

## ADVANCES IN GENETICS OF JUVENILE MYOCLONIC EPILEPSIES

Antonio V. Delgado-Escueta, MD

Professor of Neurology, David Geffen School of Medicine at University of California, Los Angeles, Comprehensive Epilepsy Program, Epilepsy Genetics/Genomics Laboratories, VA Greater Los Angeles Healthcare System, West Los Angeles, CA

*One by one, mutation-containing mendelian genes that cause monogenic juvenile myoclonic epilepsies (JME) and single nucleotide polymorphisms (SNP)-susceptibility alleles that increase risks for nonmendelian complex JME should fall to the power of molecular genetics. Of 15 chromosome loci, 3 mendelian genes ( $\alpha$ 1-subunit of the GABA<sub>A</sub> receptor [GABRA1], chloride channel 2 gene [CLCN2], and Myoclonin1/EFHC1) and 2 SNP-susceptibility alleles of putative JME genes in epistases (bromodomain-containing protein 2 [BRD2] and connexin [Cx]-36) have been identified, so far. Antiepileptic drugs now can be designed against the specific molecular defects of JME.*

Juvenile myoclonic epilepsies (JME) are primarily genetic in origin. One by one, mendelian epilepsy genes and their mutations that cause monogenic JME will unravel to linkage analyses and positional cloning, using short tandem repeat polymorphisms (microsatellites). At the same time, complex, nonmendelian JME with its susceptibility alleles should be sieved by association analyses and transmission linkage disequilibrium, using single nucleotide polymorphisms (SNPs).

In dominantly inherited epilepsies, the ideal situation for research purposes is to have one large family or a set of large families (each with a minimum of nine affected members over at least three generations) in order to independently prove linkage to the same chromosome site (logarithm of odds [LOD] score  $> 3.3$  at  $\theta=0$  during genome-wide scan, using microsatellites spaced 1 cM apart, or LOD score  $> 3.6$  at  $\theta=0$  during genome wide

scan using microsatellites spaced 0.1 cM apart—see Table 1). In recessive epilepsy traits, six affected offspring belonging to consanguineous matings in one large kindred usually will reveal homozygosities that point to the chromosome site supported by significant Lod scores. Such approaches to mendelian epilepsies have successfully identified mutation-containing genes for autosomal dominant JME (1–3), autosomal recessive Lafora disease (4), and Unverricht Lundborg disease (5).

In epilepsies that are clearly heritable yet do not follow any of the known modes of inheritance, it is thought that multiple genes interact (epistases) and produce epilepsy phenotypes. Because the heritability effects of such genes are significant enough to contribute to the epilepsy phenotype but only produce a minor proportion of the whole trait, they are undetected by traditional linkage analyses. To detect these multiple-gene epilepsy phenotypes, a large number of patients and controls (i.e., 1,200 to 2,000 unrelated index cases and an equal number of age, sex, and racial/geographically matched controls) would need to be enrolled in a genome-wide scan that uses tagging sets of SNPs (300,000 to 500,000 SNPs) and is aided by the 2005 International Haplotype Map (HapMap). This protocol statistically associates an SNP variant with a specific type of epilepsy. It allows construction of tag SNP haplotypes, which often travel together through generations of recombinations and could directly identify the many common DNA variants that underlie susceptibility to epilepsy (6). Such linkage disequilibrium-based case association studies or transmission linkage disequilibrium analyses of trios have not been executed in genetically complex epilepsies. Instead, SNP susceptibility alleles in previously linked chromosome sites have been associated with small cohorts of epilepsies, as will be discussed here.

Table 2 lists 15 JME chromosome loci that have been genetically linked to JME by genome-wide linkage analysis or by linkage analyses that tests specific candidate regions (1). Two mutation-containing ion channel genes,  $\alpha$ <sub>1</sub>-subunit of the GABA<sub>A</sub> receptor (*GABRA1*) (2), and chloride channel 2 gene (*CLCN2*) (3), as well as one non-ion channel gene, *myoclonin1/EFHC1* (4) have been identified as putative JME genes. Mutations in the two ion channel genes have, so far, been unique to a handful of families (1,2). In contrast, mutations in *myoclonin1/EFHC1* are found in 9% of consecutive JME cases from neurology clinics, including singletons, sporadics, and surprisingly also in juvenile absence epilepsy and temporal lobe epilepsy (7,8). In the last 4 years, much progress in JME research has concerned the discovery of the three mendelian JME genes mentioned and the genetic association of SNPs

---

Address correspondence to Antonio V. Delgado-Escueta, MD, Professor of Neurology, David Geffen School of Medicine at University of California, Los Angeles, Epilepsy Genetics/Genomics Laboratories, VA Greater Los Angeles Healthcare System, West Los Angeles, CA. E-mail: escueta@ucla.edu

**TABLE 1.** Identifying Chromosome Loci and Epilepsy Genes

Method for Locating Chromosome Site	Mendelian Epilepsy Genes Genetic Linkage Analyses	Complex Nonmendelian Epilepsy Genes Case Association Studies or Transmission Linkage Disequilibrium (TLD)
Subjects	For dominantly inherited epilepsies <ul style="list-style-type: none"> <li>• Large families (&gt;8 affected in at least 3 generations) and</li> <li>• Medium sized families (&gt;2 affected in at least 3 generations)</li> </ul> For recessive epilepsies <ul style="list-style-type: none"> <li>• Six offspring of consanguineous matings in a large kindred</li> </ul>	For Case Association Studies* <ul style="list-style-type: none"> <li>• 1,200 to 2,000 index cases and matched controls of equal number</li> </ul> For Transmission Linkage Disequilibrium <ul style="list-style-type: none"> <li>• &gt;300 trios (parents and index cases)</li> </ul>
Markers used	>400 microsatellites	Universal tag set of SNPs (>300,000 to 500,000 SNPs)
Refinement/reduction of size of chromosome site by recombination and haplotype analyses and linkage disequilibrium	Necessary	SNP haplotype may directly lead to the putative epilepsy variants
Mutation analyses	Mutations segregate with affected members of many generations in many unrelated families from separate cohorts, ideally of different geographic and ancestral origin	For Case Association Studies: <ul style="list-style-type: none"> <li>• SNPs associate with index cases more than by chance</li> </ul> For Transmission Linkage Disequilibrium <ul style="list-style-type: none"> <li>• SNPs are transmitted to index case more than by chance</li> </ul>
Replication	Usual	Difficult
Proof of causality	Knockout or knockin mice reproduce one or all phenotypes of epilepsy syndrome	Difficult

\*More than 50 associations with epilepsy syndromes have been reported (48); none have been replicated except the linkage based/preceded association with *BRD2* and *Cx-36*.

of bromodomain-containing protein 2 (*BRD2*) (9,10) and of Connexin (*Cx*)-36 (11) with genetically complex JME. These are the subjects of this review.

### Phenotypes of JME

JME is the most common form of idiopathic generalized epilepsies and the most common cause of primary grand mal seizures. JME accounts for 12% to 30% of all epilepsies in hospitals and clinics (12–15), while a house-to-house population survey estimates and undercounts JME at 3% (16). Two major and two minor subsyndromes form the majority of JME (17). The first major subsyndrome is classic JME with no absences and accounts for 72% of all JME patients. Awakening myoclonias start at 13 to 15 years, and grand mal convulsions appear 2 years later. Absence seizures are not present in probands and do not appear as the sole phenotype in affected nonproband family members. The second major subsyndrome, childhood absence

that evolves to JME, affects 18% of JME cases. Pyknoleptic absences with 3 Hz spike and wave complexes and 4 to 6 Hz spike or polyspike waves complexes persist into adolescence, when myoclonias and grand mal convulsions start. Nonproband affected family members can have absence only, grand mal only, or absence and grand mal but rarely have JME. The two minor JME subsyndromes consist of adolescent myoclonias and grand mal convulsions with absences that start during adolescence or after 18 years of age (7%). Even more rare (3%) are atstatic drop seizures, which appear during adolescence, together with myoclonic and grand mal seizures (17).

Neuropathology of some patients with JME reveals increased number of and diffusely distributed dystopic neurons in gray matter stratum moleculare and subcortical white matter of brains (18). Quantitative high-resolution voxel-based MRI detects such JME neuropathology as significantly larger, thicker, and increased cerebral cortical gray matter in mesial frontal lobes (19,20).

**TABLE 2.** Chromosome Loci and Genes Identified in JME

Locus	Locus Name	Gene/ Protein	Country/ Ethnic group	Mode of Inheritance	References
6p12	EJM1a	<i>Myoclonin1/ EFHCT<sup>a</sup></i>	Hispanics [LA, California]	AD	49
			Hispanics [Belize]	AD	50
			Hispanics [Mexico <sup>b</sup> ]	AD	51
			Hispanics [Honduras and Mexico]	AD	52
			Japan	AD	52
			Italy	AD	32
			Tennessee	AD	33
			Austria	AD	8
6p12	EJM1e	–	Netherlands	AD	35,39
6p21.3	EJM1b	<i>BRD2<sup>b</sup></i>	European descent [Los Angeles, California]	AD	53
			European descent [NY <sup>c</sup> ];		21,9
			Germany	AD/oligogenic	10
6p20	EJM1c	–	Germany	AD	54
6p21.2	EJM1d	–	Germany	AR	37
15q14	EJM2	<i>Cx36<sup>b</sup></i>	United Kingdom, Denmark, France, Greece, Portugal, Sweden	AR	55
6q24	EJM3	–	Saudi Arabia	AR	34
2q22-2q23	EJM4	<i>CACNB4<sup>a</sup></i>	Germany	AD	29
5q34 GABRa1	EJM5	<i>GABRA1<sup>a</sup></i>	French Canadian (Quebec, Canada);	AD	1
			France	AD	31
3q26	EJM6	<i>CLCN2<sup>a</sup></i>	Germany	AD	56
18		<i>ME2<sup>b</sup></i>	European descent (New York)	AR	26
10q25-q26	EJM7	–	India (New Delhi)	AD	36
7q32	EJM8	–	Netherlands	AD	35
16p13	EJM9	–	Netherlands	AR	35
13q31.3	EJM10	–	Germany	AR	37
19q13	EJM11	–	Europe and Australia (Germany, Netherlands, UK, France, Italy, Greece)	–	38

<sup>a</sup>Mutations; <sup>b</sup>SNP-associated variants; AD, autosomal dominant; AR, autosomal recessive; JME, juvenile myoclonic epilepsy; pCAE, pyknoleptic childhood absence epilepsy.

### Susceptibility SNPs for Complex Nonmendelian JME

After refining and narrowing a candidate chromosome locus for JME, the intent is to identify mutations that are absent in control populations and that segregate with affected members of multiplex and multigenerational families. In two candidate loci for JME, no mutations were found to segregate with affected family members. Instead, SNPs contained within putative JME genes were statistically associated with a small number of probands. These probands belong to small- or medium-sized multiplex or multigenerational JME families that had originally been genetically linked to chromosome 6p21.3 (human leukocyte antigen [HLA] region) and associated with HLA alleles, as

in New York families of European origin (21,22), and to chromosome 15q14, as in JME families from Sweden and United Kingdom (23).

In 1996, Greenberg and colleagues reported that JME families from New York had a locus within the HLA region, between HLA-DP and HLA-B loci (21,22), expressing both grand mal seizures on awakening and with JME (24). Both Greenberg et al. and Obeid et al. from Saudi Arabia noted that DR13 was significantly associated with JME families (25). These two HLA-association studies suggest that a second JME gene (i.e., aside from the *6p12* gene) may be present within the HLA complex of chromosome 6p. JME families from Los Angeles, Belize, Mexico, Honduras, and Germany do not have significant association with HLA alleles and eventually were

shown to have separate loci in chromosome 6p21.2, 6p20, and 6p12.

In 2003, Pal et al. showed that SNPs within the *BRD2* (*RING3*) gene in chromosome 6p21.3 might be susceptibility alleles (odds ratio 6.5) for autosomal recessive JME families from New York (9). Linkage disequilibrium found two strongly JME-associated SNP variants in the promoter region of *BRD2* and a common variant haplotype in over 50% of 20 probands from families that had produced positive LOD scores for 6p21 during linkage analyses (9).

Greenberg and colleagues (26) again used both case-control and family-based association studies in identifying malic enzyme 2 (*ME2*)-centered, nine-SNP haplotype in chromosome 18q. When present in the homozygous state, the *ME2*-centered nine-SNP haplotype increased the risk for idiopathic generalized epilepsy (odds ratio 6.1). The results of Pal et al. and Greenberg et al. suggest, but do not prove, that the *BRD2* gene in 6p21.3 epistatically interacts with the *ME2* gene in 18q to produce JME (9,26). In 2006, Lorenz et al. (10), tested whether alleles of *BRD2* also conferred susceptibility to the EEG photoparoxysmal response in JME/idiopathic generalized epilepsy families from Germany. Allelic and haplotypes associations were found between the photoparoxysmal response and six *BRD2* polymorphisms; however, the polymorphisms appeared to be protective against, instead of increasing risk for photoparoxysmal response.

Like the New York group, Mas et al. (11) found susceptibility SNPs in JME families from the United Kingdom and Sweden. They sequenced the gene *Cx-36* in 29 JME probands previously linked to the 15q14 locus and did not find any epilepsy-causing mutations that segregated with affected members. Instead, a case-control study found significant association between JME and the c.588T polymorphisms within exon 2 of *Cx-36*. With significant differences in both alleles ( $p=0.03$ ) and genotype ( $p=0.017$ ) frequencies, subjects with the T/T genotype at position 588 had a significantly increased risk for JME (odds ratio 4.3) compared to those with a C/C genotype. HAP2, a haplotype containing c.588T, was significantly associated with JME ( $p=0.03$ ) (8). Hempelmann and colleagues replicated association of c.588T (dbSNP:rs3743123, S196S) within *Cx-36* of 247 German JME patients versus 621 controls (27). They observed an increase in homozygotes (13.4%) in JME compared with controls (8.7%), suggesting the allele increased the risk for JME in the homozygous state or in an autosomal recessive form of transmission.

These susceptibility polymorphisms (i.e., *BRD2* in chromosome 6p21.3; *Cx-36* in chromosome 15q14; and *ME2* in chromosome 18) are hypothesized to act in epistases as minor epilepsy genes in the homozygous state, necessary but not sufficient, alone, to produce a JME phenotype. The challenge with the SNPs is to show what changes in cell function are altered

so as to produce the JME phenotypes. None so far have been tested. A second, more formidable challenge is proving causality (see Table 1). It is a difficult challenge to insert SNPs of putative JME genes in a bacterial artificial chromosome (BAC) vector—together with other SNPs of putative JME genes that they are hypothesized to interact with, in epistases—and then transgenically reproduce one or all of the JME phenotypes. The *BAC* gene targeted approach rarely has been used to prove that interacting polymorphisms can cause a given phenotype, as Wu et al. showed for the “trans” polycystin gene in the mouse model of polycystic kidney disease (28).

### Genes for Monogenic JME

As mentioned, three genes—*GABRA1* in 5q34; *CLCN2* in 3q26; and *myoclonin1/EFHC1* in 6p12—have mutations that segregate with affected members of multigenerational families and have possible mechanisms for producing JME phenotypes, which satisfy two of three criteria for a gene to be called a putative JME-causing gene. The third criterion is the generation of a knockout or knockin mouse model that replicates one or all of the electroclinical and neuropathological phenotypes of JME. Amongst the three putative JME genes, only *myoclonin1/EFHC1* so far has met the third criterion (Suzuki T, Delgado-Escueta AV, Yamakawa K, unpublished data, 2006). Major mendelian JME genes are considered necessary and alone sufficient to produce a JME phenotype.

Calcium channel  $\beta_4$  subunit (*CACNB4*) is not considered a putative JME gene because its mutation did not segregate in affected family members, was found in only one member of a JME family from Germany, and has not been replicated (29). Chloride channel 2 gene (*CLNC2*) mutations segregated in affected members of one large German JME family; mutations also segregated with affected members of two other families with idiopathic generalized epilepsies (2). Mutations in *CLCN2* also have been observed in rare families from Brussels, Belgium (30). Thus far, *GABRA1* receptor gene has been confirmed in one absence family from France and, recently, in a large French Canadian JME family (1,31).

*Myoclonin1/EFHC1* missense, nonsense, frameshift-deletion, and deletion mutations now have been found in 6 separate cohorts, including 20% of the first cohort of 30 Hispanic families from California and Mexico and 9% of a second cohort of 44 clinic index cases from Mexico and Honduras (7). In this second cohort, mutations were found in 3 families, 2 singletons, and a true sporadic with a de novo mutation. In the third cohort of 72 JME families from Japan, only 3% carried a missense mutation in transcript A and a deletion mutation in the 5'UTR promoter region of *myoclonin1/EFHC1* (7). The fourth cohort consisted of 25 JME families from Italy; 20% showed missense mutations in

*Myoclonin1/EFHC1* (32). The fifth cohort consisted of 54 JME families from Tennessee; only one mutation, *R221H*, was found (33). In the sixth cohort *myoclonin1/EFHC1* was used as a probe to define how broad its phenotype was in 61 patients from Austria with various idiopathic epilepsies and in 372 patients with temporal lobe epilepsy (8). Three novel heterozygous missense mutations and one possibly pathogenic variant in the 3'UTR (2014t > c) were found in two patients with juvenile absence, an unclassified epilepsy syndrome, and in one patient with JME. The I174V missense mutation also was found in one patient with temporal lobe epilepsy. Thus, mutations in *myoclonin1/EFHC1* are relatively more common in Hispanic JME populations from California, Mexico, and Honduras and in Caucasians from Italy and Austria. Such mutations are rarely to infrequently seen in Japan and Tennessee. Mutations in *myoclonin1/EFHC1* also may underlie idiopathic juvenile absence and temporal lobe epilepsies. JME-causing mutations or susceptibility SNPs have not yet been discovered in the eight other reported JME chromosome loci (i.e., 6q24 in Saudi Arabia families (34); 16p13 and 7q32 in Dutch photosensitive myoclonic families (35); 10q25-26 grand mal locus in New Delhi families (36); 13p in photosensitive German families; 6p20 and 6p21.2 in German families (37), and 19q13 in European and Australian families (38) or the 6p12 locus of Dutch families (39).

*Myoclonin1/EFHC1* in mouse hippocampal primary culture neurons induces apoptosis that is significantly lowered by mutations and suppressed by SNX-482, an antagonist of R-type voltage-dependent calcium channel  $Ca_v2.3$ . In patch clamp analysis, *Myoclonin1/EFHC1* specifically increases R-type calcium currents, which are reversed by JME mutations (3). In addition, *myoclonin1/EFHC1* is involved in cell division. During mitosis, both C- and N-terminus EGFP tagged *myoclonin1/EFHC1* associates with mitotic structures. In interphase cells, *myoclonin1/EFHC1* localizes with the centrosome, inter-polar microtubules, and spindle poles (40).

### Is JME a Ciliopathy?

*Myoclonin1/EFHC1* may also be involved in cell migration. Ikeda et al. demonstrated that *EFHC1* is the mammalian orthologue of *Chlamydomonas* polypeptide and the *Rib72* gene, which is found within protofilament ribbons of motile cilia (41,42). *EFHC1* expression is most robust in murine ventricular regions and cerebral aqueduct where cilia is present (43,44). Because CSF flow is driven by motile cilia of ependymal cells and establishes a chemical gradient within subventricular zones that is required for migration of neuroblasts (45), King predicts that *EFHC1* mutations will produce dominant negative effects on ciliary functions, impair the chemical gradient in the

subventricular zone, and disturb migration of neuroblasts (43). This hypothesis could provide another explanation for the thick cerebral cortex seen on MRI and for the dystopic or displaced neurons present in stratum moleculare and white matter seen on neuropathology of some JME patients.

Blast searches of *Drosophila* databases, using the human protein sequences, identified two fly homologs of *myoclonin/EFHC1*—CG11048 for *myoclonin1/EFHC1* in 6p12 and CG8959 for *myoclonin2/EFHC2* in Xp11.4 (46). In 2005, Gu et al. (47) also cloned the brain-expressed paralog *myoclonin2/EFHC2* located in chromosome Xp11.3. These authors reported a tentative association between S430y in exon 9 of *myoclonin2/EFHC2* in 97 male JME patients from Germany, providing a possible explanation for increased maternal inheritance in JME.

### Conclusion

JME accounts for at least 3% to as much as 30% of all epilepsies and is primarily genetic. Depending on racial/ethnic and geographic origin, JME trait is transmitted as a mendelian dominant or recessive trait or as complex oligogenic traits. As reviewed, 15 chromosome loci, 3 mutation-carrying major JME genes, and 2 SNP susceptibility genes in epistases have been identified so far. Among mendelian JME genes, *Myoclonin1/EFHC1* is the most common and is involved in cell division, apoptosis, and survival as well as postsynaptic calcium homeostasis via R-type voltage-dependent calcium channel function. There is great potential for improving human health if antiepileptic drugs are designed to counter major genes that cause JME. Drugs designed against genetic epilepsies generally have had broad antiepileptic actions, working against both idiopathic and lesional epilepsies.

### References

1. Cossette P, Liu L, Brisebois K, Dong H, Lortie A, Vanasse M, Saint-Hilaire JM, Carmant L, Verner A, Lu WY, Tian Wang Y, Rouleau GA. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. *Nat Genet* 2002;31:184–189.
2. Haug K, Warnstedt M, Alekov AK, et al. Mutations in *CLCN2* encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. *Nat Genet* 2003;33:527–532.
3. Suzuki T, Delgado-Escueta AV, Aguan K, Alonso ME, Shi J, Hara Y, et al. Mutations in *EFHC1* cause juvenile myoclonic epilepsy. *Nat Genet* 2004;36:842–849.
4. Minassian BA, Lee JR, Herbrick JA, Huizenga J, Soder S, Mungall AJ, Dunham I, Gardner R, Fong CY, Carpenter S, Jardim L, Satishchandra P, Andermann E, Snead OC 3rd, Lopes-Cendes I, Tsui LC, Delgado-Escueta AV, Rouleau GA, Scherer SW. Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonus epilepsy. *Nat Genet* 1998;20:171–174. PMID: 9771710 [PubMed - indexed for MEDLINE].

5. Pennacchio LA, Lehesjoki AE, Stone NE, et al. Mutations in the gene encoding cystatin B in progressive myoclonus epilepsy (EPM1). *Science* 1996;271:1731–1734.
6. De Bakker PIW, Burt NP, Graham RR, et al. Transferability of tag SNPs in genetic association studies in multiple populations. *Nat Genet* 2006;38:1298–1303.
7. Medina MT, Duron RM, Alonso ME, et al. Novel mutations in myoclonin1/EFHC1 in families from Honduras, Mexico and Japan. *Epilepsia* 2005;46(S6):156.
8. Stogman E, Lichtner P, Baumgartner C, Bonelli S, Assem-Hilger E, Leutmezer F, Schmied M, Hotzy C, Strom TM, Meitinger T, Zimprich F, Zimprich A. Idiopathic generalized epilepsy phenotypes associated with different EFHC1 mutations. *Neurology* 2006;67, 11:2029–2031.
9. Pal DK, Evgrafov OV, Tabares P, Zhang F, Durner M, Greenberg DA. BRD2 (RING3) is a probable major susceptibility gene for common juvenile myoclonic epilepsy. *Am J Hum Genet* 2003;73:261–270.
10. Lorenz S, Taylor KP, Gehrman A, Becker T, Muhle H, Gresch M, Tauer U, Sander T, Stephan U. Association of BRD2 polymorphisms with photoparoxysmal response. *Neurosci Lett* 2006;400:135–139.
11. Mas C, Taske N, Deutsch S, Guipponi M, Thomas P, Covanis A, Friis M, Kjeldsen MJ, Pizzolato GP, Villemure JG, Rees M, Malafosse A, Gardiner M, Antonarakis SE. Association of the connexin 36 gene with juvenile myoclonic epilepsy. *Am J Med Genet* 2004;41. doi:10.1136/jmg.2003.017954.
12. Janz D, Christian W. Impulsive-petit mal. *J Neurol (Zeitschrift fNervenheilkunde)* 1957;176:344–386.
13. Hauser WA, Hesdorffer DC. *Epilepsy—Frequency, Causes and Consequences*. New York: Demos Medical Publishing, 1990:1–51.
14. Panayiotopoulos CP, Obeid T, Tahan AR. Juvenile myoclonic epilepsy: a 5-year prospective study. *Epilepsia* 1994;35:285–296.
15. Delgado-Escueta AV, Enrile-Bacsal F. Juvenile myoclonic epilepsy of Janz. *Neurology* 1984;4:285–294.
16. Nicoletti A, Reggio A, Bartoloni A, Fails G, Sofia V, Bartalesi F, Roselli M, Gamboa H, Salazar E, Osinaga R, Paradisi F, Tempera G, Dumas M, Hall AJ. Prevalence of epilepsy in rural Bolivia: a door-to-door survey. *Neurology* 1999;53:2064–2069.
17. Martínez-Juárez IE, Alonso ME, Medina MT, Durón RD, Bailey JN, López-Ruiz M, et al. Juvenile myoclonic epilepsy subsyndromes: family studies and long term follow-up. *Brain* 2006;129(Pt 5):1269–1280. Epub 2006 Mar 6.
18. Meencke HJ, Veith G. The relevance of slight migrational disturbances (microdysgenesis) to the etiology of the epilepsies. In: Delgado-Escueta AV, Wilson WA, Olsen RW, Porter RJ, eds. *Jasper's Basic Mechanisms of the Epilepsies. Advances in Neurology*. 3rd ed. Vol. 79 Philadelphia: Lippincott Williams and Wilkins, 1999, pp. 123–131.
19. Woermann FG, Free SL, Koepp MJ, Sisodiya SM, Duncan JS. Abnormal cerebral structure in juvenile myoclonic epilepsy demonstrated with voxel-based analysis of MRI. *Brain* 1999;122:2101–2108.
20. Koepp MJ, Hamandi K. *Systems and Networks in Myoclonic Seizures and Epilepsies in Generalized Seizures: From Clinical Phenomenology to Underlying Systems and Networks*. Montrouge, France: John Libbey Eurotext, 2006, pp. 163–182.
21. Greenberg DA, Durner M, Keddache M, Shinnar S, Resor SR, Moshe SL, Rosenbaum D, Cohen J, Harden C, Kang H, Wallace S, Luciano D, Ballaban-Gil K, Tomasini L, Zhou G, Klotz I, Dicker E. Reproducibility and complications in gene searchers: linkage on chromosome 6, heterogeneity, association, and maternal inheritance in juvenile myoclonic epilepsy. *Am J Hum Genet* 2000;66:508–516.
22. Greenberg DA, Durner M, Shinnar S, Resor S, Rosenbaum D, Klotz I, Dicker E, et al. Association of HLA class II alleles in patients with juvenile myoclonic epilepsy compared to patients with other forms of adolescent onset generalized epilepsy. *Neurology* 1996;47:750–755.
23. Emslie FV, Ree M, Williamson MP, Kerr M, Kjeldsen MJ, Pang KA, Sundqvist A, Friis ML, Chadwick D, Richens A, Covanis A, Santos M, Arzimanoglou A, Panayiotoulos CP, Curtis D, Whitehouse WP, Gardiner RM. Genetic mapping of a major susceptibility locus for juvenile myoclonic epilepsy on chromosome 15q. *Hum Mol Genet* 1997;6:1329–1334.
24. Durner M, Sander T, Greenberg DA, Johnson K, Beck-Mannagetta G, Janz D. Localization of idiopathic generalized epilepsy on chromosome 6p in families of juvenile myoclonic epilepsy patients. *Neurology* 1991;41:1651–1655, (These are German families).
25. Obeid T, Raobb MO, Daif AK, Panayiotopoulos CP, Halin K, Bahakim H, Bamgboye E. Is HLA-DRW13 (W6) associated with juvenile myoclonic epilepsy in Arab patients? *Epilepsia* 1994;35:319–321.
26. Greenberg DA, Cayanis E, Strug L, Marathe S, Durner M, Pal DK, Alvin GB, Klotz I, Dicker E, Shinnar S, Bromfield EB, Resor S, Cohen J, Moshe SL, Harden C, Kang H. Malic Enzyme 2 may underlie susceptibility to adolescent-onset idiopathic generalized epilepsy. *Am J Hum Genet* 2005;76:139–146.
27. Hempelmann A, Heils A, Sander T. Confirmatory evidence for an association of the connexin-36 gene with juvenile myoclonic epilepsy. *Epilepsy Res* 2006;71:223–228.
28. Wu G, Tian X, Nishimura X, et al. Trans-heterozygous Pkd1 and Pkd2 mutations modify expression of polycystic kidney disease. *Hum Mol Genet* 2002;11:1845–1854.
29. Escayg A, DeWaard M, Lee DD, et al. Coding and noncoding variation of the human calcium-channel B<sub>4</sub>-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. *Am J Hum Genet* 2000;66:1531–1539.
30. D'Agostino D, Bertelli M, Gallo S, Cecchin S, Albiero E, Garofalo PG, Gambardella A, St Hilaire JM, Kwiecinski H, Andermann E, Pandolfo M. Mutations and polymorphisms of the CLCN2 gene in idiopathic epilepsy. *Neurology* 2004;63:1500–1502.
31. Cossette P, Lortie A, Vanasse M, Saint-Hilaire JM, Rouleau GA. Autosomal dominant juvenile myoclonic epilepsy and GABRA1 In: *Advances in Neurology* Vol. 95, *Myoclonic Epilepsies*. Philadelphia: Lippincott Williams and Wilkins, 2005, pp. 255–264.
32. Labate A, Tarantino P, Carrideo S, Ciro-Candiano IC, Civitelli D, Spadafora P. Mutational analysis of EFHC1 gene in families with JME et al. ASHG, Proceedings 56th meeting, New Orleans, 2006.
33. Ma S, Blair MA, Abou-Khalil B, Lagrange AH, Gurnett CA, Hedera P. Mutations in the GABRA1 and EFHC1 genes are rare in familial juvenile myoclonic epilepsy. *Epilepsy Res* 2006;71:129–34. Epub 2006 Jul 12.
34. Bate L, Mitchell W, Williamson M, Altahan AR, Obeid T, Rees M, Gardiner M. Molecular genetic analysis of juvenile myoclonic epilepsy in the Saudi Arabian population. *Epilepsia* 2000;41:72. Abstract.

35. Pinto D, Westland B, de Haan GJ, et al. Genome-wide linkage scan of epilepsy-related photoparoxysmal electroencephalographic response: evidence for linkage on chromosomes 7q32 and 16p13. *Hum Mol Genet* 2005;14:171–178.
36. Puranam RS, Jain S, Kleindienst AM, Saxena S, Kim MK, Nelly Changizi B, Padma MV, Andrews I, Lestón RC, Tiwari HK, McNamar JO. A locus for generalized tonic-clonic seizure susceptibility maps to chromosome 10q25–26. *Ann Neurol* 2005;58:449–458.
37. Tauer U, Lorenz S, Lenzen KP, Heils A, Muhle H, Gresch M, Neubauer BA, Waltz S, Rudolf G, Mattheisen M, Strauch K, Nurnberg P, Schmitz B, Stephani U, Sander T. Genetic Dissection of photosensitivity and its relation to idiopathic generalized epilepsy. *Ann Neurol* 2005;57:866–873.
38. Hempelmann A, Taylor KP, Heils A, Lorenz S, Prud'Homme JF, Nabbout R, Dulac O, Rudolf G, Zara F, Bianchi A, Robinson R, Gardiner RM, Covanis A, Lindhout D, Stephani U, Elger C, Weber Y, Lerche H, Nurnberg P, Kron K, Scheffer IE, Mulley JC, Berkovic SF, Sander T. Exploration of the genetic architecture of idiopathic generalized epilepsies. *Epilepsia* 2006;47:1682–1690.
39. Pinto D, Louwaars S, Westland B, Volkers L, de Haan GJ, Kasteleijn-Nolst Trenite DGA, Lindhout D, Koelman PC. Heterogeneity at the JME 6p11–12 locus: absence of mutations in the EFHC1 gene in linked Dutch families. *Epilepsia* 2006;47:1743–1746.
40. de Nijs L, Lakaye B, Coumans B, Leon C, Ikeda T, Delgado-Escueta AV, Grisar TM, Chanas G. EFHC1, a protein implicated in juvenile myoclonic epilepsy, associates with the mitotic spindle through its N-terminus. *Exp Cell Res* 2006;312:2872–2879.
41. Ikeda K, Brown J, Yagi T, et al. Rib72, a conserved protein associated with the ribbon compartment of flagellar A-microtubules and potentially involved in the linkage between outer boulet microtubules. *J Bio Chem* 2003;278:7725–7734.
42. Ikeda T, Ikeda K, Enomoto M, et al. The mouse ortholog of EFHC1 implicated in juvenile myoclonic epilepsy is an axonemal protein widely conserved among organisms with motile cilia and flagella. *FEBS Lett* 2005, 579:819–822.
43. King SM. Axonemal protofilament ribbons, DM10 domains and the link to juvenile myoclonic epilepsy. *Cell Motil Cytoskeleton* 2006;63:245–253.
44. Allen Brain Atlas. 2006. *Allen Institute for Brain Science*. Available at: [www.brainatlas.org](http://www.brainatlas.org).
45. Sawamoto K, Wichterle H, Gonzalez-Perez O, et al. New neurons follow the flow of cerebrospinal fluid in the adult brain. *Science* 2006;311:629–632.
46. Delgado-Escueta AV, Daga A, Suzuki T, Martinez-Juarez I, Duron RM, Alonso ME, Bailey JN, Bai DS, Medina MT, Ochoa A, Prado A, Rasmussen I, Rubio-Donnadieu F, Yamakawa K. Juvenile myoclonic epilepsy: myoclonin1/EFHC1 functions. *Epilepsia* 2005; 46(S6):256.
47. Wu W, Sander T, Heils A, Lenzen KP, Steinlein OK. A new EF-hand containing gene EFHC2 on Xp11.4: tentative evidence for association with juvenile myoclonic epilepsy. *Epilepsy Res* 2005;66:91–98.
48. Tan NC, Mulley JC, Berkovic SF. Genetic association studies in epilepsy: “The truth is out there.” *Epilepsia* 2004;45:1429–1442.
49. Liu AW, Delgado-Escueta AV, Serratosa JM, Alonso ME, Medina MT, Gee MN, Cordova S, Zhao HZ, Spellman JM, Ramos Peek JR. Juvenile myoclonic epilepsy locus in chromosome 6p21.2-p11: linkage to convulsions and EEG trait. *Amer J Hum Genet* 1995;57:368–381.
50. Serratosa JM, Delgado-Escueta AV, Posada I, Shih S, Drury I, Berciano JA, Zabala JA, Antunez MC, Sparkes RS. The gene for progressive myoclonus epilepsy of the Lafora type maps to chromosome 6q. *Hum Mol Gen* 1995;4:1657–1663.
51. Bai D, Alonso ME, Medina MT, Bailey JN, Morita R, Cordova S, Rasmussen A, Ramos-Peek J, Ochoa A, Jara A, Donnadiu FR, Cadena G, Yamakawa K, Delgado-Escueta AV. Juvenile myoclonic epilepsy: linkage to chromosome 6p12 in Mexico families. *Am J Med Genet* 2002;113:268–274. PMID: 12439895 [PubMed - indexed for MEDLINE].
52. Medina MT, Suzuki T, Alonso ME, Durón RM, Martínez-Juárez IE, Bailey JN, Bai D, Inoue Y, Yoshimura I, Kaneko S, Montoya MC, Ochoa A, Jara Prado A, Tanaka M, Machado-Salas J, Fujimoto S, Ito M, Hamano S, Sugita K, Ueda Y, Osawa M, Oguni H, Rubio-Donnadieu F, Yamakawa K, Delgado-Escueta AV. Novel Mutations in Myoclonin1/EFHC1 in JME Patients from Honduras, Mexico and Japan, submitted to *Neurology*, 2007.
53. Greenberg DA, Delgado-Escueta AV, Widelitz H, Sparkes RS, Treiman LJ, Maldonado HM, Park MS, Terasaki PI. Juvenile myoclonic epilepsy may be linked to the BF and HLA loci on human chromosome 6. *Am J Med Genet* 1988;31:185–192.
54. Sander T, Bockenkamp B, Hildmann T, et al. Refined mapping of the epilepsy susceptibility locus EJM1 on chromosome 6. *Neurology* 1997;49:842–847.
55. Elmslie FV, Rees M, Williamson MP, et al. Genetic mapping of a major susceptibility locus for juvenile myoclonic epilepsy on chromosome 15q. *Hum Mol Genet* 1997;6:1329–1334.
56. Haug K, Warnstedt M, Alekov AK, Sander T, Ramirez A, Poser B, Maljevic S, Hebeisen S, Kubisch C, Rebstock J, Horvath S, Hallmann K, Dullinger JS, Rau B, Haverkamp F, Beyenburg S, Schulz H, Janz D, Giese B, Muller-Newen G, Propping P, Elger CE, Fahlke C, Lerche H, Heils A. Mutations in CLCN2 encoding a voltage-gated chloride channel are associated with idiopathic generalized epilepsies. *Nat Genet* 2003;33:527–532.