Epileptic phenotypes related to the UNC79-UNC80-NALCN protein complex

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Abstract

The *NALCN* gene encodes a voltage-indipendent, non-selective, cation channel permeable to NA^+, K^+ and Ca^{2+} which forms a large channel complex with two other proteins, products of *UNC80* and *UNC79* genes.
The *UNC80* gene is essential for the stability and function of the *NALCN* sodium leak channel and for bridging *NALCN* to *UNC79* to form a functional complex.
This channel complex is mainly expressed in the central nervous system (CNS) and it plays a crucial role in regulating the resting membrane potentials and neuronal excitability.
Mutations in the *NALCN* gene have recently been linked to human diseases: two autosomal-recessive conditions (IHPRF1, *Infantile hypotonia with psychomotor retardation and characteristic facies;* INAD; *Infantile neuroaxonal dystrophy* with facial dysmorphisms and skeletal anomalies) and one autosomal-dominant condition (CLIFHADD; *Congenital contractures of the limbs and face, hypotonia and developmental delay).* Homozygous or compound heterozygous mutation in the *UNC80* gene are associated to the IHPRF2 syndrome *(Infantile hypotonia with psychomotor retardation and characteristic facies-2*). In addition to facial dysmorphism and psychomotor retardation, epilepsy is often associated with IHPRF1 and IHPRF2 Syndrome but the epileptic phenotype has never been accurately described.
"Recently two novel mutations in NALCN gene have been observed in two Sardinian siblings with IHPRF1 Syndrome and epilepsy. Here we describe the epileptic phenotype of these two Sardinian siblings and compare their findings with those previously reported in *NALCN* and *UNC80* recessive mutated patients.

KEY WORDS: UNC79-UNC80-NALCN protein complex, NALCN mutations, UNC80 mutations, IHPRF, epilepsy.

Introduction

The *NALCN*, *UNC80* and *UNC79* genes encode proteins that form a channel complex (1). UNC80 bridges NALCN to UNC79, and these 2 genes are involved in the folding, stabilization, cellular localization and activation of NALCN essential for channel function (2, 3). The *NALCN* gene encodes a voltage-indipendent, nonselective, cation channel permeable to NA^+, K^+ and Ca^{2+} and belongs to the 24-transmembrane domain ion channel superfamily. These channels have four homologous domains consisting of six transmembrane helices separated by three cytoplasmatic linkers. The homologous domains participate in the formation of the pores.
NALCN gene is expressed mainly in the CNS (dentate gyrus and pyramidal cell layer of the hyppocampus, granular and Purkinjie cell layers and dentate nucleus of cerebellum), but is also been found at a lower level in the heart, lymph nodes, pancreas, thyroid (4). This

channel plays a crucial role in regulation of resting membrane potentials and neuronal excitability.
Mutations in the *NALCN* gene have recently been linked to human diseases: IHPRF1 (*Infantile hypotonia with psychomotor retardation and characteristic facies*), an autosomal-recessive condition (3-7); CLIFHADD (*Congenital contractures of the limbs and face, hypotonia and developmental delay)*, an autosomal-dominant condition (5, 8); and INAD (*Infantile neuroaxonal dystrophy*) with facial dysmorphism and skeletal anomalies (9), an autosomal-recessive condition. IHPRF2 Syndrome has been associated to recessive mutations of *UNC80* gene (2, 10-12).
UNC79 so far has no corresponding human phenotype (2), although mouse knockout models for *NALCN* and *UNC79* both fail to nurse and die shortly after birth.
So far only 4 families with 11 cases have been reported in literature with *NALCN* recessive mutations (6, 7, 9) and 10 families with a total of 19 cases have been found with *UNC80* recessive mutations (2, 10-12).
Epilepsy was reported both in patients with *NALCN* and *UNC80* mutations.
Seizures, usually generalized, tended to be well controlled by AED (antiepileptic drugs).
Recently two novel bi-allelic truncating mutations in *NALCN* have been detected by Angius et al in two Sardinian siblings and they represent the fifth family reported worldwide with IHPRF1 syndrome (13).
We describe the epileptic phenotype of these two Sardinian siblings and compare them with *NALCN* and *UNC80* recessive mutated patients.

Case report

The two siblings are a 22 years old male and a 20-years-old female born from non consanguineous parents.
Clinical and epileptic phenotype of the two siblings are summarized in Table 1.
Male patient was born at the 34^ pregnancy week with a birth weight of 1790 g, while his sister was born at term with adequate birth weight.
They both show dysmorphic features: triangular face, bitemporal narrowing, high nasal bridge, downslanting palpebral fissures, posterior rotated low set-ears, large and persistently opened mouth, long fingers.
Since the first months of life hypotonia and developmental delay were evident. Now they can maintain sitting position without support but they are unable to walk, never developed speech, present a severe intellectual disability and a severe sleep disturbance. Their ophthalmic picture is characterized by convergent strabismus, lack of fixation, micropapilla, smaller retinal vessels and peripheral corioretinal sclerosis.
Both of them present severe hyperkinetic movement disorder and traits of autism spectrum disorder are evident in the male patient.
Because of feeding difficulties, during his first months of life, the male patient underwent PEG system implantation, lately removed because of swallowing improvement. The female patient was fed through nasogastric tube until age of 8 months. As for her brother, later she had an improvement, but they both showed poor growth and actually have a very low body weight. Severe chronic constipation is also present.
In the male patient, epilepsy onset was at the age of 4 years.
Seizures were generalized and polymorphic: first atypical absences and later myoclonic-atonic, tonic and tonic-clonic seizures.
EEG shows disorganized background activity, high amplitude multifocal and diffuse slow/sharp waves and spike-wave discharges intermingled with fast anterior activity (Fig. 1).
At present, despite politherapy (valproic acid, ethosuxymide, rufinamide and clonazepam) and EEG improvement, he experiences weekly tonic and tonic-clonic seizures, mainly during sleep.
The female patient experienced the first seizure at 6 years. Seizures were generalized and polymorphic: first atypical absences and later myoclonic-atonic.
At onset, EEG showed disorganized background activity both in awake and sleep state, high amplitude diffuse and multifocal slow/sharp waves, irregular spikes and spike-wave discharges (Fig. 2).
The last EEGs are characterized by irregular low voltage theta activity intermingled with diffuse high voltage theta activity.
Since the introduction of valproic acid and ethosuxymide, she is seizure free.
Their metabolic and genetic workups were normal. MRI showed diffuse atrophy and cerebellar hypoplasia.
Whole Exome Sequencing identified two novel bi-allelic truncating mutations in *NALCN* (unpublished data): c.3823C>T:p.R1275* (rs569371758) maternally derived and c.C2496insTCATA+:p.Y832fs paternally derived (Tab. 1).

Discussion

IHPRF syndrome is due to recessive mutations in the *NALCN* gene, but some cases with similar phenotype are associated with *UNC80* mutations. Until now, the syndrome associated with NALCN mutation was described only in four families worldwide (6, 7).
Neurological picture is quite similar in NALCN and UNC80 recessive mutated patients, while dysmorphic facies, autism and hyperkinetic movement disorders seem to be more frequent in UNC80 patients.
The two Sardinian siblings show clinical features more similar to the latter patients.
Among patients with recessive mutations, epilepsy is reported in 5/9 (6, 7). In the first Saudi Arabia family, seizures are not semeiologically described but it is reported a low frequency and good response to pharmacological therapy; in the second family from Israel, seizures were generalized tonic-clonic in both sisters

Table 1 - Clinical and epileptic phenotype of the two siblings.

	Patient 1	Patient 2
Age	22 y	20 y
Gender	M	F
Consanguineity	no	no
Gestational age	34^ week	42^ week
Birth weight	1.790 kg	3.250 kg
Neonatal hypotonia	yes	yes
Hypotonia	yes	yes
Feeding difficulties	yes	yes
Growth	cachetic	cachetic
Vision	convergent strabismus, lack of fixation, micropapilla, smaller retinic vessels and peripheral corioretinic sclerosis	convergent strabismus, lack of fixation, micropapilla, smaller retinic vessels and peripheral corioretinic sclerosis
Hearing	normal	normal
Dysmorphism	yes	yes
Motor delay	severe	severe
Cognitive delay	very severe	very severe
Abnormal respiratory rythm	no	no
Epilepsy	yes	yes
Seizure type	atypical absences myoclonic-atonic tonic tonic-clonic seizures	atypical absences myoclonic-atonic
Seizure onset	4 y	6 y
EEG	disorganized background activity high amplitude multifocal and diffuse slow/sharp waves spike-wave discharges fast anterior activity	disorganized background activity irregular low voltage theta activity diffuse high voltage theta activity
Movement disorder	yes	yes
Sleep disturbance	yes	yes
Hyperactivity	yes	yes
Autism	yes	no
Constipation	yes	yes
Karyotipe	46 XY	46 XX
Metabolic screen	normal	normal
Brain MRI	mild cerebellar hypoplasia	mild cerebellar hypoplasia
EMG and NC	normal	normal
NALCN mutations	c.3823C>T:p.R1275*(rs569371758) maternally derived c.2496insTCATA+:p.Y832fs (paternally derived)	c.3823C>T:p.R1275*(rs569371758) maternally derived c.2496insTCATA+:p.Y832fs (paternally derived)

with onset at about four years and good response to vigabatrin, but one of them later developed resistant status epilepticus and died for aspiration pneumonia and respiratory failure. Epilepsy is also reported in 2 patients with INAD and dysmorphic face associated with *NALCN* mutation (9) and in 9 patients with *UNC80* mutation (10-12).

In both groups, seizures are usually generalized (atypical absences, myoclonic-atonic, tonic and tonic-clonic) and only one patient experienced neonatal

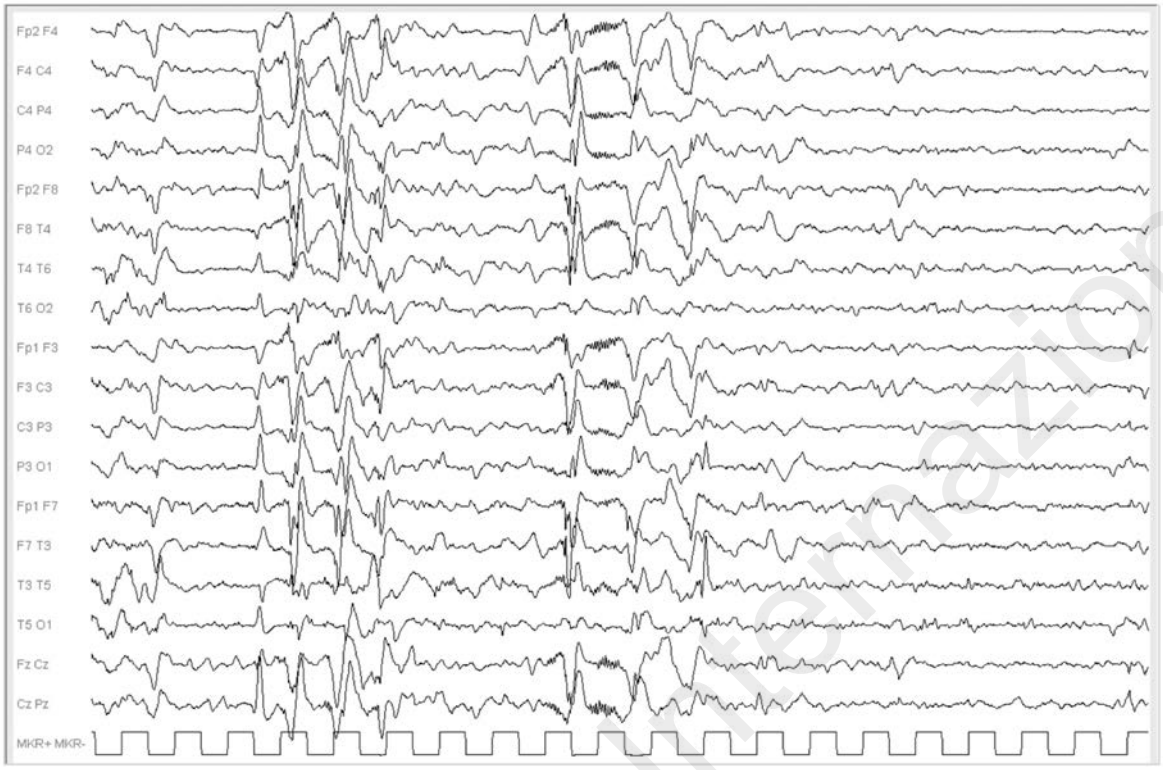

Figure 1 - Sleep EEG patient 1.

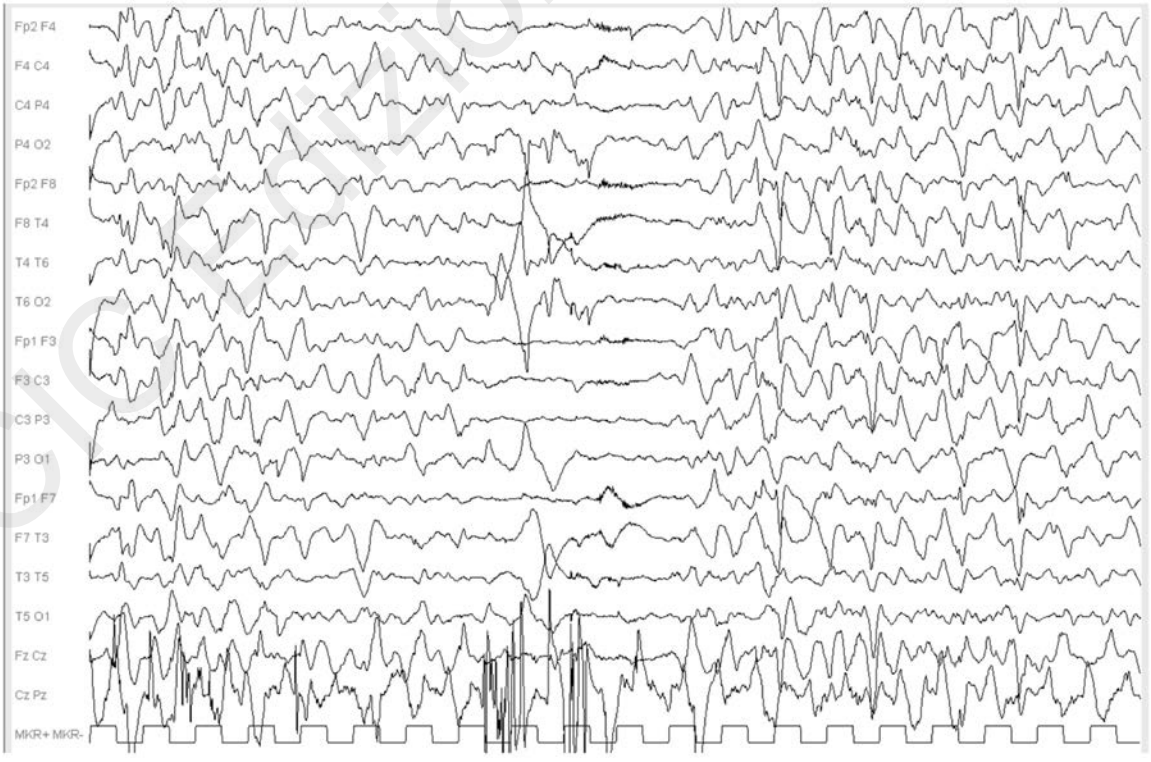

Figure 2 - Sleep EEG Patient 2.

focal seizures. Response to therapy is usually good.
EEG characteristics of patients with IHPRF Syndrome have never been accurately described. An intermittent activity with slow background, without epileptic activity was reported for some NALCN mutated patients (6).
Most *UNC80* patients show disorganized background activity, diffuse slow/sharp waves usually without epileptiform activity, but four of them present epileptic activity (spike-wave) and rarely multifocal anomalies (10-12).
The two Sardinian siblings experienced generalized polymorphic seizures and their age onset of epilepsy is similar to that reported for *NALCN* patients (6, 7), while the *UNC80* patients usually have a earlier onset during the first months of life (11, 12).
Their EEG is disorganized and bi-frontal and diffuse high amplitude sharp/slow-waves can be seen.
Particularly, EEG of the male patient has a high frequency of epileptic discharges and, despite a recent moderate improvement, his electric activity is much more impaired than his sister.
AED response has been good in the female patient with seizure freedom, while the male patient still has weekly seizures.
Epileptologic characteristics of these two Sardinian patients seem to be much more similar to that of *UNC80* mutated patients because of seizure semiology and EEG pattern.
Based on the potential implication of the UNC79-UNC80-NALCN channel complex in human physiology and diseases, *NALCN* could represent an attractive candidate gene to be tested in individuals with infantile encephalopathy with epilepsy but further investigations are needed to clarify its role.

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