ORIGINAL ARTICLE

Early infantile epileptic encephalopathy associated with a high voltage gated calcium channelopathy

Simon Edvardson,[1] Shimrit Oz,[2] Fida Aziz Abulhijaa,[3] Flora Barghouthi Taher,[4] Avraham Shaag,[1] Shamir Zenvirt,[1] Nathan Dascal,[2] Orly Elpeleg[1]

For numbered affiliations see end of article

Correspondence to
Professor Orly Elpeleg, The Monique and Jacques Roboh Department of Genetic Research, Hadassah, Hebrew University Medical Center, Jerusalem 91120, Israel; Elpeleg@hadassah.org.il and Professor Nathan Dascal, The Department of Physiology and Pharmacology, Sackler School of Medicine Tel Aviv University Ramat Aviv, Tel Aviv 69978, Israel; dascaln@post.tau.ac.il

SE and SO contributed equally

Received 7 August 2012
Revised 29 November 2012
Accepted 29 November 2012

ABSTRACT

Background Early infantile epileptic encephalopathies usually manifest as severely impaired cognitive and motor development and often result in a devastating permanent global developmental delay and intellectual disability. A large set of genes has been implicated in the aetiology of this heterogeneous group of disorders. Among these, the ion channelopathies play a prominent role. In this study, we investigated the genetic cause of infantile epilepsy in three affected siblings.

Methods and results Homozygosity mapping in DNA samples followed by exome analysis in one of the patients resulted in the identification of a homozygous mutation, p.L1040P, in the *CACNA2D2* gene. This gene encodes the auxiliary $\alpha_2\delta2$ subunit of high voltage gated calcium channels. The expression of the $\alpha_2\delta2$-L1040P mutant instead of $\alpha_2\delta2$ wild-type (WT) in *Xenopus laevis* oocytes was associated with a notable reduction of current density of both N ($Ca_V2.2$) and L ($Ca_V1.2$) type calcium channels. Western blot and confocal imaging analyses showed that the $\alpha_2\delta2$-L1040P mutant was synthesised normally in oocyte but only the $\alpha_2\delta2$-WT, and not the $\alpha_2\delta2$-L1040P mutant, increased the expression of α_{1B}, the pore forming subunit of $Ca_V2.2$, at the plasma membrane. The expression of $\alpha_2\delta2$-WT with $Ca_V2.2$ increased the surface expression of α_{1B} 2.5–3 fold and accelerated current inactivation, whereas $\alpha_2\delta2$-L1040P did not produce any of these effects.

Conclusions L1040P mutation in the *CACNA2D2* gene is associated with dysfunction of $\alpha_2\delta2$, resulting in reduced current density and slow inactivation in neuronal calcium channels. The prolonged calcium entry during depolarisation and changes in surface density of calcium channels caused by deficient $\alpha_2\delta2$ could underlie the epileptic phenotype. This is the first report of an encephalopathy caused by mutation in the auxiliary $\alpha_2\delta$ subunit of high voltage gated calcium channels in humans, illustrating the importance of this subunit in normal physiology of the human brain.

INTRODUCTION

Early infantile epileptic encephalopathies (EIEE) manifest in the neonatal or the early infantile period as severely impaired cognitive and motor development due to recurrent clinical seizures or prominent interictal epileptiform discharges. Because of the susceptibility of the developing brain to epilepsy, the result is often a devastating permanent global developmental delay and intellectual disability. With the introduction of genomic technologies, the importance of a large set of genes in the emergence of EIEE has been unravelled, including those involved with synaptogenesis, pruning, neuronal migration and differentiation, neurotransmitter synthesis and release, and the structure and function of membrane receptors and transporters (for review see Tavyev Asher and Scaglia[1]). Among these, the ion channelopathies play a prominent role, especially the voltage gated sodium channel SCN1A, with its documented 856 disease-causing mutations (HGMD 2012.2, release date 29 June 2012), which were associated with several EIEE including migrating partial seizures of infancy, severe infantile multifocal epilepsy, and Dravet syndrome.[2 3] We now report a novel calcium channelopathy identified in three infants with EIEE by a molecular and functional study.

SUBJECTS AND METHODS

Patients

The subjects were three sibs, two males and one female (3110, 3111, and 10177 in figure 1A) who suffered from epilepsy and global developmental delay. They were the offspring of healthy Arab-Palestinian first degree cousins. An older daughter (3109) was healthy. The pregnancy, delivery, and perinatal course of the three patients were uneventful. Nonetheless at birth there was generalised muscle hypotonia. Seizures were first noted at 20–60 days of age, initially consisting of eye-rolling and facial twitching, but these were later replaced by several types of seizures including atonic, clonic and tonic attacks without focality. The seizures were resistant to multiple medications including vigabatrin, clonazepam, topiramate, clobazam, phenobarbital, and valproic acid. The earliest electroencephalogram (EEG) available for review was obtained at 10 months of age and was consistent with Lennox–Gastaut syndrome. At 7 years of age, the EEG had evolved into a slow background rhythm with multifocal spikes and slow waves (figure 2).

Physical examination of the patients (the oldest was 7 years of age) revealed well nourished children, with no dysmorphic features and normal head circumference. Axial hypotonia was noted while the appendicular tone was normal. Brisk, symmetric reflexes were obtained without other pyramidal signs. Choreiform movements were noted intermittently. The children made no eye contact but the oldest appeared to track and react to noise; none of them could speak or use their hands purposefully. Repeated examinations over 3 years of follow-up revealed no major changes and the patients

To cite: Edvardson S, Oz S, Abulhijaa FA, *et al*. *J Med Genet* 2013;**50**:118–123.

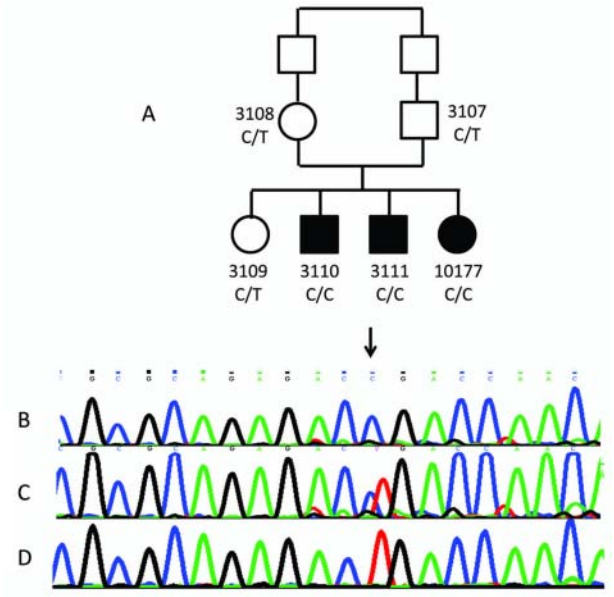

Figure 1 (A) Family pedigree. The patients are represented by filled symbols and the genotype at the mutation site is shown. The DNA sequence flanking the L1040P mutation in the *CACNA2D2* gene is shown in the DNA samples of a patient (B), a carrier (C), and a healthy control (D).

remained hypotonic, lacked psychomotor development, and suffered from pharmaco-resistant epilepsy. Growth parameters and general health remained satisfactory and there were no clinical or biochemical indications of the involvement of other systems. A thorough investigation included analysis of plasma and cerebrospinal fluid amino acids, urine organic acids, plasma very long chain fatty acids, isoelectric focusing of transferrins, serum biotinidase and carnitine values, muscle pathology, and mitochondrial respiratory chain enzymes, which were all normal. Chromosomal rearrangements were excluded by Affymetrix Genome-Wide Human Single Nucleotide Polymorphism (SNP) Array 6.0 in patient 3111 DNA. Brain MRI of patients 3111 and 3110 performed at age 4 years and 7 years, respectively, showed paucity of white matter and cerebellar atrophy (figure 2).

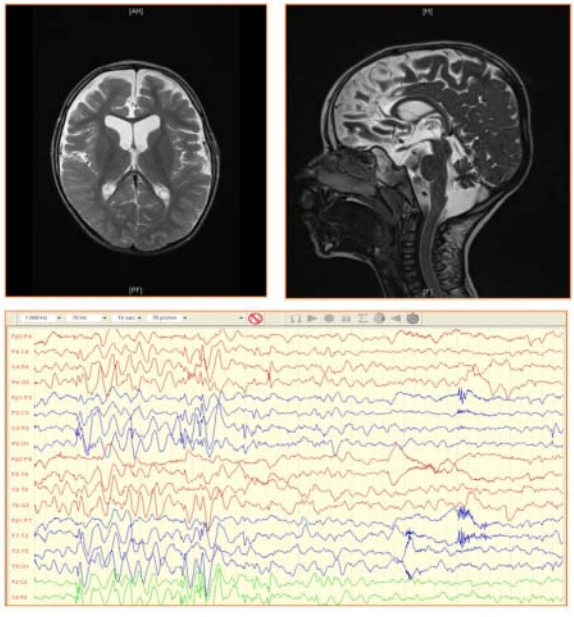

Figure 2 Patient 3111, aged 7 years. Upper panel: Axial and mid-sagittal T2 MRI showing cerebellar (vermian) atrophy and paucity of white matter. Lower panel: Awake electroencephalogram; bipolar montage demonstrating slow background activity and multifocal spikes.

Methods

Homozygosity mapping

A search for common homozygous regions in the DNA of the affected sibs and their unaffected sister was performed using the Affymetrix GeneChip Human Mapping 250K SNP Array, as previously described.[4]

Whole exome sequencing

Whole exome sequencing was performed in DNA from patient 3111 using the SureSelect Human All Exon V.2 Kit (Agilent Technologies, Santa Clara, California, USA) on HiSeq2000 (Illumina, San Diego, California, USA) as 100 bp paired-end runs. Image analysis and base calling were performed with the Genome Analyser Pipeline version 1.5 with default parameters. The reads were aligned with DNAnexus software (Palo Alto, California, USA) using the default parameters with the human genome assembly hg19 (GRCh37) as reference, as previously described.[5]

Electrophysiology

Maintenance of *Xenopus laevis* female frogs, preparation of oocytes, in vitro RNA synthesis, and measurement of currents using two-electrode voltage clamp were carried out as described.[6] Specifically, oocytes were injected 3–4 days before measurement with RNA of α_{1B} (d14157) or α_{1C} (X15539) together with β_3 (NM_012828) or β_{2b} (X64297), respectively, along with RNA of $\alpha_2\delta2$ (NM_006030)-WT or of $\alpha_2\delta2$-L1040P with the single nucleotide mutation t3119c produced by PCR (Roche). Whole cell currents were recorded in *Xenopus* oocytes using the two-electrode voltage clamp technique, as described.[6] A quantity of 30 nl of 50 mM BAPTA (Ca^{2+} chelator, to avoid endogenous Ca^{2+} dependent Cl^- currents) was routinely injected into the oocytes 0.5–2 h before the measurement of currents. The extracellular solution concentrations were adjusted in each experiment as indicated, to achieve currents ranging from 300–5000 nA using 40, 10 or 2 mM $Ba(OH)_2$ or $Ca(OH)_2$. The extracellular solution contained 50–90 mM NaOH, 2 mM KOH, and 5 mM HEPES, titrated to pH 7.5 with methanesulfonic acid (Sigma). In each oocyte, the net current was obtained by subtraction of the residual currents recorded with the same protocols after applying 200 µm $CdCl_2$ to the same solution. Recording was performed using a GeneClamp 500 amplifier (Molecular Devices). Stimulation, data acquisition and analysis were performed using pCLAMP 10.2 software (Molecular Devices). r_{400} was calculated as the fraction of current remaining after 400 ms depolarising pulse normalised to the peak current.

Western blot

Three to four days after injection of RNA, 10–20 oocytes were homogenised on ice in buffer (20 mM Tris, pH 7.4, 5 mM EGTA, 5 mM EDTA, and 100 mM NaCl) containing protease inhibitor (Roche). Debris was removed by 1000×g centrifugation for 15 min at 4°C. Protein samples were separated on SDS-8% polyacrylamide gels. Antibody against $\alpha_2\delta2$ (Alomone Labs) was used to detect the protein. Visualisation of protein bands was performed using ECL reagents (Pierce).

Confocal imaging

Oocytes expressing yellow fluorescent protein (YFP) labelled α_{1B}, YFP-α_{1B},[7] were placed in ND96 solution in a glass bottom dish. Fluorescent signals were collected from the animal hemisphere of the oocyte with a confocal microscope (Zeiss 510 META, 20× lens, digital zoom 2). YFP was excited using a 514 nm laser. Emission was collected in the 529–538 nm range in the Meta mode, and used for comparison of expression levels.

Statistical analysis

Results are shown as mean±SE; numbers within the bars represent n, number of cells measured in the same group. Two group comparisons were done using Student's t test. Asterisks indicate statistically significant differences as follows: *p<0.05; **p<0.01; ***p<0.001.

Study approval

Informed consent was granted by the parents, and the study was approved by the Hadassah ethical review committee. All heterologous expression experiments were approved by the Tel Aviv University Institutional Animal Care and Use Committee.

RESULTS AND DISCUSSION

The subjects were three sibs affected with a severe neurodevelopmental disorder of early infancy characterised by intractable epilepsy and lack of acquisition of any developmental milestones. Because of the parental consanguinity and the patients of both sexes, we assumed a founder mutation transmitted in an autosomal recessive manner. In order to identify the disease-causing mutation we searched for common homozygous regions in the patients' DNA which were not shared by their unaffected sister. This analysis, performed with DNA SNP array, resulted in the identification of only two regions larger than 2 Mb, chr3:43071923-60527479 and chr7:149752241-154181631 (numbering according to assembly HG19). The regions, spanning a total of 21.89 Mb, encompassed 277 protein coding genes, consisting of 3172 exons. Because of the large number of exons, we opted for whole exome sequencing in the DNA sample of one of the patients. Filtering of the called variants retained 204 homozygous exonic variants which were not present in dbSNP130 and had a reading depth of at least ×7. Nonetheless, within the two homozygous regions there were only six non-synonymous variants and only two of those were predicted to be pathogenic by SIFT (Sorting intolerant From ntolerant) and Mutation Taster software. These changes were on chr3: 48682550C->T (rs149614835) causing Met2630Ile in the CELSR3 protein; and on chr3:50402595 A->G resulting in c.3119 A->G (p.Leu1040Pro, L1040P) at the *CACNA2D2* gene. In mice, inactivation of CELSR3 was associated with severe malformations of the forebrain,[8] whereas a large in-frame insertion in CACNA2D2 was associated with an EEG recording indicative of absence epilepsy.[9] In view of the normal gross anatomy of the brain in our patients and the predominant seizure disorder, we focused on the CACNA2D2 mutation. The mutation segregated with the disease in the family (figure 1A–D) and affected a highly conserved codon—primates, rodents and fish all had Leu or Ile at this position, and the three other *CACNA2D* genes also had Leu or Ile at this position. The mutation was not present in 102 ethnically matched controls or in dbSNP135, nor was it found in 6503 healthy individuals whose Exome analysis results are available through the Exome Variant Server, NHLBI Exome Sequencing Project, Seattle, Washington, USA (http://evs.gs.washington.edu/EVS/) (July 2012).

The *CACNA2D2* gene encodes the $\alpha_2\delta2$ auxiliary subunit of high voltage gated calcium channels (hVGCC). hVGCC are heteromultimeric protein complexes composed of the main channel

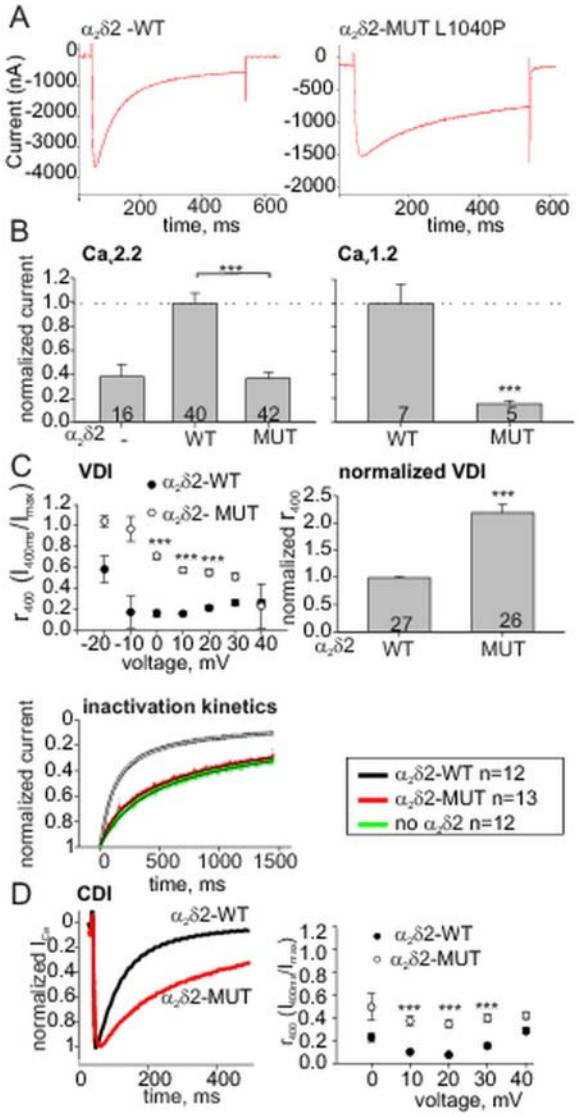

Figure 3 (A) Representative currents of $Ca_V2.2$ elicited by steps from −80 mV to 10 mV in 40 mM Ba^{2+} solution. 4 ng RNA of α_{1B}, β_3 and $\alpha_2\delta2$ (wild-type (WT); left, or L1040P; right) were injected into *Xenopus* oocytes and currents were measured using two-electrode voltage clamp. (B) Left: Normalised amplitudes of $Ca_V2.2$ in cells expressing α_{1B} and β_3, with $\alpha_2\delta2$ (WT or the mutant L1040P; MUT) or without $\alpha_2\delta2$. Right: $Ca_V1.2$ currents in cells expressing α_{1C}, β_{2b}, and WT or mutant $\alpha_2\delta2$. (C) Left: $\alpha_2\delta2$-L1040P renders a slower voltage dependent inactivation. r_{400} (% current left after 400 ms) was measured at different voltages. n=5–6 from one batch of oocytes. Open circles: channel with $\alpha_2\delta2$-WT; closed circles: channels with $\alpha_2\delta2$-L1040P. Upper right: summary of five experiments. In each experiments r_{400} was measured and normalised to the $\alpha_2\delta2$-WT group of the same experiment. Below: Inactivation kinetics in 40 mM Ba^{2+}. Currents at 10 mV were normalised to the peak in oocytes expressing α_{1B} and β_3 subunit, with or without $\alpha_2\delta2$-WT or $\alpha_2\delta2$-L1040P. (D) Left: Representative currents of $Ca_V2.2$ in 40 mM Ca^{2+} solution. Right: inactivation was evaluated as r_{400}, measured at different voltages. n=6–7 from two batches of oocytes. CDI, Ca^{2+} dependent inactivation; VDI, voltage dependent inactivation.

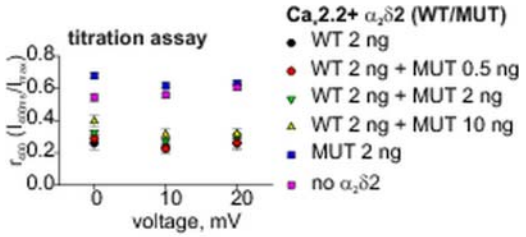

Figure 4 Four days before measurement, oocytes were injected with 2 ng RNA of α_{1B}, β_3, and wild-type (WT) $\alpha_2\delta2$ with ascending amounts of mutant $\alpha_2\delta2$ (MUT) RNA as indicated. In addition, a group of oocytes was injected with 2 ng RNA of α_{1B}, β_3, and mutant $\alpha_2\delta2$, and a group with only α_{1B} and β_3. r_{400} in 40 mM Ba^{2+} in 0–20 mV was compared. n=3–4, from one batch of oocytes.

forming α_1 subunit, which carries calcium influx across the plasma membrane, and the auxiliary subunits β, γ, and $\alpha_2\delta$. The auxiliary subunits modulate calcium currents and channel activation and inactivation kinetics.[10 11] These subunits are also involved in the proper assembly and membrane localisation of the calcium channel complexes.[12 13] $\alpha_2\delta$ is a receptor for the antiepileptic drug gabapentin,[14 15] and is associated with the enhancement of hVGCC expression and current amplitudes.[10 16 17] The $\alpha_2\delta2$ subunit is post-translationally cleaved into a long extracellular α_2 protein and a shorter membrane anchored δ polypeptide;[18] the α_2 and δ proteins are linked by disulphide bonds.[19]

In order to study the consequences of the L1040P mutation in the *CACNA2D2* gene on the properties of channels involved in neural signalling, we expressed the wild-type (WT) and the mutated *CACNA2D2* gene in *Xenopus* oocytes and monitored their effects on N ($Ca_V2.2$) and L ($Ca_V1.2$) type calcium channels. When $\alpha_2\delta2$-L1040P was expressed instead of $\alpha_2\delta2$-WT, Ba^{2+} currents of $Ca_V2.2$ ($\alpha_{1B}+\beta_3+\alpha_2\delta2$) were reduced by >65% and were comparable to currents of $\alpha_2\delta2$-less channels (figure 3A, B left). A decrease of 85% was also observed in the $Ca_V1.2$ current (figure 3B, right). $Ca_V2.2$ channels expressing $\alpha_2\delta2$-L1040P also showed a slower time dependent current decay (figure 3A). This decay reflects the inactivation process, a negative feedback mechanism that prevents an excessive entry of Ca^{2+} to the cell. Inactivation in hVGCCs is divided into voltage dependent inactivation (VDI) observed with Ba^{2+} as the charge carrier, and Ca^{2+} dependent inactivation (CDI) which occurs, along with VDI, with Ca^{2+} as the permeable ion.[11] The extent of inactivation was quantitated as the fraction of current remaining after 400 ms of depolarisation, r_{400}[20]—the higher the r_{400}, the weaker the inactivation. $Ca_V2.2$ channels expressing $\alpha_2\delta2$-L1040P showed attenuated VDI (figure 3A, C) and CDI (figure 3D) compared to $\alpha_2\delta2$-WT; the VDI became as slow as in channels expressed without $\alpha_2\delta$ subunit (figure 3C, bottom curve). We next expressed $\alpha_2\delta2$-L1040P on top of $\alpha_2\delta2$-WT, along with α_{1B} and β_3 subunits. VDI was strongly accelerated (low r_{400}) by $\alpha_2\delta2$-WT, and expression of even a fivefold excess of RNA of $\alpha_2\delta2$-L1040P did not alter r_{400} significantly (figure 4), indicating that $\alpha_2\delta2$-L1040P lacks a dominant negative effect, in correlation with the absence of epileptic symptoms in the healthy heterozygous family members.

To understand further the mechanism by which the mutation in $\alpha_2\delta2$ changes current properties, we checked whether the mutated protein was properly synthesised in the cell. We detected the $\alpha_2\delta2$ protein using a specific antibody, in cell lysates of oocytes expressing $\alpha_{1B}+\beta_3$ without $\alpha_2\delta2$ WT, with $\alpha_2\delta2$-WT or with $\alpha_2\delta2$-L1040P. In addition to an endogenous $\alpha_2\delta2$ present in native oocytes,[21] we detected a ~170 kDa band, a size that corresponds to purified $\alpha_2\delta2$ as it runs on SDS-PAGE, in the group expressing WT or mutated $\alpha_2\delta2$ (figure 5A). We concluded that the $\alpha_2\delta2$-L1040P protein is synthesised in the oocytes. To investigate whether $\alpha_2\delta2$-L1040P alters the trafficking of α_{1B} to the plasma membrane, we used a fluorescently (YFP) labelled α_{1B}, and expressed it with β_3 subunit without $\alpha_2\delta2$ or with either WT or mutant $\alpha_2\delta2$. We found that YFP-α_{1B} fluorescence signal in plasma membrane decreased by 65% when expressing $\alpha_2\delta2$-L1040P, compared to the WT $\alpha_2\delta2$ (figure 5B). A similar decrease in fluorescence was measured when the channel was expressed without $\alpha_2\delta2$. This result demonstrates that the inability of $\alpha_2\delta2$-L1040P to increase the plasma membrane expression of $Ca_V2.2$, and/or to associate with α_1, is the basis for its dysfunction.

Our results suggest that L1040P mutation in the $\alpha_2\delta2$ protein is associated with dysfunction of $\alpha_2\delta2$ rather than a decrease in

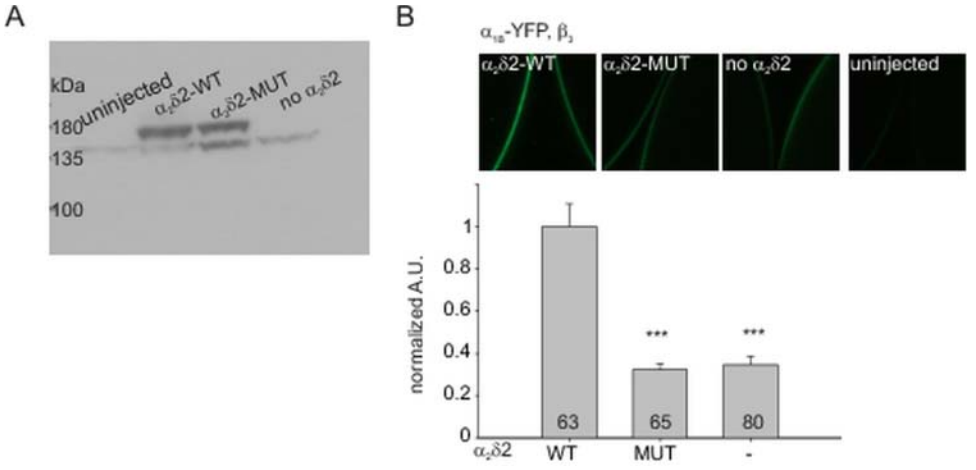

Figure 5 (A) Western blot analysis from a whole cytosolic fraction of oocytes expressing 4 ng CaV2.2 (α_{1B}, β_3)±$\alpha_2\delta2$ wild-type (WT)/mutant (MUT). Left lane, 'native' oocytes, not injected with RNA. Homogenate from 10 oocytes was loaded onto each lane. This is one representative out of two experiments. (B) Fluorescence quantification of plasma membrane level of YFP-α_{1B}, from oocytes expressing 5 ng YFP-α_{1B} and β_3 with or without $\alpha_2\delta2$ WT/mutant. Fluorescence, in arbitrary units (AU), from each experiment was normalised to signal from oocytes expressing the WT $\alpha_2\delta2$. Upper panel: Representative images of oocytes expressing YFP-α_{1B} in different composition with $\alpha_2\delta2$ and native oocytes ('uninjected'). Lower panel: Summary of three experiments (total number of oocytes is indicated within bars).

its synthesis or a dominant negative effect of the synthesised protein. The potential involvement of the mutated $\alpha_2\delta2$ protein in the epileptic phenotype may rely on retention of the α_1 subunit in the endoplasmic reticulum and/or an improper assembly of the channel, generating a channel lacking the $\alpha_2\delta2$ subunit in the neurone's plasma membrane. $\alpha_2\delta2$-L1040P is unable to reproduce the most common effects of $\alpha_2\delta2$-WT: an increase in whole cell amplitude and acceleration of inactivation.[22 23] Since $\alpha_2\delta$ increases hVGCC currents both via effects on channel gating and surface expression,[6 24 25] the $\alpha_2\delta2$-L1040P mutant appears deficient in performing one or both of these essential functions. Prolonged channel opening during depolarisation, associated with slow inactivation, could partly underlie the epileptic phenotype; low current amplitude would add to the development of an aberrant calcium signalling pattern. Furthermore, lack or dysfunction of $\alpha_2\delta2$ is expected to affect synaptogenesis and spatio-temporal calcium signalling,[13 25] aggravating the pathology. Our clinical data and the results of the electrophysiological studies are in agreement with the spike wave seizures reported in mice carrying a spontaneous mutation in $\alpha_2\delta2$ (ducky alleles) associated with an aberrant $\alpha_2\delta2$ protein.[26–29] We cannot exclude the possibility that our patients' phenotype is aggravated by the concomitant occurrence of the homozygous Met2630Ile mutation in the CELSR3 protein; this rare mutation affects a conserved codon and the protein was shown to play a role in the connectivity of the cerebral cortex.[30]

Human voltage gated calcium channelopathies with primary central nervous system manifestations have hitherto been solely reported in association with α_{1A}, the principal subunit of the voltage gated P/Q-type calcium channel encoded by *CACNA1A*. Autosomal dominant mutations in this gene were clinically manifested by episodic and progressive forms of cerebellar ataxia (EA2 and SCA6), familial hemiplegic migraine (FHM1), vertigo, and epilepsy.[31] The pathophysiology of cerebellar atrophy in patients with mutations in *CACNA1A* or *CACNA2D2* remains obscure, but may perhaps serve as a neuroimaging marker for this newly emerging group of voltage gated calcium channelopathies.

Author affiliations
[1]The Monique and Jacques Roboh Department of Genetic Research, Hadassah, Hebrew University Medical Center, Jerusalem, Israel
[2]The Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel
[3]The Specialist Clinics Jenin, Primary Health Care, Ministry of Health, Jenin, Palestine
[4]Palestine Medical Complex, Ramallah, Palestine

Acknowledgements The excellent technical assistance of Rachel Dahan, Noa Cohen and Lital Sheva is acknowledged.

Contributors SE, ND, OE conceived and designed the experiments; SO, AS, SZ performed the experiments; SE, SO, AS, ND, OE analysed the data; SE, SO, ND, OE wrote the paper; SE, FAA, FBT undertook patient management, collection of samples, and delineation of the phenotype.

Funding This work was supported in part by the Joint Research Fund of the Hebrew University and Hadassah Medical Organization to Simon Edvardson.

Competing interests None.

Ethics approval Hadassah ethical review committee and Tel Aviv University Institutional Animal Care and Use Committee.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The DNA SNP chip data and the exome data are available upon request.

Informed Consent Obtained.

REFERENCES

1 Tavyev Asher YJ, Scaglia F. Molecular bases and clinical spectrum of early infantile epileptic encephalopathies. *Eur J Med Genet* 2012;55:299–306.
2 Harkin LA, McMahon JM, Iona X, Dibbens L, Pelekanos JT, Zuberi SM, Sadleir LG, Andermann E, Gill D, Farrell K, Connolly M, Stanley T, Harbord M, Andermann F, Wang J, Batish SD, Jones JG, Seltzer WK, Gardner A. The spectrum of SCN1A-related infantile epileptic encephalopathies. *Brain* 2007;130:843–852.
3 Carranza Rojo D, Hamiwka L, McMahon JM, Dibbens LM, Arsov T, Suls A, Stödberg T, Kelley K, Wirrell E, Appleton B, Mackay M, Freeman JL, Yendle SC, Berkovic SF, Bienvenu T, De Jonghe P, Thorburn DR, Mulley JC, Mefford HC, Scheffer IE. De novo SCN1A mutations in migrating partial seizures of infancy. *Neurology* 2011;77:380–383.
4 Edvardson S, Shaag A, Kolesnikova O, Gomori JM, Tarassov I, Einbinder T, Saada A, Elpeleg O. Deleterious mutation in the mitochondrial arginyl-transfer RNA synthetase gene is associated with pontocerebellar hypoplasia. *Am J Hum Genet* 2007;81:857–862.
5 Edvardson S, Cinnamon Y, Jalas C, Shaag A, Maayan C, Axelrod FB, Elpeleg O. Hereditary sensory autonomic neuropathy caused by a mutation in dystonin. *Ann Neurol* 2012;71:569–572.
6 Shistik E, Ivanina T, Puri T, Hosey M, Dascal N. Ca2+ current enhancement by alpha 2/delta and beta subunits in Xenopus oocytes: contribution of changes in channel gating and alpha 1 protein level. *J Physiol* 1995;489:55–62.
7 Tselnicker I, Tsemakhovich VA, Dessauer CW, Dascal N. Stargazin modulates neuronal voltage-dependent Ca(2+) channel Ca(v)2.2 by a Gbetagamma-dependent mechanism. *J Biol Chem* 2010;285:20462–20471.
8 Tissir F, Bar I, Jossin Y, De Backer O, Goffinet AM. Protocadherin Celsr3 is crucial in axonal tract development. *Nat Neurosci.* 2005;8:451–457.
9 Brill J, Klocke R, Paul D, Boison D, Gouder N, Klugbauer N, Hofmann F, Becker CM, Becker K. Entla, a novel epileptic and ataxic Cacna2d2 mutant of the mouse. *J Biol Chem* 2004;279:7322–7330.
10 Dolphin AC. Calcium channel auxiliary alpha2delta and beta subunits: trafficking and one step beyond. *Nat Rev Neurosci* 2012;13:542–555.
11 Catterall WA. Structure and regulation of voltage-gated Ca^{2+} channels. *Annu Rev Cell Dev Biol* 2000;16:521–555.
12 Arikkath J, Campbell KP. Auxiliary subunits: essential components of the voltage-gated calcium channel complex. *Curr Opin Neurobiol* 2003;13:298–307.
13 Hoppa MB, Lana B, Margas W, Dolphin AC, Ryan TA. $\alpha_2\delta$ Expression sets presynaptic calcium channel abundance and release probability. *Nature* 2012;486:122–125.
14 Gong HC, Hang J, Kohler W, Li L, Su TZ. Tissue-specific expression and gabapentin-binding properties of calcium channel alpha2delta subunit subtypes. *J Membr Biol* 2001;184:35–43.
15 Dolphin AC. Calcium channel $\alpha2\delta$ subunits in epilepsy and as targets for antiepileptic drugs. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV.eds. *Jasper's Basic Mechanisms of the Epilepsies (Internet)*. 4th edn. Bethesda, MD: National Center for Biotechnology Information (US), 2012.
16 Bernstein GM, Jones OT. Kinetics of internalization and degradation of N-type voltage-gated calcium channels: role of the alpha2/delta subunit. *Cell Calcium* 2007;41:27–40.
17 Witcher DR, De Waard M, Sakamoto J, Franzini-Armstrong C, Pragnell M, Kahl SD, Campbell KP. Subunit identification and reconstitution of the N-type Ca2+ channel complex purified from brain. *Science* 1993;261:486–489.
18 Davies A, Kadurin I, Alvarez-Laviada A, Douglas L, Nieto-Rostro M, Bauer CS, Pratt WS, Dolphin AC. The alpha2delta subunits of voltage-gated calcium channels form GPI-anchored proteins, a posttranslational modification essential for function. *Proc Natl Acad Sci U S A* 2010;107:1654–1659.
19 De Jongh KS, Warner C, Catterall WA. Subunits of purified calcium channels. $\alpha2$ and δ are encoded by the same gene. *J Biol Chem* 1990;265:14738–14741.
20 Oz S, Tsemakhovich V, Christel CJ, Lee A, Dascal N. CaBP1 regulates voltage dependent inactivation and activation of CaV1.2 (L-type) calcium channels. *J Biol Chem* 2011;286:13945–13953.
21 Singer-Lahat D, Lotan I, Itagaki K, Schwartz A, Dascal N. Evidence for the existence of RNA of Ca(2+)-channel alpha 2/delta subunit in Xenopus oocytes. *Biochim Biophys Acta* 1992;1137:39–44.
22 Singer D, Biel M, Lotan I, Flockerzi V, Hofmann F, Dascal N. The roles of the subunits in the function of the calcium channel. *Science* 1991;253:1553–1557.
23 Wakamori M, Mikala G, Mori Y. Auxiliary subunits operate as a molecular switch in determining gating behaviour of the unitary N-type Ca2+ channel current in Xenopus oocytes. *J Physiol (Lond)* 1999;517:659–672.
24 Davies A, Hendrich J, Van Minh AT, Wratten J, Douglas L, Dolphin AC. Functional biology of the $\alpha2\delta$ subunits of voltage-gated calcium channels. *Trends Pharmacol Sci* 2007;28:220–228.
25 Senatore A, Colleoni S, Verderio C, Restelli E, Morini R, Condliffe SB, Bertani I, Mantovani S, Canovi M, Micotti E, Forloni G, Dolphin AC, Matteoli M, Gobbi M, Chiesa R. Mutant PrP suppresses glutamatergic neurotransmission in cerebellar granule neurons by impairing membrane delivery of VGCC $\alpha2\delta$-1 subunit. *Neuron* 2012;74:300–313.
26 Barclay J, Balaguero N, Mione M, Ackerman SL, Letts VA, Brodbeck J, Canti C, Meir A, Page KM, Kusumi K, Perez-Reyes E, Lander ES, Frankel WN, Gardiner RM, Dolphin AC, Rees M. Ducky mouse phenotype of epilepsy and ataxia is associated with mutations in the Cacna2d2 gene and decreased calcium channel current in cerebellar Purkinje cells. *J Neurosci* 2001;21:6095–6104.

27 Meier H. The neuropathology of ducky, a neurological mutation of the mouse. A pathological and preliminary histochemical study. *Acta Neuropathol* 1968;11:15–28.
28 Brodbeck J, Davies A, Courtney JM, Meir A, Balaguero N, Canti C, Moss FJ, Page KM, Pratt WS, Hunt SP, Barclay J, Rees M, Dolphin AC. The ducky mutation in Cacna2d2 results in altered Purkinje cell morphology and is associated with the expression of a truncated alpha 2 delta-2 protein with abnormal function. *J Biol Chem* 2002;277:7684–7693.
29 Donato R, Page KM, Koch D, Nieto-Rostro M, Foucault I, Davies A, Wilkinson T, Rees M, Edwards FA, Dolphin AC. The ducky(2J) mutation in Cacna2d2 results in reduced spontaneous Purkinje cell activity and altered gene expression. *J Neurosci* 2006;26:12576–12586.
30 Zhou L, Bar I, Achouri Y, Campbell K, De Backer O, Hebert JM, Jones K, Kessaris N, de Rouvroit CL, O'Leary D, Richardson WD, Goffinet AM, Tissir F. Early forebrain wiring: genetic dissection using conditional Celsr3 mutant mice. *Science* 2008;320:946–949.
31 Rajakulendran S, Kaski D, Hanna MG. Neuronal P/Q-type calcium channel dysfunction in inherited disorders of the CNS. *Nat Rev Neurol* 2012;8:86–96.

Early infantile epileptic encephalopathy associated with a high voltage gated calcium channelopathy

Simon Edvardson, Shimrit Oz, Fida Aziz Abulhijaa, et al.

J Med Genet 2013 50: 118-123
doi: 10.1136/jmedgenet-2012-101223