Multiplex families with epilepsy

Success of clinical and molecular genetic characterization

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**Supplemental data
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ABSTRACT

Objective: To analyze the clinical syndromes and inheritance patterns of multiplex families with epilepsy toward the ultimate aim of uncovering the underlying molecular genetic basis.

Methods: Following the referral of families with 2 or more relatives with epilepsy, individuals were classified into epilepsy syndromes. Families were classified into syndromes where at least 2 family members had a specific diagnosis. Pedigrees were analyzed and molecular genetic studies were performed as appropriate.

Results: A total of 211 families were ascertained over an 11-year period in Israel. A total of 169 were classified into broad familial epilepsy syndrome groups: 61 generalized, 22 focal, 24 febrile seizure syndromes, 33 special syndromes, and 29 mixed. A total of 42 families remained unclassified. Pathogenic variants were identified in 49/211 families (23%). The majority were found in established epilepsy genes (e.g., *SCN1A*, *KCNQ2*, *CSTB*), but in 11 families, this cohort contributed to the initial discovery (e.g., *KCNT1*, *PCDH19*, *TBC1D24*). We expand the phenotypic spectrum of established epilepsy genes by reporting a familial *LAMC3* homozygous variant, where the predominant phenotype was epilepsy with myoclonic-atonic seizures, and a pathogenic *SCN1A* variant in a family where in 5 siblings the phenotype was broadly consistent with Dravet syndrome, a disorder that usually occurs sporadically.

Conclusion: A total of 80% of families were successfully classified, with pathogenic variants identified in 23%. The successful characterization of familial electroclinical and inheritance patterns has highlighted the value of studying multiplex families and their contribution towards uncovering the genetic basis of the epilepsies. **Neurology® 2016;86:713-722**

GLOSSARY

BECTS = benign childhood epilepsy with centrotemporal spikes; **CNV** = copy number variant; **FS** = febrile seizures; **FTLE** = familial temporal lobe epilepsy; **GEFS+** = genetic epilepsy with febrile seizures plus; **GGE** = genetic generalized epilepsy; **IPOE** = idiopathic photosensitive occipital epilepsy; **JME** = juvenile myoclonic epilepsy.

Early epilepsy gene discoveries used the strategy of ascertaining very large families, where the family history supported the presence of simple inheritance, and success utilizing parametric linkage analysis was likely.¹ This approach led to the recognition of a number of familial epilepsies and some of their genetic determinants.²⁻⁷ However, the relevance of

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variants in these genes to common epilepsies, where inheritance is complex, remains uncertain.^{8,9}

Epidemiologic studies, including twin analyses, have shown the risk of epilepsy to be higher in the relatives of probands with generalized epilepsy than in the relatives of probands with focal epilepsy.^{10–14} The epilepsy diathesis hypothesis¹⁵ suggested that a familial predisposition for epilepsy exists due to the inheritance of susceptibility variants. In support of this was the discovery that rare inherited copy number variants can increase risk for different epilepsy syndromes.^{16,17}

To pragmatically bridge the divide between classical large family and epidemiologic approaches, this study adopted an intermediate strategy. We conducted an intensive clinical genetic survey of 211 multiplex families ascertained from the ethnically diverse Israeli population. Our objectives were to classify families into broad epilepsy groups, to analyze the inheritance patterns within each familial epilepsy group, and to determine the molecular genetic basis of familial epilepsies.

METHODS **Ascertainment of families.** Families were ascertained during 1998–2008 and molecular analysis is ongoing. Treating neurologists in Israel or surrounding Palestinian territories referred families to the study, which was not designed to be epidemiologic; no attempt towards complete ascertainment was made.

Standard protocol approvals, registrations, and patient consents. The Institutional Review Board of the Tel Aviv Sourasky Medical Center approved the study protocol. Informed consent was obtained from all participants in Hebrew, Arabic, or English, as appropriate.

Inclusion and exclusion criteria. The primary inclusion criterion for families was 2 or more affected relatives with epilepsy. It was required that at least 2 affected family members be no more than third-degree relatives.

Family members with febrile seizures (FS) were considered affected. Relatives with an abnormal EEG showing epileptiform activity but without seizures were considered unaffected.

Individual clinical evaluation. All probands and available family members underwent detailed clinical interviews. Questions were aimed at classifying each seizure type, such as age at onset, evolution, and treatment response. Eyewitness accounts of seizures were obtained wherever possible. Every attempt was made to obtain medical records, including EEG and neuroimaging results.

Seizures and syndromes of affected family members were classified according to the traditional International League Against Epilepsy classifications.^{18,19} Individuals with seizures but without adequate information to make a phenotypic diagnosis remained unclassified.

A minimum requirement for the classification of an individual with a genetic generalized epilepsy (GGE) was a positive EEG report detailing generalized spike-waves in addition to a supportive clinical history. In rare cases with a convincing clinical history (e.g., adolescent-onset myoclonus and tonic-clonic seizures), the EEG requirement was waived.

In the case of focal epilepsy, efforts were made to obtain EEG and imaging data. In the absence of diagnostic EEG data, participants were classified as focal epilepsy where there was strong clinical evidence for focal onset of seizures (e.g., rising epigastric aura with intense *déjà vu* for temporal lobe epilepsy).²⁰

Familial pedigree analysis. Pedigrees were constructed for each family, and relationships between affected family members recorded (i.e., sibling pairs or parent-child pairs). Parent-child pairs were coded as showing maternal or paternal transmission; cases of bilineal inheritance were counted twice. Families were classified as consanguineous when the proband's parents shared a common ancestor.

Familial epilepsy classification. We sought to determine whether syndromes in individuals segregated faithfully in families. Where all affected individuals had diagnoses of generalized epilepsy, focal epilepsy, or rare, yet distinct and classifiable (special) epilepsy syndromes (e.g., progressive myoclonus epilepsy), the family was classified accordingly. This classification was also used when 2 or more participants had the same syndrome despite other relatives remaining unclassified due to inadequate data (e.g., older relatives with childhood seizures). Where there was clear evidence of both generalized and focal epilepsies, the family was classified as mixed. The classification of genetic epilepsy with febrile seizures plus (GEFS+) was used when all relatives had epilepsy syndromes within the GEFS+ spectrum.^{5,21,22} Pure FS families were grouped with these families in a combined GEFS+ and pure FS cohort. A family remained unclassified when it was not possible to diagnose 2 or more family members with a specific epilepsy syndrome.

Based on earlier research,^{23,24} we classified families where individuals had both juvenile myoclonic epilepsy (JME) and idiopathic photosensitive occipital epilepsy (IPOE) as GGE.

Ethnicity classification. Families were classified by self-report and ancestral origin into 3 ethnic groups: Ashkenazi Jewish, Sephardic Jewish, and Arab. The Arab group included Muslim, Druze, and Christian Arab families, including those identified as Bedouin.

Molecular genetic analysis. The molecular genetic approach to each family during 2000–2014 varied (table e-1 on the *Neurology*[®] Web site at Neurology.org). Affected family members were analyzed for variants in genes associated with their epilepsy syndrome. For example, *SCN1A* was Sanger sequenced in all GEFS+ families. Individuals diagnosed with an epileptic encephalopathy were studied by array CGH for copy number variant (CNV) detection²⁵ and targeted resequencing of known and candidate epileptic encephalopathy genes.²⁶ Several larger families were selected for linkage analysis ($n = 18$) either using microsatellites or single nucleotide polymorphism array technologies; this was followed by massively parallel sequencing in 10 families. Variants were deemed pathogenic if they were absent in ethnically matched controls ($n > 100$), resulted in a likely deleterious functional change (e.g., splice site, missense, nonsense mutations), and importantly, familial segregation was consistent with the inheritance model. Potential susceptibility variants were considered for genes previously associated with increased

epilepsy risk. A number of these molecular discoveries have been reported previously (table e-2).

RESULTS Of 242 families ascertained, 31 families (12.8%) were excluded. Reasons for exclusion after evaluation were fewer than 2 affected relatives ($n = 16$), affected members separated by more than 3 meioses ($n = 5$), family study incomplete ($n = 9$), and nonepileptic disorder ($n = 1$).

The remaining 211 families, containing 857 affected individuals (414 male), were analyzed.

Familial epilepsy classification. We classified 169/211 families into 5 broad familial epilepsy groups: generalized ($n = 61$), focal ($n = 22$), GEFS+ and pure FS ($n = 24$), mixed ($n = 29$), and special ($n = 33$). Forty-two families (20%) remained unclassified.

Familial pedigree analysis. Family sizes ranged from 2 to 24 affected individuals (mean 4.1; median 3), although this varied according to different familial epilepsy groups (table 1).

Among the 35 families with at least 6 affected relatives, 7 pedigrees were suggestive of autosomal recessive inheritance, 26 autosomal dominant, and 2 X-linked.

Of the 211 total families, 93% consisted of at least one affected first-degree relative pair (i.e., sibling pair or parent-child pair) (table 1). A total of 89 families contributed to a total of 254 affected parent-child pairs: 139 maternal and 115 paternal.

Ethnicity classification. There were 92 Sephardic families (44%), 49 Ashkenazi (23%), 17 mixed Jewish (8%), and 53 Arab (25%). This approximates the distribution in the Israeli population, where 75.3% identify as Jewish, 20.7% as Arab, and 4.0% as other.²⁷ This distribution was reflected across the generalized, focal, GEFS+ and pure FS, and mixed syndrome groups. The 57 consanguineous families were mainly Arab ($n = 42$) or Sephardic ($n = 13$) and were overrepresented within the special syndromes and unclassified familial groups (table 1).

Molecular genetic findings. Variants deemed causative of the epilepsy syndrome were identified in 49/211 families (23%) (tables 2 and e-2). These included 48 sequence variants and 1 pathogenic CNV. Segregation of these pathogenic variants occurred in a dominant ($n = 32$ families; including 2 de novo), recessive ($n = 15$), and X-linked ($n = 2$) manner. In 3 additional families, a likely susceptibility variant was identified (tables 2 and e-2).

A specific genetic diagnosis was reached most often in families with special epilepsy syndromes (88%) (table 1). Excluding these families, considerable success was still realized, with pathogenic variants identified in 11% of families (20/178) with common epilepsy syndromes. Variants in *SCN1A* ($n = 11$) accounted for the largest proportion of all causative findings.

Clinical and molecular observations in generalized epilepsy families. Families with generalized epilepsies comprised the largest group ($n = 61$). Affected individuals ($n = 179$) were classified into syndromes of JME ($n = 54$; 30%), childhood absence epilepsy ($n = 37$; 21.5%), JME/IPOE ($n = 25$; 14%), juvenile absence epilepsy ($n = 17$; 9.5%), generalized tonic-clonic seizures alone ($n = 15$; 8%), GGE not otherwise specified ($n = 17$; 9.5%), and epilepsy with myoclonic atonic seizures ($n = 14$; 7.5%). In 24 families (39%), probands and affected relatives had concordant GGE syndromes; in 30 families (49%), 2 different GGE syndromes were present; in 6 families (10%), 3 GGE syndromes were observed; and in a single family, 4 GGE syndromes were diagnosed.

A sex bias was observed, with more female participants with GGE compared to male participants ($p = 0.0004$, $\chi^2 = 12.6$ with 1 *df*) (table 1). A greater number of affected mother-child pairs ($n = 45$), compared to affected father-child pairs ($n = 15$), was observed. However, the proportion of affected vs unaffected children for mothers with generalized

Table 1 Familial pedigree analysis results partitioned into broad epilepsy groups

Familial epilepsy group	Mean no. affected individuals per family (median)	Sex of affected individuals, M:F	Families with ≥ 1 sib pair (%)	Families with ≥ 1 parent-child pair (%)	Consanguineous families (%)	Families with pathogenic variants (%)
Generalized ($n = 61$)	3.3 (3)	74:124 ^a	48 (79)	27 (43.5)	12 (19)	1 (1.6)
Focal ($n = 22$)	2.8 (3)	32:29	14 (63.5)	12 (54.5)	1 (4.5)	4 (18)
GEFS+ and pure FS ($n = 24$)	7.6 (5)	96:87	23 (96)	16 (66.5)	5 (21)	9 (37.5)
Mixed ($n = 29$)	3.8 (3.5)	64:46	26 (89.5)	11 (39)	4 (14)	5 (17)
Special ($n = 33$)	4.6 (3.5)	74:78	30 (91)	14 (41)	18 (54.5)	29 (88)
Unclassified ($n = 42$)	3.7 (3)	74:80	31 (74)	10 (24)	17 (40.5)	1 (2.4)
Total ($n = 211$)	4.1 (3)	414:443	172 (81.5)	89 (42)	57 (27)	49 (23)

Abbreviations: FS = febrile seizures; GEFS+ = genetic epilepsy with febrile seizures plus.

^a $p = 0.0004$.

Table 2 Molecular genetic findings across broad familial syndrome groups

Broad familial group	Genetic finding	Familial syndrome solved by genetic finding	Inheritance	No. families
Generalized	<i>SLC2A1</i> mutation	Genetic generalized epilepsy ^a	AD	1
	<i>GABRA1</i> variant ^b	Genetic generalized epilepsy	Complex	1 (Family F)
	<i>SLC2A1</i> variant ^b	Genetic generalized epilepsy	Complex	1 (Family G)
Focal	<i>DEPDC5</i> mutation	Familial focal epilepsy	AD	2
	<i>KCNT1</i> mutation ^c	Autosomal dominant nocturnal frontal lobe epilepsy	AD	1
	<i>SCN1A</i> mutation	Focal epilepsy and migraine	AD	1
GEFS+ and pure FS	<i>SCN1A</i> mutation	GEFS+ and Dravet	AD/de novo	8
	Chr 7q21 deletion ^c	GEFS+	AD	1
	<i>CACNA1H</i> variant ^b	GEFS+	Complex	1 (Family H)
Mixed	<i>KCNQ2</i> mutation	Benign familial neonatal epilepsy and focal epilepsy	AD	1 (Family A)
	<i>SLC2A1</i> mutation	Epilepsy and paroxysmal dystonia ^a	AD	1 (Family B)
	<i>SCN1A</i> mutation	Dravet ^a	De novo	1 (Family C)
	<i>LG11</i> mutation	Familial temporal lobe epilepsy ^a	AD	1 (Family D)
	<i>SLC2A1</i> mutation	Genetic generalized epilepsy ^a	AD	1 (Family E)
Special	<i>PCDH19</i> mutation ^c	Epilepsy and mental retardation in females	X-linked	2
	<i>SLC2A1</i> mutation	GLUT1 deficiency	AD	1
	<i>PRRT2</i> mutation ^c	Benign familial infantile epilepsy	AD	5
	<i>KCNQ2</i> mutation	Benign familial neonatal epilepsy	AD	4
	<i>SCN2A</i> mutation	Benign familial neonatal/infantile epilepsy	AD	1
	<i>KCNC1</i> mutation ^c	PME, myoclonus epilepsy and ataxia due to potassium channel mutation	AD	1
	<i>CSTB</i> expansion	PME, Unverricht-Lundborg disease	AR	4
	<i>EPM2A</i> mutation	PME, Lafora	AR	2
	<i>NHLRC1</i> mutation	PME, Lafora	AR	1
	<i>PRICKLE1</i> mutation ^c	PME with early-onset ataxia	AR	2
	<i>CLN2</i> mutation	PME, late-infantile NCL	AR	1
	<i>LMNB2</i> mutation ^c	PME with early ataxia	AR	1
	<i>TBC1D24</i> mutation ^c	MCD, epilepsy, and mental retardation	AR	1
	<i>LAMC3</i> mutation	MCD, occipital dysplasia (presented as epilepsy with myoclonic-atonic seizures)	AR	1
	<i>GPR56</i> mutation	MCD, bilateral frontoparietal polymicrogyria	AR	1
	<i>VPS13A</i> mutation	Neuroacanthocytosis (presented as temporal lobe epilepsy)	AR	1
Unclassified	<i>SCN1A</i> mutation	Probable GEFS+ and Dravet	AD	1

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; FS = febrile seizures; GEFS+ = genetic epilepsy with febrile seizures plus; PME = progressive myoclonus epilepsy; MCD = malformation of cortical development; NCL = neuronal ceroid lipofuscinosis.

^aFamily includes members diagnosed with epilepsy not solved by genetic findings.

^bSusceptibility allele.

^cContributed to original genetic discovery.

epilepsy (30/111 affected children) vs fathers with generalized epilepsy (15/48 affected children) did not differ ($p = 0.726$, $\chi^2 = 0.1232$ with 1 *df*).

Pathogenic variants in *SLC2A1* were detected in 3 unrelated GGE probands. Importantly, 2 of the 3 families were classified as mixed, and the familial variant was not present in all affected family members (figure 1, families B and E). Likely susceptibility variants in *GABRA1* and *SLC2A1* were identified in 2 further families with generalized epilepsy; neither variant was present in all affected relatives (figure 1, families F and G).

Twelve consanguineous families with generalized epilepsy were suggestive of autosomal recessive inheritance; homozygosity mapping in the 3 largest families was unsuccessful.

Clinical and molecular observations in focal epilepsy families. Focal epilepsies ($n = 22$) accounted for 10.4% of the total cohort. Eight families comprised more than one relative diagnosed with benign childhood epilepsy with centrotemporal spikes (BECTS), benign occipital epilepsy, or atypical BECTS.

Figure 1 Understanding the genetic architecture to the epilepsies; variant segregation in select pedigrees

A. Pathogenic variants

B. Susceptibility variants

Family pedigrees with molecular findings that contribute to our understanding of the genetic basis to mixed epilepsy families (A: families A-E) with both chance occurrence and variable expressivity evident. Three families with likely susceptibility variants (B: families F-H) support the concept of an epileptic diathesis that presumably has a polygenic basis. *Family data used from previous publications; see table e-2 for full references. Family A: Heron et al. (2007) *J Med Genet*; pedigree A. Family B: Afawi et al. (2010) *Epilepsia*. Family C: Harkin et al. (2007) *Brain*; patient 48. Family D: Berkovic et al. (2004) *Neurology*; pedigree ref A. Family E: Arsov, Mullen et al. (2012) *Ann Neurol*; pedigree ref B. Family G: Arsov, Mullen et al. (2012) *Ann Neurol*; pedigree ref G. Family H: Heron et al. (2004) *Ann Neurol*; pedigree ref A.

Seven families were diagnosed with familial temporal lobe epilepsy (FTLE); no causative gene was identified in these families. The FTLE branch of

a family classified as mixed segregated a *LGI1* variant (figure 1, family D). The remaining 7 focal families comprised various focal epilepsy syndromes including

4 autosomal dominant families with pathogenic variants identified in *KCNT1* (n = 1), *DEPDC5* (n = 2), and *SCN1A* (n = 1) (table 2). The family with an *SCN1A* variant had a pattern of infrequent focal seizures associated with classical migraine.

Clinical and molecular observations in GEFS+ and pure FS families. This group comprised 24 families and had the highest proportion of large pedigrees (table 1), generally consistent with autosomal dominant inheritance.

One-third of families in this broad syndrome group were found to have pathogenic variants in *SCN1A* (n = 8) or, in a single family, a 7q21 deletion²⁵ (table 2). A likely susceptibility variant in *CACNA1H*²⁸ was identified in one additional family (figure 1, family H).

This cohort also included 6 pure FS families with 5–12 affected participants. No genetic cause has been identified in these families.

Clinical and molecular observations in special epilepsy families. There were 33 special epilepsy syndrome families, of which the genetic basis to 29 has been solved, all with Mendelian forms of inheritance (table 2). These families include those with benign familial syndromes in the first year of life (10/11 solved), the progressive myoclonus epilepsies (12/13 solved), and syndromes associated with brain malformations or intellectual disability (7/9 solved). These families contributed to the original gene discoveries of *PRICKLE1*,²⁹ *TBC1D24*,³⁰ *LMNB2*,³¹ *PCDH19*,³² *PRRT2*,³³ and *KCNK1*.³⁴

An unexpected observation for an established gene was made in a consanguineous Arab Israeli family comprising 6 affected siblings with a clinical pattern of epilepsy with myoclonic atonic seizures associated with borderline to moderate intellectual impairment and a first cousin with normal intellect and electroclinically mixed generalized and occipital seizures. CT scans in affected family members were unremarkable. Homozygosity mapping linked the potentially causative gene to chromosome 9. Targeted resequencing of the candidate region revealed a single homozygous coding variant (c.4086delA) in *LAMC3*. All living affected family members and one unaffected family member were shown to share the familial homozygous variant (figure 2). Following a report of *LAMC3* mutations in 3 unrelated Turkish patients with epilepsy and a remarkable MRI finding of occipital dysplasia,³⁵ we obtained an MRI on family member IV-13. Neuroimaging revealed the presence of an occipital malformation of cortical development, leading to the reclassification of this family from generalized to a special familial epilepsy syndrome.

Clinical and molecular observations in mixed epilepsy families. Twenty-nine families were classified as mixed, where at least 2 family members were diagnosed with a broadly different form of epilepsy (e.g., focal vs generalized). These families accounted for 14% of the total cohort.

Pathogenic variants were identified in 5 mixed families (table 2). In one family, the *KCNQ2* variant was present in all affected relatives despite the

Figure 2 Special family with occipital dysplasia due to *LAMC3* mutation

LAMC3-positive family pedigree and MRI from IV-13 show occipital dysplasia (white arrows). The primary clinical presentation in this family was that of epilepsy with myoclonic-atonic seizures.

different clinical presentations of benign neonatal epilepsy and focal epilepsy (figure 1, family A). Phenotypic variability was evident in another family with an *SLC2A1* variant where positive family members had epilepsy and/or exercise-induced dystonia; one relative with BECTS tested negative for the familial variant (figure 1, family B). This was one of the 4 mixed families where the genetic finding was relevant to one of the clinical subsets within the pedigree (figure 1, families B-E), leaving the family only partly solved.

Clinical and molecular observations in unclassified families. It was not possible to classify 42 families due to lack of electroclinical information; in one family, a genetic diagnosis was made.

An *SCN1A* variant was identified in an unclassified Arab Israeli family suspected of having a recessive familial focal epilepsy (figure 3), with both parents initially reporting their status as unaffected. One individual (II-1) was included in a screen of epileptic encephalopathy genes leading to the discovery of a heterozygous *SCN1A* variant.²⁶ The variant was found to segregate according to affected status in the children and was also found in the mother; subsequent questioning of the mother revealed a previously denied history of childhood seizures. The clinical picture for the affected children varied considerably in severity. In all but one, the age at onset was around 5–6 months of age, always with convulsive seizures apparently not associated with fever. Three of the children with severe epilepsy were found dead in bed, at 18 months (II-4), 12½ years (II-5), and 17 years (II-6). The diagnosis in 5 of the 8 affected participants was consistent with the broad definition of Dravet syndrome with prominent focal features³⁶; in the 3 remaining affected individuals, the disorder was milder.

DISCUSSION We successfully classified 169 of the 211 families (80%) into broad epilepsy groups.

Molecular genetic findings were made in 52 families: 49 with pathogenic variants and 3 with likely susceptibility variants (table 2). While the majority of findings were made in established epilepsy genes, most frequently in *SCN1A*, 13 families with pathogenic variants played a key role in the original gene discovery (tables 2 and e-2). All pathogenic variants followed a monogenic inheritance pattern and thus provide an opportunity for precise genetic counseling.

Unexpected molecular genetic findings led to clinical reevaluation in 2 families, expanding the phenotypic spectrum for 2 known genes. We report a familial case of occipital dysplasia due to *LAMC3* and expand the phenotype to include a clinical presentation of epilepsy with myoclonic-atonic seizures. We also identified an *SCN1A* pathogenic variant in a family with variable phenotypes; the phenotype for 5 siblings was broadly consistent with Dravet syndrome. This observation was particularly unexpected because Dravet syndrome usually occurs sporadically.

The generalized cohort accounted for the largest number of families (61/211); however, genetic findings in this group were infrequent. JME was the most common GGE subsyndrome and in 39% of families, relatives were concordant for the same subsyndrome. Previous reports that GGE is more common in female patients³⁷ are supported by our data; this is unlikely to be explained by ascertainment bias, as sex skewing was not seen in other broad epilepsy groups (table 1). Preferential transmission from affected mothers³⁸ was not observed.

A total of 83% of classified families comprised affected relatives concordant for the same broad epilepsy syndromic group. This is consistent with the syndromic groups being relatively distinct genetic entities and mixed families being the result of a chance occurrence. Families with variable phenotypes due to a different genetic cause provide further support for this (figure 1, families B–E). In contrast,

Figure 3 Unclassified family with *SCN1A* mutation

SCN1A-positive family pedigree with limited electroclinical data. The phenotype was broadly consistent with Dravet syndrome in severe cases.

variable expressivity of a penetrant gene is a well-established concept for the epilepsies,³⁹ and in 2 mixed families the same pathogenic variant was shown to segregate with different phenotypes (figure 1, families A and B).

Study limitations include that participants were classified with knowledge of the information collected on other relatives. The inability to blind clinicians to family history had the potential to introduce bias into the classification process and perhaps inflate similarities between family members. Ideally all families would have been subjected to the same level of molecular genetic interrogation. Had this been possible, additional genetic discoveries may have been made. A third limitation is that the relatively high rate of consanguinity may limit the generalizability of our molecular success to other populations; consanguineous families are favorable for genetic analysis in less common, recessive epilepsy syndromes. Importantly, the majority of our observations on clinical and inheritance patterns were made in families segregating common epilepsies (e.g., GGE, focal epilepsy, GEFS+), typical of epilepsy in all populations, and a molecular genetic diagnosis was still reached in 11% of these families.

This study highlights the value of focusing genetic research on an enriched sample of familial epilepsies, despite the majority of families being of only small to medium size. A better understanding of the inheritance patterns underlying the common epilepsies, including those reported here, will assist strategies to understand the elusive molecular basis of common complex epilepsies, particularly the GGEs. Outside of the generalized cohort, however, considerable molecular success was realized, emphasizing the progress made for monogenic epilepsies and reinforcing the integration of genetic testing into standard clinical care practices. As the revolutionary technology of massively parallel sequencing moves us toward large cohort studies, we expect intermediate-sized families will continue to contribute strongly to these efforts.

AUTHOR CONTRIBUTIONS

Dr. Afawi drafted/revised the manuscript for content, contributed to acquisition of the data, performed analysis/interpretation of the data, and provided study coordination. K.L. Oliver drafted/revised the manuscript for content, performed analysis/interpretation of the data, and provided study coordination. Dr. Kivity contributed to acquisition and analysis/interpretation of the data and contributed to study coordination. Dr. Mazarib contributed to acquisition and analysis/interpretation of the data and provided study coordination. Dr. Blatt contributed to acquisition and analysis/interpretation of the data and contributed to study coordination. Dr. Neufeld contributed to acquisition and analysis/interpretation of the data and contributed to study coordination. K.L. Helbig revised the manuscript for content and provided study coordination. Dr. Goldberg-Stern contributed to acquisition and analysis/interpretation of the data. Dr. Misk contributed to acquisition and analysis/interpretation of the data. Dr. Straussberg contributed to acquisition and analysis/interpretation of the data. Dr. Walid contributed to

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DISCLOSURE

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Therapeutic compound that relates to discovery of *PCDH19* gene as the cause of familial epilepsy with mental retardation limited to females; is one of the inventors listed on a patent held by Bionomics Inc. on diagnostic testing of using the *SCN1A* gene, WO2006/133508; has received speaker honoraria from UCB; has received unrestricted educational grants from UCB, Janssen-Cilag, and Sanofi-Aventis; and receives/has received research support from the National Health and Medical Research Council of Australia and NINDS. Go to Neurology.org for full disclosures.

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