De novo *SCN1A* mutations in migrating partial seizures of infancy

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ABSTRACT

Objective: To determine the genetic etiology of the severe early infantile onset syndrome of malignant migrating partial seizures of infancy (MPSI).

Methods: Fifteen unrelated children with MPSI were screened for mutations in genes associated with infantile epileptic encephalopathies: *SCN1A*, *CDKL5*, *STXBP1*, *PCDH19*, and *POLG*. Microarray studies were performed to identify copy number variations.

Results: One patient had a de novo *SCN1A* missense mutation p.R862G that affects the voltage sensor segment of *SCN1A*. A second patient had a de novo 11.06 Mb deletion of chromosome 2q24.2q31.1 encompassing more than 40 genes that included *SCN1A*. Screening of *CDKL5* (13/15 patients), *STXBP1* (13/15), *PCDH19* (9/11 females), and the 3 common European mutations of *POLG* (11/15) was negative. Pathogenic copy number variations were not detected in 11/12 cases.

Conclusion: Epilepsies associated with *SCN1A* mutations range in severity from febrile seizures to severe epileptic encephalopathies including Dravet syndrome and severe infantile multifocal epilepsy. MPSI is now the most severe *SCN1A* phenotype described to date. While not a common cause of MPSI, *SCN1A* screening should now be considered in patients with this devastating epileptic encephalopathy. *Neurology*® 2011;77:380–383

GLOSSARY

CNV = copy number variation; **EE** = epileptic encephalopathy; **MPSI** = malignant migrating partial seizures of infancy; **SIMFE** = severe infantile multifocal epilepsy.

Malignant migrating partial seizures of infancy (MPSI) is a severe early infantile epileptic encephalopathy (EE). Onset is typically from 40 days to 3 months (range 1 day–6 months).^{1–3} MPSI is characterized by multifocal bilateral independent seizures rapidly becoming very frequent, if not continuous. Refractory seizures migrate between regions.² Later, infantile spasms may occur.² Regression and severe global developmental delay are usual with cortical visual impairment and acquired microcephaly.³ MPSI is a diagnosis of exclusion.

The age-dependent onset and absence of a known etiology suggest that a genetic origin is likely. No familial cases have been described. Recently rare de novo gene mutations have been implicated in specific EEs. The clinically most relevant epilepsy gene to date, *SCN1A*, encodes the $\alpha 1$ subunit of the neuronal sodium channel. *SCN1A*-related EEs include Dravet syndrome and severe infantile multifocal epilepsy (SIMFE).⁴ Protocadherin 19 (*PCDH19*) mutations

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Study funding: Supported by National Health and Medical Research Council Australia, Thyne Reid Charitable Trusts, MS McLeod Foundation, Methusalem excellence grant of the Flemish government, and the American Epilepsy Society. D.C.R. received funding from the Instituto de Salud Carlos III, Fondo de Investigaciones Sanitarias, Spain.

Disclosure: Author disclosures are provided at the end of the article.

Table 1 Characteristics of patients with MPSI

Patient	Age, y/sex	Seizure onset	Seizure types	Max. daily seizures	Multifocal EEG	MRI	Outcome	Acquired microcephaly	Genetic findings
1	8 y/F	2 wk	MF, HC, SE	15–20	+	Normal (6 mo)	Regression, cardiac arrest, severe ID	+	SCN1A mutation p.R862G
2	20 m/F	2 d	MF, SE	40	+	Normal (18 mo)	Severe ID	NA	Del(2)(q24.2q31.1)
3	7 y/M	2 mo	MF, SE	70	+	Normal (6 mo) DM (1.5 y)	Regression, severe ID	+	Negative
4	7 y/F	3 d	MF, SE	>20	+	Increased T2 in right hemisphere (3 mo)	Regression, severe ID	+	Negative
5	10 y/F	3 mo	MF, HC, SE, spasms	100	+ Hypsarr	Moderate atrophy, DM	Regression, severe ID	+	Negative
6	9 y/F	3 wk	MF, spasms	N/A	+	Moderate atrophy, DM	Regression, severe ID	+	Negative
7	4 y/M	5 wk	MF, SE	70	+	Normal	Regression, severe ID	NA	Negative
8	4 y/F	1 d	MF	"Too many to count"	+	Normal (16 mo)	Regression, severe ID	+	Negative
9	3 y/F	1 d	MF, HC, SE	>50	+	Normal (22 mo)	Regression, severe ID	+	Negative
10	4 y/F	3 wk	"MPSI"	>100	+	Normal	Severe ID	NA	Negative
11	Deceased 5 mo/M	1 d	MF, SE	Recurrent SE	+	Normal	Regression, severe ID, deceased 5 mo	+	Negative
12	Deceased 22 mo/M	3 d	MF	30	+	Normal	Severe ID, deceased 22 mo	NA	Negative
13	3 y/F	2 wk	MF, SE	>10	+	Normal (4 mo)	Severe ID	–	Negative
14	5 y/F	3 mo	MF, SE	15–20	+	Normal (29 mo)	Severe ID, can walk	+	Negative
15	3 y/F	3 wk	"MPSI," spasms	N/A	+ Hypsarr	DM (11 mo)	Severe ID	+	Negative

Abbreviations: DM = delayed myelination; HC = hemiclonic; Hypsarr = hypsarrhythmia; ID = intellectual disability; MF = multifocal; MPSI = malignant migrating partial seizures of infancy; NA = not available; SE = status epilepticus.

have been identified in Dravet-like girls. Mutations in the cyclin-dependent kinase-like protein type 5 (*CDKL5*) gene cause a severe EE with early onset tonic seizures and spasms, mainly in girls. Mutations in the mitochondrial DNA polymerase γ type 1 (*POLG*) gene include encephalopathy and early-onset epilepsy. Ohtahara syndrome and early onset EE are associated with syntaxin binding protein 1 (*STXBPI*) mutations.⁵

We screened children with MPSI for mutations in *SCN1A*, *CDKL5*, *STXBPI*, *PCDH19* (females only), and the 3 common European mutations of *POLG*. Where possible, array CGH was performed to identify pathogenic copy number variations (CNV).

Here, we implicate *SCN1A* as causative in some patients with MPSI.

METHODS **Clinical methods.** Fifteen patients with MPSI were referred from Australia, Sweden, Canada, and the United States. The MPSI phenotype was diagnosed according to the following criteria: onset under 6 months; presentation with focal or hemiclonic seizures, with progression to frequent, almost continuous, multifocal seizures; developmental arrest at seizure onset and progressive cognitive decline with

clusters of seizures; and no etiology identified despite extensive investigation.

Molecular screening. Genomic DNA was extracted from peripheral blood leukocytes. *SCN1A* and *CDKL5* mutations were screened by denaturing high-pressure liquid chromatography and characterized by direct sequencing as previously published.^{4,6} *SCN1A* MLPA testing was also performed for negative cases. Three common *POLG* mutations of ancient European origin (p.W748S, p.A467T, and p.G848S) were analyzed using a standard multiplex single nucleotide polymerase extension reaction method.⁷ *STXBPI* was sequenced for point mutations and multiplex amplicon quantification performed for CNV as previously described.⁵ Microarray analysis was performed using commercially available exon-focused oligonucleotide arrays with either 105,000 (1 patient) or 720,000 (11 patients) probes.

Standard protocol approvals, registrations, and patient consents. The Austin Health Human Research Ethics Committee approved this study. Informed consent was obtained from the parents or guardians of patients.

RESULTS The clinical and molecular details of 15 patients with MPSI are presented in table 1 and table e-1 and appendix e-1 on the *Neurology*[®] Web site at www.neurology.org. Two patients had pathogenic de novo changes involving the *SCN1A* gene. No mutations were found in *CDKL5* (13 cases), *STXBPI* (13 cases), or *PCDH19* (9 females). The 3 most common European autosomal recessive mutations of *POLG*

were not found in 11 patients tested. Of 12 patients tested for CNV, one had a large pathogenic deletion of chromosome 2.

Patient 1. Molecular analysis detected a de novo base pair change c.2584C>G in *SCN1A* resulting in the missense mutation p.R862G in exon 14. This changes an arginine to glycine and affects charge and polarity in the voltage sensor segment S4 of the second domain of the protein.

Patient 2. Patient 2 had a de novo 11.06 Mb deletion on chromosome 2(q24.2q31.1) that resulted in the loss of more than 40 genes. The deletion includes *SCN1A* as well as other sodium channel subunit genes *SCN2A*, *SCN3A*, *SCN7A*, and *SCN9A*.

DISCUSSION There have been no genes implicated as causing MPSI in the literature. Here, we identified pathogenic genetic abnormalities in 2/15 (13%) patients, including a *SCN1A* missense mutation and a deletion that encompasses the entire *SCN1A* gene as well as other sodium channel subunit genes. Interestingly, the p.R862G missense mutation affects the same amino acid (Arg862) previously identified as a de novo mutation in a patient with Dravet syndrome (p.R862Q; unpublished data), confirming its likely pathogenic nature.

Nineteen patients with variable deletions of chromosome 2(q24-q31) have been reported, including 12 with epilepsy.⁸ While the epilepsy syndromes were generally mixed, one boy with a chromosome 2q24.3q31.1 10.4 Mb deletion shared some features with MPSI but the authors concluded that he did not fulfill the criteria.⁹ He had dysmorphic features and multiple seizure types including spasms and atonic seizures. Since one of the *SCN1A* mutations that we report is a missense mutation, this suggests that adjacent genes within the microdeletion of the other case are not necessarily involved in producing MPSI. However, modifier genes or genetic background may be responsible for the more severe phenotype in these cases with MPSI than the similar missense mutation and microdeletions that cause Dravet syndrome.

SCN1A mutations are associated with a spectrum of epilepsies, which vary markedly in severity from severe infantile epileptic encephalopathies to benign febrile seizures in genetic epilepsy with febrile seizures plus. The most severe *SCN1A* phenotype until now has been Dravet syndrome, which is less severe than MPSI and has a later onset age, normal early development, different seizure semiology, and evolution. de Novo *SCN1A* mutations are found in 70% of patients with Dravet syndrome.⁴ MPSI bears more resemblance to the *SCN1A* EE of SIMFE, as they share an electroclinical pattern of refractory multifo-

cal seizures, but patients with SIMFE have early normal development prior to later regression and a better outcome than MPSI.⁴ Our 2 cases extend the most severe end of the phenotypic spectrum of *SCN1A* epilepsies to include MPSI.

Previous genetic investigation of MPSI in 3 patients did not reveal mutations in *SCN1A*, *SCN2A*, *KCNQ2*, *KCNQ3*, and *CLCN2*.¹⁰ CNV within *SCN1A* was also excluded in our cases. However, *SCN1A* mutations explain only 2/15 (13%) of our cases, suggesting that genetic heterogeneity underlies MPSI. Although the numbers examined in MPSI remain relatively small, our findings fail to support a role for *CDKL5*, *STXBPI*, *PCDH19*, and *POLG*, but indicate that a proportion of MPSI may have pathogenic CNV.

More than 60 cases of MPSI have been published over 15 years. Increased awareness of this underrecognized syndrome and advances in molecular technology will facilitate the discovery of genetic causes that in turn will inform the neurobiology of this devastating disorder. Furthermore, genetic screening facilitates early diagnosis and avoids the burden of multiple investigations such as liver and muscle biopsies and epilepsy surgery evaluation in children with severe early-onset epilepsies. Thus early genetic screening is cost-effective in patients with MPSI and may help guide management and genetic counseling. In particular, the revolution in molecular karyotyping has been associated with an increasing yield in terms of etiologic diagnosis, is becoming less expensive, and is now a first-tier diagnostic test in severe epilepsies and mental retardation. Now, *SCN1A* screening should be considered in patients with MPSI as one of the genetic etiologies of this devastating epileptic encephalopathy.

AUTHOR CONTRIBUTIONS

Design or conceptualization of the study was performed by D. Carranza Rojo and I.E. Scheffer. Analysis or interpretation of the data was performed by D. Carranza Rojo, L. Hamiwka, J.M. McMahon, L.M. Dibbens, T. Arsov, A. Suls, T. Stödlberg, K. Kelley, E. Wirrell, B. Appleton, M. Mackay, J.L. Freeman, L.T. Bienvenu, P. De Jonghe, D.R. Thorburn, H.C. Mefford, and I.E. Scheffer. Drafting or revising the manuscript for intellectual content was performed by D. Carranza Rojo, S.C. Yendle, S.F. Berkovic, J.C. Mulley, and I.E. Scheffer.

ACKNOWLEDGMENT

The authors thank the patients and their families for participating in the research; and Dr. Christopher Burke, Royal Children's Hospital, Brisbane, and Prof. Martin Delatycki, Austin Health, Melbourne, who referred patients.

DISCLOSURE

Dr. Carranza Rojo reports no disclosures. Dr. Hamiwka receives research support from the Epilepsy Foundation. J.M. McMahon reports no disclosures. Dr. Dibbens may accrue future revenue on a pending patent re: Therapeutic compound that relates to discovery of *PCDH19* gene as the cause of familial epilepsy with mental retardation limited to females; and receives/has received research support from the National Health and

Medical Research Council of Australia, the Thyne Reid Charitable Trusts, and the MS McLeod Trustees. Dr. Arsov, Dr. Suls, Dr. Stöberg, and Dr. Kelley report no disclosures. Dr. Wirrell serves on the editorial boards of *Epilepsia*, *Canadian Journal of Neurological Sciences*, and the *Journal of Child Neurology*; and receives research support from the Mayo Foundation. Dr. Appleton has received funding for travel from UCB. Dr. Mackay has received speaker honoraria from UCB and Janssen-Cilag. Dr. Freeman and S.C. Yendle report no disclosures. Dr. Berkovic has served on scientific advisory boards for UCB and Janssen-Cilag; serves on the editorial boards of *Lancet Neurology* and *Epileptic Disorders* and the Advisory Board of *Brain*; may accrue future revenue on a pending patent re: Therapeutic compound that relates to discovery of *PCDH19* gene as the cause of familial epilepsy with mental retardation limited to females; 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 The University of Melbourne. Dr. Bienvenu reports no disclosures. Dr. De Jonghe has received speaker honoraria from Biocodex; has received funding for travel from UCB and Biocodex; serves on the editorial board of *Acta Neurologica Belgica*; and receives/has received research support from the National Fund for Scientific Research of Flanders, The University of Antwerp, and The Belgian Science and Policy Office. Dr. Thorburn serves on the editorial board of *Mitochondrion* and receives/has received support from the National Health and Medical Research Council of Australia, the Muscular Dystrophy Association (USA), The Juvenile Diabetes Research Foundation, the SMILE Foundation, the Jenour Foundation, the Australian Mitochondrial Disease Foundation, and the Foundation for Children. Dr. Mulley has served as a consultant for Athena Diagnostics; may accrue future revenue on a pending patent re: Therapeutic compound that relates to discovery of *PCDH19* gene as the cause of familial epilepsy with mental retardation limited to females; and receives/has received research support from the National Health and Medical Research Council of Australia. Dr. Mefford receives research support from the NIH/NINDS and is a recipient of the Burroughs Wellcome Fund Career Award for Medical Scientists. Dr. Scheffer has served on scientific advisory boards for UCB and Janssen-Cilag EMEA; serves on the editorial boards of the *Annals of Neurology* and *Epileptic Disorders*; may accrue future revenue on a pending patent re: Therapeutic compound that relates to discovery of *PCDH19* gene as the cause of familial epilepsy with mental retardation limited to females; has received speaker honoraria from Athena Diagnostics, UCB, Janssen-Cilag EMEA, and Eli Lilly and Company; has received funding for travel from Athena Diagnostics, UCB, and Janssen-Cilag EMEA; and receives/has received research support from the National Health and Medical Research Council of Australia, Health Research Council of New Zealand, The University of Melbourne, American

Epilepsy Society, the Jack Brockhoff Foundation, the Shepherd Foundation, and the Perpetual Charitable Trustees.

Received December 12, 2010. Accepted in final form March 31, 2011.

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