

ONLINE FIRST

Clinical Significance of Rare Copy Number Variations in Epilepsy

A Case-Control Survey Using Microarray-Based Comparative Genomic Hybridization

Pasquale Striano, MD, PhD; Antonietta Coppola, MD; Roberta Paravidino, PhD; Michela Malacarne, PhD; Stefania Gimelli, PhD; Angela Robbiano, PhD; Monica Traverso, PhD; Marianna Pezzella, MD; Vincenzo Belcastro, MD; Amedeo Bianchi, MD; Maurizio Elia, MD; Antonio Falace, PhD; Elisabetta Gazzero, MD; Edoardo Ferlazzo, MD; Elena Freri, MD; Roberta Galasso, PhD; Giuseppe Gobbi, MD; Cristina Molinatto, MD; Simona Cavani, PhD; Orsetta Zuffardi, PhD; Salvatore Striano, MD; Giovanni Battista Ferrero, MD; Margherita Silengo, MD; Maria Luigia Cavaliere, MD; Matteo Benelli, PhD; Alberto Magi, PhD; Maria Piccione, MD; Franca Dagna Bricarelli, PhD; Domenico A. Coviello, MD; Marco Fichera, PhD; Carlo Minetti; Federico Zara, PhD

Objective: To perform an extensive search for genomic rearrangements by microarray-based comparative genomic hybridization in patients with epilepsy.

Design: Prospective cohort study.

Setting: Epilepsy centers in Italy.

Patients: Two hundred seventy-nine patients with unexplained epilepsy, 265 individuals with nonsyndromic mental retardation but no epilepsy, and 246 healthy control subjects were screened by microarray-based comparative genomic hybridization.

Main Outcomes Measures: Identification of copy number variations (CNVs) and gene enrichment.

Results: Rare CNVs occurred in 26 patients (9.3%) and 16 healthy control subjects (6.5%) ($P = .26$). The CNVs identified in patients were larger ($P = .03$) and showed higher gene content ($P = .02$) than those in control subjects. The CNVs larger than 1 megabase ($P = .002$) and including more than 10 genes ($P = .005$) occurred more frequently in patients than in control subjects. Nine patients (34.6%) among those harboring rare CNVs showed rearrangements asso-

ciated with emerging microdeletion or microduplication syndromes. Mental retardation and neuropsychiatric features were associated with rare CNVs ($P = .004$), whereas epilepsy type was not. The CNV rate in patients with epilepsy and mental retardation or neuropsychiatric features is not different from that observed in patients with mental retardation only. Moreover, significant enrichment of genes involved in ion transport was observed within CNVs identified in patients with epilepsy.

Conclusions: Patients with epilepsy show a significantly increased burden of large, rare, gene-rich CNVs, particularly when associated with mental retardation and neuropsychiatric features. The limited overlap between CNVs observed in the epilepsy group and those observed in the group with mental retardation only as well as the involvement of specific (ion channel) genes indicate a specific association between the identified CNVs and epilepsy. Screening for CNVs should be performed for diagnostic purposes preferentially in patients with epilepsy and mental retardation or neuropsychiatric features.


Arch Neurol. 2012;69(3):322-330. Published online November 14, 2011. doi:10.1001/archneurol.2011.1999

IN APPROXIMATELY 30% TO 40% OF patients with epilepsy, there is an underlying cause of seizure recurrence, but it is not identifiable in most subjects.¹ Although causative mutations have been identified

likely involving combinations of variants in different genes that have proven challenging to discover.² Linkage and genome-wide association studies have failed to identify robust or unambiguous associations in large series of patients.³

*For editorial comment
see page 305*

for several epilepsy syndromes, mendelian epilepsies account for only 1% of the cases.² By contrast, common epilepsies have a complex pattern of inheritance,

 CME available online at www.jamaarchivescme.com and questions on page 295

Copy number variations (CNVs) are increasingly recognized as a source of phenotypic variation among humans, and the

Author Affiliations are listed at the end of this article.

implementation of genome-wide technologies such as microarray-based comparative genomic hybridization (array-CGH) and single-nucleotide polymorphism genotyping now allows their efficient identification.^{4,5} A number of studies have recently highlighted the role of CNVs in the etiology of various neuropsychiatric disorders.^{6,7} Targeted and genome-wide surveys of CNVs have been also performed in individuals with epilepsy, revealing recurrent deletions at 15q11.2, 15q13.3, and 16p13.11 in a small proportion of patients with different epilepsy syndromes.⁸⁻¹¹ However, the role of nonrecurrent CNVs in the etiology of epilepsy has not yet been evaluated in detail.^{2,5} We performed an array-CGH-based survey of CNVs in subjects with epilepsy of unknown cause.

METHODS

STUDY POPULATION

We prospectively collected records of patients with unexplained epilepsy referred to different centers between September 1, 2007, and August 31, 2009. Clinical evaluation included general and neurological examination, familial and personal history, electroclinical findings, 1.5-T brain magnetic resonance imaging, treatment schedule, and outcome. Seizures and epilepsy type were defined according to international recommendations.¹² The patients' IQs were defined according to *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition criteria (borderline intellectual functioning, IQ 71-84; mild mental retardation [MR], IQ 60-70; moderate MR, IQ 50-59; severe MR, IQ <50). We excluded patients with the following: (1) lesional or metabolic cause of epilepsy; (2) signs and symptoms of epileptic encephalopathy; (3) major congenital malformations or pathognomonic dysmorphisms; and (4) severe MR or major neuropsychiatric diseases (eg, schizophrenia, tic disorders, severe mood disorder). The ethics committee of each center approved the study, and informed consent was signed by participants.

CONTROL POPULATIONS

Control populations comprised subjects with nonsyndromic MR but no epilepsy (control subjects with MR) and healthy blood donors (healthy control subjects). Subjects with MR only were selected from a consecutive series of cases referred to the Laboratory of Genetics, Galliera Hospital, Genova, Italy, between January 1, 2008, and December 31, 2010, and classified according to *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition criteria. Healthy control subjects were selected among adults aged 40 to 60 years through a modified version of a structured questionnaire to exclude any seizure disorder.¹³

GENETIC STUDIES

Experimental Strategy

Genomic DNA of patients and control subjects was extracted from blood lymphocytes. Samples from patients and control subjects were screened by the Human Genome CGH Microarray 44B kit (Agilent Technologies, Inc, Santa Clara, California). Identified CNVs were checked in the Database of Genomic Variants (<http://projects.tcag.ca/variation/>) and in a data set of 790 control subjects with neurological disease who were of European descent.¹⁴ We adopted a quite conservative ap-

proach and did not follow up rearrangements overlapping more than 50% with CNVs reported in control subjects. Validation and parental origin of CNVs were obtained by fluorescence in situ hybridization, higher-density array-CGH slides (Microarray 244K kit; Agilent Technologies, Inc), or quantitative polymerase chain reaction.

Array-CGH Assay

We used array-CGH containing about 44 000 60-mer oligoprobes covering the nonredundant genome at an average distance of approximately 43 kilobases (kb) and allowing the genome-wide survey of CNVs. Labeling and hybridization were performed following the manufacturer's protocol. The arrays were scanned (Microarray Scanner G2565CA; Agilent Technologies, Inc) and analyzed by Feature Extraction version 9.5.0 software and Genomic Workbench version 5.0.14 software (Agilent Technologies, Inc). Assays showing a derivative log ratio spread score higher than 0.3 were excluded. Detection of gains and losses was performed using the Aberration Detection Method 2 algorithm with a moving average of 500 kb and a threshold of 6.0. In addition, we set the minimum number of consecutive probes to call CNVs at 8. The same protocol was applied for the higher-density Microarray 244K kit (Agilent Technologies, Inc).

Fluorescence In Situ Hybridization

Bacterial artificial chromosome clones were selected according to the UCSC Genome Browser hg18 assembly (<http://genome.ucsc.edu>; Genome Bioinformatics Group, Center for Biomolecular Science and Engineering, University of California, Santa Cruz). Bacterial artificial chromosome DNA was labeled by nick translation. Slides were counterstained with antifade Vectashield Mounting Medium with 4',6-diamidino-2-phenylindole (Vector Laboratories, Inc, Burlingame, California). Signals were visualized on a Nikon E1000 microscope (Nikon Corp, Tokyo, Japan) equipped with a charge-coupled device camera and Genikon image analysis software (Genikon, Plano, Texas).

Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction was performed using the TaqMan technology on an ABI 7500 instrument (Applied Biosystems, Inc, Foster City, California). The copy number of the unknown samples was normalized to an endogenous reference (RNase P gene). Locus-specific TaqMan probes were selected from the Applied Biosystems database.

STATISTICAL ANALYSIS AND BIOINFORMATICS

Sample power estimates were calculated assuming an α error of .05 and a β error of 0.2 (ie, a power of 80%). Two-tailed unpaired *t* test and Fisher exact tests were used for categorical sample and phenotype correlations; the nonparametric Mann-Whitney *U* test was used to evaluate numerical data. The BioMart tool (<http://www.ensembl.org/biomart>) was used to retrieve the lists of RefSeq deleted and duplicated genes in patients and control subjects. In addition, we searched for Gene Ontology (GO) annotations that are overrepresented in the group of rearranged genes using GeneCodis version 2.0 software (<http://genecodis.dacya.ucm.es>).¹⁵ This tool allows the classification of genes according to their putative biological function by screening the GO annotations included in the GO Slim database and identifying significant enrichment of specific GO

Table 1. Demographic Data of 279 Patients With Epilepsy of Unknown Cause

Characteristic	Value
Sex, No.	
Male	152
Female	127
Age, mean (range), y	
At last evaluation	39.5 (2-69)
At epilepsy onset	15.9 (0.08-38)
Epilepsy type, No.	
Generalized	188
Focal	91
Mental retardation or neuropsychiatric features, No. (%)	
Absent	198 (71.0)
Present	81 (29.0)

annotations among any set of genes by normalizing for the total number of human genes belonging to each GO group using the hypergeometric distribution and correcting resulting *P* values for multiple hypothesis testing using the false discovery rate correction.¹⁵ The DECIPHER database of genomic unbalances was used for genotype-phenotype correlations (<https://decipher.sanger.ac.uk/>).

RESULTS

Records from 279 patients with epilepsy (152 males), 265 control subjects with MR (117 males), and 246 healthy control subjects (127 males) were collected. In the patients group, epilepsy type was generalized in 188 patients and focal in 91 patients. Eighty-one subjects (29.0%) showed slight to moderate MR or additional neuropsychiatric features (**Table 1**). The initial survey led to the identification of 182 CNVs (58 in patients with epilepsy, 74 in control subjects with MR, and 50 in healthy control subjects) (data available on request). Among these, 92 CNVs (ie, 28 in 26 patients with epilepsy, 48 in 45 control subjects with MR, and 16 in 16 healthy control subjects) were absent in the Database of Genomic Variants and were further followed up (**Table 2**). The overall rate of rare CNVs in patients with epilepsy (9.3%) was similar to that in healthy control subjects (6.5%) ($P=.26$) and significantly lower than that in control subjects with MR (17.0%) ($P=.01$). Power estimates, calculated on the frequency of rare CNVs in the control population, indicate that the available sample can detect at least a 2.1-fold increase in the frequency of rare CNVs among patients.

GENOMIC REARRANGEMENTS IDENTIFIED IN PATIENTS WITH EPILEPSY AND CONTROL SUBJECTS

A total of 28 rare CNVs were identified in 26 patients with epilepsy (9.3%; 16 males) (**Figure** and **Table 2**). No recurrent CNVs were found except for the 22q13.32qter deletion (cases C012 and B004). Locus 19q13.43 was deleted in 1 case (case A022) and duplicated as part of a larger rearrangement in another one

(case A211). Twenty-four CNVs (92.3%) involved a single chromosomal segment (12 deletions, 12 duplications), whereas the other 2 involved various segments in the same (case A096) or different (case A164) chromosomes. The CNVs varied in size from 0.12 to 13 megabases (Mb). Likewise, the number of rearranged genes varied from 1 to more than 100 (**Table 2**). Two duplications composed a single gene (cases C106 and A049). The parental origin of the rearrangements was assessed in 21 patients: 10 occurred de novo, 9 were inherited from a healthy parent (5 paternal, 4 maternal), and 2 resulted from unbalanced segregation of parental balanced rearrangements. Altogether, de novo CNVs were larger ($P=.03$) and showed higher gene content ($P=.006$) compared with those inherited, whereas the type of CNV (deletions vs duplications) did not differ between the 2 groups ($P=.17$).

The clinical features of individuals carrying these CNVs are shown in **Table 3**. Age at epilepsy onset ranged from 3 months to 18 years (mean [SD], 7.1 [5.0] years). Epilepsy was generalized in 16 subjects and focal in 10. Fourteen patients (53.8%) had MR, and 10 of these had MR associated with autistic traits or attention-deficit/hyperactivity disorder. Nine patients (34.6%) showed rearrangements that have been already associated with microdeletion or microduplication syndromes (**Figure**). These rearrangements compared with the novel ones were more frequently deletions (7 of 8 vs 5 of 17, respectively; $P=.03$) and larger in size (mean, 5.2 vs 1.9 Mb, respectively; $P=.04$), whereas they did not differ in gene content (mean, 45.8 vs 20.0 genes, respectively; $P=.13$) or parental origin (de novo vs inherited, respectively; $P=.07$). No newly identified CNVs matched those reported in the DECIPHER database.

Among 246 ethnically matched healthy control subjects, we identified 16 unreported CNVs (3 deletions, 13 duplications) (**Table 2**). Largely overlapping 6q22.31 deletion (case 02957M) and 9p21.2 duplication (case 01503M) were identified in patients (cases I091 and E011), indicating 2 novel, recurrent, polymorphic CNVs.

The CNVs identified in patients compared with healthy control subjects were larger (mean, 3.04 vs 0.79 Mb, respectively; $P=.03$) and showed higher gene content (mean, 31.3 vs 7.1 genes, respectively; $P=.02$), whereas the type of unbalance (deletions vs duplications, respectively) did not differ between the 2 groups ($P=.06$). The CNVs larger than 1 Mb ($P=.002$) and including more than 10 genes ($P=.005$) occurred more frequently in patients than in healthy control subjects. Conversely, the 2 groups did not differ for CNVs smaller than 1 Mb ($P=.05$) and involving 10 or fewer genes ($P=.55$).

Genotype-phenotype correlations indicated that having MR or neuropsychiatric features was significantly associated with the occurrence of CNVs compared with not having them (14 of 81 cases [17.2%] vs 16 of 246 cases [6.5%], respectively; $P=.004$), whereas epilepsy type (focal vs generalized; $P=.52$) was not. In addition, CNVs in patients with epilepsy who have MR or neuropsychiatric features were larger ($P=.02$)

Table 2. Genomic Rearrangements Detected in Patients With Epilepsy

Patient No./ Sex	Copy Number Variation							
	Type	Chromosome	Cytoband	Start, bp	Stop, bp	Size, bp	Genes, No.	Inheritance
Disease-Associated Rearrangements								
C049/M	Deletion	6	q26-q27	163 760 006	170 734 168	6 974 162	39	De novo
A096/F	Deletion	8	p23.3-p23.1	181 330	7 290 797	7 109 467	111	Maternal balanced inversion
	Duplication	8	p23.1-p21.2	12 627 430	24 549 176	11 921 746		
A133/F	Deletion	9	q34.3	137 776 611	140 128 884	2 352 273	81	De novo
C070/M	Duplication	15	q11-q13.1	19 109 122	26 164 760	7 055 638	33	De novo
C045/M ^a	Deletion	15	q24.1-q24.3	72 253 337	75 747 080	3 493 743	54	De novo
I104/M ^a	Deletion	16	p13.11	14 851 860	16 183 756	1 331 896	17	Paternal
C012/M	Deletion	22	q13.31-q13.33	46 139 271	49 526 339	3 387 068	45	De novo
B004/F	Deletion	22	q13.32-q13.33	47 710 690	49 526 339	1 815 649	38	De novo
A040/F	Deletion	X	p22.31	6 560 955	7 992 261	1 431 306	4	Maternal
Novel Rearrangements								
A181/M	Duplication	2	p12	76 930 522	78 641 030	1 710 508	2	Paternal
A002/F	Duplication	2	q11.2	96 143 158	97 387 872	1 244 714	29	Paternal
A018/M	Deletion	4	q31.23	149 164 064	150 453 131	1 289 067	2	Maternal
C106/F	Duplication	6	q16.1	93 932 682	94 349 214	416 532	1	Paternal
A191/F	Duplication	6	q22.2	117 129 742	117 953 435	823 693	7	Maternal
I091/M ^{a,b}	Duplication	6	q22.31	123 581 123	124 208 500	627 377	2	Undetermined
A164/M	Duplication	7	q36.3	156 123 654	158 388 155	2 264 501	47	Paternal balanced translocation
	Deletion	14	q32.33	103 375 996	106 312 055	2 936 059		
E011/M ^b	Duplication	9	p21.2	26 790 566	27 940 168	1 149 602	10	Maternal
A139/F	Deletion	9	q13-q21.13	70 341 355	76 692 922	6 351 567	26	De novo
A066/M	Deletion	10	p12.33-p12.31	18 271 821	21 110 683	2 838 862	8	Paternal
I090/M	Duplication	12	q24.33	130 430 446	131 708 300	1 277 854	8	Undetermined
B061/M	Duplication	15	q26.1	88 129 144	88 253 691	124 547	4	Undetermined
A049/F	Duplication	17	q24.3	65 681 113	67 198 174	1 517 061	1	Undetermined
A154/M	Deletion	19	p13.3	1 162 440	1 377 123	214 683	12	De novo
A211/M	Duplication	19	q13.41-q13.43	57 706 964	62 901 136	5 194 172	178	De novo
A022/M	Deletion	19	q13.43	60 896 815	62 036 957	1 140 142	28	De novo
I100/F	Duplication	X	p11.3	45 364 645	46 537 389	1 172 744	4	Undetermined

Abbreviation: bp, base pairs.

^aDetected in control subjects with mental retardation only.

^bDetected in healthy control subjects.

and showed higher gene content ($P < .001$) compared with healthy control subjects, whereas the size and gene content of CNVs identified in patients without MR or neuropsychiatric features did not differ from those in healthy control subjects.

The incidence of CNVs in patients with epilepsy who have MR or neuropsychiatric features and in control subjects with MR is similar (45 of 265 cases [17.0%] vs 14 of 81 cases [17.2%]; $P > .99$). Likewise, the 2 groups did not differ in CNV size (mean, 4.51 vs 3.78 Mb, respectively; $P = .54$) or gene content (mean, 37.8 vs 29.7 genes, respectively; $P = .43$). Among CNVs identified in control subjects with MR, 3 were also observed in the epilepsy group. The 6q22.31 duplication has been also detected in 2 control subjects with MR. The 15q24.1 deletion was identified in a patient with epilepsy showing different types of generalized seizures plus mild MR with hyperactivity and in a control individual with moderate nonsyndromic MR. The 16p13.11 deletion was detected in a mentally healthy patient with idiopathic generalized epilepsy (case I104) and a control subject with MR showing moderate MR and slight dysmorphisms.

CNV VALIDATION

A set of 17 rearrangements identified in patients with epilepsy were validated by an independent assay, ie, the 244K array-CGH (cases C012, B004, C106, and A049), fluorescence in situ hybridization (cases C049, A096, C045, A018, A164, A139, A066, A211, and A022), or quantitative polymerase chain reaction (cases A181 and A002 and controls 14F and 18F). Validation assays confirmed the occurrence of CNVs in all of the cases (data available on request).

BIOINFORMATIC ANALYSIS OF GENES INCLUDED WITHIN CNVS

A total of 791 genes were involved in rearrangements identified in patients (data available on request). We classified the rearranged genes according to the putative biological role attributed by the GO database, and we searched for a significant enrichment of specific GO category using GeneCodis version 2.0. A total of 593 genes were associated with at least 1 GO annotation (**Table 4**). Genes encoding ion channels ($P = .01$) were the most significantly enriched

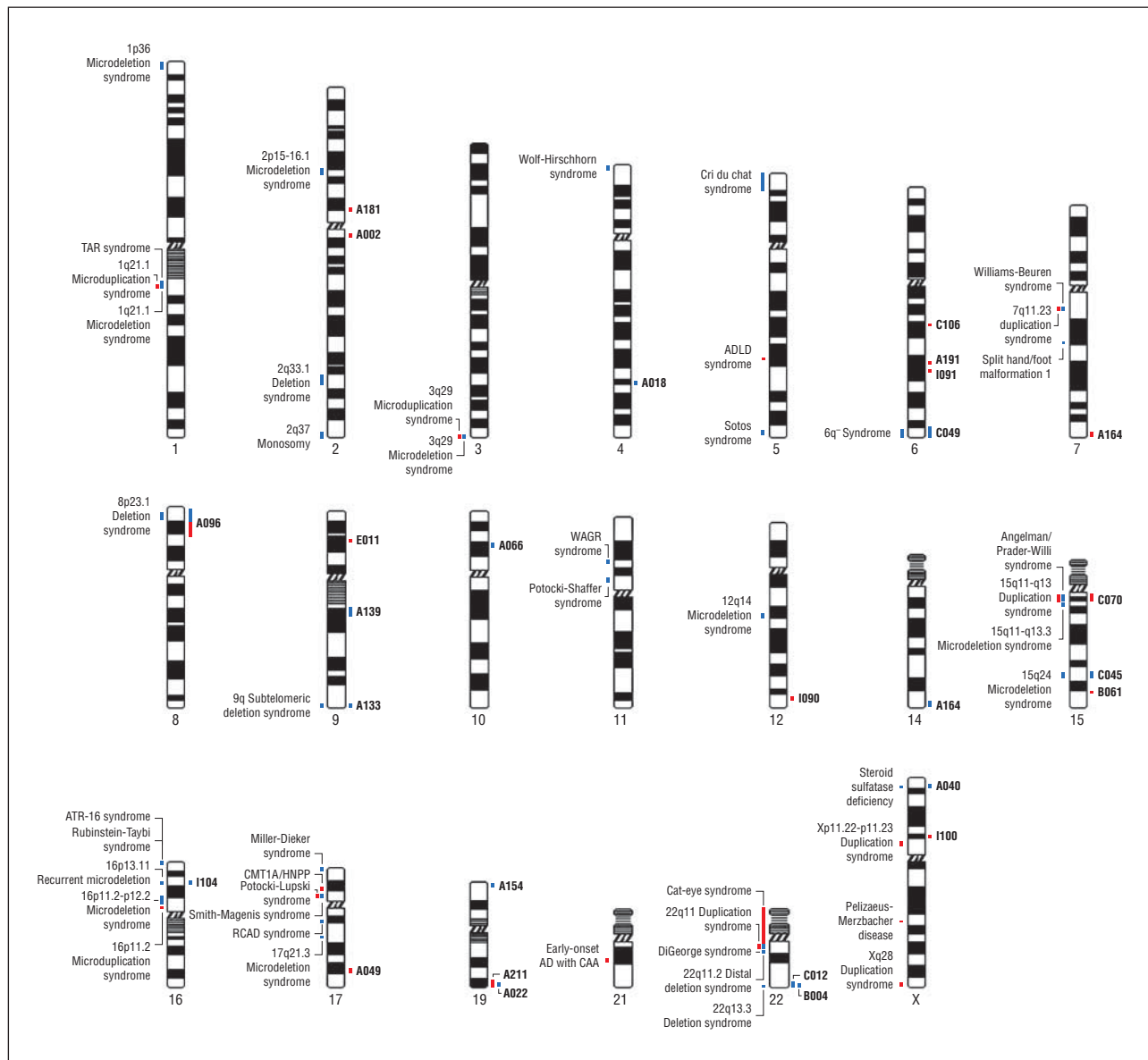


Figure. Ideograms of chromosomes showing neurological microdeletions or microduplications syndromes (left) and copy number variations identified in our study (right). Red bars indicate duplications; blue bars, deletions; AD, Alzheimer disease; ADLD, autosomal dominant leukodystrophy; ATR-16, α -thalassemia retardation associated with chromosome 16; CAA, cerebral amyloid angiopathy; CMT1A, Charcot-Marie-Tooth disease, type 1A; HNPP, hereditary neuropathy with liability to pressure palsies; RCAD, renal cysts and diabetes; TAR, thrombocytopenia-absent radius; and WAGR, Wilms tumor, aniridia, genitourinary anomalies, and mental retardation.

among rearranged genes. In addition, significant associations were detected for genes involved in posttranslational modification of proteins ($P=.01$), cell differentiation ($P=.02$), and metabolic processes ($P=.02$) (Table 4). GeneCodis analysis performed on 1327 genes (1116 with GO identification) rearranged in control subjects with MR revealed a significant enrichment of genes involved in development ($P=.004$), signal transduction ($P=.005$), metabolic processes ($P=.008$), and cell differentiation ($P=.008$) (Table 4). By contrast, we did not find any overrepresented GO category in healthy control subjects.

COMMENT

Recent studies have shown that recurrent rearrangements such as 15q11.2, 15q13.3, and 16p13.11 micro-

deletions represent a risk factor for a wide spectrum of epilepsies.⁸⁻¹¹ However, the role of nonrecurrent genomic rearrangements in patients with epilepsy of unknown cause is still unclear and the diagnostic impact of high-resolution screening of CNVs has not yet been assessed.

We showed that the cumulative incidence of rare CNVs is not significantly higher in patients with epilepsy of unknown cause compared with a healthy control population. However, CNVs identified in patients are larger and include more genes on average; in addition, rearrangements larger than 1 Mb and containing 10 or more genes are significantly overrepresented among patients with epilepsy. These data indicate that only a subgroup of rare CNVs play a role in the etiology of epilepsy.

Table 3. Clinical Data of Patients With Epilepsy and Genomic Rearrangements

Patient No./ Sex/Age, y	Epilepsy Features										
	Onset		Follow-up			Last Examination		Epilepsy Diagnosis	Mental Status	Neuropsychiatric Features	CNV
	Age	Seizure Type	Other Seizure Type (Age at Onset, y)	Previous Therapy	Seizure Frequency	Current Therapy					
Patients With Known Rearrangements											
C049/M/27	15 y	CPS	TCS (16)	NA	4/y	VPA	Cryptogenic focal	Mild MR	NA	del(6)(q26qter)	
A096/F/37	12 y	FS, CPS	TCS (12)	CBZ, LEV	Monthly	TPM, OXC	Cryptogenic focal	Moderate MR	Autistic tracts	invdup del(8)(qter→ p23.1::p23.1→ p21.2)	
A133/F/5	2 y	sGTCS, CPS	NA	VPA	Monthly	OXC	Cryptogenic focal	Moderate MR	NA	del(9)(q34.3qter)	
C070/M/28	6 mo	TCS	Atonic (6)	CBZ, OXC, FLB, TPM	Daily	VPA, CNZ, LTG	Cryptogenic generalized	Moderate MR	Autistic tracts	dup(15)(q11q13.1)	
C045/M/18	11 y	TCS	Atonic, tonic (13)	NA	1 y	VPA, LEV	Cryptogenic generalized	Mild MR	Hyperactivity	del(15)(q24.1q24.3)	
I104/M/28	12 y	Myoclonic	TCS (12)	NA	Seizure free	VPA	Idiopathic generalized	Healthy	NA	del(16)(p13.11)	
C012/M/12	6 y	Absences	NA	NA	Daily	VPA	Cryptogenic generalized	Mild MR	Autistic tracts	del(22)(q13.31qter)	
B004/F/10	2 y	FS, TCS	NA	NA	3 y	VPA	Cryptogenic generalized	Moderate MR	Hyperactivity	del(22)(q13.32qter)	
A040/F/10	1 y	TCS	Myoclonic (6), TCS (15)	PB	Monthly	VPA, FLB	Cryptogenic generalized	Moderate MR	Autistic tracts	del(X)(p22.31)	
Patients With Novel Rearrangements											
A181/M/12	6 y	Absences	NA	NA	Seizure free	VPA	Cryptogenic generalized	Mild MR	ADHD	dup(2)(p12)	
A002/F/6	3 mo	Tonic, clonic	NA	PB	Weekly	TPM	Cryptogenic generalized	Mild MR	NA	dup(2)(q11.2)	
A018/M/17	13 y	CPS	NA	NA	4 y	VPA	Cryptogenic focal	Borderline	NA	del(4)(q31.23)	
C106/F/29	5 y	Absences, TCS	Atonic (15)	PB, CBZ, CNZ	2 y	TPM, LTG, VPA	Cryptogenic generalized	Borderline	NA	dup(6)(q16.1)	
A191/M/29	10 y	SPS	CPS, sGTCS (10)	CBZ, PB	Monthly	TPM, LTG, VPA	Cryptogenic focal	Borderline	NA	dup(6)(q22.2)	
I091/M/12	10 y	FS, TCS	TCS (10)	NA	Seizure free	VPA	Idiopathic generalized	Healthy	NA	dup(6)(q22.31)	
A164/M/21	13 y	CPS	NA	NA	Seizure free	CBZ	Cryptogenic focal	Moderate MR	ADD	der(14)t(7;14) (q36.3;q32.33)	
E011/M/24	9 y	Absences	NA	NA	Seizure free	VPA	Idiopathic generalized	Healthy	NA	dup(9)(p21.2)	
A139/F/14	4 y	FS, absences	TCS (4)	NA	Seizure free	VPA, ETS	Cryptogenic generalized	Mild MR	NA	del(9)(q13q21.13)	
A066/M/24	2 y	SPS	CPS (6), TCS (17)	PB	Monthly	CBZ	Cryptogenic focal	Mild MR	Autistic tracts	del(10)(p12.33p12.31)	
I090/M/28	18 y	TCS	NA	NA	Seizure free	VPA	Idiopathic generalized	Healthy	NA	dup(12)(q24.33)	
B061/M/11	1 y	CPS, TCS	NA	VPA	Monthly	CBZ, LEV	Cryptogenic focal	Borderline	Hyperactivity	dup(15)(q26.1)	
A049/F/16	8 y	TCS	Absences (8)	PB	1 y	VPA, ETS	Idiopathic generalized	Healthy	NA	dup(17)(q24.3)	
A154/M/6	3 y	FS, TCS	NA	VPA	Seizure free	NA	Cryptogenic generalized	Mild MR	NA	del(19)(p13.3)	
A211/M/8	6 y	CPS	NA	NA	1 y	VPA	Cryptogenic focal	Healthy	NA	dup(19) (q13.41q13.43)	
A022/M/19	7 y	CPS	NA	NA	Seizure free	CBZ, LTG	Cryptogenic focal	Borderline	NA	del(19)(q13.43)	
I100/F/15	3 y	Absences	TCS (4), myoclonic (15)	NA	Seizure free	NA	Idiopathic generalized	Healthy	NA	dup(X)(p11.3)	

Abbreviations: ADD, attention-deficit disorder; ADHD, attention-deficit/hyperactivity disorder; CBZ, carbamazepine; CNV, copy number variation; CNZ, clonazepam; CPS, complex partial seizures; ETS, ethosuximide; FLB, felbamate; FS, febrile seizures; LEV, levetiracetam; LTG, lamotrigine; MR, mental retardation; NA, not applicable; OXC, oxcarbazepine; PB, phenobarbital; sGTCS, secondarily generalized tonic-clonic seizures; SPS, simple partial seizures; TCS, tonic-clonic seizures; TPM, topiramate; VPA, valproate sodium.

It should be emphasized that CNV detection is largely dependent on the type of platform (array-CGH vs single-nucleotide polymorphism genotyping), the density and design of the array, and the setting of analytic para-

eters.¹⁶ Most population-based CNV surveys have been performed by single-nucleotide polymorphism arrays, whereas array-CGH has become the elective routine diagnostic tool to identify genomic rearrangements in pa-

Table 4. Biological Processes Associated With Genes Rearranged in Patients and Control Subjects

GO Identification No.	Total Rearranged Genes With GO Annotations, No.	Total Reference Genes With GO Annotations, No.	Corrected P Value
Patients with epilepsy	(n=593)	(n=29 095)	
GO:0006811, ion transport	23	510	.01
GO:0006464, protein modification process	10	148	.01
GO:0030154, cell differentiation	20	483	.02
GO:0008152, metabolic process	20	482	.02
Healthy control subjects			
No significant association			
Control subjects with MR	(n=1116)	(n=29 095)	
GO:0007275, multicellular organismal development	56	898	.004
GO:0007165, signal transduction	100	1872	.005
GO:0008152, metabolic process	33	482	.008
GO:0030154, cell differentiation	35	483	.008

Abbreviations: GO, Gene Ontology; MR, mental retardation.

tients.¹⁷ We identified 2 previously unreported CNVs in both patients and control subjects, indicating that recurrent polymorphic CNVs may have been missed by large-scale population single-nucleotide polymorphism-based studies. Accordingly, CNV screening should include appropriate internal controls, and heterogeneous data from a public database such as the Database of Genomic Variants should be considered with caution within a diagnostic context.

Our cohort includes patients referred to third-level centers for different epilepsy types of variable severity in the absence of severe MR, major neuropsychiatric disease, or pathognomonic dysmorphisms. These patients represent a common heterogeneous phenotypic group, raising complex diagnostic and management issues.

Genotype-phenotype correlations revealed that the frequency of rare CNVs as well as their size and gene content are significantly higher in patients showing mild to moderate MR or other minor neuropsychiatric features than in healthy control subjects. On the other hand, the incidence, size, and gene content of rare CNVs in patients with epilepsy who have healthy or borderline mental status are not increased compared with our healthy control population. Notably, comparative analysis of CNVs identified in patients with epilepsy and control subjects with MR revealed a minor overlap, suggesting the involvement of specific pathogenetic mechanisms for epilepsy. Although we cannot exclude that this lack of overlap is due to a sampling issue, analysis of putative biological processes related to rearranged genes pointed out the specific role of ion channel genes in the epilepsy group. In this scenario, the analysis of genomic rearrangements emerges as a first-line diagnostic test for patients with epilepsy showing MR and/or neuropsychiatric features, even if mild.

BROADENING THE SPECTRUM OF EMERGING SYNDROMES

About one-third of the patients showed rearrangements that can be unambiguously considered pathogenetic. In these cases, epilepsy was the predominant phenotypic expression of emerging microdeletion or microduplication

syndromes, such as 6q terminal deletion (case C049),^{18,19} invdupdel(8p) (case A096),²⁰ 9q34qter deletion (case A133),²¹ 15q11-q13 duplication (case C070),²² 15q24 deletion (case C045),²³ 16p13.11 deletion (case I104),²⁴ 22q13.3 deletion (cases C012 and B004),²⁵ and Xp22.31 deletion (case A040).²⁶ These syndromes are usually characterized by complex syndromic phenotypes including dysmorphisms, brain structural abnormalities, or multiple congenital defects. Conversely, our patients showed a mild clinical expression featuring mild to moderate MR, no coarse facial features, and no structural abnormalities in the brain or other organs and could not be diagnosed on clinical grounds. The heterogeneous clinical expressivity of these syndromes may be due to several factors. Phenotypic differences among affected individuals may be explained by the parental origin of the rearrangement (15q11-q13 duplication)²² or differences in the size of the rearranged region (15q24 deletion).²³ Variable phenotypic expressivity may also be due to other factors such as unmasking of recessive mutations, somatic mosaicism, and epigenetic or environmental modifiers.^{17,27}

NOVEL REARRANGEMENTS

The assessment of the pathogenetic effect of novel, unreported rearrangements remains an open issue. The de novo origin of a rearrangement is considered an important predictor.^{17,27} Most of the novel rearrangements identified in this study were transmitted from unaffected parents. However, population studies indicate that the de novo origin of a CNV does not necessarily imply a pathogenetic effect.^{17,27} Consistently, in our series, known pathogenetic and novel CNVs do not differ for the parental origin. Moreover, rearrangements inherited from a healthy parent cannot always be considered a benign variation. Indeed, it was recently demonstrated that CNVs may represent susceptibility alleles for a number of neuropsychiatric disorders, including idiopathic epilepsy.⁸⁻¹¹

The size, the type (deletions vs duplications), and the number of genes involved in CNVs represent key factors underlying pathogenesis.²⁷ In our series, novel CNVs

are more frequently duplications and are smaller compared with known pathogenetic rearrangements, whereas the gene content is not significantly different. Moreover, we detected a tight link between the mode of transmission and the size and gene content of CNVs. Indeed, inherited rearrangements were smaller and showed lower gene content compared with de novo rearrangements. In this scenario, mode of transmission, type, size, and gene content of a novel CNV should be evaluated together to assess its pathogenetic relevance.

EMERGING EPILEPTOGENIC PATHWAYS

A comprehensive bioinformatic analysis on CNVs identified in our cohort showed significant enrichment of ion transport genes, confirming that the impairment of neuronal ion currents represents a common pathophysiological mechanism in epilepsy.^{1,2,5} In addition, our analysis revealed a significant enrichment of genes involved in cell differentiation and metabolism. This finding is consistent with emerging evidence pinpointing the role of early maturation processes,²⁸ subtle cytoarchitectural defects,^{29,30} and metabolic impairment³¹ in the etiology of epilepsies lacking detectable brain abnormalities. The implication of developmental and metabolic processes in control subjects with MR provides further evidence of a pathogenetic link between epilepsy and MR. On the other hand, the implication in patients with epilepsy of pathways related to posttranscriptional modification of proteins in epilepsy cannot be readily interpreted.

CONCLUSIONS

Patients with epilepsy have a significantly increased burden of large, rare, gene-rich CNVs, particularly when associated with MR or other neuropsychiatric features. Accordingly, we suggest that CNV screening should be performed for diagnostic purposes preferentially in such patients. Rearrangements smaller than 100 to 200 kb could have been missed in our study. However, small rearrangements involving few genes were usually inherited from healthy parents and have an uncertain clinical relevance. Therefore, the implementation of very high-resolution screening in clinical practice deserves caution.

Accepted for Publication: September 12, 2011.

Published Online: November 14, 2011. doi:10.1001/archneur.2011.1999

Author Affiliations: Muscular and Neurodegenerative Diseases Unit and Laboratory of Neurogenetics (Drs P. Striano, Robbiano, Traverso, Pezzella, Falace, Gazzo, and Zara and Mr Minetti) and Epidemiology and Statistics Unit (Dr Galasso), Institute G. Gaslini, University of Genova, and Laboratory of Genetics, Ente Ospedaliero Ospedali Galliera (Drs Paravidino, Malacarne, Cavani, Dagna Bricarelli, and Coviello), Genova, Epilepsy Center, Federico II University (Drs Coppola and S. Striano) and Medical Genetics, Azienda Ospedaliera di Rilievo Nazionale "A. Cardarelli" (Dr Cavaliere), Napoli, Ospedale "S. Anna," Como (Dr Belcastro), Epilepsy Centre, San Donato Hospital, Arezzo (Dr Bianchi), Unit of

Neurology (Dr Elia) and Laboratory of Genetics (Dr Fichera), Oasi Institute for Research on Mental Retardation and Brain Aging, Troina, Institute of Neurology, Magna Græcia University, Catanzaro and Regional Epilepsy Centre, Reggio Calabria (Dr Ferlazzo), Division of Child Neurology, Institute "C. Besta," Milano (Dr Freri), Child Neurology and Psychiatry Unit, Maggiore Hospital, Bologna (Dr Gobbi), Dipartimento di Scienze Pediatriche, University of Torino, Torino (Drs Molinatto, Ferrero, and Silengo), Biologia Generale e Genetica Medica, Università di Pavia, Pavia (Dr Zuffardi), Dipartimento Materno Infantile, Università di Palermo, Palermo (Drs Benelli and Magi), and Diagnostic Genetic Unit, Careggi Hospital, Firenze (Dr Piccione), Italy; and Genetic and Laboratory Medicine, Geneva University Hospitals, Geneva, Switzerland (Dr Gimelli).

Correspondence: Federico Zara, PhD, Laboratory of Neurogenetics, Department of Neuroscience, Institute G. Gaslini, Largo Gaslini 5, Genova 16147, Italy (federicozara@ospedale-gaslini.ge.it).

Author Contributions: *Acquisition of data:* P. Striano, Coppola, Paravidino, Malacarne, Gimelli, Robbiano, Traverso, Pezzella, Belcastro, Bianchi, Elia, Falace, Gazzo, Ferlazzo, Freri, Galasso, Gobbi, Molinatto, Cavani, Zuffardi, S. Striano, Ferrero, Silengo, Cavaliere, Benelli, Magi, Piccione, Dagna Bricarelli, Coviello, Fichera, Minetti, and Zara. *Critical revision of the manuscript for important intellectual content:* P. Striano, Coppola, Paravidino, Malacarne, Gimelli, Robbiano, Traverso, Pezzella, Belcastro, Bianchi, Elia, Falace, Gazzo, Ferlazzo, Freri, Galasso, Gobbi, Molinatto, Cavani, Zuffardi, S. Striano, Ferrero, Silengo, Cavaliere, Benelli, Magi, Piccione, Dagna Bricarelli, Coviello, Fichera, Minetti, and Zara. *Statistical analysis:* P. Striano and Zara. *Obtained funding:* Zara. *Administrative, technical, and material support:* Coppola, Robbiano, and Zara.

Financial Disclosure: None reported.

Funding/Support: This work was supported in part by the Pierfranco and Luisa Mariani Foundation. Dr P. Striano received the Italian Paediatrics Association Prize 2009 for his research on "Application of Novel Genomics Technologies for Diagnosis of Patients With Epilepsy."

Additional Contributions: The Collaborative Group on Genetics of the Italian League Against Epilepsy provided helpful discussion of data.

REFERENCES

1. Baulac S, Baulac M. Advances on the genetics of Mendelian idiopathic epilepsies. *Clin Lab Med.* 2010;30(4):911-929.
2. Pal DK, Pong AW, Chung WK. Genetic evaluation and counseling for epilepsy. *Nat Rev Neurol.* 2010;6(8):445-453.
3. Cavalleri GL, Weale ME, Shianna KV, et al. Multicentre search for genetic susceptibility loci in sporadic epilepsy syndrome and seizure types: a case-control study. *Lancet Neurol.* 2007;6(11):970-980.
4. Koolen DA, Pfundt R, de Leeuw N, et al. Genomic microarrays in mental retardation: a practical workflow for diagnostic applications. *Hum Mutat.* 2009;30(3):283-292.
5. Gurnett CA, Hadera P. New ideas in epilepsy genetics: novel epilepsy genes, copy number alterations, and gene regulation. *Arch Neurol.* 2007;64(3):324-328.
6. Cook EH Jr, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. *Nature.* 2008;455(7215):919-923.
7. McMullan DJ, Bonin M, Hehir-Kwa JY, et al. Molecular karyotyping of patients

- with unexplained mental retardation by SNP arrays: a multicenter study. *Hum Mutat.* 2009;30(7):1082-1092.
8. Helbig I, Mefford HC, Sharp AJ, et al. 15q13.3 microdeletions increase risk of idiopathic generalized epilepsy. *Nat Genet.* 2009;41(2):160-162.
 9. de Kovel CG, Trucks H, Helbig I, et al. Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies. *Brain.* 2010;133(pt 1):23-32.
 10. Heinzen EL, Radtke RA, Urban TJ, et al. Rare deletions at 16p13.11 predispose to a diverse spectrum of sporadic epilepsy syndromes. *Am J Hum Genet.* 2010;86(5):707-718.
 11. Mefford HC, Muhle H, Ostertag P, et al. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet.* 2010;6(5):e1000962.
 12. Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia.* 1989;30(4):389-399.
 13. Ottman R, Barker-Cummings C, Leibson CL, Vasoli VM, Hauser WA, Buchhalter JR. Validation of a brief screening instrument for the ascertainment of epilepsy. *Epilepsia.* 2010;51(2):191-197.
 14. Itsara A, Cooper GM, Baker C, et al. Population analysis of large copy number variants and hotspots of human genetic disease. *Am J Hum Genet.* 2009;84(2):148-161.
 15. Carmona-Saez P, Chagoyen M, Tirado F, Carazo JM, Pascual-Montano A. GENE-CODIS: a web-based tool for finding significant concurrent annotations in gene lists. *Genome Biol.* 2007;8(1):R3.
 16. Cooper GM, Zerr T, Kidd JM, Eichler EE, Nickerson DA. Systematic assessment of copy number variant detection via genome-wide SNP genotyping. *Nat Genet.* 2008;40(10):1199-1203.
 17. Lee C, Iafrate AJ, Brothman AR. Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. *Nat Genet.* 2007;39(7)(suppl):S48-S54.
 18. Elia M, Striano P, Fichera M, et al. 6q terminal deletion syndrome associated with a distinctive EEG and clinical pattern: a report of five cases. *Epilepsia.* 2006;47(5):830-838.
 19. Striano P, Malacarne M, Cavani S, et al. Clinical phenotype and molecular characterization of 6q terminal deletion syndrome: five new cases. *Am J Med Genet A.* 2006;140(18):1944-1949.
 20. Shimokawa O, Kurosawa K, Ida T, et al. Molecular characterization of inv dup del(8p): analysis of five cases. *Am J Med Genet A.* 2004;128A(2):133-137.
 21. Stewart DR, Kleefstra T. The chromosome 9q subtelomere deletion syndrome. *Am J Med Genet C Semin Med Genet.* 2007;145C(4):383-392.
 22. Orrico A, Zollino M, Galli L, Buoni S, Marangi G, Sorrentino V. Late-onset Lennox-Gastaut syndrome in a patient with 15q11.2-q13.1 duplication. *Am J Med Genet A.* 2009;149A(5):1033-1035.
 23. Sharp AJ, Selzer RR, Veltman JA, et al. Characterization of a recurrent 15q24 microdeletion syndrome. *Hum Mol Genet.* 2007;16(5):567-572.
 24. Hannes FD, Sharp AJ, Mefford HC, et al. Recurrent reciprocal deletions and duplications of 16p13.11: the deletion is a risk factor for MR/MCA while the duplication may be a rare benign variant. *J Med Genet.* 2009;46(4):223-232.
 25. Phelan MC. Deletion 22q13.3 syndrome. *Orphanet J Rare Dis.* 2008;3:14.
 26. Van Esch H, Hollanders K, Badisco L, et al. Deletion of VCX-A due to NAHR plays a major role in the occurrence of mental retardation in patients with X-linked ichthyosis. *Hum Mol Genet.* 2005;14(13):1795-1803.
 27. Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet.* 2010;86(5):749-764.
 28. Zhou YD, Lee S, Jin Z, Wright M, Smith SE, Anderson MP. Arrested maturation of excitatory synapses in autosomal dominant lateral temporal lobe epilepsy. *Nat Med.* 2009;15(10):1208-1214.
 29. de Nijis L, Léon C, Nguyen L, et al. EFHC1 interacts with microtubules to regulate cell division and cortical development. *Nat Neurosci.* 2009;12(10):1266-1274.
 30. Falace A, Filipello F, La Padula V, et al. TBC1D24, an ARF6-interacting protein, is mutated in familial infantile myoclonic epilepsy. *Am J Hum Genet.* 2010;87(3):365-370.
 31. Suls A, Mullen SA, Weber YG, et al. Early-onset absence epilepsy caused by mutations in the glucose transporter GLUT1. *Ann Neurol.* 2009;66(3):415-419.