



# Genetic Diagnosis of Chromosomal Congenital Anomalies in Albanian Pediatric Patients by Array CGH

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## Abstract

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**AIM:** The aim of our study was to identify chromosomal imbalances by whole-genome microarray-based comparative genomic hybridization (array CGH) in DNA samples of children in which karyotype results cannot be obtained. The present paper describes the first Albanian experience of an array CGH application.

**MATERIAL AND METHODS:** The cohort included seven children with developmental delay or intellectual disability, facial dysmorphism and congenital anomalies according to clinical criteria, suggestive of chromosomal anomalies. The age range was from newborn to five years old. The cytogenetic analysis determined by a standard method of G-banding according to the International System for Human Cytogenetic Nomenclature (ISCN 2005) was performed for all our patients, while array CGH was performed on genomic DNA isolated from the blood of 7 cases.

**RESULTS:** Among the seven patients analysed with array CGH, three patients resulted in duplication and one deletion, one patient with a microdeletion and three patients with duplication. Array CGH facilitated the recognition of submicroscopic deletions and duplications as risk factors for genetic diagnosis in all our patients.

**CONCLUSIONS:** Our case series with congenital chromosomal anomalies confirms the high diagnostic value of the method, as suggested by previous studies. The technique must be available also in less developed countries, to significantly improve the genetic diagnosis of paediatric patients with developmental delay or intellectual disability, congenital anomalies and dysmorphic features. The identification of chromosomal abnormalities in these patients and the genetic counselling will provide family members with an explanation for their child's developmental disability or birth defect, allowing better information about recurrence risks, and permit the anticipation of certain medical problems that require intervention.

## Introduction

Laboratory evaluation of patients with developmental delay/intellectual disability, congenital anomalies and dysmorphic features has changed significantly in the last years with the introduction of microarray technologies [1].

Array Comparative Genomic Hybridization (array CGH) is rapidly becoming the first tier clinical genetic test for patients with developmental delay/intellectual disability and multiple congenital anomalies. Numerous studies have demonstrated that array CGH offers a much higher diagnostic yield for this group of patients in respect to conventional karyotyping with a G-banded karyotype [2]. This increased resolution of microarray technology over conventional cytogenetic analysis allows for

identification of chromosomal imbalances with greater precision, accuracy, and technical sensitivity, primarily because of its higher sensitivity for sub-microscopic deletions and duplications.

The aim of our study was to identify chromosomal imbalances by array CGH in DNA samples of children in which karyotype results cannot be obtained. We describe the first Albanian experience from a cohort of 7 children with developmental delay or intellectual disability, congenital anomalies and dysmorphic features, according to clinical criteria.

## Material and Methods

The cohort included seven children with developmental delay/intellectual disability, congenital

anomalies and dysmorphic features, according to clinical criteria, suggestive of chromosomal anomalies. The age range was from newborn to five years old. The case series has only a small number of cases because array CGH is not yet performed in Albania.

The cytogenetic analysis, as determined by a standard method of G banding according to the International System for Human Cytogenetics Nomenclature (ISCN 2013), was performed in all our patients; while array CGH according to the International Standard Cytogenomic Array (ISCA) Consortium statement 2010 was performed on genomic DNA isolated from the blood of 6 cases. All patients were scored using a clinical scoring system. Written informed consent was obtained from the parents of all children.

### **DNA isolation and Array CGH**

Blood samples with EDTA were collected from peripheral blood of patients. DNA extraction was carried out using the Qiagen QIAamp® DNA blood mini kit (QIAGEN, Valencia, CA, USA). Array-CGH analysis was performed using 4 × 44 K, 2 × 105 K and 4 × 180 K commercial arrays (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. This platform contains 60-mer oligonucleotide probes spanning the entire human genome with an overall median probe spacing of 22 Kb (19 Kb in Refseq genes). After hybridization, the arrays were scanned in a dual-laser scanner (DNA Microarray Scanner with Sure Scan High-Resolution Technology, Model G2565CA; Agilent Technologies) and the images were extracted and analysed through Agilent Feature Extraction software (v10.5.1.1) and DNA Analytics software (v4.0.73), respectively. Fluorescence in situ hybridization (FISH) analysis was performed to confirm the results of array-CGH and the mode of inheritance.

## **Results**

All seven patients with suggestive features of a chromosomal anomaly had been analysed by conventional karyotyping by standard GTG banding (550 band resolution per haploid karyotype). Most of the patients had an apparently normal karyotype, or in some cases, the karyotype analysis did not yield significant results. In these patients, the detection of gains or losses of genetic material were small to be detectable by standard G-banded chromosome, and array CGH method was applied.

The blood sample of one patient, not included in this case series (*findings from this patient are not*

*described in Table one*), was analysed only with conventional karyotyping. GTG banding revealed a karyotype 46, XY, del (7) (q21q22.2). He was a newborn boy with dysmorphic facial features, bilateral limbs ectrodactyly, growth retardation and developmental delay. His karyotype was 46, XY, del (7) (q21-q22.2), with one interstitial deletion of chromosome 7. The deletion was de novo because the parental karyotype was normal. Syndromic ectrodactyly is expressed as an autosomal dominant trait with reduced penetrance and variable expression. Several studies have pointed out the probable role of three genes present in this region, DLX5, DLX6 and DSS1 genes, in limb development [3].

Among the seven patients of our *case series* (Table 1) analysed with karyotype and array CGH, three patients resulted with duplication and a deletion (cases 2, 3, 7); 3 patients with a duplication (cases 1, 5, 6) and one patient with a deletion (case 4). The results were confirmed by FISH analysis. Clinical information, karyotype and array-CGH results of our patients are summarised in Table 1. For all patients, karyotype and FISH analysis has been extended to parental samples, so to establish if an aberration was inherited or de novo. In 2 out of the seven patients, the chromosomal aberration had been inherited from one of the healthy parents who were carriers of balanced reciprocal translocation.

One male patient (**case 2**) with karyotype 46, XY, der (21), was diagnosed with a duplication involving 16p13.3-16p13.2, which spans a region of about 8, 7 Mb and deletion involving 21q23.3-21q22.3 of about 280,8Kb. The duplication was confirmed by FISH analysis using locus specific probe 16pter that has confirmed the derivative material of chromosome 16 in the terminal end of the long arm of rearranged chromosome 21. This rearrangement was the result of an unbalanced translocation between the short arm of a chromosome 16 and the long arm of a chromosome 21. A balanced translocation t (16; 21) (p13.3-13.2; q23.3-22.3) inherited from a healthy mother was found by FISH analysis.

Another case, a male patient (**case 7**) with karyotype 46, XY, der (4), showed a duplication involving 4q27 and a deletion involving 21q22. FISH analysis using specific probes confirmed an unbalanced translocation between the subtelomeric region of the long arm of a chromosome 4 and the subtelomeric region of the short arm of a chromosome 21. A balanced translocation t (4; 21) (q27; q22) inherited from a healthy father was found by FISH analysis.

It is important to emphasise that these parents were phenotypically normal and all translocations were balanced. The other chromosomal rearrangements which were observed in the remaining patients were a de novo occurrence.

**Table 1: Clinical information, karyotype and array-CGH results of our patients**

Case/No	Gender	Array-CGH results	Karyotype results	Origin	Estimated size	Clinical features
1	M	dup(10)(q22)	46, XY, dup(10)(q22)	de novo	6 Mb	moderate mental retardation, hypotonia, microcephaly, epicanthus, low-set, misshapen ears with overfolding of angulated upper helix, relatively short metatarsals and phalanges and hypoplasia of midphalanx of the fifth finger with clinodactyly of both feet
2	M	dup 16p13.3-16p13.2 del 21q23.3-21q22.3	46, XY, der (21)	maternal	8,7 Mb 280,8Kb	micrognathia, labioschisis, hypospadias, congenital heart disease and pes equinovarus
3	F	dup 7p22.37p21.2 del 9p24.3p24.1	46, XY, der (9)	de novo	5,1 Mb 4 Mb	delayed psychomotor development, trigonocephaly, broad flat nasal bridge, epicanthus, anteverted nares, malformed external ears, hypertelorism, omphalocele, palatoschisis
4	F	del 22q11.1-q11.21	46, XX	de novo	2,5 Mb	congenital heart disease
5	F	dup 7p22.3-p22.1	46, XX, der(20)	de novo	4,2 Mb	microphthalmia, ocular hypertelorism and low-set ears
6	F	dup 1p36.33	46, XX	de novo	3,9 Mb	psychomotor delay, trigonocephaly, microcephaly, epicanthus, hypertelorism, wide nose, convergent strabismus, and spastic cerebral palsy
7	M	dup 4q27 del subtle 21q22	46, XY, der (4)	paternal	750Kb 1 Mb	dysmorphic facial features, complex heart disorder and cryptorchidism

## Discussion

In this study, the array-CGH analysis yielded more results than did karyotype analysis, provided better detection of genetic abnormalities and a better opportunity for genotype/phenotype correlations. We used DNA oligonucleotides to analyse other patients by array CGH, where the chromosomal abnormality was suspected due to the combination of clinical features. Five of the observed chromosomal aberrations were de novo, and two aberrations were inherited from one of the phenotypically normal parents (cases 2, 7 in Table 1). In the setting of multiple anomalies, a balanced translocation in one of the parents could be the explanation for unbalanced offspring, and G-banded karyotyping should still be the standard of care for this indication. In the clinical setting, probands are more likely to carry an imbalance, and parents or other family members would subsequently be tested through traditional

cytogenetic methods [3, 4].

The first patient (*Case one in Table 1*) was a boy five years old with moderate mental retardation, hypotonia, microcephaly, epicanthus, low-set, misshapen ears with overfolding of angulated upper helix, relatively short metatarsals and phalanges and hypoplasia of midphalanx of the fifth finger with clinodactyly of both feet. His karyotype was 46, XY, dup (10) (q22) with one duplication of the long arm of chromosome 10. The range and severity of symptoms and physical findings in dup (10) (q22) may vary from person to person, depending upon the exact length and location of the duplicated portion of chromosome 10q. The previous studies have reported that the use of array-CGH would provide an insight of genetic abnormality [5].

The second patient was a newborn boy, who presented a facial dysmorphic phenotype with micrognathia, labioschisis, hypospadias, congenital heart disease and pes equinovarus. The high-resolution G-banding, revealed a karyotype with 46 chromosomes, XY chromosome complement and a structurally abnormal chromosome 21. Array-CGH revealed one 8, 7 Mb duplication in the short arm of chromosome 16, involving 16p13.3-16p13.2 and a 280, one 8 Kb deletion in the long arm of chromosome 21, involving 21q23.3-21q22.3. These anomalies were inherited from his mother who had a balanced translocation (16; 21) with 46, XX, der (21) t (16; 21) (p13.3-13.2; q23.3-22.3) karyotype. Previous studies have suggested that the *CREBBP* gene was dosage sensitive, and also responsible for the phenotype of chromosome 16p13.3 duplication syndrome. Chromosome 16p13.3 duplication syndrome is due to the duplication of chromosome 16p13.3; encompassing the *CREBBP* gene and characterised by frequent clinical findings such as mild to moderate intellectual disability, facial dysmorphism, anomalies of the extremities, and occasional developmental defect of the eyes, palate, genitalia, and heart (OMIM 613458). Both the genotype and phenotype of our case overlapped with chromosome 16p13.3 duplication syndrome, suggesting that the duplication of 16p13.3 was the pathogenic copy number variation (CNV) in our case and the *CREBBP* gene was the most critical candidate gene responsible for the phenotype of this patient [6].

The third patient was a female; one-year-old presented delayed psychomotor development, trigonocephaly, broad flat nasal bridge, epicanthus, anteverted nares, malformed external ears, hypertelorism, omphalocele, and palatoschisis. Her cytogenetic analysis revealed 46, XX, der (9) karyotype; but array CGH identified a 5, 1 Mb duplication involving 7p22.3-p21.2 and a deletion involving a 9p24.3-p24.1 region of about 4 Mb. This rearrangement was the result of an unbalanced translocation between the short arm of a chromosome 7 and the short arm of chromosome 9, confirmed by FISH analysis. The presence of craniofacial

dysmorphism, trigonocephaly, prominent forehead, anteverted nostrils with long philtrum, psychomotor retardation, and congenital malformations such as omphalocele and cardiopulmonary anomalies should alert clinicians to the possibility of deletion 9p syndrome and refer such patients for cytogenetic confirmation, as in our case. Cytogenetic analysis is vital as it aids in the precise and early diagnosis of this syndrome and helps to exclude other causes and provide appropriate genetic counselling [7].

The fourth patient was a girl, two months old, with congenital heart disease (interrupted aortic arch). Her cytogenetic analysis revealed a normal karyotype 46, XX; but array CGH identified a 2, 5 Mb deletion located in 22q11.1-q11.2, that encompassed 12 HGNC genes (Hugo Gene Nomenclature Committee) and 10 OMIM genes (Online Mendelian Inheritance in Man). This microdeletion determines DiGeorge syndrome. Although the genes responsible for the clinical features associated with 22q11.2 distal deletion syndrome have not been clearly defined, several potential candidate genes have been suggested. *CRKL* and *MAPK1* genes have been suggested to play a role in the heart anomalies that are common in 22q11.2 distal deletion syndrome. *MAPK1* has also been suggested to be associated with placental development. Therefore, having one copy of this gene missing in 22q11.2 distal deletion syndrome may be linked to the tendency for premature birth and intrauterine growth restriction. The *MAPK1* gene in mice has been shown to contribute to social behaviour and therefore may play a role in the behavioural problems found in some people with 22q11.2 distal deletion syndrome [8].

The fifth patient was a girl four months old presented microphthalmia, ocular hypertelorism and low-set ears. Her cytogenetic analysis revealed a euploid karyotype with the extra chromosomal material in the telomeric area of the short arm of chromosome 20 with 46, XX, der (20) karyotype. Array CGH identified a duplication involving 7p22.3-p21.2 of about 4, 2 Mb. There are some reports of patients with duplications that include overlapping chromosomal sub-domains. 7p22.1 duplication syndrome is described in the literature, and speech delay with recognisable facial features is observed in these patients. The described duplication is approximately 1.7 Mb and fifteen genes are involved in the duplicated segment [9].

The sixth patient was a girl, seven-month-old presented psychomotor delay, trigonocephaly, microcephaly, epicanthus, hypertelorism, wide nose, convergent strabismus, and spastic cerebral palsy. Her cytogenetic analysis revealed a normal karyotype 46, XX; but array CGH identified an extra chromosomal material in the short arm of chromosome 1, dup1p36.33. Few cases of 1p36 duplications have been reported, and data regarding the genotype-phenotype correlations are emerging.

The phenotype includes intellectual disability, developmental delay, feeding difficulties, hyperactivity and seizures. The distal duplication region contains the putative gene for epilepsy, *KCNAB2*, and the *MMP 23A* and *B* genes. Overexpression of *MMP 23A* and *B* genes has been proposed as possible candidate genes for craniosynostosis [10-12].

The seventh patient was a boy, one-month-old, with dysmorphic features, complex heart disorder and cryptorchidism. His karyotype resulted 46, XY, der (21) t (4; 21) (q27; q22). Array CGH revealed duplication at 4q27q35 and a deletion 21q22. This rearrangement was the result of one balanced translocation between the long arm of chromosome 4 and the distal area of the long arm of chromosome 21, exactly in 4q27 and 21q22 that were inherited from his healthy father. Partial trisomy distal 4q is a rare chromosomal disorder, commonly characterised by a low birth weight and growth deficiency. About fifty percent of cases suffering from this syndrome also will have heart defects and vascular abnormalities. According to reports in the medical literature, severe cardiac and/or renal defects may lead to potentially life-threatening complications in some cases [13].

Genetic counselling about the risk of an unbalanced offspring in the future pregnancies was considered in dealing with the parents. The identification of chromosomal abnormalities provided family members with an explanation for their child's developmental disability or birth defect allowed for better information about recurrence risks and permitted the anticipation of certain medical problems that may require intervention.

We may conclude that the emergence of array CGH as a diagnostic tool in molecular genetics has facilitated recognition of submicroscopic deletions and duplications as risk factors for genetic diagnosis in all our patients. This analysis allowed for a higher rate of detection the chromosomal anomalies and this determination is especially valuable in patients with congenital anomalies of unknown aetiology, or in cases in which karyotype results cannot be obtained. It can be used in the first-line investigation as a practical and scientific value and has improved significantly the genetic diagnosis of paediatric patients with developmental delay/intellectual disability, congenital anomalies and dysmorphic features.

In conclusion, clarification of the genetic profile generated by array-CGH analysis may result in detailed follow-ups and, in the long-term, a better overall outcome for these patients. The short-term perspective allows for family counselling and prenatal diagnosis.

We have reported a small number of cases with congenital chromosomal anomalies and confirmed the high diagnostic value of the previous studies. It is important that the technique becomes

available also in less developed countries improving significantly the genetic diagnosis of paediatric patients with developmental delay/intellectual disability, congenital anomalies and dysmorphic features.

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