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Vlaskamp, Danique R M; Callenbach, Petronella; Rump, Patrick; Giannini, Lucia A A; Dijkhuizen, Trijntje; Brouwer, Oebele; Arts, Cornelia

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Copy number variation in a hospital-based cohort of children with epilepsy

*†Danique R. M. Vlaskamp , *¹Petra M. C. Callenbach , †¹Patrick Rump, †Lucia A. A. Giannini, †Trijnie Dijkhuizen, *Oebele F. Brouwer, and †Conny M. A. van Ravenswaaij-Arts 

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SUMMARY

Objective: To evaluate the diagnostic yield of microarray analysis in a hospital-based cohort of children with seizures and to identify novel candidate genes and susceptibility loci for epilepsy.

Methods: Of all children who presented with their first seizure in the University Medical Center Groningen (January 2000 through May 2013) ($n = 1,368$), we included 226 (17%) children who underwent microarray analysis before June 2014. All 226 children had a definite diagnosis of epilepsy. All their copy number variants (CNVs) on chromosomes 1–22 and X that contain protein-coding genes and have a prevalence of <1% in healthy controls were evaluated for their pathogenicity.

Results: Children selected for microarray analysis more often had developmental problems (82% vs. 25%, $p < 0.001$), facial dysmorphisms (49% vs. 8%, $p < 0.001$), or behavioral problems (41% vs. 13%, $p < 0.001$) than children who were not selected. We found known clinically relevant CNVs for epilepsy in 24 of the 226 children (11%). Seventeen of these 24 children had been diagnosed with symptomatic focal epilepsy not otherwise specified (71%) and five with West syndrome (21%). Of these 24 children, many had developmental problems (100%), behavioral problems (54%) or facial dysmorphisms (46%). We further identified five novel CNVs comprising four potential candidate genes for epilepsy: *MYTIL*, *UNC5D*, *SCN4B*, and *NRXN3*.

Significance: The 11% yield in our hospital-based cohort underscores the importance of microarray analysis in diagnostic evaluation of children with epilepsy.

KEY WORDS: Microarray, Deletions, Duplications, Genetics, Seizures.



Danique R. M. Vlaskamp, MD, is a PhD student in epilepsy genetics at the University Medical Center Groningen.

Genetic factors play an important role in the etiology of epilepsy,¹ as demonstrated by the large number of genes and regions that cause or predispose to epilepsy newly identified by various genome-wide technologies.² Chromosomal

microarray analysis, in particular, enables the identification of chromosomal deletions (losses) or duplications (gains), called copy number variants (CNVs).³ CNVs may contribute to epilepsy in two ways. First, CNVs that include epilepsy-related genes could lead to epilepsy following a Mendelian inheritance. For example, both *KCNQ2* sequence variants and whole gene deletions can cause benign familial neonatal seizures.^{4,5} Second, CNVs that occur more frequently in patients compared to in healthy controls may increase an individual's susceptibility to developing epilepsy, with the responsible haploinsufficient gene(s) often being unknown. Large cohort studies have identified such susceptibility CNVs in several chromosomal regions, including well-known CNVs located at 15q11.2 (BP1-BP2), 15q13.3 and 16p13.11.^{6–8}

Studies using microarray analysis have most often been performed in research cohorts of children who were selected

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*Departments of Neurology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands; and †Department of Genetics, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

Address correspondence to Conny M. A. van Ravenswaaij-Arts, Department of Genetics, University Medical Center Groningen, University of Groningen, CB50, P.O. Box 30.001, 9700 RB Groningen, the Netherlands. E-mail: c.m.a.van.ravenswaaij@umcg.nl

¹These authors contributed equally to this work.

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KEY POINTS

- Microarray in our hospital-based cohort of children with epilepsy had a 11% yield of clinically relevant CNVs
- The yield of microarray in children with epilepsy is largely based on the selection of individuals by the clinician
- Novel CNVs were identified, including four epilepsy candidate genes: *MYT1L*, *UNC5D*, *SCN4B*, and *NRXN3*

on the basis of their epilepsy diagnosis, for example, idiopathic generalized epilepsies, focal epilepsies, and/or fever-associated epilepsies.^{6–8} Only a few studies have addressed the yield of microarray analysis in clinical cohorts of all children presenting with any type of seizures in a clinical setting.^{9–11} We therefore aimed to evaluate the diagnostic yield of microarray analysis in a hospital-based cohort of children with epilepsy for whom detailed phenotypic information was available, with the further goal of identifying novel candidate genes or susceptibility loci for epilepsy.

MATERIAL AND METHODS

Study cohort

The study cohort was derived from the childhood seizure database of the University Medical Center Groningen (UMCG), a regional referral center for children with epilepsy. In this database, we retrospectively included all children who presented with their first febrile or afebrile seizure before the age of 18 years between January 2000 and June 2013, and who were seen and/or treated by a child neurologist of the UMCG (n = 1,368). Epilepsy was diagnosed in 91% of these children using the current International League Against Epilepsy (ILAE) practical clinical definition of epilepsy.¹² Of the remaining children (9%), 7% had febrile seizures only and 2% had only one afebrile seizure. The UMCG database contains phenotype information and was independently completed by two researchers (DRMV and PMCC). Phenotypic inconsistencies and epilepsy classification were discussed until agreement was reached using the information in the database as well as in the original medical records (PMCC and OFB). Epilepsy syndromes and seizure types were classified according to the 2006 ILAE classification.¹³ Children were included in this study if they underwent microarray analysis in the context of their diagnostic work-up before June 2014. Formal independent review board evaluation was waived by the Institutional Medical Ethical Committee of the UMCG because of the retrospective and observational character of this study.

Chromosomal microarray analysis and data interpretation

Microarray analyses were performed using an oligonucleotide array (Agilent 105K or 180K custom HD-DGH microarray; Agilent Technologies, Santa Clara, CA, U.S.A.) or a single nucleotide polymorphism (SNP) array (Illumina Omni Express 12-V1.0; Illumina, San Diego, CA, U.S.A.). Cartagenia Bench Lab CNV software was used for storage, analysis and reporting of the structural genomic data (Cartagenia, Leuven, Belgium; part of Agilent Technologies). The chromosomal coordinates of CNVs were reported relative to the Genome Reference Consortium Human Reference genome version 37 (GRCh37/hg19).

CNVs on chromosome 1–22 or X identified by at least three (SNP microarray) or four (oligonucleotide microarray) consecutive probes were evaluated for their pathogenicity (Fig. 1). CNVs were excluded from further analysis when they did not contain (protein-coding) genes or had $\geq 90\%$ overlap with CNVs seen in $\geq 1\%$ of healthy controls. The prevalence of CNVs in healthy controls was calculated using the International Database of Genomic Variance (n = 14,316, last updated February 2013),¹⁴ the Low Lands Consortium database of oligonucleotides (n = 2,402, last updated December 2012), and SNP microarray results of healthy parents of children who underwent microarray analysis in five Dutch genetic centers (n = 749, last updated October 2014). Remaining CNVs were categorized into two groups: (1) CNVs with $< 90\%$ overlap with CNVs observed in healthy controls, and (2) CNVs with $\geq 90\%$ overlap with CNVs observed in $< 1\%$ of healthy controls (Fig. 1). CNVs in both groups were marked as potentially clinically relevant if they had overlap with genetic regions previously associated with epilepsy. These regions were identified by performing a literature search using PubMed, complemented with information from the Decipher database and Cartagenia Bench Lab CNV software. The remaining CNVs in both groups were evaluated for novel candidate genes or susceptibility loci for epilepsy. CNVs with $< 90\%$ overlap with CNVs of healthy controls were of interest if they contained a gene with an expression or function in the brain or a gene associated with an autosomal dominant or X-linked neuropsychiatric disease, and if they occurred in at least one (for deletions) or two (for duplications) unrelated children in our cohort. In the group of CNVs with $\geq 90\%$ overlap with CNVs observed in $< 1\%$ of healthy controls, overlapping regions between CNVs in at least two unrelated children were of interest if these regions contained protein-coding genes and were 10 times more prevalent in our cohort compared to healthy controls.

Statistical analyses

SPSS Statistics Version 22.0 (IBM Corporation, NY, U.S.A.) was used to perform descriptive and comparative statistics. Differences in categorical and ordinal phenotypic

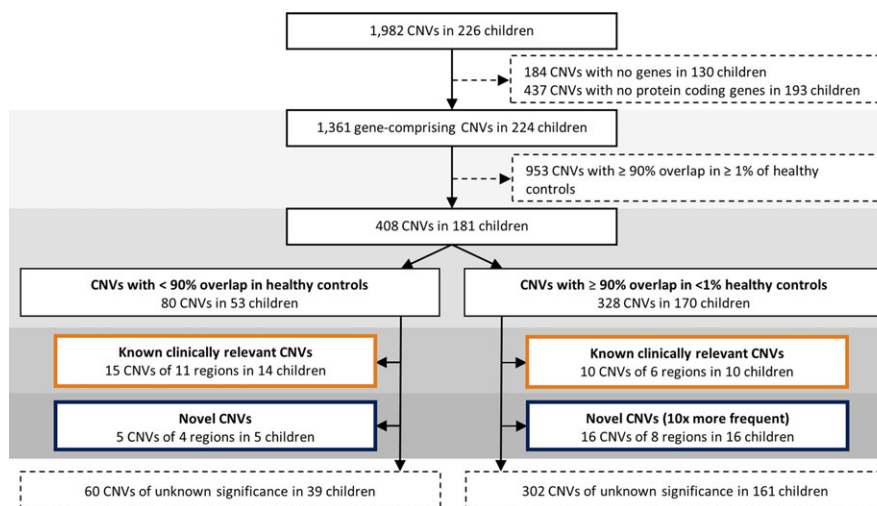


Figure 1.

Flow chart for evaluating copy number variants (CNVs) in our hospital-based cohort of children with epilepsy. *Epilepsia Open* © ILAE

data between children were analyzed using Fisher's exact and Mann-Whitney U tests, respectively.

RESULTS

Characteristics of the study cohort

In 226 (17%) children, microarray analysis was performed in the context of their diagnostic work-up. Their phenotypic characteristics are summarized in Table 1. All children had a definite diagnosis of epilepsy, except for one who had a single febrile status epilepticus.

Children with epilepsy who underwent microarray analysis had significantly more often developmental problems (82% vs. 25%, $p < 0.001$), facial dysmorphisms (49% vs. 8%, $p < 0.001$), or behavioral problems (41% vs. 13%, $p < 0.001$) than children in our database who did not undergo microarray analysis. To reduce bias, our comparisons were limited to children with epilepsy onset after December 31, 2005, when microarray analysis was introduced in our center ($n = 158$ for children with array; $n = 271$ for children without array or another identified genetic cause). The presence of a positive family history for epilepsy, known in 141/158 children who did and in 206/271 children who did not undergo microarray, did not differ significantly (33% vs. 30%, $p = 0.56$) between the two groups.

Diagnostic yield of microarray analysis

Microarray analysis revealed 1,982 CNVs in 226 children (Fig. 1). After excluding CNVs that contained no (protein-coding) genes and/or were identified as most likely benign polymorphisms ($\geq 90\%$ overlap in $\geq 1\%$ of controls), 408 CNVs in 181 children remained to be evaluated for their pathogenicity. These 408 CNVs included 233 (57.1%)

duplications with a median size of 198.8 kb (range 17.9 kb–21.0 Mb) and 175 (42.9%) deletions with a median size of 168.4 kb (range 22.4 kb–21.0 Mb). Inheritance could be analyzed for 102 (25.0%) CNVs, with 23 (22.5%) occurring de novo and 79 (77.5%) being inherited.

Known clinically relevant CNVs for epilepsy were identified in 24 of the 226 (11%) children with epilepsy (Fig. 1, Tables 2 and 3). Their epilepsy was most often classified as symptomatic focal epilepsy not otherwise specified (71%) or West syndrome (21%). All children had developmental problems (100%), and many had behavioral problems (54%) and facial dysmorphisms (46%). Overall, no significant differences were found between children with and without clinically relevant CNVs for epilepsy syndrome diagnosis or the presence of other phenotypic characteristics (data not shown).

In 14 (7%) children, 15 known clinically relevant CNVs were found that do not occur in healthy controls (Table 2). Ten of these CNVs occurred de novo. For the remaining CNVs, inheritance was unknown. The phenotypes of these 14 children were compatible with previously reported phenotypes associated with these CNVs and included the well-established diagnoses: chromosome 1p36 deletion syndrome (MIM 607872), chromosome 2q23.1 deletion syndrome (MIM 156200), chromosome 18q deletion syndrome (MIM 601808), Angelman syndrome (MIM 105830), chromosome 15q11q13 duplication syndrome (MIM 608636), chromosome 16p11.2 deletion syndrome (MIM 611913), lissencephaly type 1 (MIM 607432), Phelan-McDermid syndrome (MIM 606232), and Juberg-Hellman syndrome (MIM 300088; epilepsy, female restricted, with mental retardation) (Table 2). One patient had a 2.6 Mb 8q22 deletion; comparable deletions have been published in six other cases.^{16,17}

Table 1. Characteristics of the study cohort (n = 226)

Characteristics	
Male (%)	132 (58.4)
Deceased (%)	17 (7.5)
Median age at evaluation (range)	8 years 10 months (1 year 8 months–23 years 3 months)
Median age at epilepsy onset (range)	1 year 1 month (0 days–15 years 11 months)
Seizure types (%)	
GTCS	10 (4.4)
Absences	5 (2.2)
Myoclonic seizures	18 (8.0)
Epileptic spasms	36 (15.9)
Atonic seizures	12 (5.3)
Focal seizures	176 (77.9)
Secondarily generalized seizures	123 (54.4)
Neonatal seizures	33 (14.6)
Unclassified	1 (0.4)
Status epilepticus (%)	74 (32.7)
Epilepsy syndrome (%)	
Benign (familial) neonatal seizures	7 (3.1)
Neonatal seizures (not benign)	1 (0.4)
Ohtahara syndrome	1 (0.4)
Benign familial infantile seizures	4 (1.8)
West syndrome	36 (15.9)
Myoclonic epilepsy in infancy	3 (1.3)
Myoclonic encephalopathy in nonprogressive disorders	1 (0.4)
Benign epilepsy with centrotemporal spikes	5 (2.2)
Childhood absence epilepsy	1 (0.4)
Epilepsy with myoclonic absences	1 (0.4)
CSWS/Landau-Kleffner syndrome	9 (4.0)
Lennox-Gastaut syndrome	5 (2.2)
Juvenile absence epilepsy	1 (0.4)
Symptomatic focal epilepsies n.o.s.	148 (65.5)
Localization-related cryptogenic epilepsy	24 (10.6)
Other symptomatic generalized epilepsy	2 (0.9)
Epilepsy with both generalized and focal seizures	3 (1.3)
Febrile seizures plus	5 (2.2)
Febrile infection related epilepsy syndrome	1 (0.4)
One febrile status epilepticus	1 (0.4)
One seizure likely to reoccur	2 (0.9)
Epilepsies of unknown cause	1 (0.4)
Seizure free ^a (%)	89/192 (46.4)
Family history of epilepsy ^b (%)	68/200 (34.0)
Developmental problems in speech, language, motor skills, and/or cognition (%)	195 (86.3)
Behavioral/psychiatric problems (%)	104 (46.0)
Microcephaly (≤ -2 SD) (%)	40 (17.7)
Macrocephaly (≥ 2 SD) (%)	13 (5.8)
Short stature (≤ -2 SD) (%)	39 (17.3)
Tall stature (≥ 2 SD) (%)	13 (5.8)
Facial dysmorphisms (%)	109 (48.2)
Congenital anomalies (%)	72 (31.9)
MRI abnormalities (%)	118/218 (54.1)

CSWS, continuous spike during slow-wave sleep; GTCS, generalized seizures with tonic and/or clonic manifestations; MRI, magnetic resonance imaging; n.o.s., not otherwise specified; SD, standard deviation.

Epilepsy syndromes and seizure types were classified according to the International League Against Epilepsy (ILAE) classification of 2006.¹³

^aSeizure freedom was defined as present if a patient had no clinical seizures for at least 1 year at the time of evaluation.

^bFamily history of epilepsy was positive if a first- or second-degree relative has epilepsy.

In 10 (4%) children, known clinically relevant CNVs were found that also occur in healthy controls, albeit in <1% (Table 3). Six of these CNVs (60%) were inherited from an affected (n = 2) or non-affected (n = 4) parent, and two

(20%) CNVs occurred de novo (the deletions including *NRXN1* and *CHRNA7*). In three (30%) children, another cause for epilepsy—one not associated with the CNVs—was identified. These were developmental anomalies of

Table 2. Clinically relevant CNVs with <90% overlap with CNVs observed in healthy controls (n = 14)

Patient	Sex, age (years)	Microarray results	CNV size in kilobases	Inheritance (parental phenotype)	Relevant genes	Epilepsy syndrome	Age at epilepsy onset	Age at last seizure	Developmental problems	Behavioral problems	Microcephaly ($\leq -2SD$)	Macrocephaly ($\geq 2SD$)	Short stature ($\leq -2SD$)	Tall stature ($\geq 2SD$)	Facial dysmorphisms	Congenital anomalies	MRI abnormalities
1,040	F, 3.5 [†]	arr 1p34.1p33 (46,089,475–46,738,333)×3 ^o	649	De novo	Unknown	Focal ^k	1 years	3.5 years [†]	+	–	–	–	+	–	+	–	–
1,032	M, 8	arr 1p36.33p36.31 (746,419–5,696,745)×1 ^b	4,950	De novo	GABRD KJHL17	WS	3 months	21 months	+	–	–	–	+	–	+	–	–
590	F, 10	arr 1p36.33p36.23 (564,224–8,104,812)×1 ^b	7,541	Unknown	GABRD KJHL17 KGNAB2	WS, focal ^k	1 month	NA	+	–	–	–	–	–	+	+	+
183	M, 11	arr 2q23.1 (148,775,316–149,002,634)×1 ^c	227	De novo	MBDS	Focal ^k	2 years 3 months	2 years 3 months	+	+	–	–	–	–	–	+	–
1,105	F, 2	arr 2q22.3q23.3 (146,506,579–151,355,790)×1 ^c	4,849	Unknown	MBDS	Focal ^k	2 months	9 months	+	–	–	–	+	–	+	+	+
575	F, 6	arr 8q22.3 (101,795,020–104,406,406)×1	2,611	De novo	Unknown	Undet ^l	1 year	4.5 years	+	+	–	–	–	–	+	+	+
1,037	M, 4 [†]	arr 13q31.3q34 (94,017,655–115,105,959)×3,	21,088	Unknown	Unknown	WS, focal ^k	2 years	4 years [†]	+	–	–	–	+	–	+	+	U
1,079	M, 4	arr 15q11.2q13.1 (22,285,091–28,940,239)×1 ^e	6,655	De novo	GABRB3 UBE3A	Focal ^k	7 months	NA	+	–	–	–	–	–	–	+	U
356	M, 16	arr 15q11.2q13.1 (22,668,852–29,045,487)×3 ^f	6,377	De novo	GABRB3 UBE3A	Focal ^k	7 years	14 years	+	+	–	–	–	–	+	–	–
1,062	M, 5	arr 15q11.2q13.1 (22,668,852–29,060,634)×3 ^f	6,392	De novo	GABRB3 UBE3A	Focal ^k	8 months	NA	+	+	–	–	–	–	–	–	–
319	F, 13	arr 16p11.2 (29,620,489–30,199,507)×1 ^l	579	De novo	PRT2	BFS	4 months	4 years	+	+	–	–	–	–	–	–	–

Continued

Table 2. Continued.

Patient	Sex, age (years)	Microarray results	CNV size in kilobases	Inheritance (parental phenotype)	Relevant genes	Epilepsy syndrome	Age at epilepsy onset	Age at last seizure	Developmental problems	Behavioral problems	Microcephaly ($\leq -2SD$)	Macrocephaly ($\geq 2SD$)	Short stature ($\leq -2SD$)	Tall stature ($\geq 2SD$)	Facial dysmorphism	Congenital anomalies	MRI abnormalities
1,081	M, 4	arr 17p13.3 (2,355,353–3,322,779) × 1 ^b	967	De novo	PFAFH1/B1	WS, focal ^k	3 months	NA	+	–	+	–	–	–	–	–	+
201	M, 16	arr 22q13.3 (51,125,351–51,219,150) × 1 ^l	94	Unknown	SHANK3	Undet. ^l	9 years	NA	+	+	–	–	–	–	+	+	–
831	F, 11	arr Xq22.1(99,582,921–99,671,028) × 1 ^l	88	De novo	PCDH19	Focal ^k	2 years	NA	+	–	–	–	–	–	+	+	–

BFIS, benign familial infantile seizures; CNVs, copy number variants; F, female; M, male; MRI, magnetic resonance imaging; NA, not applicable (not seizure-free); SD, standard deviation; U, unknown; WS, West syndrome; †, deceased; +, phenotype is present in the child; –, phenotype is absent in the child.
 The chromosomal coordinates are reported relative to the Genome Reference Consortium Human Reference genome version 37 (GRCCh37/hg19).
^aPreviously published by some of us.
^bChromosome 1p36 deletion syndrome (MIM 607872).
^cChromosome 2q23.1 deletion syndrome (MIM 156200).
^dChromosome 18q deletion syndrome (MIM 601808).
^eAngelman syndrome (MIM 105830).
^fChromosome 15q11–15q13 duplication syndrome (MIM 608636).
^gChromosome 16p11.2 deletion syndrome (MIM 611913).
^hLissencephaly type I (MIM 607432).
ⁱPhelan-McDermid syndrome (MIM 606232).
^jJuberg-Hellman syndrome (MIM 300088; epilepsy, female restricted with mental retardation [EFMR]).
^kSymptomatic focal epilepsy not otherwise specified.
^lOther undetermined epilepsy with both generalized and focal seizures.

Table 3. Clinically relevant CNVs with ≥90% overlap with CNVs observed in <1% of healthy controls (n = 10)

Patient	Sex, age (years)	Microarray results	CNV size in kilobases	Inheritance (parental phenotype)	Relevant genes	Epilepsy syndrome	Age at epilepsy onset	Age at last seizure	Developmental problems	Behavioral problems	Microcephaly (< -2SD)	Macrocephaly (> 2SD)	Short stature (< -2SD)	Tall stature (> 2SD)	Facial dysmorphisms	Congenital anomalies	MRI abnormalities
822	F, 6	arr:1q21.1 (145,395,197-146,089,261)×3	694	Pat (none)	Unknown	VES, focal ^a	6 months	NA	+	+	+	-	+	-	-	-	+
337	F, 13	arr:2p16.3 (50,968,252-51,579,862)×1 ^b	612	Denovo	NRXN1	Focal ^a	3.5 years	NA	+	-	+	-	+	-	+	+	+
1,000	M, 16	arr:15q11.2 (20,279,343-23,300,438)×1	3,021	Pat (none)	CYFIP1, NIPA1, NIPAZ	Focal ^a	11 years	13 years	+	+	-	-	-	-	-	+	-
225	M, 11	arr:15q11.2 (22,698,322-23,217,655)×1 ^c	519	Pat (none)	CYFIP1, NIPA1, NIPAZ	Focal ^a	6 years	NA	+	+	-	-	-	-	-	-	-
1,099	F, 8	arr:15q13.3 (30,921,717-32,515,121)×1	1,593	Denovo	CHRNA7	Focal ^a	4 years	NA	+	-	-	-	-	-	-	-	-
372	M, 9	arr:15q13.3 (30,833,546-32,861,767)×3	2,028	Mat (none)	CHRNA7	Focal ^a	11 months	6 years	+	+	-	-	-	-	-	-	+
761	F, 13	arr:16p11.2 (29,620,488-30,198,752)×3	578	Unknown	PRRT2	CSWS	4 years	NA	+	+	-	-	-	-	-	-	+
730	M, 12	arr:16p11.2 (29,592,582-30,198,752)×3	606	Unknown	PRRT2	Focal ^a	1 months	8 years	+	+	-	-	-	-	-	-	+
270	M, 14	arr:16p13.11 (14,944,359-16,525,488)×1	1,581	Pat (FS)	NDE1	One FS	2.5 years	2.5 years	+	+	-	-	-	+	-	-	U
1,045	M, 6	arr:16p13.11 (14,944,360-16,561,292)×1	1,617	Mat (FS)	NDE1	MAE	1.5 years	NA	+	-	-	-	-	-	+	-	-

BECTS, benign epilepsy with centrotemporal spikes; CAE, childhood absence epilepsy; CNVs, copy number variants; CSWS, continuous spike waves during slow-wave sleep syndrome; F, female; FS, febrile seizures; JME, juvenile myoclonic epilepsy; M, male; mat., maternal; MAE, epilepsy with myoclonic absences; NA, not applicable (not seizure-free); SD, standard deviation; U, unknown; pat, paternal; WS, West syndrome; +, phenotype is present in the child; -, phenotype is absent in the child.
 The chromosomal coordinates are reported relative to the Genome Reference Consortium Human Reference genome version 37 (GRCh37/hg19).
^aSymptomatic focal epilepsy not otherwise specified.
^bThis patient also carries a chromosome 14q31.1 deletion including the NRXN3 gene.
^cThis patient also carries a likely pathogenic sequence variant in the SLC2A1 gene associated with GLUT-1 deficiency.

Table 4. Novel CNVs in our cohort of children with epilepsy (n = 5)

Patient (sex, age in years)	Microarray results, inheritance	CNV size in kilobases	Relevant genes	Seizure types (estimated number of seizures)	Age at active epilepsy	Epileptiform activity on EEG (localization)	Anti-epileptic drugs (effectiveness)	Epilepsy syndrome	Additional features	MRI abnormalities
981 (M, 16)	arr 2p25.3 (1,711,399–2,078,557) × 1, de novo	376	MYT1L	Frontal absences (3/day), sec. gen. sz. (4)	3–12 years	Gen. 3-Hz SWC, focal spikes and SWC (fronto-temp., L > R)	ETX (–) LEV (+)	Focal ^b	Developmental problems, autism, pubertas praecox, upslanting palpebral fissures	Normal
626 (F, 9)	arr 8p12 (35,120,621–35,358,315) × 1, paternal father has migraine	238	UNC5D	Focal SE (1), focal sz. (10)	3 years–ongoing	Gen. SWC (max. bifront.), focal sharp waves and SWC (L temp.)	VPA (side effects) CBZ (+)	Focal ^b	Developmental and behavioral problems	MTS and possible left cortical dysplasia
31 (M, 23)	arr 11q23.3 (117,951,629–118,022,700) × 1, unknown	71	SCN4B	Focal sz. (unknown), sec. gen. sz. (1)	14 years–unknown	Normal	VPA (side effects) TPM (+) LTG (unknown) None	Focal ^b	Perinatal asphyxia, developmental and behavioral problems, dyskinesia	Diffuse white matter abnormality
337 (F, 13)	arr 14q31.1 (79,335,493–79,654,245) × 1 ^a , de novo	319	NRXN3	Focal sz. (unknown)	3.5 years–ongoing	Focal sharp waves (occ.)	None	Focal ^b	Developmental problems, hearing loss, microcephaly, thick eyebrows, deeply set eyes, entropion, thin lips, high nasal bridge, abnormal position of ears, short stature, pectus excavatum	Megacisterna magna
969 (F, 8)	arr 14q24.3q31.1 (76,621,116–79,828,269) × 1, de novo	3,207	NRXN3	Focal SE (1), focal sz. (2–4/month)	2–4 years	Normal	VPA (+)	Focal ^b	PDD-NOS, epicanthal fold, fifth finger clinodactyly	Normal

bifront., bifrontal; CBZ, carbamazepine; CNVs, copy number variants; EEG, electroencephalogram; ETX, ethosuximide; F, female; fronto-temp., fronto-temporal; gen., generalized; Hz, Hertz; L, left; LEV, levetiracetam; LTG, lamotrigine; M, male; max., maximum; MRI, magnetic resonance imaging; MTS, mesiotemporal sclerosis; occ., occipital; Pat, paternal; PDD-NOS, pervasive developmental disorder not otherwise specific; R, right; SE, status epilepticus; sec. gen., secondarily generalized; SWC, spike-wave complexes; sz., seizures; temp., temporal; TPM, topiramate; VPA, valproic acid; +, >50% seizure frequency reduction; –, <50% seizure frequency reduction.

The chromosomal coordinates are reported relative to the Genome Reference Consortium Human Reference genome version 37 (GRCh37/hg19).

^aThis patient also carries a chromosome 2p16.3 deletion including the NRXN1 gene.

^bSymptomatic focal epilepsy not otherwise specified.

cerebral structure (Patient 822), a GLUT1-deficiency (Patient 225), and polymicrogyria (Patient 761) (Table 3).

Novel CNVs of interest for epilepsy

In 5 (2%) children, we identified novel CNVs of interest that were not found in healthy controls (Table 4). These CNVs comprised four potential candidate genes for epilepsy: *MYTIL*, *UNC5D*, *SCN4B* and *NRXN3* (see Table S1 for more information on these genes).

In 16 (7%) children, eight different overlapping deletions (n = 4) and duplications (n = 4) occurred at a 10 times higher frequency in our cohort than in healthy controls (Table S2). However, another cause for epilepsy was identified in 7 (44%) of these children, with CNVs involving six of the eight regions. The two remaining regions did not contain genes that seem of particular interest for epilepsy (Table S2).

DISCUSSION

Our study was performed in a university hospital-based cohort of children with epilepsy, who were selected on the basis of their doctors' preference to undergo microarray analysis as part of their diagnostic work-up. We found that microarray analysis yielded known clinically relevant CNVs for epilepsy in 11% of the children. We further identified five novel CNVs of interest for epilepsy in 2% of the children.

Diagnostic yield of microarray analysis

The 11% yield of microarray analysis in our cohort is comparable with the 9% yield found in another clinical cohort of American individuals with epilepsy.⁹ Higher yields of 36–40% have been reported in smaller cohorts of Saudi individuals with epilepsy.^{10,11} Differences in yield between studies is probably due to the selection of children for microarray analysis, which is often based on the presence of additional features other than epilepsy. For example, a higher yield of microarray analysis is found in individuals with epilepsy when the epilepsy is accompanied by global developmental delay or cognitive dysfunction.¹⁸ In our database, children who underwent microarray analysis more often had developmental problems, facial dysmorphisms and behavioral problems when compared to those who did not undergo microarray analysis. Thus, the presence of such comorbidities prompted the treating physicians to request a microarray analysis. Probably because of this selection, we found no differences in epilepsy syndrome diagnosis or the presence of other phenotypic characteristics between children with and without clinically relevant CNVs.

We found a 2.6 Mb 8q22 deletion in one child. She (Patient 575) is the seventh individual reported with such a deletion so far and shares the combination of absence seizures and focal seizures with two of the previously reported

children.^{16,17} An eighth individual, listed in the DECIPHER database (Case 2846), also has an 8q22 deletion and absence seizures. Thus, both focal and generalized (especially absences) seizures may occur in patients with 8q22 deletions. The smallest region of overlap harbors two candidate genes for epilepsy, *NCALD* (MIM 606722) and *RRM2B* (MIM 604712), which both have a function in the brain (Figure S1).¹⁷

A large proportion of identified CNVs are also observed in <1% of the healthy controls. Among these CNVs were chromosome 15q11.2 and 15q13.3 deletions in three children with symptomatic focal epilepsy, while similar deletions are known to predispose to idiopathic generalized epilepsies (Table 3).^{6–8,19,20} We also found a chromosome 15q13.3 duplication in a child with focal seizures. An association between 15q13.3 duplications and epilepsy has only been reported in a few cases so far.^{9,21} The observations in our cohort suggest that chromosome 15q11.2 and 15q13.3 deletions and duplications might predispose to both generalized and focal epilepsies. Although these CNVs are regarded as susceptibility CNVs for epilepsy, one should always consider that other causes of epilepsy may also be present, as seen in 30% of the children with susceptibility CNVs in our cohort (Table 3).

Novel CNVs of interest for epilepsy

In five children, we identified novel CNVs comprising four candidate genes for epilepsy (*MYTIL*, *UNC5D*, *SCN4B* and *NRXN3*) with either expression or function in the brain or a previous association with neurodevelopmental disorders (Table S1).

We found a 376-kb deletion involving the *MYTIL* gene (MIM 613084) in a child with focal epilepsy and intellectual disability. *MYTIL* codes for a transcription factor that has an important role in the differentiation of cells to functional neurons.²² It has been identified as a candidate gene for intellectual disability in patients with 2p25.3 deletions,^{23,24} and seizures have been reported in 8/21 patients with such a deletion.^{7,23,25} The DECIPHER database includes another individual (Case 259324) with absence seizures, intellectual disability, autism and a large chromosome 2p25 deletion including *MYTIL*. Thus, based on our and previous observations, *MYTIL* deletions are not only associated with intellectual disability but also with epilepsy.

We found a deletion of *UNC5D* in a child with focal epilepsy, mesiotemporal sclerosis and developmental and behavioral problems, as well as in his father who had migraine. *UNC5D* (MIM 616466) on chromosome 8p12 has been shown to be involved in cortical development and p53-dependent apoptosis in neuroblastoma cells.^{26,27} *UNC5D* was considered as a candidate gene for neurodevelopmental phenotypes in a family with a t(6;8) balanced translocation that disrupted this gene in two affected siblings with developmental delay (one with schizencephaly) and their asymptomatic mother.²⁸

Further confirmation that deletion of the *UNC5D* gene may cause or predispose to epilepsy and developmental problems is, however, needed.

In one child from our cohort with focal epilepsy and developmental problems, we found a deletion of the sodium channel voltage-gated type IV beta subunit gene, *SCN4B* (MIM 608256). *SCN4B* is expressed in rat brain and spinal cords, and its protein has been shown to influence *SCN2A* by altering channel properties and shifting the voltage dependence of activation in the hyperpolarizing direction.²⁹ Variants in the *SCN2A* gene are a well-known cause of benign familial neonatal and infantile seizures⁵ and early infantile epileptic encephalopathy.³⁰ Haploinsufficiency of *SCN4B* may cause epilepsy indirectly by influencing *SCN2A* function.

Last, the *NRXN3* gene (MIM 600567) was deleted in two unrelated children in our cohort. We had described one of them in a previous study: she (Patient 337) has a severe developmental delay and a concomitant deletion of *NRXN1* with no second *NRXN1* sequence variant on the other allele.³¹ *NRXN3* encodes a polymorphic cell surface protein, neurexin III, that is expressed in neurons and is necessary for neurotransmission. Deletions and variants in the neurexin I gene, *NRXN1*, have been associated with moderate to severe intellectual disability, language delay, autism spectrum disorder, and seizures.³¹ The severe phenotype of our patient was not in line with the milder phenotypes previously reported in children with heterozygous *NRXN1* deletions so far, and we speculated that her severe phenotype might be explained by the additional deletion of *NRXN3*.³¹ In the current study, we found a second *NRXN3* deletion in another unrelated child with symptomatic focal epilepsy and a pervasive developmental disorder not otherwise specified (Table 2). *NRXN3* has been associated with bipolar disorder³² and autism spectrum disorder.³³ Recently, *NRXN3* deletions were reported in four individuals of three different families with epilepsy (unclassified in three, progressive myoclonic epilepsy in one), behavioral problems and developmental delay, or intellectual disability.¹⁰ The observation of *NRXN3* deletions in two children in our cohort supports the idea that *NRXN3* haploinsufficiency can be associated with epilepsy.

For all four candidate genes, *MYTIL*, *UNC5D*, *SCN4B*, and *NRXN3*, additional patients with compatible genotypes and phenotypes, and/or supporting evidence from functional studies, are needed to confirm their role in the pathophysiology of epilepsy.

In eight different regions, we found CNVs that occurred 10 times more often in our study cohort than in healthy controls. The pathogenicity of these CNVs in epilepsy is doubtful because these children either had other identified epilepsy causes and/or the CNVs lacked genes of interest for epilepsy.

CONCLUSION

Our study demonstrates the importance of microarray analysis in the diagnostic work-up of epilepsy in childhood. We identified known clinically relevant CNVs for epilepsy in 11% of the children investigated. This yield was obviously influenced by the clinical selection of children, which was largely based on the presence of additional developmental or behavioral problems and/or facial dysmorphisms. Furthermore, we identified novel CNVs that include four new candidate genes for epilepsy: *MYTIL*, *UNC5D*, *SCN4B* and *NRXN3*. Analysis of these genes in larger study cohorts is warranted to further confirm their role in the etiology of epilepsy.

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DISCLOSURE

None of the authors has any conflicts of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

LINKING TO DATABASES

UCSC Genome Bioinformatics

DatabaseE of genomic variation and Phenotype in Humans using Ensembl Resources (DECIPHER)

Online Mendelian Inheritance in Man (OMIM)

Database of Genomic Variants (DGV)

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Overview of chromosome 8q22 deletions.

Table S1. Information on novel candidate genes.

Table S2. CNVs with a 10 times higher frequency in the study cohort compared to controls.