Original article

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Autosomal dominant lateral temporal lobe epilepsy associated with a novel reelin mutation

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ABSTRACT – *Aims*. Reelin mutations are responsible for a minority of families with autosomal dominant lateral temporal lobe epilepsy. Here, we report a novel nuclear family with distinct clinical and neuroradiological findings.
Methods. We studied the proband and her mother by means of EEG, video-EEG, 3T MRI, FDG-PET and genetic testing.
Results. Both patients had a focal drug-resistant epilepsy with onset at the age of 16 and focal seizures with typical auditory features combined with fear, followed by loss of contact or evolving to bilateral tonic-clonic seizures. The proband's ictal EEG showed clear left temporal seizure onset, and cerebral MRI revealed subtle left temporal changes (mild hypotrophy, slight blurring of the white and grey matter and hyperintensity) with corresponding left temporal mesial focal hypometabolism on FDG-PET. Genetic testing identified a missense variant, c.6631C>T (p.Arg2211Cys), in reelin repeat #5 in both patients, which markedly affected the secretion of the protein.
Conclusion. The data from this family support previous findings indicating that reelin mutations are a cause of autosomal dominant lateral temporal lobe epilepsy which has a clinical spectrum that may also encompass drug-resistant epilepsy associated with mild MRI temporal changes.

Key words: autosomal dominant lateral temporal lobe epilepsy, autosomal dominant epilepsy with auditory features, reelin, lateral temporal lobe seizures

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Autosomal dominant lateral temporal epilepsy (ADLTE), otherwise known as autosomal dominant epilepsy with auditory features (ADEAF), is a rare genetic condition characterized by onset, in adolescence or early adulthood, of focal seizures that are believed to originate from the lateral temporal lobe, based on prominent auditory auras, complex visual auras or aphasic seizures (Ottman *et al*., 1995; Michelucci *et al*., 2003). The patients show a normal neurological status, negative conventional magnetic resonance imaging (MRI), good response to antiepileptic treatment, and overall benign outcome, and belong to families in which two or more members show a similar phenotype. The inheritance pattern is compatible with autosomal dominance with reduced penetrance. The most common gene associated with ADLTE is *LGI1*, of which mutations account for around 30% of ADLTE families and 2% of sporadic cases (Michelucci *et al*., 2003; Michelucci *et al*., 2013). More recently, two further genes, *RELN* (Reelin) and *MICAL-1*, have been implicated in the aetiology of ADLTE and together may account for an additional 30% of typical pedigrees. Moreover, *RELN* and *MICAL-1* mutations have been detected, respectively, in seven and three Italian ADLTE families with a phenotype indistinguishable from *LGI1*-related pedigrees (Dazzo *et al*., 2015; Dazzo *et al*., 2018). In these cases, conventional MRI also showed normal findings (Michelucci *et al*., 2017).

Here, we report a small ADLTE family linked to a novel *RELN* mutation, characterized by pharmacoresistant focal epilepsy, with video-EEG documentation of seizures and neuroradiological data.

Case study

The family pedigree is reported in *figure 1A*.

Case 1

The proband was a 40-year-old female who had her first tonic-clonic seizure, preceded by the experience of a sudden noise, inability to speak and fear, at the age of 16. In the following months, she also had elementary auditory hallucinations associated with fear, without loss of consciousness. After a six-year seizure-free period on carbamazepine, the seizures recurred. At the age of 35, the attacks were characterized by complex auditory hallucinations ("..a phrase, always the same, I can't remember..") associated with aphasia and fear and sometimes loss of contact. The seizures increased over time and were refractory to multiple AEDs, including lamotrigine, levetiracetam, topiramate, zonisamide and perampanel.

The patient was admitted to our hospital to perform long-term video-EEG monitoring and MRI. Neurological examination and neuropsychological assessment were normal. Sleep interictal EEGs showed focal slowing and spikes over the left temporal region. Video-EEG monitoring captured a number of typical seizures. The shortest events (lasting up to 2-4 seconds), with simple perception of a noise or fear, were not associated with overt EEG changes, whilst longer seizures (up to 20-30 seconds), with loss of contact, were associated with rhythmic focal discharges in the left temporal region (*figure 2A*). MRI showed mild left temporal hypotrophy, slight blurring of white and grey matter in the left temporal lobe and hyperintensity of the left hippocampus (*figure 2B*). FDG-PET showed focal hypometabolism in the left temporal lobe, mostly in the polar and mesial regions (*figure 2C*).

Case 2

The mother was a 69-year-old female with onset, at age 16, of tonic-clonic seizures preceded by elementary auditory auras (humming sensation). The epilepsy was partially controlled by therapy and the patient continued to report, over time, elementary hallucinations on a monthly basis. The interictal EEG showed mild left temporal slow activity along with rare spikes. The MRI only disclosed mild left temporal hypotrophy (*see supplementary material*).

Molecular genetic analysis

High-throughput DNA sequencing of the proband, using a custom multi-gene panel containing all the genes associated with familial focal epilepsies (*LGI1, RELN, DEPDC5*), led to identification of a missense variant, c.6631C>T (p.Arg2211Cys), in reelin repeat # 5. The variant, validated by Sanger sequencing, was also detected in the proband's mother (*figure 1B*). This was found only once in the gnomAD population database (frequency: 0.000004) and is predicted to be pathogenic/damaging based on the most commonly utilized computational prediction tools, such as CADD, Polyphen-2, SIFT, Mutation Taster and Grantham matrix score. The residue, Arg2211, is highly conserved across species (PhyloP score: 5.881).

Cell transfection assay

To ascertain the functional consequences of the p.Arg2211Cys variant, we transfected mutant and wild-type *RELN* expression constructs into embryonic kidney 293T (HEK293T) cells, which do not express endogenous reelin, and analysed both the cell lysates and concentrated (about 20x) serum-free media by western blot using anti-reelin antibodies. The three

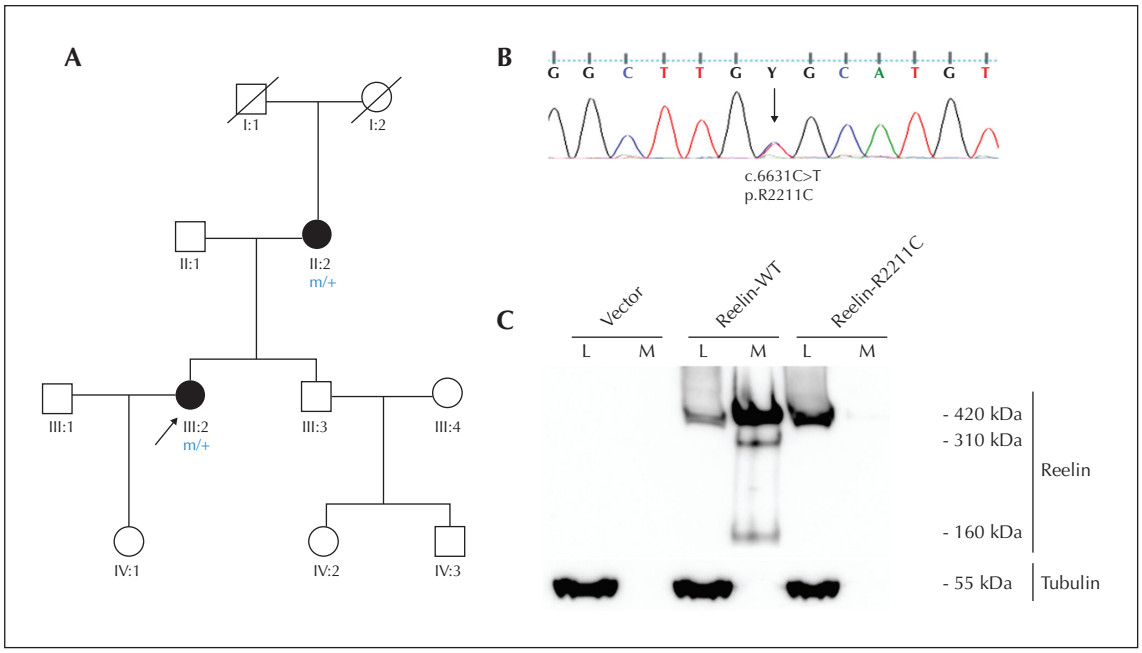

Figure 1. Family pedigree and mutation characterization. (A) Family pedigree segregating with the *RELN* mutation. Filled symbols: affected family members; open symbols: healthy family members; circle: female; square: male. Individuals carrying the mutation are indicated by m/+. (B) chromatograms of mutated family members. The mutated nucleotide is indicated by an arrow. (C) Secretion test of mutant reelin. Cell lysates (L) and concentrated media (M) of human embryonic kidney 293 T cells transfected with *RELN* wild-type or mutant expression constructs were analysed by western blot using anti-reelin antibodies. Anti-tubulin antibodies were used as loading control. The mutant protein is hardly seen in the culture medium, indicating that its secretion is almost completely suppressed.

wild-type reelin isoforms, of 420, 360, and 180 kDa, were detected in the medium of transfected cells, whereas only the full-length protein was retained in the cell lysate, as expected. The mutant protein, instead, was detected in the cell lysate but was almost completely absent in the medium (*figure 1C*), clearly indicating that the mutant reelin was barely secreted by transfected cells.

Discussion

The nuclear family described in this paper showed a typical clinical ADLTE phenotype, with onset in adolescence (at the same age in both the proband and her mother) of seizures with prominent auditory hallucinations, fear and tonic-clonic seizures. Some of these seizures were recorded on video-EEG showing a clear left temporal onset.

Despite auditory hallucinations are the usual aura type in ADLTE, psychic symptoms (such as fear) have been occasionally reported in association with auditory features (Michelucci *et al*., 2013) and exceptionally as the only symptom in one *LGI1*-mutated pedigree (Striano *et al*., 2011), suggesting that mutations in ADLTE-related genes may encompass a wider range of auras of temporal origin.

Video-EEG recording of focal seizures in ADLTE have been rarely described, mostly because of the good seizure control in most cases. Brodtkorb *et al*. (2005) provided EEG documentation of a left temporal speech-induced aphasic seizure in a patient belonging to a large family with *LGI1* mutation. Di Bonaventura *et al*. (2009) reported a *LGI1*-mutated large pedigree in which the proband had frequent episodes of aphasic focal status originating from the left posterior lateral temporal region, as demonstrated by ictal EEG-fMRI.

At variance with the typical pedigrees, in our family, both cases (but particularly the proband) had a refractory epilepsy requiring a complex therapy. Pharmacoresistance is described in a minority of ADLTE families and does not exclude the diagnosis (Di Bonaventura *et al*., 2009).

The brain MRI was also peculiar in the proband who showed subtle left temporal changes (mild hypotrophy, slight blurring of the white and grey matter and hyperintensity), suggesting an underlying focal dysplasia. In agreement with these findings, FDG-PET disclosed a left mesial and polar temporal hypometabolism. Structural conventional MRI are usually normal in ADLTE. Kobayashi *et al*. (2003) described a large Brazilian family associated with *LGI1* mutations in which various family members had MRI focal temporal dysplastic features, but these abnormalities did not fully segregate with the mutation. Tessa *et al*. (2007), using non-conventional MRI techniques (voxel-based analysis of diffusion tensor MR images), in eight

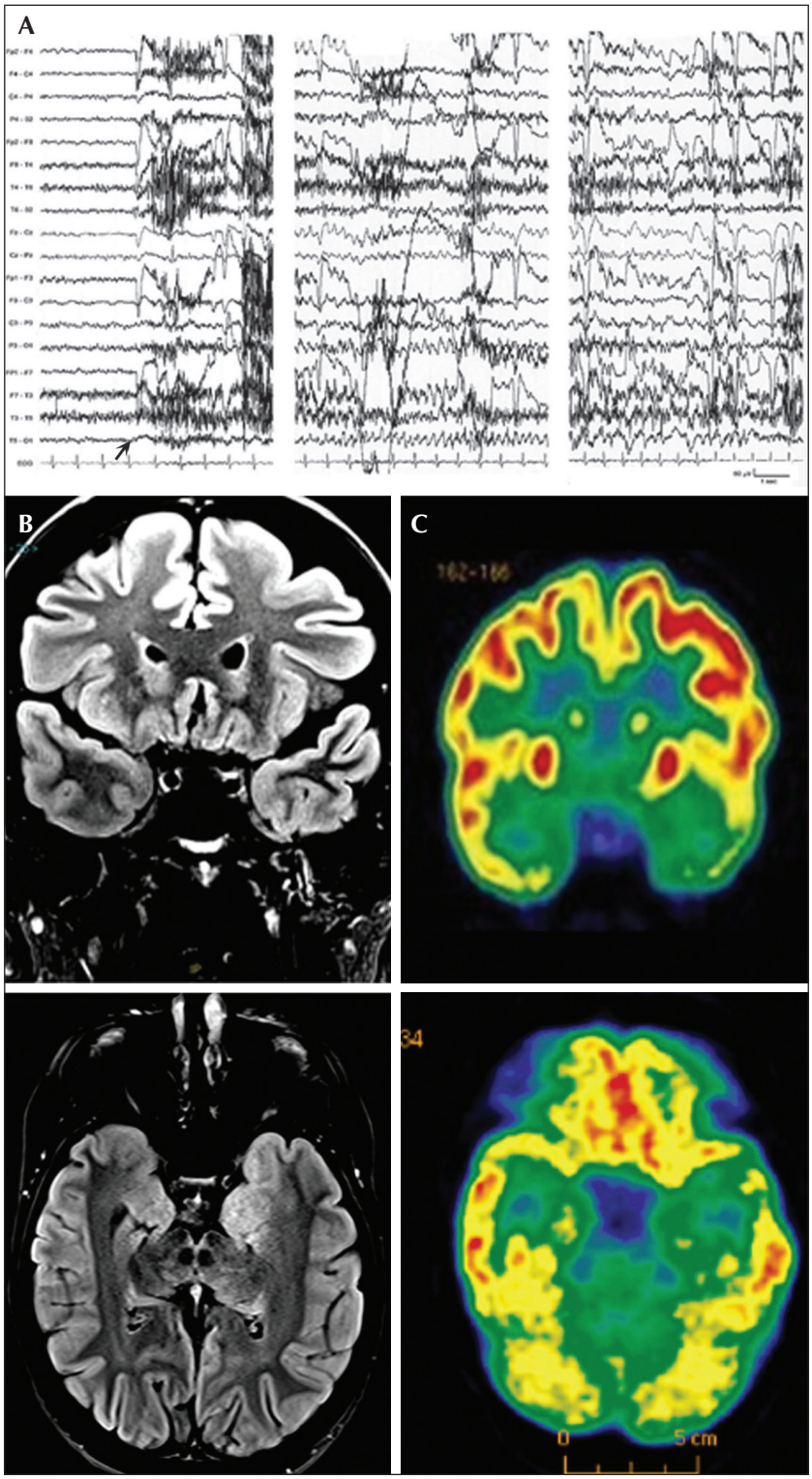

Figure 2. Case 1. (A) EEG recording of a typical seizure clinically characterized by sudden auditory aura and fear followed by loss of contact. The EEG shows a 4-Hz rhythmic theta activity involving the left temporal regions with posterior predominance. The seizure onset is indicated by the arrow. (B) 3T MRI (T2 TSE DARK FLUID sequence) showing mild left temporal hypotrophy, slight blurring of the white and grey matter in the left temporal lobe and hyperintensity of the left hippocampus on coronal (upper panel) and axial (lower panel) planes. (C) FDG-PET showing left temporal (polar and mesial) hypometabolism on coronal (upper panel) and axial (lower panel) planes.

ADLTE-mutated patients with a left temporal EEG focus, demonstrated a cluster of fractional anisotropy in the left lateral temporal cortex, suggesting a malformative origin of the abnormality. Ottman *et al*. (2008) performed fMRI with an auditory description decision task and MEG in 17 *LGI1*-mutated patients and found functional impairment in language processing. Finally, Ceska *et al*.(2019) reported a morphometric MRI analysis in a *RELN*-mutated patient with typical clinical findings and failed to disclose focal structural changes, however the functional connectivity analysis revealed higher local synchrony in the left temporal, frontal, and parietal regions as well as cingulated region, as compared to healthy controls.

Our study suggests that high-resolution structural MRI may disclose focal temporal abnormalities in ADLTE, possibly related to the underlying genetic aetiology in this family. In our study, both patients showed a novel *RELN* mutation, p.Arg2011Cys, causing severe reduction of protein secretion, which strongly supports its pathogenic nature together with the extremely low frequency in the general population and consistent computational predictions of a damaging effect on protein structure/function. Reelin is a secreted brain protein that, during embryogenesis, controls neuronal migration by specifically directing the radial migration and formation of cellular layers by excitatory cortical neurons. Homozygous mutations that abolish reelin expression cause lissencephaly with cerebellar hypoplasia, a condition characterized by severe neuronal dysplasia in several brain regions including the neocortex and hippocampus (Hong *et al*., 2000). Moreover, reelin is reported to be involved in human heterotopic nodular formation based on surgical specimens (Rossini *et al*., 2012) and focal cortical dysplasia by interacting with the m-TOR cascade in animal models (Baek *et al*., 2015). These data support the view that a severe defect of secretion of the mutant reelin protein in the proband may have at least contributed to the development of subtle MRI changes as well as epilepsy.

In conclusion, the data from our family support previous findings indicating that reelin mutations are a cause of ADLTE, the clinical spectrum of which may also encompass drug-resistant epilepsy associated with mild MRI temporal changes. □

Supplementary data.

Summary didactic slides and supplementary material are available on the www.epilepticdisorders.com website.

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TEST YOURSELF

(1) Which is the most frequent seizure type in autosomal dominant lateral temporal lobe epilepsy?

(2) Variants of which genes are associated with autosomal dominant lateral temporal lobe epilepsy?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".