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ORIGINAL ARTICLE

Variant Intestinal-Cell Kinase in Juvenile Myoclonic Epilepsy

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ABSTRACT

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BACKGROUND

In juvenile myoclonic epilepsy, data are limited on the genetic basis of networks promoting convulsions with diffuse polyspikes on electroencephalography (EEG) and the subtle microscopic brain dysplasia called microdysgenesis.

METHODS

Using Sanger sequencing, we sequenced the exomes of six members of a large family affected with juvenile myoclonic epilepsy and confirmed cosegregation in all 37 family members. We screened an additional 310 patients with this disorder for variants on DNA melting-curve analysis and targeted real-time DNA sequencing of the gene encoding intestinal-cell kinase (*ICK*). We calculated Bayesian logarithm of the odds (LOD) scores for cosegregating variants, odds ratios in case–control associations, and allele frequencies in the Genome Aggregation Database. We performed functional tests of the effects of variants on mitosis, apoptosis, and radial neuroblast migration in vitro and conducted video-EEG studies in mice lacking a copy of *Ick*.

RESULTS

A variant, K305T (c.914A→C), cosegregated with epilepsy or polyspikes on EEG in 12 members of the family affected with juvenile myoclonic epilepsy. We identified 21 pathogenic *ICK* variants in 22 of 310 additional patients (7%). Four strongly linked variants (K220E, K305T, A615T, and R632X) impaired mitosis, cell-cycle exit, and radial neuroblast migration while promoting apoptosis. Tonic–clonic convulsions and polyspikes on EEG resembling seizures in human juvenile myoclonic epilepsy occurred more often in knockout heterozygous mice than in wild-type mice (P=0.02) during light sleep with isoflurane anesthesia.

CONCLUSIONS

Our data provide evidence that heterozygous variants in *ICK* caused juvenile myoclonic epilepsy in 7% of the patients included in our analysis. Variant *ICK* affects cell processes that help explain microdysgenesis and polyspike networks observed on EEG in juvenile myoclonic epilepsy. (Funded by the National Institutes of Health and others.)

JUVENILE MYOCLONIC EPILEPSY IS THE MOST common form of genetic generalized epilepsy and the most common cause of seizures among adolescents and young adults.[1,2] The disorder is distinguished by the clinical features of myoclonus when the patient awakens from sleep after being sleep-deprived or exposed to alcohol and by a characteristic electroencephalographic (EEG) pattern of bursting, high-amplitude polyspikes, which may be elicited by photic stimulation.[1-4] The disorder affects approximately 8.5 million persons worldwide[5-7] and is treatable with antiepileptic medications, which must often be administered for the patient's lifetime.[4,6] Approximately 16 to 17% of patients have resistance to antiepileptic medications and have intermittent seizures despite treatment.[4-7] Data are limited on the molecular pathology underlying the networks that promote grand mal myoclonic–tonic–clonic convulsions,[3] diffuse high-amplitude polyspikes on EEG,[4] and the subtle microscopic brain dysplasia (microdysgenesis) that occur in patients with juvenile myoclonic epilepsy. (Microdysgenesis is characterized by an increased number of displaced heterotopic neurons in the molecular layer of gray matter and in white matter.[8])

A total of 22 chromosomal loci have been associated with susceptibility to juvenile myoclonic epilepsy.[9,10] Of these loci, 7 house genes that, when variant, cause rare mendelian forms of juvenile myoclonic epilepsy. Of these genes, 6 encode subunits that make up ion channels; the seventh, *EFHC1*, encodes a calcium-binding protein with an EF-hand motif and three domains that have sequences homologous to *Drosophila melanogaster* domains.[10] In most patients with juvenile myoclonic epilepsy, there is no known cause. We investigated whether variants in the gene encoding intestinal-cell kinase (*ICK*)[11] and in genes located close to *EFHC1* on chromosome 6p12 cause juvenile myoclonic epilepsy in Hispanic, European-American, and Japanese patients.

METHODS

ASCERTAINMENT OF FAMILIES

Researchers at the GENESS (Genetic Epilepsies Studies) consortium sites (located in the United States, Mexico, Honduras, Brazil, and Japan) ascertained 334 families through a proband with genetic generalized epilepsy. These patients included 310 probands who had adolescent-onset, awakening myoclonic seizures and polyspike waves measuring 3.5 to 5 Hz or who had juvenile myoclonic epilepsy.[3,4,9,10]

DISCOVERING VARIANTS ASSOCIATED WITH JUVENILE MYOCLONIC EPILEPSY

The first step was to look for a variant that segregated with affected family members of a large pedigree with juvenile myoclonic epilepsy (Family A), which we found among the 334 families with genetic generalized epilepsy (Table 1; and Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). We sequenced the exomes[12] of 4 affected family members (Patients 9, 25, 27, and 30) and two persons who married family members, who were considered to be controls. After observing the presence of a variant in each of the 4 affected members and not in the controls, we went back to Family A and performed bidirectional Sanger sequencing[13] in samples obtained from all 37 members, including spouses, to find out whether the same variant cosegregated with affected status.

The second step was to screen for more variants of the candidate epilepsy gene in the 334 families with genetic generalized epilepsy. We used DNA melting-curve analysis (an assessment of the dissociation characteristics of double-stranded DNA during heating)[14] and targeted real-time DNA sequencing,[15] followed by confirmation on Sanger sequencing.[13]

PROVING PATHOGENICITY OF VARIANTS

The third step was to vet all discovered variants through the guidelines of the National Human Genome Research Institute[16] for investigating causality of sequence variants in human disease and the evidentiary criteria recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG–AMP) for classifying variants as pathogenic or benign.[17]

For each variant discovered in a patient with juvenile myoclonic epilepsy, we calculated Bayesian factor logarithm of the odds (LOD) scores for cosegregating variants,[18] unconditional exact tests and odds ratios for case–control associations,[19] and allele frequencies in the databases of the 1000 Genomes Project,[20] the Genome Aggregation Database (gnomAD),[21,22] and the National Heart, Lung, and Blood Institute GO Exome Sequencing Project (ESP).[23] We also made theoretical

Table 1. Families with Pathogenic *ICK* Variants, According to ACMG–AMP Guidelines.*

Family and Patient Number	Sex	Variant	Clinical Seizure Phenotype and Age at Onset				
			Febrile	Awakening Myoclonic	Awakening Grand Mal TC or MTC	Absence Petit Mal	TC or MTC Only
Family A							
Proband: Patient 9	Male	K305T	1 yr	14 yr	MTC, 14 yr and 18 yr		
Nonproband†							
Patient 1	Female		2 yr			sAbs, 7 yr	
Patient 13	Male		1 yr				
Patient 14	Female		1 yr	1–2 wk			
Patient 23	Female			14 yr	MTC, 14 yr	sAbs, 14 yr	
Patient 25	Male			11 yr	MTC, 20 yr	sAbs, 7 yr	
Patient 30	Female						MTC, 13 yr
Patient 40	Female						MTC, 10 yr
Family D							
Proband: Patient 17	Female	K220E		16 yr	MTC, 16 yr	pAbs, 4–5 yr	
Nonproband: Patient 20	Female					pAbs, 7 yr	
Family E							
Proband: Patient 1	Female	A615T		14 yr			
Nonproband: Patient 4	Male						TC, 14 yr
Family R							
Proband singleton	Male	R632X		20 yr	TC and MTC, 25 yr		

* ACMG denotes American College of Medical Genetics and Genomics, AMP Association for Molecular Pathology, MTC myoclonic–tonic–clonic, pAbs pyknoleptic absence, sAbs spanioleptic absence, and TC tonic–clonic. Expanded versions of these data are provided in Figure S1 and Table S1 in the Supplementary Appendix. Blank cells indicate that the clinical phenotype was not present.
† Among the affected members of Family A, Patients 4, 27, 29, and 33 were clinically asymptomatic with polyspike waves on electroencephalographic recording.

predictions with respect to pathogenicity using algorithms that measure evolutionary conservation at the level of DNA base pairs and that calculate deleterious effects on encoded amino acids.[24] We observed allele frequencies of noncoding regions in the Kaviar Genomic Variant Database,[25] the Haplotype Reference Consortium,[26] and gnomAD.[21,22] We tested variants for their ability to impair the function of the candidate epilepsy gene[27] and determined whether mice that are deficient in the candidate epilepsy gene had convulsions resembling those in patients with juvenile myoclonic epilepsy.[28] Additional details regarding the methods that were used are provided in the Supplementary Appendix.

RESULTS

LINKAGE ANALYSES AND DNA SEQUENCING

Two-point linkage analysis and fine mapping[29,30] in the 37-member Family A with 12 living affected members across four generations confirmed significant linkage to chromosome 6p12.2 with a maximum LOD score of 3.35 for variant D6S257 and of 3.27 for variant D6S1573. (Table 1, and Table S1 and Fig. S1 in the Supplementary Appendix, show the affected status of Family A; Fig. S2 shows the results of linkage analysis.) Haplotype analysis[31] identified a single region at chromosome 6p12.2 that was identical by descent across the affected family members (i.e.,

those who had either juvenile myoclonic epilepsy or polyspike waves observed on EEG) (Fig. S3 in the Supplementary Appendix).

We mapped reads of FASTQ files (a text-based format for storing both a biologic sequence and its corresponding quality scores) from the exomes of four affected members of Family A (Patients 9, 25, 27, and 30) and two unaffected spouses to a reference genome, GRCh37/hg19, and used the Genome Analysis Toolkit[32] to improve alignment quality and variant calling (Fig. S1 in the Supplementary Appendix). We filtered 165,642 single-nucleotide variants (SNVs) and 12,865 insertion–deletion variants (indels) from the proband of Family A with ANNOVAR (a software tool to functionally annotate genetic variants detected from diverse genomes),[33] which left 287 SNVs and 98 indels. On removing variants found in the exomes of the two unaffected spouses, three nonsynonymous SNVs remained for analysis. Variants in TGFBI and PKG2 lay outside the candidate 6p12.2 region. Only variant K305T (c.914A→C) in ICK was located at the chromosome 6p12.2 locus that we identified by linkage, fine mapping, and haplotype analysis[29-31] in Family A.

Sanger sequencing[13] then confirmed cosegregation of the K305T variant with each of the 12 affected members and 3 unaffected members. Of the affected family members, 3 had received a diagnosis of juvenile myoclonic epilepsy, 2 had myoclonic–tonic–clonic convulsions only, 2 had febrile convulsions plus childhood absence seizures or neonatal myoclonias, 1 had febrile convulsions only, and 4 had polyspikes on EEG and were clinically asymptomatic. These results genetically implicated K305T as an autosomal dominant, possibly disease-causing trait.[16,17]

ICK VARIANTS IN OTHER AFFECTED FAMILIES

Among the 334 index patients with genetic generalized epilepsy, including 310 probands with juvenile myoclonic epilepsy, we identified ICK variants in 24 of the 310 probands (8%), using DNA melting-curve analysis[14] and targeted real-time DNA sequencing,[15] followed by confirmation on Sanger sequencing.[13] All 24 probands had juvenile myoclonic epilepsy (Tables S2 and S3 in the Supplementary Appendix). Of the 24 probands, 17 had mixed European and American Indian ancestry, 4 had European ancestry, and 3 had Japanese ancestry (Table S6A in the

Supplementary Appendix). Table S3 lists 25 ICK variants because it includes R272Q, which had been reported in Amish infants with the endocrine-cerebro-osteodysplasia syndrome. Of the 24 patients with juvenile myoclonic epilepsy who had ICK variants, 9 had families with other affected members; in these nine families, we again observed the implicated ICK variant to cosegregate with affected status. Five other persons were singleton probands (Fig. S1 in the Supplementary Appendix). Three other pedigrees (Families N, O, and P) had nonsegregating ICK variants (Fig. S4 in the Supplementary Appendix).
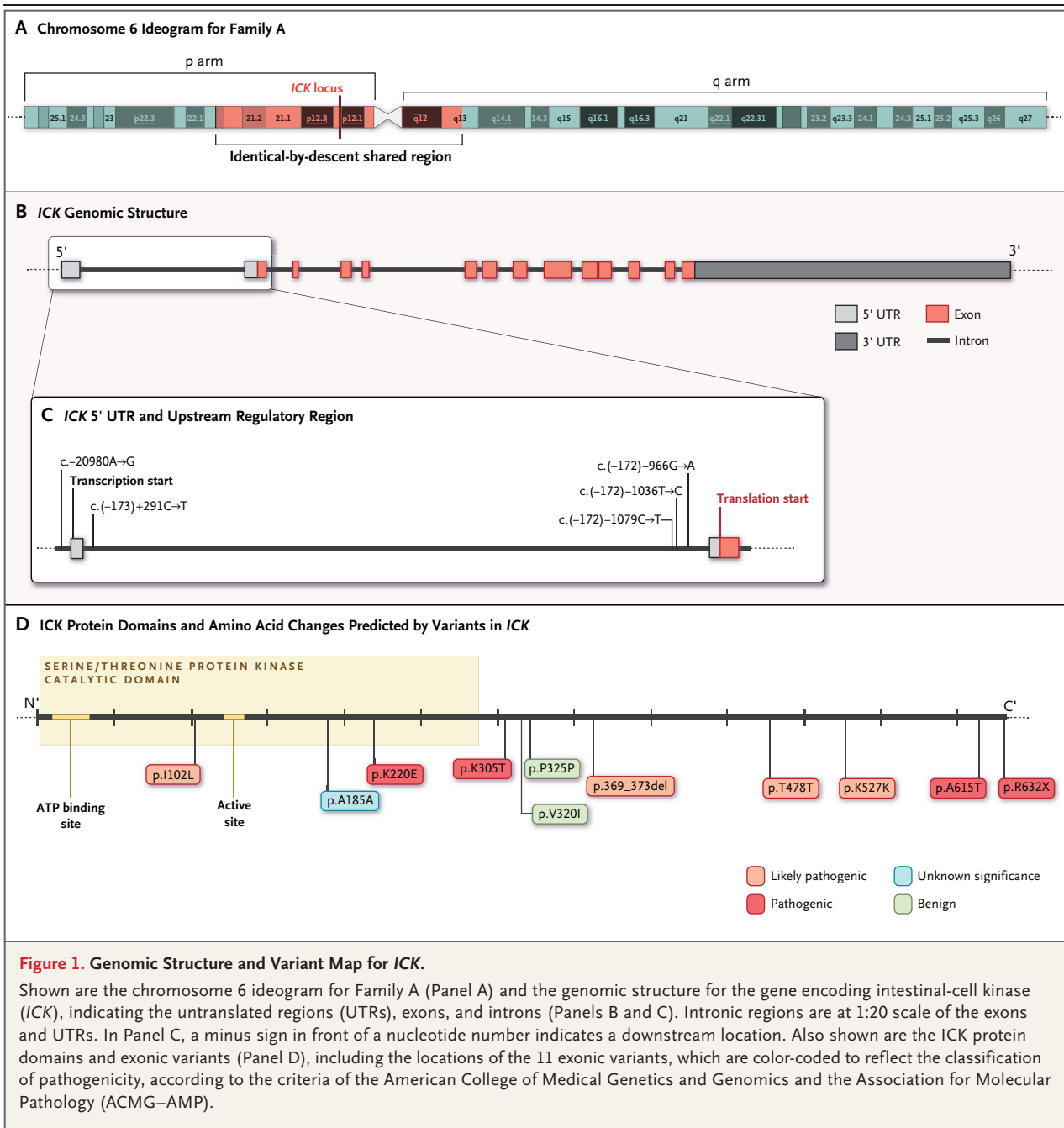
Figure 1 shows the genomic structure of ICK and its encoded protein,[34,35] along with the position and pathogenicity status of 11 variants.[16,17] (A classification of each variant according to ACMG–AMP criteria[17] is provided in Tables S8, S9, and S10 in the Supplementary Appendix.) An expanded view of the region of chromosome 6p that was identical by descent in Family A, including genomic and gene structure and location of variants in ICK,[34,35] is provided in Figure S5 in the Supplementary Appendix.

COINHERITANCE AND BAYESIAN FACTOR LOD SCORES

We used a Bayesian method[18] to evaluate the causality of variants. Only four of the families that we evaluated were sufficiently large (>2 members with epilepsy) to yield significant P values: Family A with K305T (c.914A→C), Family B with I102L (c.304A→C), and Family I with intron variant c.(−172)−966G→A (all with juvenile myoclonic epilepsy) (Fig. S1 in the Supplementary Appendix) and the Amish family with the endocrine-cerebro-osteodysplasia syndrome.[36] According to the guidelines of the National Human Genome Research Institute,[16] these Bayesian factor LOD scores[18] implicate ICK variants in causing juvenile myoclonic epilepsy in Families A, B, and I; the cosegregation in three unrelated families is rated as strong evidence by the ACMG–AMP criteria[17] that variant ICK is causal.

CASE–CONTROL STUDY

A total of 24 variants were tested by the unconditional exact test[19] of homogeneity (binomial model) or independence (multinomial model) with the use of gnomAD population alleles as controls. Of these 24 variants, 13 reached statistical significance and had an odds ratio exceed-
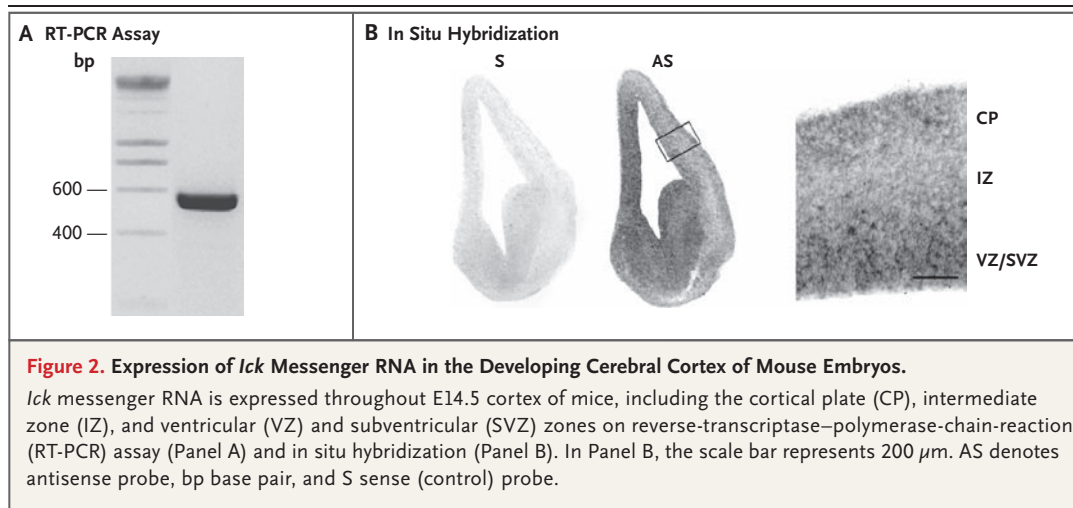
Figure 1. Genomic Structure and Variant Map for *ICK*.

Shown are the chromosome 6 ideogram for Family A (Panel A) and the genomic structure for the gene encoding intestinal-cell kinase (*ICK*), indicating the untranslated regions (UTRs), exons, and introns (Panels B and C). Intronic regions are at 1:20 scale of the exons and UTRs. In Panel C, a minus sign in front of a nucleotide number indicates a downstream location. Also shown are the ICK protein domains and exonic variants (Panel D), including the locations of the 11 exonic variants, which are color-coded to reflect the classification of pathogenicity, according to the criteria of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG–AMP).

ing 5 for the risk of juvenile myoclonic epilepsy (Table S5 in the Supplementary Appendix). In these analyses, we again found strong evidence that *ICK* variants cause juvenile myoclonic epilepsy, according to the ACMG–AMP criteria.[17]

MINOR ALLELE FREQUENCIES

Of the 24 *ICK* variants found in family members with juvenile myoclonic epilepsy, 8 were absent and 12 were extremely rare in the ethnically matched population group in gnomAD.[21,22] One other *ICK* variant (c.1745-73C→G, found in a Mexican patient with juvenile myoclonic epilepsy) is also extremely rare in American Indians but is a benign polymorphism in Africans. Thus, there are 21 *ICK* variants that are absent or extremely rare in the gnomAD in which the allele was found. These 21 *ICK* variants were found in 22 patients with juvenile myoclonic epilepsy out of 310 cases (7%). The presence of *ICK* variant

Figure 2. Expression of *Ick* Messenger RNA in the Developing Cerebral Cortex of Mouse Embryos.

Ick messenger RNA is expressed throughout E14.5 cortex of mice, including the cortical plate (CP), intermediate zone (IZ), and ventricular (VZ) and subventricular (SVZ) zones on reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay (Panel A) and in situ hybridization (Panel B). In Panel B, the scale bar represents 200 μm. AS denotes antisense probe, bp base pair, and S sense (control) probe.

A615T (c.1843G→A), which was present in probands of Families E and F, brings the number of patients with juvenile myoclonic epilepsy who have novel or extremely rare *ICK* variants to 22. Three other *ICK* variants were benign polymorphisms, according to the gnomAD population database.[21,22]

Three variants (c.658A→G, c.304A→C, and c.1843G→A) that were found in Japanese patients are extremely rare in East Asians in gnomAD[21,22] and absent in the 1000 Genomes Project.[20] Two exonic *ICK* variants, c.914A→C in Family A and c.1894C→T in a Japanese family, are absent in almost all populations of gnomAD except among East Asians and American Indians, among whom they are extremely rare (0.0006 for c.1894C→T among East Asians and 0.0012 for c.914A→C among American Indians). In the ESP database,[23] c.1894C→T was also extremely rare (<0.000077), as was c.1843G→A (Table S6A and S6B in the Supplementary Appendix). Allele frequencies of variants in noncoding regions of *ICK* in public databases[21,22,25,26] are provided in Table S6, and predictions of protein pathogenicity, evolutionary nucleotide conservation, splicing consensus, and microRNA binding[24] are provided in Table S7.

EXPERIMENTAL STUDIES IN MICE

ICK was discovered almost two decades ago.[11,34,35] The ICK protein phosphorylates the regulatory-associated protein of the mechanistic target of rapamycin (mTOR) and thereby activates mTOR complex 1,[35] which in turn controls progression from the G1 phase of the cell cycle.[34] It also phosphorylates Scythe (BAT3), a reaper-binding nuclear protein that inhibits apoptosis and cell proliferation.[37] Because *ICK* lies 0.58 Mb away from *EFHC1* in 6p12.2 (in *cis*), we asked whether *ICK* and *EFHC1* share disease pathways and assessed the effects of *ICK* variants K220E, K305T, A615T, R632X, and R272Q on mitosis, neuroblast migration, and apoptosis during cerebral neocortex development.[27] In an analysis of extracts obtained from E14.5 cortexes of mice, we found *Ick* messenger RNA expression in the telencephalon, its various cortical regions, cortical plate, intermediate zone, and ventricular and subventricular zones on reverse-transcriptase–polymerase-chain-reaction assay (Fig. 2A) and in situ hybridization (Fig. 2B).

We introduced constructs encoding wild-type or variant Ick proteins, tagged with enhanced green fluorescent protein (EGFP), and also a construct encoding EGFP on its own, into neural progenitor cells in mouse neocortex by ex vivo electroporation at E14.5.[27] Three days later, we examined the ventricular and subventricular zone, intermediate zone, and cortical plate and observed no significant mean (±SE) differences between brains transfected with EGFP alone (10.7±1.0%) and those transfected with EGFP-tagged Ick (9.7±0.8%) (Fig. 3A and 3B). In contrast, mouse brains transfected with variant Ick showed impaired radial migration. Fewer neurons reached the cortical plate and intermediate zone, with the exception of those transfected with the variant A615T.[27] In the mice transfected with EGFP-tagged variant Ick, there were more electroporated cells in the ventricular and subventricular zone than in mice transfected with EGFP-tagged Ick or EGFP alone.[27] The impaired migration of neural progenitor cells can be ex-

plained by haploinsufficiency or by a dominant-negative effect of *ICK* variants.[27] Our experiments in mice that were lacking a copy of *Ick* support the conclusion that such haplosufficiency impairs the migration of progenitor cells.

Because cortical progenitor proliferation, division, and cell-cycle exit (i.e., the point at which neuronal progenitor cells stop dividing, enter a quiescent resting state, become an immature neuron, and withdraw from the active cell cycle) are tightly coupled to neuronal migration,[34,37] we studied the influence of Ick variants on mitotic progression and cell-cycle exit of cortical progenitor cells. Two days after ex vivo electroporation at E14.5, we stained sections of electroporated brain using an antibody against phosphohistone H3, an M-phase marker for mitotic progression (Fig. 3C).[27] Cells that were electroporated with Ick variants had a significantly lower mitotic index than cells electroporated with wild-type Ick or EGFP (Fig. 3D).

To better understand the effect of variant Ick on cell-cycle exit, we exposed brain slices to a short (1-hour) pulse of bromodeoxyuridine (BrdU) 24 hours after ex vivo electroporation.[27] We fixed brain slices 24 hours later. Triple immunolabeling with antibodies against GFP, BrdU, and Ki67 established the cell-cycle exit index (i.e., the percentage of progenitor cells exiting the cell cycle to become immature neurons in 24 hours) (Fig. 3E). Cells that were electroporated with variant Ick had a significantly lower index than those electroporated with wild-type Ick or EGFP (Fig. 3F).

Because Scythe, a physiologic substrate of Ick, inhibits apoptosis,[37] we analyzed apoptosis in brain slices 2 days after ex vivo electroporation at E14.5 using activated caspase-3 staining (Fig. 3G). Overexpression of EGFP-Ick resulted in a significantly higher rate of apoptosis ($18.8\pm0.8\%$) than EGFP ($8.3\pm0.8\%$) (Fig. 3H). Electroporation of Ick variants K220E, R272Q, K305T, and R632X (but not A615T) further increased apoptosis induced by wild-type protein.[27]

VIDEO AND ELECTROCORTICOGRAPHIC MONITORING OF MOUSE MODEL

We next investigated whether mice lacking a copy of *Ick*[38,39] model human juvenile myoclonic epilepsy. Because sleep that is induced by isoflurane is known to cause seizures in some mouse strains,[28] we videotaped mice that were heterozy-

*A video showing a seizure in a knockout mouse is available at NEJM.org*

Figure 3. Association between *Ick* Mutations and Impairment of Radial Migration, Mitosis, and Cell-Cycle Exit and Increased Apoptosis in Cerebral Cortex of Mouse Embryos.

Panel A shows the distribution of enhanced green fluorescent protein (EGFP), EGFP-tagged wild-type (EGFP-Ick), and mutant Ick (EGFPmut-Ick) in different cortical regions of mice 3 days after ex vivo electroporation at E14.5, along with a scale bar indicating that the percentage of neurons reaching the cortical plate (CP) is significantly lower with mutant Ick than with wild-type Ick (Panel B). Panel C shows the results of immunostaining for phosphohistone H3 (PHH3) (red), a mitotic marker, and a mitotic index showing a significantly lower percentage of mitotic cells with Ick mutations (Panel D). Panel E shows immunolabeling images for bromodeoxyuridine (BrdU) (red) and Ki67 (blue) establishing the percentage of cells exiting the cell cycle in 24 hours and an index for cell-cycle exit than is significantly lower for Ick mutants than for wild-type Ick and EGFP (Panel F). Panel G shows immunostaining with antibody against activated caspase-3 and quantifications showing a higher percentage of apoptosis with wild-type Ick than with EGFP; the percentage is higher with mutant proteins than with wild-type Ick, except for A615T (Panel H). EGFP-mut-Ick images are representative of all the mutations that were tested. In Panels C through H, brain slices that were obtained from E14.5 embryos and electroporated ex vivo with EGFP, EGFP-Ick, or EGFP-mut-Ick were cultured for 2 days. Data are means from three to five independent experiments with 8 to 10 embryos per condition. Error bars indicate standard errors. Scale bars represent 200 μm. In Panels B, D, F, and H, one asterisk indicates a P value of less than 0.05, and two asterisks a P value of less than 0.01.

gous for *Ick* (i.e., carrying a single copy instead of two copies of the gene) and wild-type mice using an infrared camera while the mice were in a state of light sleep induced by 2% isoflurane. Tonic–clonic convulsions occurred significantly more often in mice that were heterozygous for *Ick* than in wild-type mice (P=0.02 by Fisher's exact test) (see video).[28] One week later, we implanted intracranial electrodes over both somatosensory cortexes and electromyography electrodes into cervical muscles of mice that were heterozygous for *Ick* and wild-type mice, which were anesthetized with 2% isoflurane. One week later, we recorded electrocorticograms of freely moving heterozygous *Ick* mice and wild-type mice for 3 days. The freely moving heterozygous mice were the same mice that had previously had tonic–clonic seizures during isoflurane anesthesia, whereas freely moving wild-type mice had not
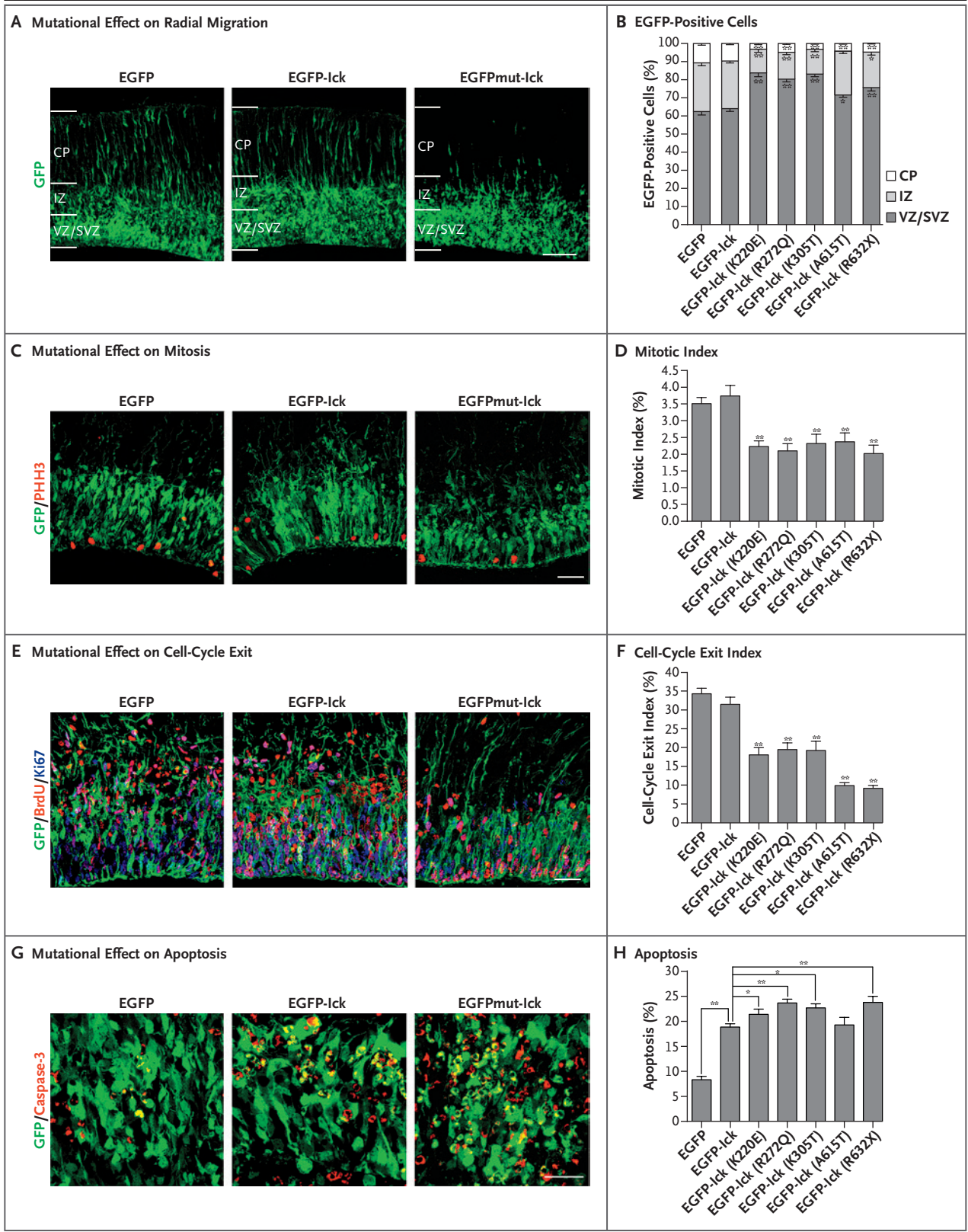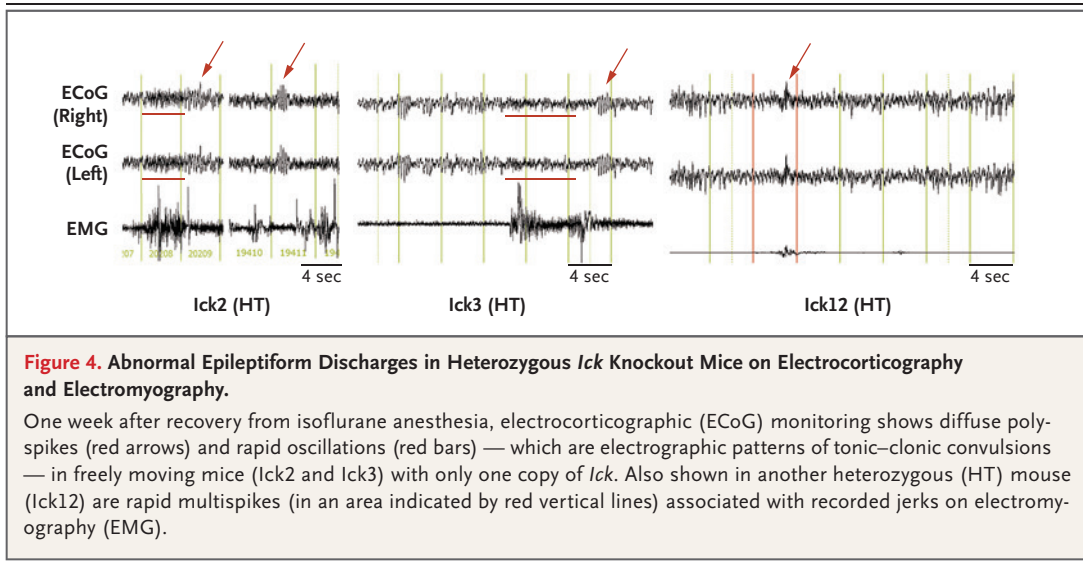
Figure 4. Abnormal Epileptiform Discharges in Heterozygous *Ick* Knockout Mice on Electrocorticography and Electromyography.

One week after recovery from isoflurane anesthesia, electrocorticographic (ECoG) monitoring shows diffuse polyspikes (red arrows) and rapid oscillations (red bars) — which are electrographic patterns of tonic–clonic convulsions — in freely moving mice (Ick2 and Ick3) with only one copy of *Ick*. Also shown in another heterozygous (HT) mouse (Ick12) are rapid multispikes (in an area indicated by red vertical lines) associated with recorded jerks on electromyography (EMG).

had seizures. The heterozygous mice had more diffuse polyspike waves than the wild-type mice (P=0.047) (Fig. 4; and Fig. S8A, S8B, and S8C in the Supplementary Appendix). Diffuse polyspikes and high-frequency oscillations, which are known electrographic patterns of tonic–clonic convulsions, were recorded on the electrocorticograms. Brief diffuse polyspikes, the electrographic signature of myoclonias, were also recorded (Fig. S8D in Supplementary Appendix).

DISCUSSION

The data we obtained through the use of electroporated slices of mouse brain support the conclusion that pathogenic variants in *ICK* cause 7% of cases of juvenile myoclonic epilepsy by disrupting mitosis, neuroblast migration, and apoptosis. Pathogenic variants in *ICK*, similar to pathogenic variants in *EFHC1*,[10,27] cause disease by impairing progenitor-cell migration, which manifests as microdysgenesis.[8] This process results in focal orbitofrontal cortical thickenings, as seen on voxel-based morphometry on magnetic resonance imaging.[10] The orbitofrontal cortical thickening could be a consequence of impaired apoptosis[10] or represent the cerebral cortical component of the cortical–subcortical network (i.e., consisting of the primary motor cortex, orbitofrontal cortex, caudate nucleus head, and cerebellum), which serve to promote myoclonic seizures and diffuse polyspikes on EEG (Fig. S9 in the Supplementary Appendix). However, the

timing of disruption differs between these two genetic forms of juvenile myoclonic epilepsy. Pathogenic variants in *ICK* affect progenitor-cell proliferation, mitosis, and cell-cycle exit and so act at an earlier stage than pathogenic variants in *EFHC1*.[10]

Pathogenic variants in *EFHC1* and *ICK* share some similarities in expressed phenotypes. Homozygous *ICK* R272Q variants produce the fatal neonatal encephalopathy called the endocrine-cerebro-osteodysplasia syndrome[36]; the *EFHC1* F229L variant, when homozygous, also produces a lethal epileptic encephalopathy of infancy.[40] Heterozygous pathogenic variants in *ICK* and *EFHC1* can cause juvenile myoclonic epilepsy with myoclonic–tonic–clonic convulsions in patients who are otherwise neurologically normal. Three carriers in our study were entirely unaffected, as far as we could determine. These results suggest that some pathogenic variants in *ICK* have variable penetrance.

Of the 24 *ICK* variants that we detected in 310 patients with juvenile myoclonic epilepsy, 13 met the criteria for causality according to case association studies, whereas 21 were either absent or extremely rare in the gnomAD population in which the *ICK* allele was found. These significant genetic implications and associations were observed only in 24 patients with juvenile myoclonic epilepsy. None of the patients had photosensitivity,[4] a condition that would not be expected in patients of mixed European and American Indian ancestry with juvenile myoclonic

epilepsy. As observed by other investigators,[1,4,9,10] we report striking variation with respect to epilepsy phenotypes both within and among families. Of 34 affected nonproband family members, 5 (15%) had juvenile myoclonic epilepsy, 10 (29%) had myoclonic–tonic–clonic seizures, 4 (12%) had pyknoleptic petit mal seizures alone or with myoclonic–tonic–clonic seizures, 4 (12%) had febrile seizures alone or with absence seizures or myoclonias, and 11 (32%) were clinically asymptomatic but had polyspikes or focal spikes on EEG. These results strongly suggest that *ICK* is pleiotropic (i.e., a single mutation has many different phenotypic consequences in a patient or among patients) and that epistatic loci with different genes are present in affected family members and interact with *ICK* and contribute to pleiotropism and clinical heterogeneity.

APPENDIX

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