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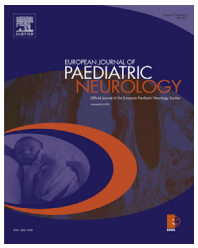

Original article

Phenotype profiling of patients with intellectual disability and copy number variations

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ABSTRACT

Background: Nowadays the microarray technology allows whole-genome analysis with a high resolution and performance for the genetic diagnosis in any patient with intellectual disability or autism spectrum disorder. However in the immediate future, with the development of massive sequencing systems for application at clinical diagnosis, it will be necessary to have clinical criteria to guide studies.

Aim: To perform an exhaustive clinical definition of patients with pathogenic copy number variations in order to establish the clinical criteria most suggestive of this kind of genomic rearrangements.

Method: We designed and implemented a database to collect 190 different clinical variables (pregnancy, neonatal, facial dysmorphism, congenital anomalies, neurological features and family history) in a series of 246 patients, with developmental delay/intellectual disability. All cases were studied with array comparative genomic hybridization.

Results: We have found a pathogenic genomic imbalance in 73 patients. Frequency analysis of all clinical variables showed that growth disorder, abnormalities of hands, low-set ears and hypertelorism are the more frequent features among patients with genomic rearrangements. However other clinical features, such as genital abnormalities and aggressiveness, are more specifically associated with pathogenic copy number variations in spite of their low frequencies in the overall series, yielding higher statistical significance values than other traits.

Conclusions: The genotype–phenotype comparison may be useful to set in the future the main clinical manifestations associated with deletions, duplications and unbalanced translocations. Theses analyses will improve the clinical indications and protocols to implement genomic arrays in the genetic study of patients with neurodevelopment disorders.

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1. Introduction

The study of the developmental delay/intellectual disability (DD/ID) is one of the most complex fields because of their high clinical and genetic heterogeneity. Genetic causes include chromosomal abnormalities, microduplication or microdeletion syndromes, X-linked forms, monogenic autosomal dominant or recessive forms and inheritance modified by genetic imprinting. Most patients remain without a specific diagnosis, with the corresponding consequences for the patients and their families. Ignorance of its etiology does not allow to offer an appropriate genetic counseling, carrier identification and prenatal or preimplantational diagnosis to the families.[1,2]

Patients with chromosomal abnormalities frequently present concomitant clinical features, in addition to the ID: growth retardation, dysmorphic features (DF) and multiple congenital abnormalities (CA). When they affect the function of internal organs worsen the prognosis and patient survival. In addition, patients' families sometimes have a higher incidence of abortions, infertility and premature infants with multiple malformations that usually die shortly after birth.[3]

With the development of high resolution genetic techniques[4–7] and particularly after the introduction of array-based comparative genomic hybridization (aCGH), the study of chromosomes has enabled dramatic progress in the field of clinical genetics in relation to routine karyotyping. Currently there are studies that show the relevance of subtelomeric chromosomal rearrangements and interstitial submicroscopic disbalances in the etiology of ID.[8–10] The application of these molecular techniques to the study of patients with ID has been a major advance in the understanding of their etiology and characterization of new syndromes due to its higher resolution and accuracy. However, very few studies on clinical criteria maximizing the diagnostic yield of this technology have been published.[11,12] It is necessary to define the clinical criteria to allow the selection of patients with high probability of harboring relevant copy number variations (CNVs).

Nowadays all patients with no specific clinical suspicion are tested by chromosomal microarray because only this technology allows whole-genome analysis with a high resolution and performance. Consequently there is a trend to apply these studies of arrays for the genetic diagnosis in any patient with intellectual disability or autism spectrum disorder. However in the immediate future, with the development of massive sequencing systems for application at clinical diagnosis, it will be necessary to develop new working algorithm in a rational way.

The aim of this work is the exhaustive clinical definition and the genotype–phenotype comparison to ascertain the main clinical manifestations specifically associated with pathogenic CNVs. The aim is to establish the clinical indications and protocols to implement arrays in the genetic study of intellectual disability. At the same time, achievement of a specific diagnosis allows accurate genetic counseling that helps the family to take informed reproductive decisions.

2. Method

2.1. Participants

A series of 246 children (136 males; 110 females), with a mean age of 6 years and 9 months, were recruited for genetic investigation of unexplained DD/ID during a 10-years period (January 2001–December 2010). These patients were tested because of a suspicion of chromosomal abnormalities based on their clinical presentation. We selected the patients on the basis of the following criteria: DD/ID associated with CA, DF and/or a positive family history for ID, CA or miscarriages.

All patients except one (bearing an apparently balanced chromosomal translocation inherited from his father) had a normal karyotype and most of them were negative for other genetic conditions. Exclusion criteria were the presence of a pathological cytogenetic abnormality or a definitive clinical and genetic diagnosis of a specific syndrome.

Patients were referred to our reference Unit by 32 different health professionals from 13 hospitals (Comunidad Valenciana; East Spain). Patients were assessed by two different health professionals. Detailed clinical data were recorded in a data base (190 clinical traits). Consent was obtained from all the participants in the study, according to the ethical board of our hospital. Peripheral blood samples were obtained from both the patients and their parents.

This study was reviewed and approved by the Institutional Ethics Committee of our hospital, thus complying with the treaty agreed in 1964 in Helsinki, by the World Medical Association on ethical principles of human research for medical purposes and subsequent revisions of the same (2013).

2.2. Genetic studies

Extraction of genomic DNA was performed from peripheral blood samples collected in EDTA (1 mM) in a volume of about 5 ml. We used the phenol extraction protocol described by Miller.[13]

Array-CGH was performed in most patients with two types of arrays. The first one consists of 6000 bacterial artificial chromosome (BAC) clones evenly distributed throughout the human genome at a resolution of 0.5–1 Mb. This array was applied to 50 DNA samples.[14] The second one was an oligonucleotide array consisting of 44,000 60-mer probes evenly distributed throughout the human genome with a resolution of 50–150 Kb (44K Agilent Technologies). This array was applied to all samples except those showing a well-characterized pathological alteration in previous BAC microarray study.

Fluorescence *in situ* hybridization (FISH), microsatellite markers, quantitative PCR, multiplex ligation-dependent probe amplification (MLPA), X inactivation and custom aCGH designs were used to confirm the alterations and establish the parental origin thereof. The technical protocols and the analysis were performed as recommended by the manufacturer with minor modifications.[4,5]

Clinical and genetic findings were compared to known CNVs listed in the Database of Genomic Variants (DGV), in the International Standard Cytogenomic Array Consortium Da-