Pharmacoresistant epilepsy associated with mutations in the KCNB1 and RELN genes. A case report

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SUMMARY
Introduction. Epilepsy is one of the most common neurological disorders worldwide. In most cases, epilepsy can be well managed. However, there is a number of patients who do not respond well enough to common medical treatments; a situation known as pharmacoresistant epilepsy. It can be caused by mechanisms that may involve environmental and genetic factors, as well as disease or drug related factors.

Case presentation. Herein we present a case report of a six-year-old girl who has been diagnosed with pharmacoresistant epilepsy, characterized by generalized and focal seizures while she was on two antiepileptic drugs. Molecular testing, with Next Generation Sequencing (NGS) technique, revealed mutations at KCNB1 and RELN genes.

Key words: pharmacoresistant epilepsy • KCNB1 gene • RELN gene • delayed speech

INTRODUCTION
Epilepsy is one of the most common neurological diseases worldwide and is considered a major public health problem, with socioeconomic problems (Camfield and Camfield, 2015; López González et al., 2015). Generally, this disease can be successfully treated in most cases. However, there is a high percent of patients that does not respond favorably to common antiepileptic drugs (AED) despite the continued development of new AED the last two decades, resulting in pharmacoresistance (Kwan and Brodie, 2000). It is considered that via biochemical and biological sciences new AED can be developed and perhaps with different mechanisms of action compared to the AED currently on the market.

Pharmacoresistant epilepsy is defined as an epilepsy that does not respond to at least two AED, used either in monotherapy or in combination therapy (bi- or polytherapy) and failed to control seizures for a sufficient period of time (Kwan et al., 2010). However, in 2011, the International League Against Epilepsy (ILAE) modified the definition of drug-resistant epilepsy to the presence of seizures for a period of 6 months, even under suitable treatment (either monotherapy or combination therapy) (Kwan et al., 2010).

Various prognostic factors, associated with the development of drug resistance, have been investigated in various studies. Special abnormalities at electroencephalogram (EEG) such as asymmetric generalized spike-wave discharges (Szaflarski et al., 2010) or gen-
eralized polyspikes during sleep (Sun et al., 2018), sta-
tus epilepticus, febrile seizures, neurological disorders 
and mental retardation were identified as factors asso-
ociated with worse outcomes (Kalilani et al., 2018). Oth-
er factors which have been analyzed in multivariate 
studies, and are associated with poor prognosis, are the 
pre-treatment seizure frequency, the recreational drug 
use and the family history of epilepsy in 1st degree rel-
atives (Téllez-Zenteno et al., 2014; Fiest et al., 2017), as 
well as the failure of previous AED and the duration of 
epilepsy (Luciano et al., 2007). Moreover, another pro-
spective incident study of newly diagnosed drug-resis-
ant patients found that the largest percentage of patients 
with bad prognosis was those with temporal onset com-
pared to occipital or unclear onset (Choi et al., 2016). 

Regarding the biological basis of AED pharmacore-
sistance, the data is poorly understood. Epilepsy is a het-
erogeneous condition with multiple etiologies, and it is 
considered that the pathogenesis of AED pharmacore-
sistance is multifactorial and may involve both genet-
ic and environmental factors (Cárdenas-Rodríguez et 
al., 2020).

**CASE PRESENTATION**

We report a case of a six-year-old female who was re-
ferred to our Neurology Department at the age of three 
because of frequent episodes of altered level of con-
osis. The patient’s perinatal history was normal; she was term 
and her delivery was vaginal and an APGAR score of 
9–10. She had a birth weight of 2840 gram and a head 
circumference of 36.1 cm. However, from the pa-
tient’s personal history it is described that at the age of 
9 months she had further developed complex febrile 
seizures, while at the age of three-years-old she de-
veloped the first afebrile seizures, with disturbance of 
the level of consciousness and lip-smacking/chewing 
movements, without the episode developing into gen-
eralized tonic-clonic seizures, lasting up to 5 minutes. 
From her family history, her father described episodes 
of convulsions in infancy which were self-resolved with-
out any treatment requirement. All her family mem-
bers are healthy.

On the first physical examination, neither patholog-
ic findings nor dysmorphic facial features were no-
ticed. EEG was compatible with focal epilepsy. Espe-
cially, the main characteristic of EEG is the recording 
of sharp waves, spikes and spike-and-wave complexes at the centrotemporal regions (Figure 1). Polyspikes 
have been recorded to a lesser extent. Brain magnetic 
resonance imaging (MRI) did not reveal any patholog-
ical findings. Therefore, she was commenced on levetiracetam. Moreover, in the future, on follow up, significant speech delay was also reported, which required speech and language therapy sessions. A developmental assessment was also recommended to parents and performed which revealed a mild developmental disorder.

For the next two and a half years, she continued to present with similar episodes of seizures, which partially treated AED dosage adjustments. Thus, levetiracetam was discontinued and oxcarbazepine with sodium valproate were added to her treatment plan, without any improvement. At that time, different type of seizures, with tonic-clonic movements, occurred.

Due to the combination of speech delay and pharmacoresistant epilepsy, Next Generation Sequencing (NGS) technique with DNA isolation from the patient was performed. Oligonucleotide-based target capture analysis and nucleotide sequencing were undertaken using the Blueprint Genetics (BpG) Beyond Pediatric Epilepsy Panel Plus and the NGS (Illumina NextSeq), respectively, examining 283 disease-causing genes. The Burrows-Wheeler Aligner (BWA-MEM) software was used for the data analysis [reference genome UCSC hg19 and reference database Human Gene Mutation Database (HGMD v.2017.1)]. Two nucleotide changes were detected. The first one c.1234A> G, p. (Ile412Val) in the KCNB1 gene, in heterozygous state (NM_004975.3), (Figure 2) and the second one, c.6770C> G, p. (Ser2257Trp) in the RELN gene, in heterozygous state (NM_005045.3) (Figure 3).

Nucleotide change c.1234A> G to KCNB1 gene leads to replacement of the amino acid isoleucine by valine at 412 position of amino acid chain and the c.6770C> G change in RELN gene causes the replacement of the amino acid serine by tryptophan at 2257 position of the amino acid chain. These result in disruption of the protein’s structure and function.

We believe that the above genetic changes have not been previously reported in the scientific literature or in the ClinVar and HGMD databases.

However, it should be noted that it remains unknown whether these mutations have been inherited from the parent or were de novo, because the parents did not agree to undergo genetic testing themselves.

At her most recent re-evaluation (at the age of 6 years old), she was seen more sociable and smiley. However, deficits in speech perception still persist, while she is still under speech and language therapy. The likelihood of borderline mental retardation is increased but no mental quotient measurement has been performed yet. Presently, she is on two AED (sodium valproate and lacosamide) and is free of seizures.

**DISCUSSION**

This case report highlights the difficulties that neurologists often face when managing patients with the drug-resistant epilepsy. The developing genomic technologies have dramatically increased the understanding of the genetic background in epilepsy (Myers, Mefford, 2015). However, underlying mechanisms about pharmacological resistance, have not yet been fully exemplified (Depondt, 2006).

The first mutation in our patient’s DNA is at the KCNB1 gene which is located on the chromosomal region 20q13.13. This encodes the KV2.1-ion channel which contributes to the exit of potassium (K) from
the cell and plays an important role in the cell’s ability to generate and transmit electrical signals. Because KCNB1 is a voltage gated potassium channel, its function is based on the charge around it. A change (variant/mutation) at the one copy of the KCNB1 gene stops it from working properly (Torkamani et al., 2014; de Kovel et al., 2017).

Patients have developmental delay starting in infancy or early childhood, often with prominent language impairment. Most children develop multiple types of seizures that can be frequent and hard to control with standard treatments. However, a few patients do not have seizures but may still have abnormal patterns on their EEG. Some other children may have features of autism or Rett syndrome or have a diagnosis of Lennox-Gastaut syndrome. Generally, patients with KCNB1 gene mutations may be classified as having a developmental and epileptic encephalopathy since features of developmental delay and epilepsy are the most common (Marini et al., 2017).

In the literature, KCNB1 mutations are commonly associated with diffuse brain dysfunction combining seizures, motor, and cognitive impairment (Marini et al., 2017; Kang et al., 2019).

The second mutation is at the RELN gene which encodes the protein reelin. It is located on human chromosome 7q22 and is considered as a potential gene for childhood epilepsy (Dutta et al., 2011). Reelin is a protein that plays a crucial role in regulating the processes of neuronal migration and their installation in the developing brain along with the adult brain (Bosch et al., 2016).

Unfortunately, there are not many studies linking epilepsy with mutations in the RELN gene. However, homozygous RELN mutations have been reported in three families, causing lissencephaly and cerebellar hypoplasia, which led to epilepsy and severe cognitive defect. Moreover, in other studies, heterozygous mutations of RELN gene are associated with the onset of autosomal dominant lateral temporal lobe epilepsy (Michelucci et al., 2017). Thus, as in other genetic epilepsy syndromes, RELN mutations might result in different clinical phenotypes, depending on the mode of inheritance. Heterozygous mutations might cause autosomal dominant lateral temporal epilepsy (ADLTE) and homozygous mutations might result in more severe disorder, as lissencephaly with cerebellar hypoplasia (Hong et al., 2000).

In our case, the patient certainly has the features described above such as speech delay, mild mental retardation and seizures. However, we cannot be certain if both described mutations are pathogenic or not because, as highlighted above, there are no genetic examinations of the parents. Thus, at this stage, the genetic epidemiology of our patient is only a suspicion.

CONCLUSION

In this case report we describe a six-year-old girl with speech delay and drug resistant epilepsy, with mutations in two different genes associated with epilepsy. New genetic techniques are very useful in clinical practice and may help clinicians to explain the clinical presentation of drug resistant epilepsy. In addition, this will probably help us understand the impact of pharmacological treatment on epilepsy. That is why cooperation with clinical geneticists is important.
CONSENT
The patient’s parents gave written, informed consent for publication of this case report and the accompanying Figures.

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CONFLICT OF INTEREST
Non declared.

REFERENCES