

De Novo Loss-of-Function Mutations in *CHD2* Cause a Fever-Sensitive Myoclonic Epileptic Encephalopathy Sharing Features with Dravet Syndrome

Arvid Suls,^{1,2,38} Johanna A. Jaehn,^{3,38} Angela Kecskés,^{4,38} Yvonne Weber,^{5,38} Sarah Weckhuysen,^{1,2} Dana C. Craiu,^{6,7} Aleksandra Siekierska,⁴ Tania Djémié,^{1,2} Tatiana Afrikanova,⁴ Padhraig Gormley,⁸ Sarah von Spiczak,³ Gerhard Kluger,⁹ Catrinel M. Iliescu,^{6,7} Tiina Talvik,^{10,11} Inga Talvik,^{10,11} Cihan Meral,¹² Hande S. Caglayan,¹³ Beatriz G. Giraldez,¹⁴ José Serratos,¹⁴ Johannes R. Lemke,¹⁵ Dorota Hoffman-Zacharska,¹⁶ Elzbieta Szczepanik,¹⁷ Nina Barisic,¹⁸ Vladimir Komarek,¹⁹ Helle Hjalgrim,^{20,21} Rikke S. Møller,²⁰ Tarja Linnankivi,²² Petia Dimova,²³ Pasquale Striano,²⁴ Federico Zara,²⁵ Carla Marini,²⁶ Renzo Guerrini,²⁶ Christel Depienne,^{27,28,30} Stéphanie Baulac,^{27,28,29} Gregor Kuhlenbäumer,³¹ Alexander D. Crawford,^{4,32} Anna-Elina Lehesjoki,^{33,34,35} Peter A.M. de Witte,⁴ Aarno Palotie,^{8,36,37} Holger Lerche,⁵ Camila V. Esguerra,^{4,39} Peter De Jonghe,^{1,2,39,*} Ingo Helbig,^{3,39} and the EuroEPINOMICS RES Consortium

Dravet syndrome is a severe epilepsy syndrome characterized by infantile onset of therapy-resistant, fever-sensitive seizures followed by cognitive decline. Mutations in *SCN1A* explain about 75% of cases with Dravet syndrome; 90% of these mutations arise de novo. We studied a cohort of nine Dravet-syndrome-affected individuals without an *SCN1A* mutation (these included some atypical cases with onset at up to 2 years of age) by using whole-exome sequencing in proband-parent trios. In two individuals, we identified a de novo loss-of-function mutation in *CHD2* (encoding chromodomain helicase DNA binding protein 2). A third *CHD2* mutation was identified in an epileptic proband of a second (stage 2) cohort. All three individuals with a *CHD2* mutation had intellectual disability and fever-sensitive generalized seizures, as well as prominent myoclonic seizures starting in the second year of life or later. To explore the functional relevance of *CHD2* haploinsufficiency in an in vivo model system, we knocked down *chd2* in zebrafish by using targeted morpholino antisense oligomers. *chd2*-knockdown larvae exhibited altered locomotor activity, and the epileptic nature of this seizure-like behavior was confirmed by field-potential recordings that revealed epileptiform discharges similar to seizures in affected persons. Both altered locomotor activity and epileptiform discharges were absent in appropriate control larvae. Our study provides evidence that de novo loss-of-function mutations in *CHD2* are a cause of epileptic encephalopathy with generalized seizures.

¹Neurogenetics group, Department of Molecular Genetics, VIB, 2610 Antwerp, Belgium; ²Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, 2610 Antwerp, Belgium; ³University Medical Center Schleswig-Holstein, Christian-Albrechts University, 24105 Kiel, Germany; ⁴Laboratory for Molecular Biodiscovery, Department of Pharmaceutical and Pharmacological Sciences, University of Leuven, 3000 Leuven, Belgium; ⁵Department of Neurology and Epileptology, Hertie Institute for Clinical Brain Research, University of Tübingen, 72076 Tübingen, Germany; ⁶Pediatric Neurology Clinic II, Departments of Neurology, Pediatric Neurology, Psychiatry, and Neurosurgery, “Carol Davila” University of Medicine, Sector 4, 050474 Bucharest, Romania; ⁷Pediatric Neurology Clinic, “Professor Doctor Alexandru Obregia” Clinical Hospital, Sector 4, 041914 Bucharest, Romania; ⁸Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire CB10 1SA, UK; ⁹Neuropädiatrie und Neurologische Rehabilitation, Epilepsiezentrum für Kinder und Jugendliche, Tagesklinik für Neuropädiatrie, Schön Klinik Vogtareuth, 83569 Vogtareuth, Germany; ¹⁰Department of Pediatrics, University of Tartu, 51014 Tartu, Estonia; ¹¹Department of Neurology and Neurorehabilitation, Children’s Clinic, Tartu University Hospital, 50406 Tartu, Estonia; ¹²Department of Pediatric Neurology, GATA Haydarpasa Teaching Hospital, 34668 Istanbul, Turkey; ¹³Department of Molecular Biology and Genetics, Bogazici University, 34342 Istanbul, Turkey; ¹⁴Epilepsy Unit, Hospital Universitario Fundación Jiménez Díaz and Centro De Investigación Biomédica En Red De Enfermedades Raras, 28040 Madrid, Spain; ¹⁵Division of Human Genetics, University Children’s Hospital Inselspital, 3010 Bern, Switzerland; ¹⁶Department of Medical Genetics, Institute of Mother and Child, 01211 Warsaw, Poland; ¹⁷Clinic of Neurology of Child and Adolescents, Institute of Mother and Child, 01211 Warsaw, Poland; ¹⁸Department of Paediatrics, University of Zagreb School of Medicine, University Hospital Centre Zagreb, 10000 Zagreb, Croatia; ¹⁹Child Neurology Department, University Hospital Motol, 150 06 Praha, Czech Republic; ²⁰Danish Epilepsy Centre, 4293 Dianalund, Denmark; ²¹Institute for Regional Health research, University of Southern Denmark, 5230 Odense, Denmark; ²²Pediatric Neurology, Children’s Hospital, University of Helsinki and Helsinki University Central Hospital, 00029 Helsinki, Finland; ²³Clinic of Child Neurology, St. Naum University Hospital of Neurology and Psychiatry, 1113 Sofia, Bulgaria; ²⁴Pediatric Neurology and Muscular Diseases Unit, Departments of Neurosciences, Rehabilitation, Ophthalmology, Genetics, and Maternal and Child Health, University of Genova and Gaslini Institute, 16147 Genova, Italy; ²⁵Laboratory of Neurogenetics, Pediatric Neurology and Muscular Diseases Unit, Department of Neurosciences, Gaslini Institute, 16147 Genova, Italy; ²⁶Pediatric Neurology Unit and Laboratories, Meyer Children’s Hospital, University of Florence, 50132 Florence, Italy; ²⁷Institut National de la Santé et de la Recherche Médicale U975, Centre de Recherche de l’Institut du Cerveau et de la Moelle Epinière, Hôpital Pitié-Salpêtrière, 75013 Paris, France; ²⁸Centre National de la Recherche Scientifique 7225, Centre de Recherche de l’Institut du Cerveau et de la Moelle Epinière, Hôpital Pitié-Salpêtrière, 75013 Paris, France; ²⁹Université Pierre et Marie Curie (Paris VI), UMR_S 975, 75013 Paris, France; ³⁰Département de Génétique et de Cytogénétique, Unité Fonctionnelle de Neurogénétique Moléculaire et Cellulaire, Hôpital Pitié-Salpêtrière, Assistance Publique – Hôpitaux de Paris, 75013 Paris, France; ³¹Department of Neurology, Institute of Experimental Medicine, Christian-Albrechts University of Kiel, 24105 Kiel, Germany; ³²Luxembourg Center for Systems Biomedicine, University of Luxembourg, L-4362 Esch-sur-Alzette, Luxembourg; ³³Folkhälsan Institute of Genetics, 00290 Helsinki, Finland; ³⁴Research Programs Unit, Molecular Neurology, University of Helsinki, 00290 Helsinki, Finland; ³⁵Neuroscience Center, University of Helsinki, 00290 Helsinki, Finland; ³⁶Institute for Molecular Medicine Finland, University of Helsinki, 00290 Helsinki, Finland; ³⁷Program in Medical and Population Genetics and Genetic Analysis Platform, The Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

³⁸These authors contributed equally to the work

³⁹These authors contributed equally to the work

*Correspondence: peter.dejonghe@molgen.vib-ua.be

<http://dx.doi.org/10.1016/j.ajhg.2013.09.017>. ©2013 by The American Society of Human Genetics. All rights reserved.

Epileptic encephalopathies (EEs) are severe, intractable childhood epilepsies with concomitant cognitive impairment and other associated comorbidities. Although a broad range of exogenous factors can lead to EE, in a significant subset of affected individuals the etiology remains unidentified and might be endogenous. Consequently, a genetic cause is assumed.¹ Among the various EEs of genetic origin, Dravet syndrome (MIM 607208) has emerged as one of the best-defined phenotypes and the one with the highest mutation detection yield. Children with Dravet syndrome are prone to repetitive and prolonged epileptic seizures in the setting of fever.² Although these fever-induced seizures start around the age of 6 months (range = 3–16 months),³ other seizure types occur during the course of the disease, and developmental plateauing is typical in the second year of life.

Taking into account *SCN1A* abnormalities due to single or multiple exons deletions, de novo mutations in *SCN1A* (MIM 182389) are known to cause Dravet syndrome in ~75% of affected probands.⁴ Despite this high probability of identifying a mutation in an individual with a typical phenotype, up to 25% of affected persons do not carry *SCN1A* mutations, suggesting the involvement of other genes. Mutations in *PCDH19* (MIM 300460) can result in a Dravet-syndrome-like phenotype in females and explain some of the cases without an *SCN1A* mutation.⁵ For a relevant subset of probands, however, a causative mutation cannot be identified in either gene. We therefore explored the involvement of mutations in additional genes in these probands by using whole-exome sequencing in proband-parent trios. This approach provides the possibility of querying the entire exome for de novo mutations in simplex cases, which has been proven to be successful for intellectual disability, schizophrenia, autism, and some forms of epilepsy or epilepsy-related disorders.^{6,7}

We first studied nine Dravet-syndrome-affected individuals in whom *SCN1A* sequence mutations or copy-number variations had been excluded. These probands were selected on the basis of broad inclusion criteria, extending the onset age of seizures to an age between 3 months and 2 years of life. Further inclusion criteria consisted of (1) the presence of both febrile and afebrile seizures; (2) multiple seizure types including tonic-clonic, hemiclonic, myoclonic, absence, and/or focal seizures; (3) pharmacoresistant epilepsy at least during childhood or frequent status epilepticus; (4) normal development prior to epilepsy onset, although some minor degree of developmental delay could be present; and (5) slowing or stagnation of development after onset of seizures and absence of epileptogenic lesions on brain MRI.

Signed informed consent was obtained from all study participants or their legal representatives. The local ethical committees of the University of Antwerp and Antwerp University Hospital, University of Kiel, and other collaborating centers approved this study. Genomic DNA was extracted from peripheral blood according to standard procedures.

We performed whole-exome sequencing on genomic DNA of the nine selected individuals with Dravet syndrome and both unaffected parents at the Wellcome Trust Sanger Institute (Hinxton, Cambridgeshire). In brief, genomic DNA (~3 µg) was fragmented by sonication, and fragments with a length of 150–200 bp were purified. After a paired-end DNA library was prepared from the DNA fragments (with the TruSeq DNA Sample Preparation Kit from Illumina), targeted enrichment was performed with the SureSelect Human All Exon 50Mb Kit (Agilent Technologies). Captured DNA was then sequenced on a HiSeq2000 (Illumina) as paired-end 75 bp reads according to the manufacturer's protocol. Sequencing reads passing quality filtering were aligned to the human reference genome (hg19, UCSC Genome Browser) with the Burrows-Wheeler Aligner.⁸ The Genome Analysis Toolkit (GATK)⁹ was used to recalibrate base quality scores, realign around indels, and mark duplicate reads. Independent variant calling was performed on the mapped reads with SAMtools¹⁰ mpileup, GATK UnifiedGenotyper, and Dindel.¹¹ The GenomeComb¹² program was used for annotating, comparing, and filtering the data. For de novo variant calling, the DeNovoGear¹³ program by Conrad and colleagues was used and double-checked by GenomeComb analysis.

Our analysis revealed a heterozygous de novo mutation in *CHD2* (MIM 302119; RefSeq accession number NM_001271.3), encoding chromodomain helicase DNA binding protein 2 (CHD2; RefSeq NP_001262.3), in two out of nine probands. One individual carried a nonsense mutation, c.4971G>A (p.Trp1657*), in exon 38; the second person carried a splice-site mutation, c.1810–2A>C, affecting the splice acceptor site of exon 16. Presence or absence of the mutations was confirmed on genomic DNA of the probands or parents, respectively, by bidirectional Sanger sequencing using the ABI BigDye Terminator v.3.1 cycle sequencing kit on an ABI 3730xl automated DNA Analyzer (Applied Biosystems).

To obtain further genetic evidence of pathogenicity, we performed a mutation analysis of all 39 coding exons and intron-exon boundaries of *CHD2* by using bidirectional sequencing (primer sequences are available upon request) in a cohort of 150 EE probands similar to the two individuals carrying a *CHD2* mutation: all selected probands had infantile- or childhood-onset epilepsy with subsequent developmental delay. All individuals had normal brain MRI and at least one of the following seizure types: tonic-clonic seizures, myoclonic seizures, (atypical) absence seizures, or atonic seizures. In this cohort, we identified a third person carrying a de novo mutation in *CHD2* (c.1396C>T [p.Arg466*]) in exon 13.

The two identified premature stop codons are predicted to result in degradation of the mutant transcript by means of nonsense-mediated mRNA decay (NMD). The effect of the splice-site mutation on transcript level is unclear, given that both exon skipping and (partial) intron retention are possible. Most likely, the splice acceptor mutation results in skipping of exon 16 and a subsequent out-of-frame

deletion of exon 16, leading to a premature stop codon in exon 17 (p.Thr604Valfs2*). Therefore, NMD could also be active in this case. To test the NMD hypothesis, we extracted RNA from fresh blood (QIAGEN RNeasy Micro Kit) of the three probands and a parent as a control individual, removed contaminated DNA by Ambion DNA-free DNase treatment (Life Technologies), and synthesized cDNA by Superscript III reverse transcriptase (Life Technologies). For the two nonsense mutations, we performed bidirectional sequencing on cDNA with primers flanking the mutation. With these experiments, we were able to detect the mutation with a 50/50 ratio, proving that the aberrant allele was not degraded by NMD. These results were confirmed by Sybr-Green-based quantitative PCR (qPCR) experiments (Sigma-Aldrich) with six primer pairs complementary to *CHD2* cDNA (data not shown). For the splice-site mutation, we performed qPCR and developed sequencing primers flanking exon 16 on cDNA. Also here, qPCR showed the presence of both alleles. Additionally, the sequencing experiment showed an abundance of alternative splicing events in the probands compared to the control individual, but the consequence of the alternative splicing remained unknown. Although skipping of exon 16 appeared to be a logical consequence, additional investigations revealed more complex alternative splicing events. Whereas definite identification of all alternatively spliced transcripts was not possible, we were able to rule out NMD. We assumed that either the aberrant proteins were degraded by the proteasome or the shorter proteins were not fully functional; both of these cases would result in loss of function. In support of the pathogenicity of these mutations, none of the identified mutations have been observed in the 1000 Genomes Project, National Heart, Lung, and Blood Institute Exome Sequencing Project Exome Variant Server (EVS), or dbSNP (build 137). Furthermore, splice-site, frameshift, or nonsense variations are absent in the EVS and 1000 Genomes Project cohorts.

Between the age of 14 months and 3.5 years, the three persons carrying a de novo *CHD2* mutation presented with febrile seizures followed by therapy-resistant generalized seizures (Table 1). Frequent myoclonic seizures, generalized tonic-clonic seizures (GTCs), and absences were seen in all three individuals. Proband 1 developed normally during the first year of life. The first febrile seizure occurred when he was 14 months old and was soon followed by afebrile head drops occurring several times a day. When he was 2 years old, therapy-resistant myoclonic seizures, atypical absences, and GTCs rarely associated with fever developed. On one occasion, he had a status epilepticus. Electroencephalography (EEG) showed generalized polyspike wave discharges and, later, focal epileptic discharges. He has been treated with valproic acid, levetiracetam, phenytoin, topiramate, bromide, phenobarbital, ethosuximide, topiramate, vitamin B6, and prednisolone. He now has moderate intellectual disability (ID), dysarthria, and ataxia. Proband 2 had a normal early development. At the age of 2 years, she had a cluster of febrile

Table 1. Clinical and Genetic Characteristics of Persons with a *CHD2* Mutation

Proband	Sex	Age at Inclusion	Mutation	Development Prior to Epilepsy	Age of Seizure Onset	First Seizure Type	Further Seizure Types	Fever Sensitivity	EEG	Imaging	Clinical Exam	Cognitive Outcome
1	male	6 years	c.1810-2A>C (p.?)	normal	14 months	simple FSs	head drops, GTCs, myoclonic seizures, atypical absences, status epilepticus	+	frequent generalized (poly)SWs	normal	ataxia, dysarthria	mild ID
2	female	24 years	c.4971G>A (p.Trp1657*)	normal	2 years	cluster of FSs	myoclonic absences, myoclonic seizures, GTCs	+	frequent generalized SWs and polyspikes	normal	normal	mild ID
3	male	6 years	c.1396C>T (p.Arg466*)	subtle delay in motor and speech development	3 years, 6 months	two FSs during single fever episode	GTCs and hemiclonic, atonic, myoclonic, atypical absences	+	generalized (poly)SWs	nonspecific atrophy	mild ataxia	mild ID, ASD, ADHD

Abbreviations are as follows: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; FS, febrile seizure; GTCs, generalized tonic-clonic seizure; ID, intellectual disability; and SW, spike-wave complex.

seizures. At the age of 2.5 years, she developed therapy-resistant absence seizures accompanied by eyelid myoclonias, myoclonic seizures, and febrile and afebrile GTCs. EEG showed frequent generalized spike-wave complexes and polyspikes. She has been treated with vigabatrin, valproate, bromide, ethosuximide, lamotrigine, and levetiracetam and is currently taking a combination of topiramate and valproic acid. She still has afebrile GTCs and moderate ID. Proband 3 had slightly delayed early motor and speech development, given that he walked at the age of 1.5 years and produced his first words at the age of 2 years. At the age of 3.5 years, he had two GTCs during an episode of high fever. During the following 2 years, he had pharmacoresistant febrile and afebrile GTCs, hemiclonic, atonic, and myoclonic seizures, and atypical absences. He was treated with valproic acid, topiramate, and levetiracetam. After the start of clobazam at the age of 5.5 years, he became seizure free. EEG showed generalized (poly)spike-wave complexes. After seizure onset, a cognitive decline was seen but partially improved when seizures were controlled. He now has mild ID, autism spectrum disorder (ASD), attention deficit hyperactivity disorder, and mild ataxia. Brain MRI was normal in probands 1 and 2 and showed atrophic changes in proband 3.

In addition to our three individuals with a *CHD2* mutation, one simplex case with ID and absence epilepsy and one simplex ASD case both carrying a mutation in *CHD2* have been reported in the literature.^{14,15} Recently, two other research groups have also shown the involvement of *CHD2* mutations in EEs.^{16,17} Furthermore, several individuals affected by ID and generalized epilepsy and carrying a multigenic chromosomal deletion of 15q26.2, including *CHD2*, have been described (Table 2).^{18–22} The presumed haploinsufficiency of *CHD2* in these probands prompted us to screen all individuals without a previous detected mutation from both studied cohorts for copy-number variants in *CHD2* with the multiplex amplicon quantification (MAQ) technique, an in-house-developed technique based on a semiquantified multiplex PCR. The multiplex PCR reaction consisted of six target amplicons located in the genomic region of *CHD2* and four reference amplicons randomly located on different chromosomes. The MAQ analysis was performed as described previously.²³ A partial or full deletion or duplication of the gene was not identified in any of the remaining studied persons (7 of the initial cohort and 149 of the second-stage cohort).

To establish additional evidence of the implication of *CHD2* in the development of epilepsy, we examined the functional consequence of *CHD2* haploinsufficiency by knocking down *chd2* in zebrafish by using targeted morpholino (MO) antisense oligomers.²⁴ All zebrafish experiments carried out were approved by the ethics committee of the University of Leuven (Ethische Commissie van de KU Leuven, approval number P05090) and by the Belgian Federal Public Service of Health, Food Chain Safety, and Environment (Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu, approval

number LA1210199). In order to mimic loss-of-function mutations, we designed a MO (E2I2 MO, 5'-GATCAGA CTGGCCTTTTGTGTACC-3') to target the splice donor site of exon 2 and interfere with normal pre-mRNA splicing of zebrafish *chd2* (ENSART00000127730). Targeting of the exon 2-intron 2 boundary should result in abnormal exon 2 splicing, leading to its complete or partial deletion together with its flanking introns. This should result in an mRNA shorter than the wild-type transcript (Figure 1). A control MO (randomized 25 N oligomer) was used as a negative control (ctrl MO). All MOs were designed and synthesized by GeneTools. Gene knockdowns were achieved through microinjection of MOs into 1- to 2-cell-stage embryos from the AB (wild-type) strain according to the method previously described.²⁵ In order to mimic haploinsufficiency, we titrated the amount of E2I2 MO to 9 ng per injection so as to reduce correctly spliced *chd2* mRNA levels by approximately 50%. The same amount of ctrl MO was injected into sibling control embryos. To evaluate the level of knockdown in zebrafish embryos and larvae, we performed qPCR on splice-blocked pre-mRNA. Total mRNA was purified from each of 10 ctrl-MO- and E2I2-MO-injected embryos and larvae between 1 and 5 days postfertilization (dpf) with TRIzol reagent (Life Technologies) according to the recommended protocol. Reverse transcription of 2 µg total RNA to single-stranded cDNA was performed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The PCR reaction was performed with Phusion Polymerase (ThermoScientific) and *chd2*-specific primers (5'-AAGAC GAAGGCTCGACTCAA-3' and 5'-TGCAGGCTCTTGCTA CTGC-3') for detecting the amplicon with or without the partially deleted exon 2. PCR products were visualized with standard agarose gel electrophoresis. As expected, the ctrl MO did not have any effect on splicing and resulted in the predicted wild-type mRNA corresponding to a 384 bp PCR product (Figure 1). *chd2* knockdown using the E2I2 MO resulted in two products: (1) a 384 bp band corresponding to the wild-type mRNA and (2) a shorter product (~300 bp) probably corresponding to an abnormally spliced mRNA, suggesting a partial knockdown. The discrepancy between the length of predicted (149 bp) versus observed (~300 bp) abnormally spliced mRNA was most likely a result of the activation of a cryptic splice site in exon 2 instead of splicing to the 5' end of the upstream splice site (this could cause a partial deletion of exon 2). This abnormal product appeared after 1 dpf and was sustained until 5 dpf. Adult zebrafish (*Danio rerio*) of the AB strain (Zebrafish International Resource Center, Eugene) were maintained at 28.5°C on a 14/10 hr light/dark cycle under standard aquaculture conditions, and fertilized eggs were collected via natural spawning. Embryos were raised in embryo medium consisting of 1.5 mM HEPES, pH 7.6, 17.4 mM NaCl, 0.21 mM KCl, 0.12 mM MgSO₄, and 0.18 mM Ca(NO₃)₂ in an incubator on a 14/10 hr light/dark cycle at 28.5°C. For all experiments described, larvae of 1–5 dpf were used.

Table 2. Clinical Phenotype of Probands Reported in Literature with *CHD2* Deletions or Mutations

	Reference							
	Capelli et al. ¹⁸	Dhamija et al. ¹⁹	Veredice et al. ²⁰	Li et al. ²¹	Lund et al. ²²	Rauch et al. ¹⁴	Carvill et al. ¹⁶	Allen et al. ¹⁷
Number of individuals	1	1	1	1	1	1	6	1
Genetic findings	de novo 0.5 Mb deletion including <i>CHD2</i> and <i>RGMA</i>	de novo 0.9 Mb deletion including <i>CHD2</i> and three other genes	de novo 5 Mb deletion including <i>CHD2</i> and 55 other genes	3.3 Mb deletion including <i>CHD2</i> and 17 other genes (no segregation analysis)	2 Mb deletion including <i>CHD2</i> and seven other genes. Also carried five additional deletions and duplications, including a total of 100 genes (paternal DNA not available)	de novo frameshift mutation c.1809 del (p.Thr604Leufs*19)	four de novo frameshift and two de novo missense alterations: p.Glu1412Glyfs*64, p.Arg121*, p.Gly491Valfs*13, p.Arg1644Lysfs*22, p.Trp548Arg, p.Leu823Pro	de novo splice mutation c.1502+1G>A
Age at seizure onset	2 years	3.5 years	6 months	not specified	4 years	5 years	1–3 years	6 months
Seizure type at onset	not specified	CPSs	febrile generalized clonic SE	two episodes of FSs	atypical ASs, MSs	ASs	atypical ASs, AtSs, MSs, GTCs, FSs, FDSs	unknown
Further seizure types	not specified	therapy-resistant ASs with eyelid flutter, TSs, MSs, GTCs	therapy-resistant massive MSs with head drop, eyelid MSs, prolonged hemiclonic FSs	none	TSs, MSs, atypical ASs, nonconvulsive SE	not specified	FSs, AtSs, MSs, GTCs, NCS, SE, TSs, HSs, FDSs, MAS, atypical ASs	MSs, FDSs, GTCs, atypical ASs, AtSs
Fever sensitivity	not specified	no	yes	yes	no	not specified	one patient	no
EEG	generalized spike waves and focal discharges	generalized spike waves, PPR	irregular generalized spike waves, PPR	not specified	generalized slow spike waves and runs of fast spikes	not specified	generalized (poly)spike waves, slow spike waves, multifocal discharges, generalized paroxysmal fast activity, diffuse slowing	slow background, generalized spike waves
MRI	normal	normal	vermis hypoplasia, cisterna magna	normal	partial agenesis of vermis	unknown	unknown	normal
Development prior to epilepsy	not specified	delayed	delayed	delayed	delayed	delayed	normal or delayed	normal
Developmental outcome	globally delayed, severe speech impairment	mild ID	mild ID	mild to moderate ID, speech impairment	severe ID	mild ID	moderate to severe ID	unspecified delay
Other clinical findings	ataxia, relative microcephaly, mild facial dysmorphisms	microcephaly, short stature, mild facial dysmorphisms	microcephaly, congenital hypothyroidism, bicuspid aortic valve, hypotonia	microcephaly, short stature, mild facial dysmorphisms	short stature, hypertelorism, epicanthal fold, micropenis, single palmar creases	Duane anomaly		

Abbreviations are as follows: AS, absence seizure; AtS, atonic seizure; CPS, complex partial seizure; FDS, focal dyscognitive seizure; FS, febrile seizure; GTCs, generalized tonic-clonic seizure; HS, hemiclonic seizure; ID, intellectual disability; MA, myoclonic absence; MS, myoclonic seizure; NCS, nonconvulsive status epilepticus; PPR, photo paroxysmal response; SE, status epilepticus; and TS, tonic seizure.

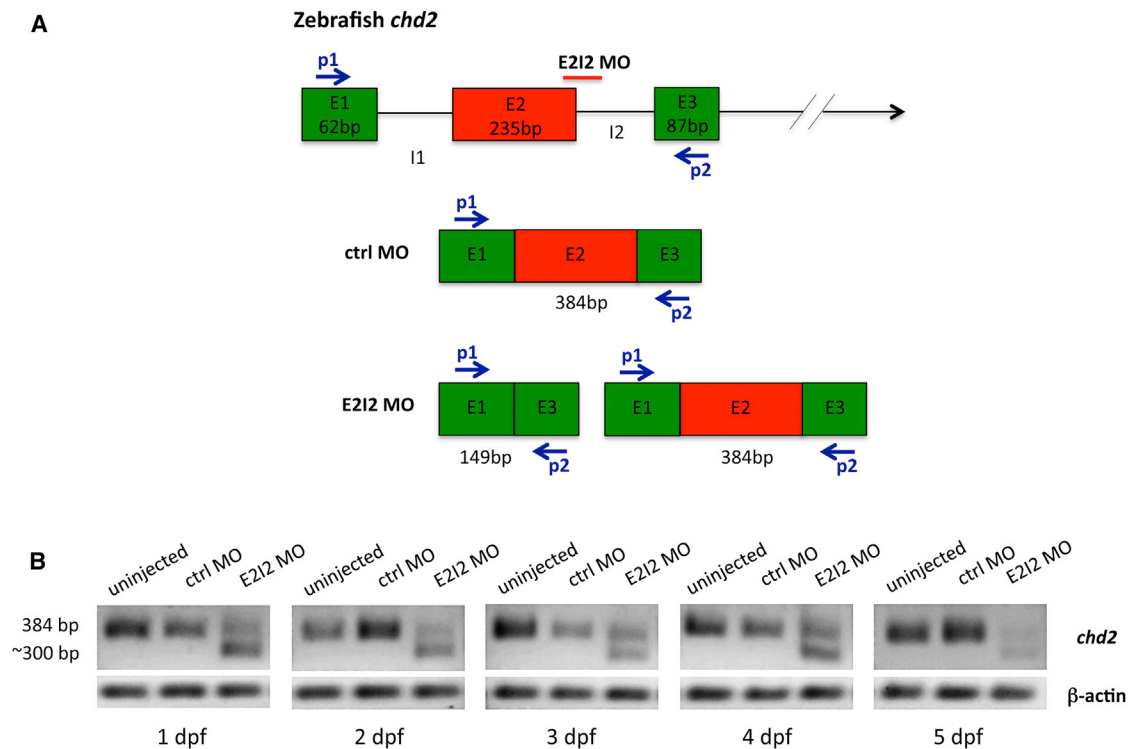


Figure 1. *chd2* Knockdown: E2I2 MO Causes Missplicing of Zebrafish *chd2*

(A) Schematic cartoon showing predicted splicing events in the pre-mRNA of *chd2* E2I2-MO- and ctrl-MO-injected fish. The exons are represented by colored boxes (E1, E2, and E3), and the introns are represented by solid black lines (I1 and I2). The splice MO binding site (E2I2 MO) and the primer binding sites (P1 and P2) used for qPCR are indicated. According to the predictions, PCR products of 384 and 149 bp were expected for ctrl-MO- and E2I2-MO-injected larvae, respectively. In the case of a partial knockdown, both products should be present.

(B) qPCR analysis of MO-knockdown larvae. Normal splicing in ctrl-MO-injected larvae resulted in the predicted 384 bp product, indicating the presence of wild-type mRNA. Abnormal splicing in E2I2-MO-injected larvae resulted in two products: 384 bp (corresponding to the wild-type mRNA) and ~300 bp (an abnormal mRNA), indicating partial knockdown. The larger-than-expected size of the PCR product could have been a result of the activation of a cryptic splice site. The aberrant mRNA was present from 1–5 dpf and was accompanied by the wild-type 384 bp fragment.

E2I2-MO-injected larvae exhibited prominent morphological and behavioral alterations. The E2I2-MO-injected larvae showed from 2 dpf onward multiple developmental abnormalities: pericardial edema, microcephaly, body curvature, absent swim bladder, and stunted growth (Fig-

ure 2). The ctrl-MO-injected larvae were morphologically indistinguishable from the uninjected ones. Visual observation of *chd2* knockdown larvae by means of high-magnification stereomicroscopy revealed that all 4 dpf larvae displayed abnormal movement patterns with frequent

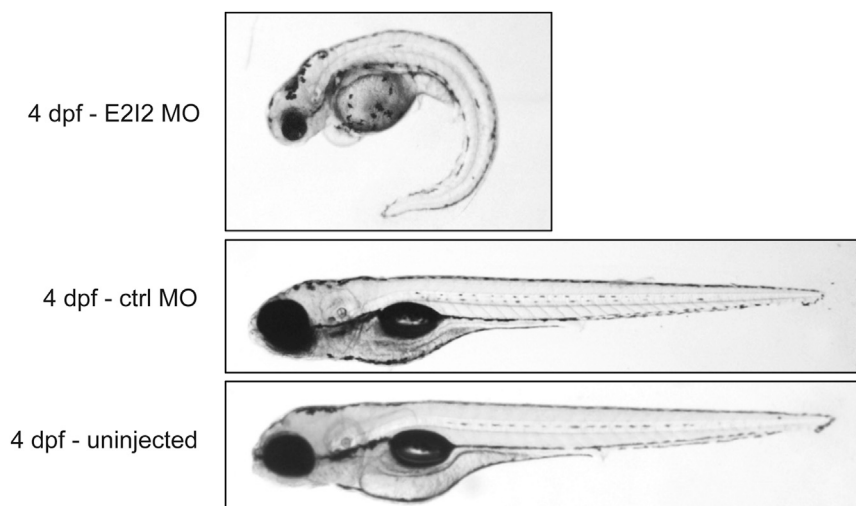


Figure 2. Representative Pictures of 4-Day-Old *chd2* Knockdown and Control Larvae

chd2 E2I2-MO-injected larvae displayed pericardial edema, microcephaly, body curvature, absent swim bladder, and stunted growth (A). Ctrl-MO-injected (B) and uninjected (C) larvae developed normally. The experiment was performed at least three times with 100 embryos injected per condition.

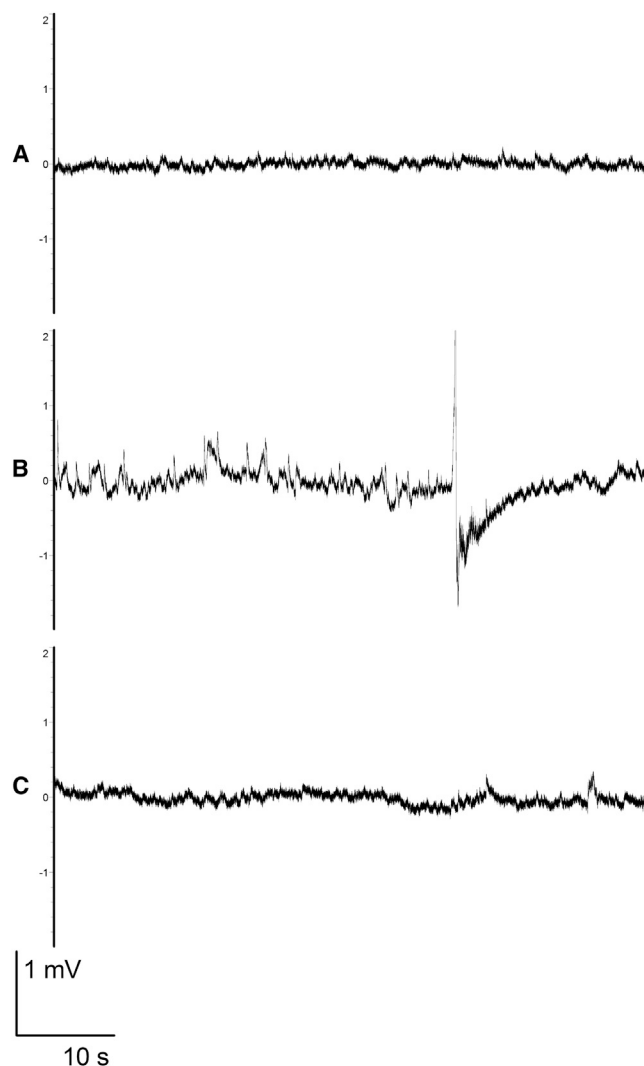


Figure 3. Electrographic Activity of 4-Day-Old *chd2* Knockdown and Control Larvae

(A) Uninjected larva.

(B) *chd2* E2I2-MO-injected larva displaying ictal-like discharges.

(C) Ctrl-MO-injected larva.

One-minute fragments are shown. The y axis shows a 4 mV span. Ictal-like events shown in (B) (more than 3 s in duration) were observed in five out of seven fish larvae in the *chd2* E2I2-MO-injected group.²⁶ Seven, six, and five fish were analyzed for the *chd2* E2I2 MO, ctrl MO, and uninjected larvae, respectively.

whirlpool-like events. Occasionally, we also noticed pectoral-fin and jaw twitching and whole-body trembling. To confirm whether this behavior could have been a result of seizure activity, we performed field-potential recordings on larval tecta as described previously.²⁶ Already from 4 dpf, E2I2-MO-injected larvae displayed epileptiform discharges (Figure 3). Discharges consisted of multiple upward spikes of amplitudes several times larger than those of ctrl-MO-injected or uninjected larvae and with occasional ictal-like patterns. This spiking pattern resembled preictal discharges observed in immature hippocampi of a mouse model of temporal lobe epilepsy.²⁷ These experi-

mental findings suggest that loss of *CHD2* results in an epilepsy phenotype.

CHD2 encodes CHD2, a protein that does not have an established link to neuronal function or hyperexcitability. CHD proteins are assumed to modify gene transcription by affecting chromatin structure through helicase function. A *Chd2*-deficient mouse model has been reported to show spinal abnormalities, renal dysfunction, growth retardation, and susceptibility to tumors, but not epileptic seizures.^{28,29} None of the features described in the mouse model were seen in the probands with de novo *CHD2* mutations, suggesting that in contrast to the zebrafish model presented here, the existing mouse model insufficiently replicates the human phenotype. On the other hand, in addition to showing electrographic seizures observed in zebrafish *chd2* knockdowns, these larvae displayed edemas, body curvature, and stunted growth that could be considered equivalent to the cardiovascular and renal defects, lord kyphosis, and postnatal stunted-growth phenotypes described in *Chd2* mouse mutants.^{28,30,31} Thus, the zebrafish phenocopies aspects of both the human and the mouse spectra. Why no seizure phenotype has been reported for *Chd2* mutant mice remains to be determined. Perhaps these mice have to be monitored with EEG for seizure detection and/or challenged before (presumably) spontaneous seizure behavior can be detected. Additionally, mutations in other genes encoding members of the CHD family have been identified, such as *CHD7* mutations in persons with CHARGE syndrome³² and de novo mutations in *CHD3*, *CHD7*, and *CHD8* in individuals with ASD.^{33,34} Although the exact role of CHD2 in neuronal hyperexcitability remains to be determined, our findings suggest that helicase dysfunction might be a mechanism involved in epileptogenesis and neurodevelopment.

In summary, we identified *CHD2* nonsense mutations as the underlying genetic defect for a fever-sensitive myoclonic EE. In our study, individuals carrying a *CHD2* mutation displayed a spectrum of fever-sensitive generalized seizures similar to those in Dravet syndrome, but seizure onset was clearly later, and unlike in Dravet syndrome, developmental delay could be seen prior to epilepsy onset. Future studies will reveal whether *CHD2* mutations also account for a subset of individuals with more typical Dravet syndrome. We showed that a MO-based knockdown of *chd2* in zebrafish resulted in clinical and electrographic seizures paralleling the human phenotype. Our study further suggests that helicase dysfunction in humans might specifically result in neuronal hyperexcitability in the absence of syndromic or dysmorphic features.

Consortia

Additional members of the EuroEPINOMICS RES Consortium are Rik Hendrickx, Philip Holmgren, Ulrich Stephani, Hiltrud Muhle, Manuela Pendiziwiat, Silke Appenzeller, Kaja Selmer, Eva Brilstra, Bobby Koeleman, Felix Rosenow, Eric Leguern, Katalin Sterbova,

Budisteanu Magdalena, Gherghiceanu Rodica, Oana Tarta Arsene, Barca Diana, Rosa Guerrero-Lopez, Laura Ortega, Albena P. Todorova, Andrey V. Kirov, Angela Robbiano, Mutluay Arslan, Uluç Yiş, and Vanja Ivanović

Acknowledgments

We thank the probands and their families for their cooperation. The research was supported by the EUROCORES program EuroEPINOMICS of the European Science Foundation; funds from the state budget of Romania, managed by the Executive Agency for Higher Education, Research, Development, and Innovation Funding for Project 6-EUROC; the Scientific and Technological Research Council of Turkey; National Science Centre Poland funding for project 800/N-ESF-EuroEPINOMICS/10/2011/0; MH CZ-DRO, University Hospital Motol, Prague (00064203); “Investissements d’avenir” ANR-10-IAIHU-06; the Academy of Finland (grant 141549) and Folkhälsan Research Foundation; Wellcome Trust grants WT089062 and 098051; Academy of Finland grant 251704; Academy of Finland Center of Excellence in Complex Disease Genetics grants 213506 and 129680; the European Community’s Seventh Framework Programme (FP7/2007-2013) ENGAGE Consortium (grant HEALTH-F4-2007-201413); Synaptic Systems grant 242167; the Sigrid Juselius Foundation; European Commission FP7 project 261123 (gEUVADIS); Federal Ministry of Education and Research NGFNplus/EMINet 01GS08123; IonNeuroNet 01GM1105A; German Research Foundation (DFG) Le1030/11-1; the German Society for Epileptology; the Fund for Scientific Research Flanders (FWO); the Flemish government Methusalem excellence grant; and the University of Antwerp. We thank the VIB Genetic Service Facility and the institute of Clinical Molecular Biology in Kiel for providing Sanger sequencing, supported in part by DFG Cluster of Excellence “Inflammation at Interfaces” and “Future Ocean.” We thank technicians S. Greve, S. Arndt, and T. Henke for technical support. G.K. is a member of the DFG-funded Cluster of Excellence “Inflammation at Interfaces.” A.S. is a FWO postdoctoral fellow. T.D. and A.K. are Institute of Science and Technology PhD fellows.

Received: May 8, 2013

Revised: May 28, 2013

Accepted: September 30, 2013

Published: October 24, 2013

Web Resources

The URLs for the data presented herein are as follows:

1000 Genomes Project, <http://www.1000genomes.org>

Beyond the Ion Channel (EuroEPINOMICS blog), <http://channelopathist.net/>

Burrows-Wheeler Aligner (BWA), <http://bio-bwa.sourceforge.net/>

dbSNP, <http://www.ncbi.nlm.nih.gov/projects/SNP>

Decipher, <http://decipher.sanger.ac.uk/>

DeNovoGear, <http://sourceforge.net/projects/denovogear/>

Dindel, <http://www.sanger.ac.uk/resources/software/dindel/>

EuroEPINOMICS Consortium, <http://www.euroepinomics.org/>

Genome Analysis Toolkit (GATK), <http://www.broadinstitute.org/gatk/>

GenomeComb, <http://genomecomb.sourceforge.net>

Multiplex Amplicon Quantification (MAQ), <http://www.multiplicom.com/multiplex-amplicon-quantification-maq>

NHLBI Exome Sequencing Project (ESP) Exome Variant Server, <http://evs.gs.washington.edu/>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>

RefSeq, <http://www.ncbi.nlm.nih.gov/RefSeq>

SAMtools, <http://samtools.sourceforge.net>

References

1. Cross, J.H., and Guerrini, R. (2013). The epileptic encephalopathies. *Handb. Clin. Neurol.* **111**, 619–626.
2. Dravet, C. (2011). The core Dravet syndrome phenotype. *Epilepsia* **52**(Suppl 2), 3–9.
3. Kearney, J.A., Wiste, A.K., Stephani, U., Trudeau, M.M., Siegel, A., RamachandranNair, R., Elterman, R.D., Muhle, H., Reinsdorf, J., Shields, W.D., et al. (2006). Recurrent de novo mutations of SCN1A in severe myoclonic epilepsy of infancy. *Pediatr. Neurol.* **34**, 116–120.
4. Depienne, C., Trouillard, O., Saint-Martin, C., Gourfinkel-An, I., Bouteiller, D., Carpentier, W., Keren, B., Abert, B., Gautier, A., Baulac, S., et al. (2009). Spectrum of SCN1A gene mutations associated with Dravet syndrome: analysis of 333 patients. *J. Med. Genet.* **46**, 183–191.
5. Depienne, C., Trouillard, O., Bouteiller, D., Gourfinkel-An, I., Poirier, K., Rivier, F., Berquin, P., Nabbout, R., Chaigne, D., Steschenko, D., et al. (2011). Mutations and deletions in PCDH19 account for various familial or isolated epilepsies in females. *Hum. Mutat.* **32**, E1959–E1975.
6. Vissers, L.E., de Ligt, J., Gilissen, C., Janssen, I., Stehouwer, M., de Vries, P., van Lier, B., Arts, P., Wieskamp, N., del Rosario, M., et al. (2010). A de novo paradigm for mental retardation. *Nat. Genet.* **42**, 1109–1112.
7. de Ligt, J., Veltman, J.A., and Vissers, L.E. (2013). Point mutations as a source of de novo genetic disease. *Curr. Opin. Genet. Dev.* **23**, 257–263.
8. Li, H., and Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* **26**, 589–595.
9. McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., and DePristo, M.A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303.
10. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R.; 1000 Genome Project Data Processing Subgroup. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079.
11. Albers, C.A., Lunter, G., MacArthur, D.G., McVean, G., Ouwehand, W.H., and Durbin, R. (2011). Dindel: accurate indel calls from short-read data. *Genome Res.* **21**, 961–973.
12. Reumers, J., De Rijk, P., Zhao, H., Liekens, A., Smeets, D., Cleary, J., Van Loo, P., Van Den Bossche, M., Catthoor, K., Sabbe, B., et al. (2012). Optimized filtering reduces the error rate in detecting genomic variants by short-read sequencing. *Nat. Biotechnol.* **30**, 61–68.
13. Conrad, D.F., Keebler, J.E.M., DePristo, M.A., Lindsay, S.J., Zhang, Y., Casals, F., Idaghdour, Y., Hartl, C.L., Torroja, C., Garimella, K.V., et al.; 1000 Genomes Project. (2011). Variation in genome-wide mutation rates within and between human families. *Nat. Genet.* **43**, 712–714.

14. Rauch, A., Wieczorek, D., Graf, E., Wieland, T., Ende, S., Schwarzmayr, T., Albrecht, B., Bartholdi, D., Beygo, J., Di Donato, N., et al. (2012). Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. *Lancet* 380, 1674–1682.
15. Neale, B.M., Kou, Y., Liu, L., Ma'ayan, A., Samocha, K.E., Sabo, A., Lin, C.-F., Stevens, C., Wang, L.-S., Makarov, V., et al. (2012). Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature* 485, 242–245.
16. Carvill, G.L., Heavin, S.B., Yendle, S.C., McMahon, J.M., O'Roak, B.J., Cook, J., Khan, A., Dorschner, M.O., Weaver, M., Calvert, S., et al. (2013). Targeted resequencing in epileptic encephalopathies identifies de novo mutations in *CHD2* and *SYNGAP1*. *Nat. Genet.* 45, 825–830.
17. Allen, A.S., Berkovic, S.F., Cossette, P., Delanty, N., Dlugos, D., Eichler, E.E., Epstein, M.P., Glauser, T., Goldstein, D.B., Han, Y., et al.; Epi4K Consortium; Epilepsy Phenome/Genome Project. (2013). De novo mutations in epileptic encephalopathies. *Nature* 501, 217–221.
18. Capelli, L.P., Krepisch, A.C., Gurgel-Giannetti, J., Mendes, M.F., Rodrigues, T., Varela, M.C., Koiffmann, C.P., and Rosenberg, C. (2012). Deletion of the *RMGA* and *CHD2* genes in a child with epilepsy and mental deficiency. *Eur. J. Med. Genet.* 55, 132–134.
19. Dhamija, R., Patterson, M.C., and Wirrell, E.C. (2012). Epilepsy in children—when should we think neurometabolic disease? *J. Child Neurol.* 27, 663–671.
20. Veredice, C., Bianco, F., Contaldo, I., Orteschi, D., Stefanini, M.C., Battaglia, D., Lettori, D., Guzzetta, F., and Zollino, M. (2009). Early onset myoclonic epilepsy and 15q26 microdeletion: observation of the first case. *Epilepsia* 50, 1810–1815.
21. Li, M.M., Nimmakayalu, M.A., Mercer, D., Andersson, H.C., and Emanuel, B.S. (2008). Characterization of a cryptic 3.3 Mb deletion in a patient with a “balanced t(15;22) translocation” using high density oligo array CGH and gene expression arrays. *Am. J. Med. Genet. A.* 146, 368–375.
22. Lund, C., Brodtkorb, E., Røsbj, O., Rødningen, O.K., and Selmer, K.K. (2013). Copy number variants in adult patients with Lennox-Gastaut syndrome features. *Epilepsy Res.* 105, 110–117.
23. Suls, A., Claeys, K.G., Goossens, D., Harding, B., Van Luijk, R., Scheers, S., Deprez, L., Audenaert, D., Van Dyck, T., Beeckmans, S., et al. (2006). Microdeletions involving the *SCN1A* gene may be common in *SCN1A*-mutation-negative SMEI patients. *Hum. Mutat.* 27, 914–920.
24. Nasevicius, A., and Ekker, S.C. (2000). Effective targeted gene ‘knockdown’ in zebrafish. *Nat. Genet.* 26, 216–220.
25. Summerton, J., and Weller, D. (1997). Morpholino antisense oligomers: design, preparation, and properties. *Antisense Nucleic Acid Drug Dev.* 7, 187–195.
26. Afrikanova, T., Serruys, A.-S.K., Buenafe, O.E.M., Clinckers, R., Smolders, I., de Witte, P.A.M., Crawford, A.D., and Eguerra, C.V. (2013). Validation of the zebrafish pentylenetetrazol seizure model: locomotor versus electrographic responses to antiepileptic drugs. *PLoS ONE* 8, e54166.
27. Derchansky, M., Jahromi, S.S., Mamani, M., Shin, D.S., Sik, A., and Carlen, P.L. (2008). Transition to seizures in the isolated immature mouse hippocampus: a switch from dominant phasic inhibition to dominant phasic excitation. *J. Physiol.* 586, 477–494.
28. Kulkarni, S., Nagarajan, P., Wall, J., Donovan, D.J., Donnell, R.L., Ligon, A.H., Venkatachalam, S., and Quade, B.J. (2008). Disruption of chromodomain helicase DNA binding protein 2 (*CHD2*) causes scoliosis. *Am. J. Med. Genet. A.* 146A, 1117–1127.
29. Marfella, C.G., Henninger, N., LeBlanc, S.E., Krishnan, N., Garlick, D.S., Holzman, L.B., and Imbalzano, A.N. (2008). A mutation in the mouse *Chd2* chromatin remodeling enzyme results in a complex renal phenotype. *Kidney Blood Press. Res.* 31, 421–432.
30. Marfella, C.G., Ohkawa, Y., Coles, A.H., Garlick, D.S., Jones, S.N., and Imbalzano, A.N. (2006). Mutation of the *SNF2* family member *Chd2* affects mouse development and survival. *J. Cell. Physiol.* 209, 162–171.
31. Nagarajan, P., Onami, T.M., Rajagopalan, S., Kania, S., Donnell, R., and Venkatachalam, S. (2009). Role of chromodomain helicase DNA-binding protein 2 in DNA damage response signaling and tumorigenesis. *Oncogene* 28, 1053–1062.
32. Vissers, L.E.L.M., van Ravenswaaij, C.M.A., Admiraal, R., Hurst, J.A., de Vries, B.B.A., Janssen, I.M., van der Vliet, W.A., Huys, E.H.L.P.G., de Jong, P.J., Hamel, B.C.J., et al. (2004). Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat. Genet.* 36, 955–957.
33. O'Roak, B.J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B.P., Levy, R., Ko, A., Lee, C., Smith, J.D., et al. (2012). Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature* 485, 246–250.
34. O'Roak, B.J., Vives, L., Fu, W., Egerton, J.D., Stanaway, I.B., Phelps, I.G., Carvill, G., Kumar, A., Lee, C., Ankenman, K., et al. (2012). Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science* 338, 1619–1622.