uniport activity (Fig. S2, supporting information online), further supporting the key role of this glycine residue for SLC25A22 protein functions.

Discussion

We describe a novel missense mutation of the SLC25A22 gene identified in a patient with EIEE syndrome. This substitution alters a highly conserved amino acid that is crucial for SLC25A22 activity as demonstrated by the complete loss of transport and uniport activity of the SLC25A22 (G236W) mutant protein. The previously described P206L mutation in the SLC25A22 protein also completely abolished uniport and exchange activity in vitro, whereas cell respiration was intact indicating normal respiratory chain activity (14). Therefore, Pro206 and Gly236, which reside in the face of helix IV and V, respectively, lining the internal channel of the carrier through which the substrate is translocated, do not tolerate substitution demonstrating the key role of these residues in the transport mechanism catalyzed by the SLC25A22 protein.

The clinical presentation of the patient described in this report is very similar to the other infants described by Molinari et al. (14): epileptic spasms and focal seizures associated with SB beginning in the first days of life, microcephaly, hypotonia, abnormal ERG recording and psychomotor retardation (Table 1). Imaging of patients IV.3 at 7 years and II.1 at 3 years (previously described family) revealed cerebellar hypoplasia, callosal dysmorphia, abnormal gyration of temporo-parietal regions and abnormal myelination of temporal poles (Fig. S1, supporting information online).

In situ hybridization in human embryos showed strong SLC25A22 expression in the brain (in the cortex, the hippocampi, the brain stem and the cerebellum), mainly in territories involved in
Table 1. Main clinical features of patients with SLC25A22 mutation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Seizure onset</th>
<th>EEG with SB</th>
<th>OFC</th>
<th>ERG</th>
<th>VEP</th>
<th>Brain MRI</th>
<th>Hypotonia</th>
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<tbody>
<tr>
<td>Family 1, Molinari et al. (14)</td>
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<tr>
<td>II.1</td>
<td>Birth</td>
<td>N/A</td>
<td>32.5 cm at birth (−2 SD)</td>
<td>Normal (2 years 6 months)</td>
<td>Weak (2 years 6 months)</td>
<td>Brain atrophy Callosal dysmorphia</td>
<td>+</td>
</tr>
<tr>
<td>II.2</td>
<td>Birth</td>
<td>N/A</td>
<td>32 cm at birth (−2 SD)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>+</td>
</tr>
<tr>
<td>II.4</td>
<td>Birth</td>
<td>+</td>
<td>33 cm at birth (−1 SD)</td>
<td>N/A</td>
<td>Altered (7 weeks)</td>
<td>N/A</td>
<td>+</td>
</tr>
<tr>
<td>II.7</td>
<td>Birth</td>
<td>+</td>
<td>45.5 cm at 3 years 9 months (−2 SD)</td>
<td>N/A</td>
<td>Altered (7 weeks)</td>
<td>N/A</td>
<td>+</td>
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<tr>
<td>Family 2, reported here</td>
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<tr>
<td>IV.3</td>
<td>Day 5</td>
<td>+</td>
<td>50 cm at 10 years (−2 SD)</td>
<td>Weak (7 months)</td>
<td>No signal (7 months)</td>
<td>Brain atrophy Callosal dysmorphia Abnormal myelination (temporal poles) Abnormal gyration (temporo-parietal regions)</td>
<td>+</td>
</tr>
</tbody>
</table>

EEG, electroencephalogram; SB, suppression burst; OFC, occipitofrontal circumference; ERG, electroretinogram; VEP, visual evoked potentials; MRI, magnetic resonance imaging; N/A, not available; SD, standard deviation; +/−, presence/absence.
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References


