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CNV analysis from exome data in 349 patients with epileptic encephalopathy

EPGP Investigators & Epi4K Consortium[*,^]

Abstract

Infantile spasms (IS) and Lennox Gastaut syndrome (LGS) are epileptic encephalopathies characterized by early-onset, intractable seizures and poor developmental outcomes. *De novo* sequence mutations and copy number variants (CNVs) are causative in a subset of cases. We used exome sequence data in 349 trios with IS or LGS to identify putative *de novo* CNVs. We confirm 18 *de novo* CNVs in 17 patients (4.8%), 10 of which are likely pathogenic, giving a firm genetic diagnosis for 2.9% of patients. Confirmation of exome-predicted CNVs by array-based methods is still required due to false positive rates of prediction algorithms. Our exome-based results are consistent with recent array-based studies in similar cohorts and highlight novel candidate genes for IS and LGS.

The epileptic encephalopathies are a devastating group of epilepsies in which epileptic activity and seizures contribute to cognitive impairment or regression [1]. Most epileptic encephalopathies begin in infancy or early childhood and are associated with poor developmental outcome. Though the cause is unknown in the majority of cases, recent studies confirm that *de novo* mutations and copy number variants (CNVs) play an important role [2, 3]. We recently reported exome sequencing data in 264 parent-proband trios with infantile spasms (n=149) or Lennox-Gastaut syndrome (LGS; n=115) without syndromic features or MRI abnormalities from the Epilepsy Phenome/Genome Project (EPGP) cohort, identifying likely pathogenic, *de novo* sequence changes in >10% of patients [2]. Here we report results of copy number analysis derived from the exome data of this cohort and 85 additional patients to further elucidate the genetic architecture of these paradigmatic epileptic encephalopathies. Our exome-based CNV calling yields similar results to array-based studies for confirmed, *de novo*, likely pathogenic CNVs.

Correspondence to epi4k@duke.edu.

[^]A full list of authors appears at the end of the manuscript

Author Contributions

Initial Design of EPGP: B.K.A., E.A., O.D., D.D., M.P.E., Ru.Ku., D.H.L., R.O., E.S., M.R.W. **EPGP Patient Recruitment and Phenotyping:** B.A., J.F.B., S.F.B., G.C., D.C., P.C., O.D., D.D., M.F., N.B.F., D.F., E.B.G., T.G,. S.G., S.R.H., J.H., S.L.H., H.E.K., R.C.Kn., E.K., Ra.Ku., Ru.Ku, D.H.L., S.M.M., P.V.M., E.J.N., J.M.P., J.P., K.P., A.P., I.E.S., J.J.S., R.A.S., J.Si., M.S., L.L.T., A.V., E.P.G.V., G.K.V., J.W., P.W. **Phenotype Data Analysis**: B.A., B.K.A., A.B., G.C., O.D., D.D., J.F., T.G., S.J., A.K., R.C.Kn., Ru.Ku., D.H.L., R.O., J.M.P., A.P., I.E.S., R.A.S., E.S., J.J.S., J.Su., P.W., M.R.W. **Epi4K Steering Committee:** S.F.B., P.C., N.D., D.D., E.E.E., M.P.E., T.G., D.B.G., E.L.H., M.R.J., R.K., D.H.L., A.G.M., H.C.M., T.J.O., R.O., St.P, Sl.P., A.P., I.E.S., E.S. **Epileptic Encephalopathy Phenotyping Strategy:** S.F.B., P.C., D.D., R.K., D.H.L., R.O., I.E.S., E.S. **Encephalopathy Phenotyping:** D.D., M.R.Z.M., H.C.M., A.P., I.E.S., E.S. **Sequence Data Generation, Array Data Generation, Analysis & Statistical Interpretation:** A.S.A., B.P.C., J.C., E.L.H., N.K., H.C.M., B.K.N., A.R. **Writing & Editing of Manuscript:** S.F.B., E.E.E., H.C.M., E.L.H., D.H.L., A.P., A.R., E.S.

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PATIENTS & METHODS

Samples

Probands and family members were collected as part of the EPGP cohort (Supplementary Table 1) as described previously [2, 4] with approval by site-specific Institutional Review Boards; 1047 individuals comprising 349 parent-child trios were included in the present analysis. Of these, 264 were previously analyzed for *de novo* single nucleotide variants [2]. Prior clinical CNV testing included chromosome microarray and/or karyotype analysis in 131/349 patients (38% of cohort). Detailed inclusion criteria are published [4]; briefly, participants were required to have EEG findings consistent with LGS (slow or disorganized background, and slow spike and wave <2.7 Hz or generalized paroxysmal fast activity) or IS (hypsarrhythmia or hypsarrhythmia variant or electrodecremental discharge) [4]. Exclusion criteria included evidence of a known genetic syndrome or chromosome abnormality. Extensive phenotype analysis of patients enrolled in the study are published elsewhere [5] (and Madou *et al.*, manuscript in preparation). All available clinical records were re-reviewed for those patients found to have a *de novo* CNV and evidence of syndromic features was often noted upon reexamination of the medical records.

CNV calling and validation

Copy number variations (CNVs) were detected by analyzing exome data using the CoNIFER pipeline, a depth-of-coverage based algorithm using the conifer-tools package, which implements DNACopy [67] (Supplementary Methods). The following filtering criteria were applied: CNVs of 3–5 probes average singular value decomposition (SVD)-transformed signal >1; CNVs of 6 or greater probes, average signal > 0.5. CNVs more than 50% in repetitive or duplicated genomic space were removed. CNV calls were manually curated, and curated calls were compared to control CNV datasets to filter out common CNVs present in >1% of the general population. Control CNV datasets included (i) CNV calls from the Atherosclerosis Risk in Communities (ARIC) Study (n=11,305) analyzed using Affymetrix AFFY_6.0 SNP microarray and (ii) CNV calls from the NLHBI GO Exome Sequencing Project (ESP, n=2,972) from CoNIFER analysis of exome sequence data. CoNIFER-predicted *de novo* CNVs and a subset of predicted large (>500 kb), inherited CNVs were validated using oligonucleotide (Agilent) and/or SNP (Illumina HumanCore 12v1; n=295,393 probes) microarray. *De novo* CNVs were considered pathogenic if the CNV (or largely overlapping CNV) was previously associated with epilepsy or related neurodevelopmental disorders or contained a known epilepsy gene.

RESULTS

CNV discovery and validation

As CNV detection from exome data is still an emerging technique, we initially performed comprehensive validation studies in 43 probands to estimate our overall validation rate for CoNIFER calls in this dataset. We validated 53/80 (66%) predicted inherited CNVs, consistent with our previous studies [8] (Supplementary Table 2). Twenty-four were paternally inherited and 29 were maternally inherited, with a size range of 5.2 kb to 8.8 Mb (mean 377 kb). For the same 43 probands, we validated 5/21 (24%) predicted *de novo* CNVs

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(Supplementary Table 3). The lower validation rate is not unexpected, given that any false positive call in a proband will appear to be *de novo*, whereas inherited CNV predictions are supported by the same predicted CNV in two individuals (proband and one parent).

As the majority of causative CNVs in this cohort were expected to be *de novo*, we targeted the remainder of our validation studies to predicted *de novo* CNVs. We confirmed a total of 18 *de novo* CNVs in 17 patients (Table 1). The *de novo* CNVs range in size from 94 kb to 16 Mb and involve 1 to 163 genes. Notably, none of these 17 individuals had clearly pathogenic *de novo* SNVs by exome sequencing. In ten patients, the *de novo* CNV(s) is likely pathogenic based on size, previous association with epilepsy or gene content [9]. One pathogenic CNV (15q11 dup) recurred in 3 cases. In seven patients, the *de novo* CNV is of uncertain clinical significance (Table 1).

Because whole genome array CGH was used to validate *de novo* CNVs, we also confirmed a large number of inherited CNVs across the cohort. We confirmed 69 inherited CNVs in 54 individuals ranging from 5.2 kb to 8.8 Mb (mean 305 kb; Supplementary Table 4). Eight individuals (2.3%) each have an inherited CNV >500 kb; four (1.2%) of these are >1 Mb (Table 2). We also looked specifically for inherited CNVs within three recurrent deletion regions that have been previously associated with risk for epilepsy [10, 11]: 15q11.2, 15q13.3 and 16p13.11. Two patients have inherited deletions of 15q11.2 that may contribute to their phenotype; another two patients each have a small, inherited duplication within the 16p13.11 region of uncertain significance. Aside from the large 15q11-q13 duplications described above, there were no additional CNVs within the 15q13.3 region. Though *de novo* CNVs are more likely to be pathogenic [12], it is possible that one or more of the inherited CNVs in our cohort is contributory. Three individuals with rare inherited CNVs had a pathogenic SNV and one has a de novo 15q11 duplication, making it less likely that the inherited CNV is causative (Table 2).

There are 540 unique genes within the 18 *de novo* CNV regions in our cohort (Supplemental Table 5), three of which are known EE genes: *SCN1A, SCN2A* and *GABRB3*. All five individuals with CNVs involving these genes have phenotypes consistent with those described for the CNVs they carry (Supplementary Table 7). Eight additional genes (*GLIS3, KIAA1324L, NIPA1, PLCG2, RCL1, RFX3, SPG7, YWHAG*) within *de novo* CNV regions were also found to have a *de novo* sequence variant by trio exome sequencing in the same cohort (Supplementary table 5, reference [2] & unpublished data); these cannot be regarded as confirmed EE genes, but finding both a *de novo* SNV and CNV involving each of them suggests that follow-up in a larger cohort is warranted. In addition, three and 30 genes within *de novo* CNVs were found to have *de novo* mutations by trio exome sequencing in ID [13, 14] and autism [15–18], respectively; these genes may warrant follow-up given the overlapping genetic susceptibility of these disorders.

DISCUSSION

We detected CNVs from exome sequencing data in 349 trios from patients with IS or LGS. We confirmed 18 *de novo* CNVs in 17/349 probands (4.8%), providing a definitive diagnosis in 2.9% of patients and a possible explanation for another 2.0%. Notably, 38% of the current

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cohort had already undergone karyotype and/or chromosome microarray testing prior to enrollment in the study and had not arrived at a diagnosis through clinical testing. Evaluation of patients without prior screening may result in a higher yield; indeed, we observed a *de novo* CNV in 5.6% of the 218 participants in our cohort without previous clinical testing. These results are similar to our prior studies in a broader spectrum of EE where 4.1% had a definitely pathogenic CNV [3] and to our recently reported findings in a large clinically ascertained cohort with a broad range of epilepsy diagnoses where 5% of cases had a causative CNV [19].

Three individuals each have a *de novo* duplication consistent with 15q11q13 duplication syndrome, characterized by hypotonia, seizures, developmental delay and behavior problems. A “late-onset LGS” phenotype has been described in some patients. Other *de novo* CNVs in our cohort that have been previously associated with epilepsy include 7q11 deletion, 9p terminal deletion, 2q24 duplication and *SCN1A* deletion. One patient harbors a *de novo* intragenic deletion of the *GPHN* gene, which encodes a protein that is responsible for the clustering of glycine and GABA receptors at inhibitory synapses. Inherited or *de novo* deletions involving *GPHN* were recently described in six patients with autism, schizophrenia or seizures [20]. The deletion in our patient is the largest of those described and also involves the *FAM17D* and *MIPP5* genes.

Comparison of the genes within *de novo* CNV regions in our cohort to those in which at least one other patient in this cohort had a *de novo* sequence variant identifies several novel candidate genes that deserve follow-up in a larger cohort. Furthermore, several patients harbor *de novo* CNVs involving only 1–4 genes. While these CNVs are of uncertain significance, identification of *de novo* SNVs in the same genes encompassed by certain CNVs would support the fact that these CNVs are related to disease.

In the large EPGP cohort of IS and LGS patients, the addition of this CNV data to the *de novo* SNV findings shows that a definitive genetic diagnosis can be reached in >15% of cases for which there was previously no known cause. As whole exome sequencing is becoming widely used, one might ask if CNV data can be efficiently and reliably extracted in a clinical setting, thus bypassing the need for array-based CNV assays. Our experience, especially as shown by the false positive rate, suggests that array-based technologies are currently still required. A logical clinical approach to a patient with IS or LGS of unknown etiology should include a chromosome microarray for patients with epilepsy and additional findings such as abnormal MRI, developmental delays or dysmorphic features, followed by an epilepsy-focused targeted gene panel and then whole exome sequencing in cases that remain undiagnosed. As prediction algorithms improve, exome and, eventually, whole genome sequencing will provide a genetic diagnosis in an even greater proportion of patients in the clinical setting, improving medical management and genetic counseling in this patient population.

Supplementary Material

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Appendix

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Andrew S. Allen [1], Samuel F. Berkovic [2], Bradley P. Coe [6], Joseph Cook [14], Patrick Cossette [3], Norman Delanty [4], Dennis Dlugos [5], Evan E. Eichler [6], Michael P. Epstein [7], Tracy Glauser [8], David B. Goldstein [9], Erin L. Heinzen [9], Michael R. Johnson [10], Nik Krumm [6], Ruben Kuzniecky [11], Daniel H. Lowenstein [12], Anthony G. Marson [13], Heather C. Mefford [14], Ben Nelson [6], Sahar Esmaeeli Nieh [15], Terence J. O'Brien [16], Ruth Ottman [17], Stephen Petrou [18,19], Slavé Petrovski [2,9,16], Annapurna Poduri [20], Archana Raja [63], Elizabeth K. Ruzzo [9], Ingrid E. Scheffer [21], Elliott Sherr [22]

EPGP

Bassel Abou-Khalil [23], Brian K. Alldredge [24], Eva Andermann[25], Frederick Andermann[25], Dina Amron[25], Jocelyn F. Bautista [26], Samuel F. Berkovic [2], Alex Boro [27], Gregory Cascino [28], Damian Consalvo [29], Patricia Crumrine [30], Orrin Devinsky [31], Dennis Dlugos [5], Michael P. Epstein [7], Miguel Fiol [32], Nathan B. Fountain [33], Jacqueline French [34], Daniel Friedman [35], Eric B. Geller [36], Tracy Glauser [8], Simon Glynn [37], Sheryl R. Haut [38], Jean Hayward [39], Sandra L. Helmers [40], Sucheta Joshi [41], Andres Kanner [42], Heidi E. Kirsch [43], Robert C. Knowlton [44], Eric H. Kossoff [45], Rachel Kuperman [46], Ruben Kuzniecky [11], Daniel H. Lowenstein [12], Shannon M. McGuire [47], Paul V. Motika [48], Edward J. Novotny [49], Ruth Ottman [17], Juliann M. Paolicchi [50], Jack Parent [51], Kristen Park [52], Annapurna Poduri [20], Ingrid E. Scheffer [21], Renée A. Shellhaas [53], Elliott Sherr [22], Jerry J. Shih [54], Rani Singh [55], Joseph Sirven [56], Michael C. Smith [42] , Joe Sullivan [12], Liu Lin Thio [57], Anu Venkat [58], Eileen P.G. Vining [59], Gretchen K. Von Allmen [60], Judith L. Weisenberg [61], Peter Widdess-Walsh [36], Melodie R. Winawer [62]

1. Department of Biostatistics and Bioinformatics, Duke Clinical Research Institute, and Center for Human Genome Variation, Duke University Medical Center, Durham, North Carolina 27710 USA.
2. Epilepsy Research Centre, Department of Medicine, University of Melbourne (Austin Health), Heidelberg, Victoria 3084, Australia.
3. Centre of Excellence in Neuromics and CHUM Research Center, Université de Montréal, CHUM-Hôpital Notre-Dam Montréal, Quebec H2L 4M1e, Canada.
4. Department of Neurology, Beaumont Hospital and Royal College of Surgeons, Dublin 9 Ireland.
5. Department of Neurology and Pediatrics, The Children's Hospital of Philadelphia, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania 19104 USA.
6. Howard Hughes Medical Institute, Department of Genome Sciences, University of Washington School of Medicine, Seattle, Washington 98195 USA.

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7. Department of Human Genetics, Emory University School of Medicine, Atlanta, Georgia 30322, USA.

8. Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio 45229 USA.

9. Center for Human Genome Variation, Duke University School of Medicine, Durham, North Carolina 27708 USA.

10. Centre for Clinical Translation Division of Brain Sciences, Imperial College London, London, SW7 2AZ United Kingdom.

11. NYU School of Medicine, New York University, New York, New York 10016 USA.

12. Department of Neurology, University of California, San Francisco, San Francisco, California 94143 USA.

13. Department of Molecular and Clinical Pharmacology, University of Liverpool, Clinical Sciences Centre, Lower Lane, Liverpool, L9 7LJ, United Kingdom.

14. Department of Pediatrics, Division of Genetic Medicine, University of Washington, Seattle, Washington 98115 USA.

15. University of California, San Francisco, California 94143 USA.

16. Departments of Medicine and Neurology, The Royal Melbourne Hospital, Parkville, Victoria, 3146 Australia.

17. Departments of Epidemiology and Neurology, and the G.H. Sergievsky Center, Columbia University; and Division of Epidemiology, New York State Psychiatric Institute, New York, New York 10032 USA.

18. Florey Institute for Neuroscience and Mental Health. The University of Melbourne, VIC 3010, Australia.

19. Centre for Neural Engineering. The University of Melbourne, VIC 3010, Australia.

20. Division of Epilepsy and Clinical Neurophysiology, Department of Neurology Boston Children's Hospital, Boston, Massachusetts 02115 USA.

21. Epilepsy Research Centre, Department of Medicine, University of Melbourne (Austin Health), Heidelberg, Victoria 3084, Australia, Florey Institute and Department of Pediatrics, Royal Children' s Hospital, University of Melbourne, Victoria 3052, Australia.

22. Departments of Neurology, Pediatrics and Institute of Human Genetics, University of California, San Francisco, San Francisco, California 94158 USA.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

23. Department of Neurology, Vanderbilt University Medical Center, Nashville, Tennessee 37232 USA.

24. Department of Clinical Pharmacy, UCSF School of Pharmacy, Department of Neurology, UCSF School of Medicine 94143 USA.

25. Departments of Neurology, Neurosurgery and Human Genetics, McGill University, Montreal, Quebec H3A 2B4 Canada.

26. Department of Neurology, Cleveland Clinic Lerner College of Medicine & Epilepsy Center of the Cleveland Clinic Neurological Institute, Cleveland, Ohio 44195 USA.

27. Department of Neurology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York, 10467 USA.

28. Divison of Epilepsy, Mayo Clinic, Rochester, Minnesota 55905 USA.

29. Epilepsy Center, Neurology Division, Ramos Mejía Hospital, Buenos Aires, 1221, Argentina.

30. Medical Epilepsy Program & EEG & Child Neurology, Children's Hospital of Pittsburgh of UPMC, Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15224 USA.

31. NYU and Saint Barnabas Epilepsy Centers, NYU School of Medicine, New York, New York 10016 USA.

32. Department of Neurology, Epilepsy Care Center, University of Minnesota Medical School, Minneapolis, Minnesota 55414 USA.

33. FE Dreifuss Comprehensive Epilepsy Program, University of Virginia, Charlottesville, Virginia 22908 USA.

34. NYU Comprehensive Epilepsy Center, New York, New York 10016 USA.

35. Department of Neurology, NYU School of Medicine, New York, New York, 10016 USA.

36. Division of Neurology, Saint Barnabas Medical Center, Livingston, New Jersey 07039 USA.

37. Department of Neurology, Comprehensive Epilepsy Program, University of Michigan Health System, Ann Arbor, Michigan 48109 USA.

38. Comprehensive Epilepsy Center, Montefiore Medical Center, Bronx, New York 10467 USA.

39. The Kaiser Permanente Group, Oakland, California 94618 USA.

40. Neurology and Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322 USA.

41. Pediatrics & Communicable Diseases, University of Michigan, Ann Arbor, Michigan 48109 USA.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

42. Department of Neurological Sciences, Rush Epilepsy Center, Rush University Medical Center, Chicago, Illinois 60612 USA.

43. Departments of Neurology and Radiology, University of California, San Francisco, California 94143 USA.

44. Neurology, University of Texas Medical School, Houston, Texas 77030 USA.

45. Neurology and Pediatrics, Child Neurology, Pediatric Neurology Residency Program, Johns Hopkins Hospital, Baltimore, Maryland 21287 USA.

46. Epilepsy Program, Children's Hospital & Research Center Oakland, Oakland, California 94609 USA.

47. Clinical Neurology, Children's Hospital Epilepsy Center of New Orleans, New Orleans, Louisiana 70118 USA.

48. Comprehensive Epilepsy Center, Oregon Health and Science University, Portland, Oregon 97239 USA.

49. Departments of Neurology and Pediatrics, University of Washington School of Medicine, Seattle Children's Hospital, Seattle, Washington 98105 USA.

50. Weill Cornell Medical Center, New York, New York 10065 USA.

51. Department of Neurology and Neuroscience Graduate Program, University of Michigan Medical Center, Ann Arbor, MI 49108 and Ann Arbor Veterans Administration Healthcare System, Ann Arbor, Michigan 48105 USA.

52. University of Colorado School of Medicine, Aurora, Colorado 80045, U.S.A.; Division of Neurology, Department of Pediatrics, Children's Hospital Colorado, Aurora, Colorado 80045 USA.

53. University of Michigan, Pediatric Neurology, Ann Arbor, Michigan 48109 USA.

54. Department of Neurology, Mayo Clinic, Jacksonville, Florida 32224 USA.

55. Division of Pediatric Neurology, University of Michigan Health System, Ann Arbor, Michigan 48109 USA.

56. Department of Neurology, Mayo Clinic, Scottsdale, Arizona 85259 USA.

57. Department of Neurology, Washington University School of Medicine, St. Louis, Missouri 63110.

58. The Children's Hospital at Saint Peter's University Hospital, New Brunswick, New Jersey 08901 USA.

59. Department of Neurology, Johns Hopkins Hospital, Baltimore, Maryland 21287 USA.

60. Division of Child & Adolescent Neurology, Departments of Pediatrics , University of Texas Medical School, Houston, Texas 77030 USA.

61. Department of Neurology, Division of Pediatric Neurology, Washington University School of Medicine, St. Louis, Missouri 63110 USA.

62. Department of Neurology and the G.H. Sergievsky Center, Columbia University, New York, New York, 10032 USA.

63. Department of Genome Sciences, University of Washington School of Medicine, Seattle, Washington, 98195 USA.

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Table 1

de novo CNVs in 349 trios

Trio	CNV	Size	Candidate or known epilepsy genes or known disease association	*De novo* SNV calls from exome (REF)	Validation platform	Gene(s) enriched in CNVs found in patients with neurodevelopmental phenotypes*	Age at onset	Seizure types%
Likely pathogenic CNVs								
fx	2q24 dup	7.5 Mb	*SCN2A, SCN1A*	*SMG9 (mis), EPHB1 (synon)*	CGH	*SCN1A SCN2A*	7 mo	IS
iq	2q24 del	296 kb	*SCN1A*	None	CGH, SNP	*SCN1A*	<1 yr	GTC, aA
hj	5p15 del	3.8 Mb	*SEMA5A, CTNND2*	*SDCBP2* (mis)	CGH	*TAS2R1, FAM173B, CCT5, MTRR*	6 mo	FS, focal, GTC, aA, SE
cy	7q11 del	11.4 Mb	*MAGI2, YWHAG, HIP1*	*ZNF12* (UTR), FPGT-TNNI3K (mis), FAM50A	CGH, SNP	*HIP1*	3 mo	IS, aA
aia	9p ter del	8.7 Mb	9p deletion syndrome	None	SNP	*DMRT2, DMRT3*	5 mo	IS
iz	14q23 del	585 kb	*GPHN*	*HRG* (mis), *PCDHB13* (mis)	CGH	--	2.5 yrs	FS+SE, T, drop
eh	15q11 dup	5.0 Mb	15q11q13 dup syndrome; *GABRB3*	*PAQR8* (synon)	CGH	*GABRB3*	2 wks	IS, multiple other
ag	15q11 dup#	12.0 Mb	15q11q13 dup syndrome; *GABRB3*	*MLL4* (mis)	CGH, karyo	*GABRB3*	8 mo	IS
gq^	15q11 dup	8.4 Mb	15q11q13 dup syndrome; *GABRB3*	None	CGH, SNP	*GABRB3*	8 mo	GTC, T, atonic
fu	t(15;16)#	1.8 Mb del, 16.3 Mb dup	Large unbalanced translocation	None	CGH, karyo	*SNRPA1 FANCA*	8 mo	IS
CNVs of uncertain clinical significance								
ig	1p22 dup	140 kb	1 gene: *ZNF644*	*IQSEC1* (mis)	CGH, SNP	--	2 yrs	A, GTC, M, T, drop
ad	1q21 dup	249 kb	TAR region dup	*NFE2L1* (mis)	SNP	*LIX1L*	8 mo	IS

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Trio	CNV	Size	Candidate or known epilepsy genes or known disease association	*De novo* SNV calls from exome (REF)	Validation platform	Gene(s) enriched in CNVs found in patients with neurodevelopmental phenotypes*	Age at onset	Seizure types%
aib	2q37 del	154 kb	4 genes: *PPP1R7, HDLBP, ANO7, SEPT2*	*CXXC11* (synon)	SNP	*PPP1R7*	5 mo	IS, T
gc	7q22 del	622 kb	15 genes in region	*NR1H2* (mis)	CGH	*MUC17, MYL10, TRIM56*	8 mo	IS
ahp	7q31 dup	94 kb	2 genes: *CPED1, ING3*	*ADAMSL4* (mis), *PPP6R2* (mis)	SNP	--	7 mo	IS
le	8p23 del	140 kb	2 genes: *MCPH1, AGTP2*	*DACH2* (mis)	CGH, SNP	*ANGPT2*	3y 10mo	GTC, drop, T, M, A, aA
bda	17q12 del	1.5 Mb	15 genes in region	None	h.c.		8 mo	IS, M, SE, GTC

*genes listed represent those with mean p-value <0.05 for known disease gene(s) in region or peak p-value <0.05 for novel regions as described by Cooper and colleagues[21], see Supplementary Table 5 for details; --, no gene within region with p<0.05;

#upon review of records, diagnosis made prior to enrollment; h.c., high confidence CNV call by CoNIFER;

%seizure types include all reported, first type listed was the initial seizure type: IS, infantile spasms; GTC, generalized tonic clonic; aA, atypical absence; FS, febrile seizures; SE, status epilepticus; T, tonic; A, absence; M, myoclonic. Additional information available in Supplementary Table 7.

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Table 2

Selected Inherited CNVs

Trio	CNV (inher)	Size (kb)	# of genes; possible EE candidates	Causative d.n. SNV?	Validation platform
Large (>500 kb) inherited CNVs					
jp	2p22 dup (pat)	620	3 genes: *BIRC6, TTC27, LTBP1*	No	SNP
ip	17q dup (pat)	737	13 genes	No	CGH
ad	10q21 del (mat)	858	1 gene: *PCDH15*	No	SNP
jg	4p16 dup (mat)	885	5 genes	*WDR45* frameshift	SNP
ki	7q11 dup (pat)	1000	9 genes	*DNM1* missense	SNP
dg	Xp22 del (pat)	1900	8 genes	*ALG13* missense	h.c.
bj	Xp22 dup (mat)	2000	9 genes	No	h.c.
gq	1q31 dup (pat)	8800	23 genes	No; *de novo* 15q11 dup	CGH, SNP
Recurrent CNV regions previously associated with epilepsy					
j	16p13 dup (pat)	30	*NTAN1, PDXDC1 (16p13.11)*	No	h.c.
r	16p13 dup (mat)	58	*ABCC1, ABCC6 (16p13.11)*	No	h.c.
d	15q11.2 del (mat)	213	*NIPA2, CYFIP1*	No	h.c.
in	15q11.2 del (pat)	213	*NIPA2, CYFIP1*	No	SNP

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