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The epilepsy phenotype in adult patients with intellectual disability and pathogenic copy number variants

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ABSTRACT

Purpose: To characterize the electroclinical features of epilepsy associated with intellectual disability and pathogenic copy number variations (CNVs)

Methods: we prospectively investigated 61 adult patients with epilepsy and intellectual disability or other neurodevelopmental disorders. We performed high resolution SNP-Array analysis in order to detect clinical relevant chromosomal microdeletions and microduplications. An ordinal logistic regression model was fitted with 34 demographic, clinical and EEG-related variables in order to identify the epilepsy phenotype of patients with pathogenic CNVs.

Results: chromosome microarray analysis identify non-polymorphic CNVs in 33 patients analyzed: 11 had an established pathogenic microdeletion/microduplication, 22 were carriers of CNVs of unknown clinical significance. Univariate analysis revealed a significant association between pathogenic CNVs and 3 electroclinical variables considered, specifically atypical absence seizures ($p < 0.05$), tonic seizures ($p < 0.05$), epileptic spasms ($p < 0.01$).

Conclusions: high resolution SNP-Array analysis should be evaluated in adult patients with intellectual disability and epilepsy with peculiar electroclinical features, specifically atypical absence seizures, tonic seizures, and epileptic spasms, resembling a Lennox-Gastaut syndrome without a clear structural lesion.

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1. Introduction

During the last decade, genome-wide chromosomal microarray analysis (CMA) has enhanced the knowledge of neuropsychiatric and neurodevelopmental disorders by uncovering genomic microdeletions and microduplications, defined copy number variations (CNVs), undetectable by conventional karyotyping.

CNVs are associated with a broad spectrum of developmental disorders, including intellectual disability (ID), multiple congenital anomalies, learning difficulties and autism spectrum disorders (ASDs), schizophrenia, and it is currently assumed that CMA should be the first genetic test offered to detect genomic imbalances in these patients [1,2]. Although no formal guidelines include CMA in the diagnosis workflow of patient affected by epilepsy, molecular studies and recommendations for clinical practice [3–6] suggest that array-CGH or SNP arrays may represent a powerful first-line screening tool in epilepsy, especially if associated with ID and other developmental disorders. In fact, three microdeletions, also important contributors to ID, ASDs, and schizophrenia, are established as risk factors for genetic generalized epilepsy (GGE): microdeletion of chromosomal regions 15q13.3, 15q11.2 and 16p13.11 [6]. Each of these CNVs is present in 0,5 to 1% of patients with GGE, but, when GGE is associated with ID, 10% of these patients had one of the three recurrent CNVs, confirming that different neurocognitive phenotypes can be seen in different patients, as well as multiple phenotypes in a single carrier [7]. Therefore, the role and the use of CMA in patients with epilepsy associated with ID or other developmental disorders should be considered. Case reports, small case series and large cohorts studies of patients with epilepsy and ID or developmental disorders have been reported [3,4,7–11], usually revealing the molecular features and the overall characteristics of CNVs (e.g. type, number, size, burden, inheritance) and their influence on ID phenotypes and syndromic pictures, confirming the utility of CMA. On the other hand, the epilepsy phenotypes in patients with ID and pathogenic role of CNVs has not yet been fully explored. In fact, detailed electroclinical features of epilepsy (i.e. family history of

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epilepsy; type and frequency seizures; temporal pattern of seizures; neurophysiological findings; response to anti-epileptic drugs and drug-resistance) were often limited or missing in previous studies, and patients, also with pathogenic CNVs, were generically indicated as affected by "epilepsy" or "seizures" without other information. However, the knowledge of the specific electroclinical features of epilepsy associated with ID may be helpful in as to as to select epilepsy patients which are likely to carrier a pathogenic CNVs, increasing the diagnostic yields of this investigation. Moreover, prognosis studies are essential to understand the effect of epilepsy on the patient with ID, leading to improved management decisions. Most genetic epilepsies begin in childhood, and often persist into adolescence and adulthood, and electroclinical features from childhood to adulthood are needed to know the natural history and prognosis [12,13]. With improved pediatric care, the population of adults with ID and epilepsy will expand, but only a limited number of previous genome-wide CNV studies have focused on these patients to date [14,15]. In fact, the majority of studies have been performed entirely or predominantly among pediatric cohorts, and the natural history and long-term outcome of epilepsy associated with ID and pathogenic CNVs are often unknown. Finally, chromosomal disorders may be associated with drug-resistant epilepsy, and long-term cognitive outcome may be also impaired [16]. Therefore, it is essential to characterize the electroclinical features of epilepsy associated to ID in patients with chromosomal disorders in order to improve the diagnosis and management.

To highlight the epilepsy phenotype mostly associated to CNVs, in order to classify patients with epilepsy and ID recruitable for CMA analysis, we reported a prospective study based on 61 adult patients who were consecutively presented to the Epilepsy Centre – Clinic of Nervous System Diseases, Riuniti Hospital, Foggia, Italy. We hypothesized that the knowledge about the specific electroclinical features of epilepsy associated with ID and pathogenic CNVs may be helpful to a neurologist, especially epileptology experts, to define the convenience and the correct indications to perform CMA analysis in adult patients with epilepsy and ID.

2. Methods

The mean age of the population at the start of the study was 29.16 ± 10.09 years (median 26.5, range 18–62). The age at seizure onset was 5.51 ± 5.21 years (median 4, range 1 month-19 years), whereas the mean duration of follow-up after epilepsy onset was 23.65 ± 11.55 years (median 21.83, range 4–62).

2.1. Criteria inclusion

Inclusion criteria were epilepsy associated with ID, and one or more of the following characteristic: 1) dysmorphic features; 2) learning disability, autistic features, or developmental delay; 3) family history of epilepsy, neurodevelopmental and neuropsychiatric disorder; 4) abnormal neuroimaging (brain malformations or other congenital abnormalities).

2.2. Ethics statement

The local ethics committee on human experimentation approved the study, and a written informed consent was obtained from relatives or a legal guardian of the patients.

2.3. Patient samples

The data from each patient were tabulated and included a) demographic information: age, gender, family history, personal antecedents, systemic disorders; b) details of the epilepsy features: family history of epilepsy; febrile convulsions; age at seizure onset; seizure type; epilepsy type; frequency at onset and during the epilepsy evolution; follow-up duration; response to the therapy; ictal and interictal video- electroencephalography (EEG)/polygraphic recordings; awake and sleep EEG abnormalities. Other items tabulated were: abnormal pregnancy history and neonatal abnormality, presence of other neurodevelopmental disorder (i.e. ASDs, schizophrenia), presence of malformations, particularly as major defects (i.e. those affecting organs like the heart and the urogenital tract), ID based on intelligence quotient scoring (IQs); neuroradiological findings.

Epilepsy and seizures were classified according to the International League Against Epilepsy (ILAE) Commission on Classification and Terminology, 2017 [17,18]. Lennox-Gastaut syndrome diagnosis is based in two key criteria: (i) multiple seizure types, to include tonic, atonic, and atypical absence seizures, with tonic seizures predominantly occurring at night and (ii) abnormal EEG, consisting of an interictal pattern of diffuse, slow spike-wave (SSW) complexes at < 3 Hz, occurring during wakefulness, and paroxysmal fast rhythms (10–20 Hz) during sleep (tonic seizures). The patients' IQs were defined according to Diagnostic and Statistical manual of mental Disorders (DSM-IV): mild mental retardation (IQ 60–70), moderate mental retardation (IQ 50–59) and severe mental retardation (IQ < 50).

Each patient underwent brain magnetic resonance imaging (MRI; 1.5-T System).

2.4. Genetic analysis

All the patients enrolled for the study are negative to standard karyotype.

Genomic DNA was extracted from peripheral blood cells using automated BioRobot EZ1 (Qiagen, Solna,Sweden). It was checked for quantity and purity using the NanoDropND-1000 Spectrophotometer (Thermo Fisher Scientific,Wilmington, DE). Whole genome chromosomal microarray analysis was performed by using the CytoScan HD array platform (Affymetrix, Santa Clara, CA). This array contains more than 2.6 million markers for copy number analysis and approximately 750,000 SNPs that fully genotype with greater than 99% accuracy. The CytoScan HD assay was performed according to the manufacturer's protocol and as previously described [19], starting with 250 ng DNA. Copy number analysis was performed using the Chromosome Analysis Suite Software version 3.1: (i) the raw data file (.CEL) was normalized using the default options; (ii) an unpaired analysis was performed using as baseline 270 HapMap samples in order to obtain Copy numbers value from CEL files. An additional 200 samples (100 females and 100 males), which were hybridized in our facility, were used as controls. Since 2010, we have analyzed 3500 patients with syndromic and non- syndromic forms of neurodevelopmental disorders. The 200 controls reported are parents and non affected members of the families which are healthy, carriers of no pathogenic CNVs. (iii) The amplified and/or deleted regions were detected using a standard Hidden Markov Model (HMM) method.

CNVs were selected by the number of consecutive probes >25 and their size >50 kb. Karyotype was designated according to ISCN 2009 [20] and base pair position were derived from the University of California Santa Cruz (UCSC) Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway), build GRCh37 (hg19). The significance of each CNV detected was determined by comparison with public databases of copy number variants such as the Database of Genomic Variant (DGV; http://dgv.tcag.ca/dgv/app/home), the ICCG (The International Collaboration for Clinical Genomics; http://www.iccg.org/) and DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans Using Ensemble Resources; http://decipher.sanger.ac.uk/) databases. When available, blood

samples were obtained from patient's parents and the same analysis was done to investigate CNV inheritance. Also, to predict the pathogenic role of the identified microdeletions/microduplications, we considered the presence of functionally related genes with neurodevelopmental clinical phenotypes and followed the American College of Medical Genetics guidelines [20].

2.5. Statistical analysis

Data were expressed as mean ± SD, unless otherwise indicated, and analyzed using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria). Crude differences across groups were evaluated by the chi-square test, *t*-test and one-way analysis of variance as appropriate. P-value < 0.05 were considered as statistically significant.

3. Results

3.1. Genetic findings

36 non-polymorphic copy number changes were detected in 33 of 61 patients (54.6%), ranging in size from 20 Kb to 5.7 Mb. Following the international guidelines of the American Collage of Medical Genetics (ACMG) [20], we divided them into two groups. The first group contained pathogenic CNVs (i.e. CNVs documented as clinically significant in multiple peer-reviewed publications, even if penetrance and expressivity of the variant are known to be variable); the second group consisted of variants of uncertain clinical significance (VOUS) (i.e. CNV containing genes, but it is not known whether the genes in the interval are dosage sensitive or CNVs described in multiple contradictory publications and/or databases, and firm conclusions regarding clinical significance are not yet established). In detail, a pathogenic rearrangement was observed in 11 cases (17.7% of the study population) (Table 1). Among those latter, 3 had a de novo rearrangement, 5 were inherited and 3 were of unknown origin. Finally, in 22 patients (25.8%) a variant of unknown clinical significance (VOUS) was observed (Table 2).

3.2. Electroclinical findings

Tables 3 and 4 show the results of the univariate analysis, with summarized covariates' results. Among demographic and clinical covariates, age at visit was greater among patients with negative result at SNP array analyses ($p = 0.06$). Autism was present among carriers of a pathogenic CNV (27.3%) and patients without CNVs (7.1%, $p < 0.05$). Intellectual disability was more severe among patients with CNVs, although not statistically significant ($p > 0.15$). Finally, cerebral malformations were more frequent among patients with a pathogenic CNV and patients without CNV ($p = 0.09$), while cortical malformations were associated with negative result at SNP array analyses ($p = 0.05$).

Univariate analyses of covariates relating to epileptic features revealed a significant association for atypical absence ($p < 0.05$), tonic seizure ($p < 0.05$) and epileptic spasms ($p < 0.01$). These three variables are more frequent in the pathogenic CNVs group. Univariate analyses of covariates related to EEG pattern showed a greater frequency of diffuse abnormalities among patients with pathogenic CNVs ($p > 0.07$), while generalized abnormalities were present among patients with VOUS CNVs ($p < 0.05$).

4. Discussion

Clinical features associated with microdeletion/microduplication syndromes are highly heterogeneous and complex, and patients with syndromic ID (e.g., congenital anomalies, malformations and dysmorphisms) have a higher likelihood to be carriers of pathogenic CNVs. In fact, several papers described specific clinical features which may be associated with clinically relevant CNVs: onset of the symptoms before one year of life, presence of malformations and of dysmorphisms [22], congenital malformations of the corpus callosum, minor anomalies of the ear, and brachydactyly [23]; heart defects, primary microcephaly, short stature and failure to thrive [24]; multiple congenital anomalies and severe phenotypes [25]. The association of pathogenic CNVs with ID and epilepsy has been documented in reports on individual, small groups or large cohorts of patients [3,4,7–13]. Large cohort studies, also including thousands of cases, disclosed the overall characteristics of CNVs (e.g., size and burden) and their influence on syndromic Pictures [3,4,7,9–11]. Nevertheless, detailed electroclinical descriptions of epilepsy phenotypes which have a higher likelihood to be carriers of pathogenic CNVs has been rarely explored. Muhle et al. analyzed a cohort of 570 patients with various pediatric epilepsies and identified four patients with 15q13.3 microdeletions presenting with absence epilepsy associated with ID [26]. Similar features were also described in two families with 15q13.3 deletions in which the electroclinical features showed a picture of absence epilepsy with mild ID [27]. A study realized by Mullen et al. [7] showed that CNVs are common in patients with GGE and ID, with recurrent deletions at 15q13.3, 15q11.2 and 16p13.11 respect to patients with GGE alone (10% vs 3%). Later, in a large European case-control cohorts of 1366 GGE patients and 5234 ancestry-matched controls analyzed by high resolution SNP array platform (Affymetrix SNP 6.0 array), Lal et al. [28] found a significant excess of microdeletions in 7.3% of GGE patients compared to 4.0% in controls ($P = 1.8 \times 10{-}7$; OR = 1.9) and a 7-fold increased burden for known hotspot microdeletions (15q11.2, 15q13.3, 16p13.11, 22q11.2) previously associated with a wide range of neurodevelopmental disorders in cases relative to the population controls.

At least 5% of the pediatric patients with epilepsy in Olson et al. series [3] harbor pathogenic CNVs, and additional patients harbor possibly pathogenic CNVs. In 4.1% of patients with epileptic encephalopathies (focal epilepsy with regression, epilepsy-aphasia syndrome and symptomatic generalized epilepsy) Mefford et al. [8] identified clearly pathogenic CNVs, while Helbig et al. [5] found a high frequency of rare CNVs in 31.8% of patients with a complex childhood phenotype, including patients with or without ID, dysmorphic features, or MRI abnormalities. Rare CNVs occurred in 9.3% of patients with epilepsy described by Striano et al. [4], significantly associated with ID or neuropsychiatric features, whereas epilepsy type (focal vs generalized) was not. Finally, other studies of patients with epilepsy or epileptic seizures with or without additional neurodevelopmental abnormalities, and without electroclinical information about epilepsy phenotype, identified pathogenic CNVs in almost 10% [9–11,22,29]. All these studies usually included childhood-onset epilepsy and pediatric series, with limited knowledge about the epilepsy evolution and prognosis in adult patients with additional ID. In fact, published data on the use of CMA in adult patients with epilepsy and ID are rare. Galizia et al. [14] studied 82 adult patients with drug-resistant epilepsy and co-morbidities, and identified pathogenic CNVs in almost 15%; nevertheless, the electroclinical and the prognostic features of epilepsy were not fully examined, whereas the genetic findings were clearly characterized. Lund et al. [15] found rare CNV in eight adult patients (38%) with Lennox-Gastaut syndrome (LGS), in four patients (19%) the detected CNvs was considered highly pathogenic, and three of these (14%) had CNVs and clinical features consistent with specific genetic syndromes (22q13.3 deletion, 2q23.1 deletion and MECP2 duplication).

In our study, evaluation was carried out using univariate analyses 61 patients with epilepsy and ID in which clinical-

Table 1
Pathogenic CNVs.

Cases	Sex	SNP-Array Results	Gain/ Loss	Inheritance	Size	Genes involved	References
#1	M	arr[hg19]3p26.3 (152,842–336,148) × 3; align="center"	gain;	Unk.	183 Kb align="center"	***CHL1*** align="center"	1
		arr[hg19]3p26.3 (430,682–624,201) × 3	gain		194 Kb align="center"		
#2	M	arr[hg19]5p15.2p15.1 (13,872,756–16,174,863) × 3	gain	Mat.	2.30 Mb align="center"	*DNAH5*, ***TRIO***, *ANKH*, *FBXL7*, *MARCH11* align="center"	2,3
#3	M	arr[hg19]7q35 (146,410,469–146,609,067) × 1	loss	Pat.	198 Kb align="center"	***CNTNAP2*** align="center"	4
#4	F	arr[hg19]15q11.2q13.1 (22,770,422–28,545,601) × 4	gain	dn	5.77 Mb align="center"	*116* align="center"	
#5	M	arr[hg19]15q11.2q13.1 (22,770,422–28,545,601) × 4	gain	dn	5.77 Mb align="center"	*116* align="center"	
#6	M	arr[hg19]15q13.2q13.3 (30,374,369–32,446,830) × 3	gain	Pat.	2.07 Mb align="center"	*ULK4P3*, *LOC653075*, *DKFZP434L187*, *CHRFAM7A*, *ULK4P1*, *ULK4P2*, *ARHGAP11B*, *LOC100288637*, *HERC2P10*, *FAN1*, *MTMR10*, *TRPM1*, *MIR211*, *LOC283710*, *KLF13*, ***OTUD7A***, ***CHRNA7*** align="center"	5,6, 7
#7	M	arr[hg19]22q13.33 (51,121,147–51,183,840) × 1 align="center"	loss	dn	62 Kb align="center"	***SHANK3***, *ACR* align="center"	8
#8	F	arr[hg19]Xp22.31 (6,455,152–8,128,200) × 3 align="center"	gain	Unk.	1.67 Mb align="center"	*HDHD1*, *MIR4767*, *STS*, *VCX*, *PNPLA4*, *MIR651* align="center"	9
#9	M	arr[hg19] Xp22.31 (6,455,151–8,135,644) × 2 align="center"	gain	Mat.	1.68 Mb align="center"	*HDHD1*, *MIR4767*, *STS*, *VCX*, *PNPLA4*, *MIR651* align="center"	9
#10	F	arr[hg19]Xp22.31 (7,514,751–8,135,644) × 3 align="center"	gain	Unk.	620 Kb align="center"	*VCX*, *PNPLA4*, *MIR651* align="center"	9
#11	M	arr[hg19]Xq28 (151,537,594–153,731,581) × 2 align="center"	gain	Mat.	2.19 Mb align="center"	*82* align="center"	10

M, male; F, female; dn, de novo; Mat., inherited from the mother; Pat., inherited from the father; Unk., parents not tested.
The candidate genes for the clinical phenotype observed in the carrier patients are reported in bold.

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radiologic features and, especially, several electroclinical variable referring to epilepsy were compared between patients showing pathogenic CNVs (Group A) vs. all the others, i.e. CNVs of VOUS (Group B) and absent CNVs (Group C). Univariate analyses of the clinical-radiological features of patients revealed no statistically significant differences between these three groups (see Table 3). Although ID severe was more frequent in pathogenic group (63.6% in group A vs 50% in group B and 32.1% in group C), as well as

Table 2
Variants of uncertain clinical significance (VOUS).

Cases	Sex	SNP-Array Results	Gain/ Loss	Inh.	Size	Genes involved	References
#1	M	arr[hg19]1q42.12(225,252,427–225,320,495) × 1	loss	Mat.	68 Kb	*DNAH14*	–
#2	M	arr[hg19]4q25(108,632,540–108,771,899) × 1	loss	Mat.	140 Kb	*PAPSS1, SGMS2*	–
#3	M	arr[hg19]4p16.3(152,726–419,601) × 3	gain	Unk.	267 Kb	*ZNF718, ZNF876P, ZNF732, ZNF141*	–
#4	M	arr[hg19]4p16.2(4,964,637–5,492,259) × 3	gain	Mat.	527 Kb	*CYTL1, STK32B*	–
#5	F	arr[hg19]5q33.1(151,270,938–151,365,881) × 3	gain	Mat.	95 Kb	***GLRA1***	*1,2*
#6	F	arr[hg19]8p22p21.3(18,669,680–19,164,832) × 3	gain	Mat.	495 Kb	*PSD3, LOC100128993*	–
#7	F	arr[hg19]8p21.2(24,987,322–25,077,779) × 1;	loss;	No mat.;	90 Kb	*DOCK5*	*3,4*
		arr[hg19]9q34.3(140,994,217–141,020,389) × 3	gain	Mat.	26 Kb	***CACNA1B***	
#8	M	arr[hg19]9q21.12(73,228,442–73,527,570) × 1	loss	Unk.	302 Kb	***TRPM3***, *MIR204*	*5,6*
#9	F	arr[hg19]9q31.1(106,805,218–106,865,548) × 1	loss	Mat	60 Kb	*SMC2*	–
#10	F	arr[hg19]9q31.3(114,706,412–114,883,382) × 3	gain	Mat.	177 Kb	*SUSD1 MIR3134*	–
#11	M	arr[hg19]10q21.1(58,928,650–59,170,118) × 3	gain	Unk.	241 Kb	*MIR3924*	–
#12	F	arr[hg19]10q22.3(81,617,261–81,984,775) × 1	loss	Unk.	367 Kb	*LOC100288974, MBL1P, SFTPD, TMEM254-AS1, TMEM254, PLAC9, ANXA11, LINC00857*	–
#13	F	arr[hg19]10q25.1(109,269,433–109,834,672) × 1	loss	Unk.	565 Kb		–
#14	M	arr[hg19]12q13.13(51,688,851–51,775,950) × 1	loss	Pat	87 Kb	*BIN2, CELA1, GALNT6*	–
#15	M	arr[hg19]13q12.11(20,543,431–20,741,201) × 3	gain	Pat.	198 Kb	*ZMYM2, GJA3*	–
#16	F	arr[hg19]13q12.12(24,886,198–25,210,903) × 1	loss	Mat	325 Kb	*C1QTNF9, PARP4, TPTE2P6*	–
#17	F	arr[hg19]15q15.1q15.2(42,785,144–42,850,434) × 1	loss	No mat.	65 Kb	***SNAP23,*** *LRRC57, HAUS2*	*7,8*
#18	M	arr[hg19]16p13.2(8,740,518–8,878,007) × 3	gain	Pat.	137 Kb	*ABAT*	–
#19	F	arr[hg19]21q22.3(44,275,740–44,340,179) × 1	loss	Unk.	65 Kb	*WDR4, NDUFV3*	–
#20	M	arr[hg19]22q13.31(46,502,870–46,914,974) × 1	loss	No mat.	412 Kb	*MIRLET7A3, MIRLET7B, PPARA, PKDREJ, GTSE1, TRMU,* ***CELSR1***	*9,10*
#21	F	arr[hg19]22q13.31(46,502,870–46,914,974) × 1	loss	No mat.	412 Kb	*MIRLET7A3, MIRLET7B, PPARA, PKDREJ, GTSE1, TRMU,* ***CELSR1***	*9,10*
#22	F	arr[hg19]Xp22.11(24,505,311–24,525,694) × 1	loss	Unk.	20 Kb	*PDK3*	–

M, male; F, female; Mat., inherited from the mother; Unk., parents not tested; Pat., inherited from the father.
In bold there are brain expressed genes that have been implicated in neurological/psychiatric phenotypes.
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psychiatric disturbances (54.5% in Group A vs 40.9% in group B and 42.8% in group C), significant differences were not disclosed; moreover, autism was present among pathogenic group and patients with absent CNVs (27.3% group A, 0% in group B, 7.1% in group C). Cerebral malformations (72.7% in group A vs 36.4% in group B and 60.7% in group C), especially cortical malformations (0% in group A vs 13.6% in group B and 32.1% in group C), were more frequent in patients with absent CNVs ($p = 0.05$). Comparing the frequency by univariate analysis of clinical features referring to epilepsy in patients with pathogenic CNVs vs. those with VOUS or absent CNVs (see Table 4), a statistically significant association with the presence of pathogenic CNVs was found for the atypical absence seizures ($p < 0.05$), tonic seizures ($p < 0.05$) and epileptic spasms ($p < 0.01$). Conversely, febrile convulsions (36.4% in group

Table 3
Demographic features in patients with pathogenic CNVs, VOUS CNVs and without CNVs (negative).

	Pathogenic CNVs	VOUS CNVs	Negative	p-value (*)
Number of Patients	11	22	28	/
Gender (F/M)	5/6	10/12	18/10	$p > 0.3$
Mean Age ± SD (y) (range)	25.6 ± 8.10 (18–43)	27.0 ± 7.78 (18–54)	32.5 ± 11.65 (18–62)	p = 0.06 (**)
Pre-perinatal Risk factors	7 (63.6 %)	11 (50.0 %)	18 (64.3 %)	$p > 0.5$
ID				
Mild	0	6 (27.3 %)	9 (32.1 %)	$p > 0.15$
Moderate	4 (36.4 %)	5 (22.7 %)	10 (35.7 %)	
Severe	7 (63.6 %)	11 (50 %)	9 (32.1 %)	
Neurodevelopmental disorder associated				
Autism	3 (27.3 %)	0	2 (7.1 %)	$p < 0.05$
Psychiatric disturbances	6 (54.5 %)	9 (40.9 %)	12 (42.8 %)	$p > 0.9$
Dysmorphisms	8 (72.7%)	15 (68.2 %)	19 (67.8 %)	$p > 0.9$
Comorbidities	0	5 (22.7 %)	3 (10.7 %)	$p > 0.15$
Radiological Abnormalities				
Cerebral Malformations	8 (72.7 %)	8 (36.4 %)	17 (60.7 %)	$p = 0.09$
Cortical malformations	0	3 (13.6 %)	9 (32.1 %)	$p = 0.05$

F: female. **M:** male. **y:** years. **SD:** standard deviation. **ID:** intellectual disability.
*Univariate analysis by means of Person's Chi-squared test.
** One-way between subjects ANOVA [F(2, 59) = 3.207, p = 0.0476].
Post hoc comparisons using the Tukey HSD test indicated that the mean
age for the Pathogenic CNVs group was significantly greater than the Negative group at $p < 0.05$.

Table 4
The epilepsy phenotypes in patients with pathogenic CNVs, VOUS CNVs and without CNVs (negative).

	Pathogenic CNVs	VOUS CNVs	Negative	p-value (*)
Family history of epilepsy	6 (54.5%)	8 (36.4%)	6 (21.4%)	$p > 0.1$
Febrile Convulsion	4 (36.4%)	3 (13.6%)	2 (7.1%)	$p = 0.07$
Mean Age ± SD at Seizure Onset (y) (range)	4.9 ± 5.7 (0.08–19.0)	6.99 ± 4.88 (0.25–18.0)	4.8 ± 5.23 (0.08–18.0)	$p > 0.25$ (**)
Seizure Type (****)				
Absence	1 (9.1%)	3 (13.6%)	1 (3.6%)	$p > 0.4$
Atypical Absence	4 (36.4%)	7 (31.8%)	2 (7.1%)	$p < 0.05$
Tonic	6 (54.5%)	5 (22.7%)	3 (10.7%)	$p < 0.05$
Myoclonic	0	2 (18.2%)	3 (10.7%)	$p > 0.5$
Tonic-Clonic	2 (18.2%)	5 (22.7%)	5 (17.8%)	$p > 0.9$
Drop (Tonic/Atonic)	5 (45.4%)	5 (22.7%)	4 (14.3%)	$p > 0.1$
Spasms	5 (45.4%)	5 (22.7%)	1 (3.6%)	$p < 0.01$
Epilepsy Type				
Focal	4 (36.4%)	9 (40.9%)	18 (64.3%)	$p > 0.1$
Generalized	1 (9.1%)	5 (22.7%)	5 (17.8%)	
Encephalopathy	6 (54.5%)	8 (36.4%)	5 (17.8%)	
Seizure Frequency				
High (***) at onset	7 (63.6%)	13 (59.1%)	21 (75%)	$p > 0.4$
High (***) at last visit	8 (72.7%)	9 (40.9%)	14 (50%)	$p > 0.2$
Worsening seizure frequency between onset and last visit	6 (54.5%)	10 (45.4%)	14 (50%)	$p > 0.8$
Nocturnal Seizures	4 (36.4%)	12 (54.5%)	9 (32.1%)	$p > 0.2$
Status Epilepticus	0	2 (9.1%)	3 (10.7%)	$p > 0.5$
Drug Resistance	8 (72.7%)	12 (54.5%)	22 (78.6%)	$p > 0.1$
EEG Features (****)				
Awake Epileptiform Abnormalities	5 (41.2%)	9 (47.1%)	9 (32.1%)	$p > 0.6$
Sleep Epileptiform Abnormalities	8 (64.7%)	13 (64.7%)	14 (50%)	$p > 0.4$
Focal Abnormalities	4 (23.5%)	3 (17.6%)	10 (35.7%)	$p > 0.1$
Multi-focal Abnormalities	1 (11.8%)	6 (35.3%)	6 (21.4%)	$p > 0.4$
Diffuse Abnormalities	7 (58.8%)	8 (35.3%)	7 (25%)	$p > 0.07$
Generalized Abnormalities	0	4 (17.6%)	0	$p < 0.05$
Sleep fast polyspikes	4 (41.2%)	8 (35.3%)	4 (14.3%)	$p > 0.1$

Y: year. SD: standard deviation.
(*) Univariate analysis by means of Person's Chi-squared test.
(**) One-way between subjects ANOVA [F(2, 59) = 0.751, p = 0.476].
(***) High seizure frequency is defined as multi-monthly, weekly, multi-weekly, daily or multi-daily. Annual, multi-annual and monthly seizure frequency are considered as low seizure frequency.
(****) The total number of patients may not be identical to the sum of subcategories, since some patients may experience more than one seizure type or more than one EEG feature.

A vs 13.6% in group B and 7.1% in group C), drop seizures (45.4% in group A vs 22.7% in group B and 14.3% in group C) and epileptic encephalopathy (54.5% in group A vs 36.4% in group B and 17.8% in group C) were more frequent in patients with pathogenic CNVs, but without significant differences. Moreover, focal seizures (36.4% in group A vs 40.9% in group B and 64.3% in group C) and drug resistance (72.7% Group A vs 54.5% in group B and 78.6% in group C) were more frequent in patients with absent CNVs, but without significant differences. Finally, univariate analyses of the EEG features of patients revealed a non-significant trend for diffuse EEG abnormalities (58.8% Group A vs 35.3% in group B and 25% in group C) and a statistically significant difference for generalized EEG abnormalities (0% in group A and C, 17.6% in group B). Although sleep fast polyspikes (41.2% in group A vs 35.3% in group B and 14.3% in group C) were more frequent in pathogenic group, and focal EEG abnormalities (23.5% Group A vs 17.6% in group B and 35.7% in group C) were more frequent in absent CNV group, significant differences were not disclosed.

In the light of the electroclinical features referring to epilepsy in adult patients with ID, 3 variables revealed a significant association with pathogenic CNVs, specifically atypical absence seizures, tonic seizures, and epileptic spasms. These variables evoke a peculiar electroclinical pattern resembling a LGS, a childhood-onset epileptic encephalopathy [30–32]. The classic LGS triad comprises intractable, multiple and generalized seizures that include absences seizures, epileptic spasms and, especially, tonic seizures, ID, and an interictal EEG showing bursts of slow spike-and-wave, paroxysmal bursts of generalized polyspikes, and a slow background [31,32]. Approximately 75% of LGS patients are thought to be symptomatic, suggesting an identifiable cause that is the result of a static brain disorder such as a cerebral-cortical malformation or hypoxic–ischemic injury [31]. When LGS has no apparent etiology, approximately 25% of cases [33], a genetic predisposition [34] influence has been also hypothesized, but without strong evidence due to little progress in understanding the genetic contributions. In our series, clear structural lesions, and especially cortical malformation, showed a statistically significant increased probability with absent CNVs. Therefore, adult patient with electroclinical features suggestive of LGS and without clear structural lesion seem to show the indications to perform CMA because of a statistically significant increased probability for pathogenic CNVs results.

To conclude, the results of our study enhanced the knowledge about the phenotypic features of adult patients with pathogenic CNVs, particularly disclosing the electroclinical features of epilepsy in patients with ID. The information about the specific electroclinical features of epilepsy may be helpful for neurologists, especially epileptology experts, in selecting adult patients for molecular studies and the adequate use of CMA increasing the diagnostic yields of this investigation. High resolution SNP-Array analysis should be evaluated in adult patients with ID and epilepsy with peculiar electroclinical features, specifically atypical absence seizures, tonic seizures, and epileptic spasms, resembling a LGS without a clear structural lesion.

Conflicts of interest

We declare that there are no conflicts of interest.

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.

Authors contribution

Giuseppe d'Orsi: study concept and design, acquisition of data, analysis and interpretation.

Tommaso Martino: statistical analysis

Orazio Palumbo, Pietro Palumbo Massimo Carella

genetic analysis

Maria Grazia Pascarella, Maria Teresa Di Claudio, Carlo Avolio: acquisition of data, analysis and interpretation.

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