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Array CGH identified rare chromosomal micro-imbalances in three patients with congenital malformations and mental retardation

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Introduction

In Bulgaria, about 2,100 children are born with congenital malformations annually. Most malformations are present at delivery and are due to genetic and non genetic factors. Submicroscopic chromosome aberrations can cause mental retardation (MR), congenital malformations and miscarriage. The etiology of intellectual and developmental impairment remains unidentified in about 50% of the patients despite extensive clinical examinations and laboratory investigations.

Materials and methods:

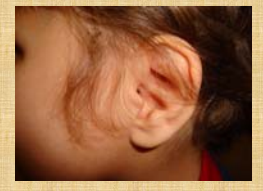
Eleven patients with DD/CM of unknown etiology were selected for high-resolution array-CGH screening for genomic imbalances. We found chromosome micro-imbalances in 3 patients from the study group.

I. Karyotype analysis- peripheral blood lymphocytes culture, conventional cytogenetic analysis, GTG banding at 550 band level

II. Array CGH- analysis. We have used genomic array CytoChip (BlueGnome, Cambridge, UK), covering the entire genome at a median 565Kb. It investigate sub-telomeres at a median 250Kb resolution, reliably detect mosaicism and examine 90 known genetic conditions at a median 100Kb resolution. This resulted in an average density of 1clone/0.5Mb by 4400 clones. Test and sex-matched reference genomic DNA was labeled by random-priming, using BlueGnome Fluorescent Labeling System.

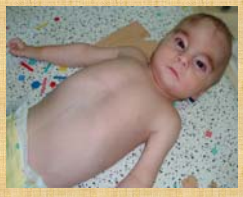
III. FISH- analysis. Confirmatory interphase FISH studies with BAC clone was performed on interphase and metaphase from cultured lymphocytes for accurate confirmation of CytoChip result.

Case 1: Clinical summary



We report the details of 15 months old boy. The patient was born from fourth uneventful pregnancy. He was referred for genetic diagnosis, because of developmental delay and dismorphic features. His facial features were distinct with hypertelorism , depressed nasal bridge, epicant, facial asymmetry with right facial hyperthrophy; malformed auricles and bilateral simian creases.

Case 2: Clinical summary

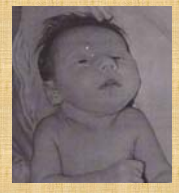


We present here details of six months old boy who was referred for genetic evaluation due to the developmental delay and dysmorphic features (Fig. 1- 4):

- **Facial dismorphism: dolichocephaly, metopic sutural synostosis, up slanting palpebral fissures, short nose with anteverted nares, long philtrum, micrognathia, thin upper lip;**
- **Low-set malformed auricles with prominent helix;**
- **Proximal placement of thumb, incomplete simian creases;**
- **Hammer thumbs, pes equinovarus;**
- **Hypospadia, scrotal hypoplasia, cryptorchidism;**
- **Omphalocele, cardiac defect,moderate hypotonia.**

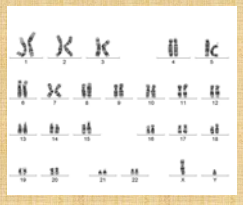
Case 3: Clinical summary

We describe a 14 months old girl referred to our genetics center because of severe developmental delay, mental retardation (IQ 48) and dysmorphic features. The patient was born from second uneventful pregnancy. Her facial features were distinct with low set ears, short philtrum, up slanting palpebral fissures, frontal upsweep; microcephaly, tapering fingers.

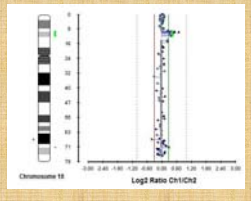


Results

Cytogenetic analysis: 550 band GTG chromosome analysis revealed a normal male karyotype.

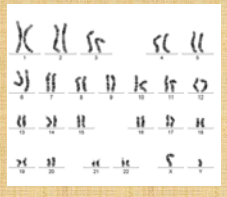


Array CGH analysis: Microarray study revealed a cryptic microdeletion encompassing an approximate 2,2 Mb in the (18)(p11.22;p11.21) region

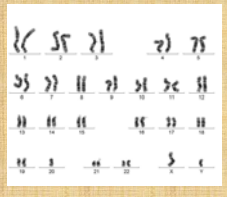


Further work at a molecular level is needed. This findings have to be validated by MLPA or FISH for validation.

Cytogenetic analysis: 550 band GTG chromosome analysis revealed a visible balanced pericentric inversion of chromosome 9- inv (9)(p11; q13) in the patient. The cytogenetic analysis of the parents ascertained the same inversion in the father .

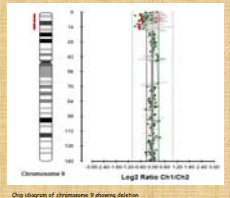


Patient's karyotype

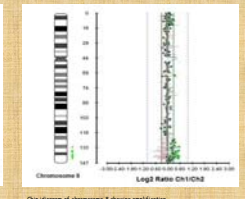


Father's karyotype

Array CGH analysis: Microarray study revealed cryptic deletion of (9)(p24.2;p23) region encompassing an approximate 8Mb and duplication of chromosome 8q24 region expanding from 8q24.22 to 8q24.3 and involving 11Mb.

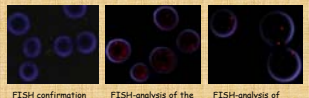


Chip idiogram of chromosome 9 showing deletion



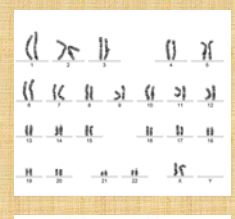
Chip idiogram of chromosome 8 showing amplification

FISH- analysis: FISH study of the patient chromosomes with locus specific blueFISH probe confirmed the deletion in 9p23 region on metaphases and interphases from peripheral blood lymphocytes. FISH analysis of the parents proved a de novo deletion in the boy.

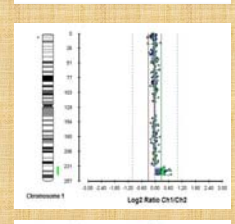


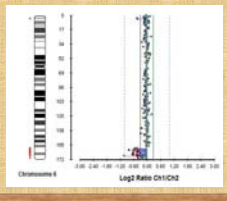
FISH confirmation of the 9p23 deletion in the patient | FISH-analysis of the mother | FISH-analysis of the father

Cytogenetic analysis: 550 band GTG chromosome analysis revealed a normal female karyotype.



Array CGH analysis: Microarray study revealed cryptic deletion of (6)(q25.3;q27) region encompassing an approximate 9,4 Mb and duplication of chromosome 1q44 region expanding from 1q42.3 to 1q44 and involving 12Mb. Further work at a molecular level is needed. This findings have to be validated by MLPA or FISH for validation.





Discussion and conclusion

These cases:

- show that array CGH could improve the prenatal diagnosis for carriers of chromosomal rearrangements.
- . illustrate the ability of this methodology to interrogate many regions in one assay is valuable when studying patients with nonspecific clinical findings;
- show how the molecular delineation enables improved genotype–phenotype correlations of chromosomal abnormalities to be made and may improve the genetic counselling in individuals with chromosomal imbalances;
- serves as reminder that not all cases with inherited visible balanced pericentric inversion of chromosome 9 will have normal phenotype;
- show that array CGH could improve the prenatal diagnosis for carriers of chromosomal rearrangements.

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