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Array-CGH in pediatric neurology: A prospective observational study

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Abstract

Array-comparative genomic hybridization (Array-CGH) has been proposed as the first efficient approach to scan the entire genome for variations in DNA copy number. This diagnostic method is based on the study of total genomic DNA isolated from any sample and reference cell populations, differentially labelled and hybridized to DNA microarrays. The method has been initially applied in clinical genetics research and has recently also been used in cancer research, as tumor genomes have a wide variety of copy number phenotypes, indicating different types of genetic instability. In this field, array-CGH has been demonstrated to be an efficient diagnostic method to provide information on the location of important cancer genes. Recently the use of array-CGH has been expanded, including the analysis of constitutional abnormalities, to diagnose subtending mutations of neurologic diseases, with promising results in diagnosing genetic mutations otherwise not evident with other genetic tests. We performed a prospective study on the efficiency of array-CGH in the genetic-molecular diagnosis of pediatric patients affected by developmental delay and mental retardation associated with clinical signs of dimorphism and/or other relevant neurological symptoms. In our study, we had a detection rate of 22.71% by array-CGH analysis and we were able to take more precise genetic information for microdeletion and microduplication of our cohort of patients with developmental delay and/or idiopathic mental retardation and/or dimorphic face and/or epilepsy. In our opinion, we think that these findings would be helpful in early diagnosis and family genetic counseling, above all in those clinical neurologic cases in which other diagnostic tests have not succeed in performing a diagnosis.

Keywords

array-comparative genomic hybridization (array-CGH), observational study, pediatric neurology

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Introduction

Array-comparative genomic hybridization (Array-CGH) has been proposed as an efficient diagnostic method to scan the entire genome in order to study variations in DNA copy number.[1] In array-CGH measurement, total genomic DNA is isolated from any cell populations, differentially labeled, and hybridized to DNA microarrays.[1] The test for relative hybridization intensity and reference signals at a given location ideally should be proportional to the relative copy number of those sequences in the test and reference

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genomes. If the reference genome is normal, then increases and decreases in the intensity ratio directly indicate DNA copy number variation (CNV) in the genome of the test cells.[2]

This genomic method has been initially applied for diagnosis in clinical genetics research and has also recently been used in cancer research, as tumor genomes have a wide variety of copy number phenotypes, indicating different types of genetic instability. The wide range of genomic phenotypes in cancer means that, for some sets of specimens, array-CGH will provide information on the location of important cancer genes, even if in others may be uninformative.[3] In those cases of ongoing genomic instability, array-CGH may result in insufficient diagnosis and should be associated with techniques that examines individual cells.[3]

Recently, array-CGH has been often used for the analysis of constitutional abnormalities and it has been useful to diagnose subtending mutations of neurologic diseases or pathologies of various origin, also in the pediatric age group,[4–6] with promising results in diagnosing genetic mutations otherwise not evident with other genetic tests.

The aim of our study was the analysis of the efficiency of array-CGH in the genetic-molecular diagnosis of pediatric patients affected by developmental delay and mental retardation associated with clinical signs of dimorphism and/or other relevant neurological symptoms.

Materials and methods

Our study was a prospective study on children referring to our General Paediatrics Operative Unit, University of Catania, Italy, for developmental delay and mental retardation associated with dimorphisms and eventual neurological signs and/or symptoms.

The study period was for two years (from January 2012 to January 2014) and we included all pediatric patients (age range, 2–14 years) affected by developmental delay, mental retardation, dimorphic features, and/or seizures of unknown origin.

We excluded those patients aged under 2 year or over 14 years, with neurologic disorders associated to known syndromes, affected by systemic chronic diseases of an already known origin.

All patients underwent blood routine tests, metabolic screens, and diagnostic instrumental exams (cardiac and abdomen ultrasound scans, brain MRI, visual function tests) in order to exclude metabolic causes of disease and multi-organ failure.

All patients underwent a venous withdraw at admission, in order to perform a karyotyping by array-CGH. The patient DNA was labeled with Cy3 and Cy5, each through a random priming method (Human-DNA Promega). An Array Human Genome CGH Microarray kit 8×60 K (Agilent), with a Cytogenomic analysis (by genomic assemblance h19), quality score (DLRS): <0.3, analysis parameters: 8 consecutive probes, algorithm ADM-2, threshold 6.0, resolution 100–200 Kb was then used for hybridization and washing. The genetic test was performed in both children and respective parents, including the most proximate relatives (mother, father, and brothers and/or sisters).

Public databases such as the Database of Genomic Variants (DGV) and the International Standard for Cytogenomic Arrays were used to classify whether CNVs were benign (on uncertain clinical significance) or pathogenic. In the present study, the size of 500 kb was designated as relative criteria to distinguish benign from pathogenic CNVs.

Results were then consecutively collected and analyzed by specific statistical software.

Statistical analysis

All data with numeric values have been expressed as absolute values in mean and standard deviation (SD).

The percentage statistic values were obtained using the frequency calculation test.

Results

In our study we included 44 pediatric patients (26 boys, 18 girls; mean age, 5.62 ± 2.32 [SD] years. All patients were affected by developmental delay and/or mental retardation of various grades associated with facial dimorphisms. Apart from the described clinical signs, 6/44 patients displayed behavioral disturbs; in four patients, epileptic disease was diagnosed. Results are summarized in Table 1.

None of the included patients had a diagnosis of known genetic disease, metabolic disease, and/or organ failure.

The array-CGH exam gave a positive result in ten patients (22.72% of cases), resulting in chromosomal duplications in 70% and in chromosomal deletion in 30% of positive cases, 15.90% and

Table 1. Description of the diagnosed mutations by array-CGH in 10/44 patients of our study cohort.

Patients' initials	Signs and symptoms	Diagnosed mutation	Proband study	Associated disease
FS	DD, DS	Dup cr 17p12-p11.2	De novo	Potocki-Lupski syndrome
FC	DD, DS	Dup cr 16p13.11 - p12–3	Proband carrier	ND
CG	DD, myoclonic epilepsy	Dup cr 7q11.23 - q21.11.11	Proband carrier	ND
DGN	DD, DS	Dup cr 19p13.11	De novo	ND
FC	DS	Dup cr 18q11.2	De novo	ND
TA	DD, behavior disturbs	Dup cr 16p13.3	De novo	ND
DAM	DD, hypotonia	Dup cr 19q13.11	De novo	ND
FG	DD, DS	Partial Del- cr 8p12.4	De novo	ND
TS	DD, DS, febrile seizures, behavior disturbs	Partial Del- cr 6p21.2	De novo	ND
LA	DD, DS	Partial Del- cr 16	De novo	ND

DD, developmental delay; DS, dimorphic signs, ND, never described.

6.81% of all included patients, respectively. In 9/10 positive cases, the resulted mutations have been until now never described in the International Database of Polymorphisms (IDP), thus they have not been associated with specific genetic diseases until now. In these cases, we detected more than one benign aberration for a total of 385 CNVs with an average size of 132.16 ± 180.60 SD. These aberrations have not been listed in the Database of Genomic Variants (DGV) and might thus be specific for the Mediterranean population. Only in two cases was the same genetic mutation present in one of the probands, while in all the other cases the described mutations were de novo.

Discussion

In our study, the array-CGH was diagnostic in 22.71% of cases, showing the presence of aneuploidy mutations, mostly of de novo origin.

Patients with mental retardation or developmental delay constitute 2–3% of the total population. Chromosomal anomalies detected by G-band karyotype account for 10–15% of mentioned patients;[7,8] and their causes could be recognized by the FISH test in 2–5% of previously diagnostically unexplained cases.[8–10] In spite of this fact, in 60% of the patients the etiology is undetermined.

A review by Hochstebach et al. showed an additional 13.6% detection by array-CGH when added to traditional genetic tests.[11] A study by Byeon et al.,[12] showed an additional 24.4% detection rate by array-CGH for 78 cases which were recognized as normal in G-band karyotyping. In our study, this rate is even higher, with 22.71% diagnosed cases for 44 studied patients. Furthermore (according to Byeon et al.), we have to consider that a normal array-CGH does not convey that most of the genetic abnormalities are excluded. This is due to the fact that many of these genetic abnormalities could not be identified by array-CGH, namely "balanced chromosomal rearrangements, less than 20–30% mosaicism, severe ploidy, single and multiple base polymorphism, numerical variations and tribasic repeat sequence." Other genetic abnormalities including "uniparental disomy, a 2:1 allele ratio which can be shown in single nucleotide polymorphism (SNP) array" may not be identified by this method as well. In our study, similar to Byeon et al., array-CGH was beneficial in gathering genetic details of microdeletion and microduplicaiton of our cohort patients suffering from developmental delay and/or idiopathic mental retardation and/or dimorphic face and/or epilepsy.

The current study found a detection rate of 22.71%; and we believe that result of this study would be beneficial to help patients in early detection of the disease and family genetic consultation. Novel treatments (including medications and gene therapy) could be accomplished according to these findings as well. Although applying array-CGH in clinical cases is accompanied with some restrictions, it should be considered as a diagnosis tool due to its ability for determining the etiology of several idiopathic neurological diseases.

A limitation of this study is that the difficult interpretation of results of CNVs should have been confirmed by FISH of specific locus, real-time PCR, multiplex ligation dependent probe amplification, and function tests for verification. Nevertheless, in the present study the aforementioned tests were performed in only two cases due to cost limitations.

Array-CGH provides further knowledge on normal chromosomal assessment variations and addresses the presence of new genetic mutations that have never been described in literature previously. Clinical application of array-CGH still has limitations, such as the difficult interpretation of CNVs, cost, and no specific treatment for most of the genetic diseases. With regard to further studies, a larger sample size is necessary to provide further information on species specificity, identification of the cause of idiopathic neurologic diseases in childhood, and further development of gene therapy.

Declaration of conflicting interest

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