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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.

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