

UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL



**PhD THESIS
ABSTRACT**

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UTILITY OF CLASSICAL AND MOLECULAR
CYTOGENETIC TECHNIQUES IN
PRE- AND POSTNATAL DIAGNOSIS

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Keywords: array CGH, classical cytogenetics, prenatal diagnosis, microdeletion, microduplication

INTRODUCTION

Pregnancy is a physiological process characterized by both resistance (most of term pregnancies result in live births with complete genetic structure) and vulnerability (a percentage of conception products show changes in number or structure of the genetic material which, depending on their amplitude, can or cannot result in pregnancy loss). Considering the major impact of fetal malformations and genetic disorders on families and society, pregnancy-associated pathology represents a major area of interest for specialists.

Pre- and postnatal diagnosis aims to detect changes of genetic material and congenital malformations.

Changes of genetic material can be benign, potentially pathogenic or lethal. In the last decade, the interest of specialists was directed towards developing methods and techniques for prenatal screening and diagnosis of fetal genetic pathology. In this context, this study aimed to improve the organizational framework of prenatal diagnosis at a regional level by achieving two main objectives:

1. to evaluate the cases with anomalies identified by means of prenatal screening, through various genetic techniques (both classical and molecular) and correlate between the existing methods of screening and diagnosis.
2. to determine correlations between the phenotype and the genetic modifications identified pre- and postnatally, by analyzing the potential of using the comparative genomic hybridization technique in prenatal genetic diagnosis in Romania.

The aim and objectives of this study are justified by the economic, social and psychological impact of the genetic pathology (increased perinatal mortality, motor or intellectual disabilities, anxiety, depression, stigma).

I. BACKGROUND

Chapter 1, entitled "*Prenatal diagnosis*", describes the prenatal screening methods, indications for prenatal genetic diagnosis, invasive methods used in prenatal diagnosis, as well as classical and new generation cytogenetic and molecular techniques used in prenatal testing.

Chapter 2, entitled "*Array-CGH in pre-and postnatal diagnosis*", presents the general principles of microarray-based comparative genomic hybridization techniques, criteria used to choose the platform and types of samples used, as well as the difficulties which arise during results interpretation, in particular in prenatal diagnosis.

Chapter 3, entitled "*Genetic counseling and prenatal diagnosis*", describes the history of genetic counselling, its necessity before testing and the importance of obtaining the parental consent in prenatal diagnosis. Prenatal diagnosis requires a multidisciplinary approach that can be achieved by establishing a good collaboration between different specialists (physician, geneticist, obstetrician, etc.).

II. PERSONAL CONTRIBUTIONS

Chapter 4. Purpose and objectives of the study

We aimed to evaluate pregnancies considered to have an increased risk for genetic defects before birth and children with suggestive clinical genetic syndromes after birth. The assessment was performed by array CGH and / or conventional karyotyping.

Chapter 5. Material and methods

In this study we included 23 prenatal and postnatal cases with suspected genetic disorders based on clinical assessment, ultrasound screening and prenatal biochemical tests. The cases included in this study were evaluated in the Prenatal Diagnostic Unit of the Department of Obstetrics and Gynecology of the Emergency Clinical County Hospital Craiova, in the Pediatrics Department and the Medical Genetics Ambulatory of the same institution, as well as in the Human Genomics Laboratory of UMF Craiova (HGL).

The biological material collected was represented by peripheral venous blood, amniotic fluid, chorionic villus samples or fragments of fetal tissue.

Assessment was made through either classical cytogenetics (conventional karyotyping), or molecular methods (array CGH), and in some cases by associating the two techniques (performed in Human Genomics Laboratory – HGL).

Conventional karyotyping required freshly collected biological samples, stored in appropriate conditions to set up cell cultures, in order to obtain chromosomes and analyze them under the microscope.

For array CGH, both fresh and frozen samples were used. DNA was isolated, purified, assessed in terms of quality, followed by fluorescent labeling and hybridization on microarray slides.

Chapter 6. Results and discussion

In the first analyzed case, both biochemical and ultrasound abnormalities were found at prenatal screening. Initially, it was assessed through conventional karyotyping and after that through array CGH. The karyotype revealed an interstitial deletion on the short arm of chromosome 10.

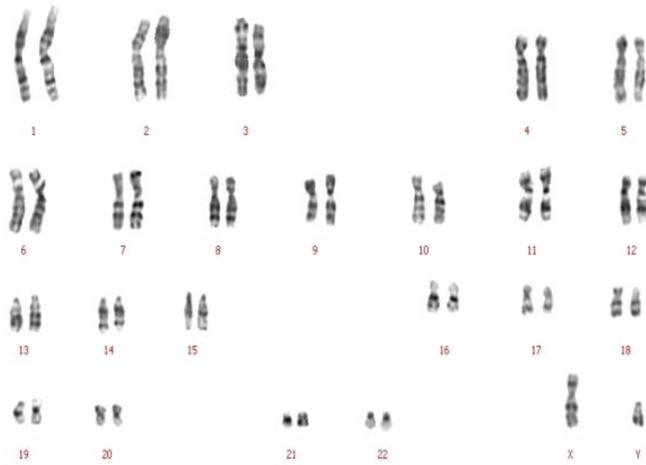


Figure 6.4. Karyotype: 46,XY,del(10p)

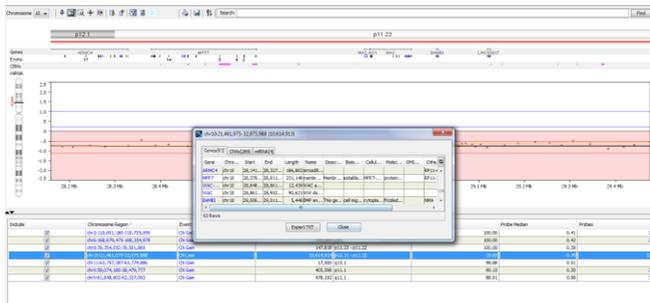


Figure 6.9. Chromosome 10 assessment by array CGH
arr 10p12.32p11.22(21,461,075-32,075,988)x1dn

The second case that we evaluated was a patient with Klippel-Trenaunay-Weber syndrome and mental retardation. Postnatal classical cytogenetic testing revealed a normal karyotype (46,XY), while array CGH identified a submicroscopic deletion on the long arm of chromosome 2.

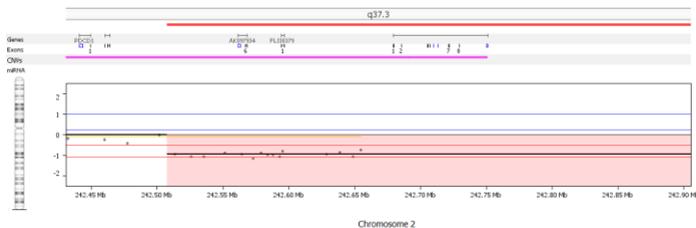


Figure 6.16. Chromosome 2 assessment by array CGH

Three cases assessed through array CGH presented the following aneuploidies: trisomy 21, trisomy 18 and monosomy X.

Regarding the case diagnosed with trisomy 18, we also investigated the parents through classical cytogenetics. The chromosomal analysis showed that the father is carrier of a balanced translocation between the chromosomes 8 and 10.

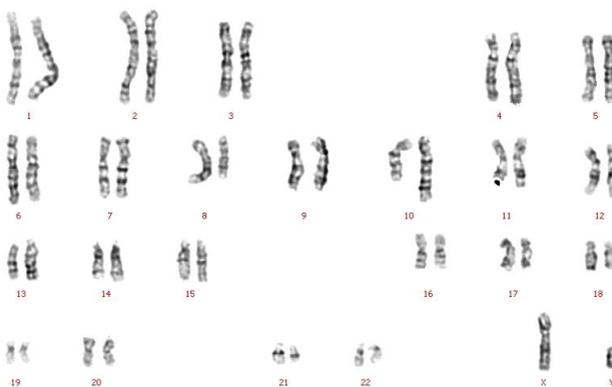


Figure 6.24. Karyotype: 46,XY,t(8;10)(q22;q26)

Another prenatal case evaluated through array CGH, presented a neural tube defect detected through ultrasonography. Array CGH revealed the presence of a microdeletion on the chromosome 13, in the region containing the GPC5 gene (Glypican 5). GPC5 expression was identified in fibrous cartilage. Cell culture studies showed the importance of GPC5 in Hedgehog (Hh) signaling, suggesting its involvement in the development process of the skeleton and limbs.

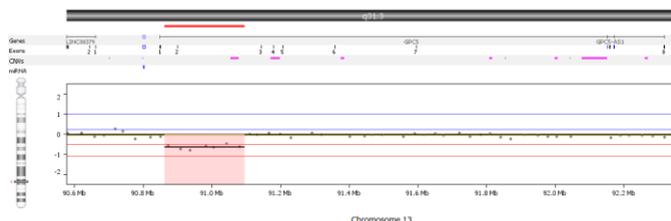


Figura 6.36. Rezultatul evaluării cromozomului 13 prin array CGH

Regarding other cases evaluated prenatally through array CGH, three of these had negative findings, and other twelve cases showed microdeletions or microduplications which, at this point, have no proven involvement in embryonic development.

In another case, classical cytogenetics identified the monosomy X, but the array CGH investigation did not reveal any changes. A possible explanation could be the contamination of the sample with maternal DNA, in which case the source of the sample cannot be accurately known.

The last analyzed case was that of a couple with no children, but with three consecutive pregnancies with autosomal trisomies (trisomy 21, trisomy 9 and trisomy 18). The pregnancies were assessed through conventional karyotyping.

Chapter 7. Final conclusions

1. In this thesis, the array CGH method identified genetic modifications in a significant number of investigated cases.

2. In the case with a interstitial deletion on the short arm of chromosome 10 identified through conventional karyotype, array CGH analyze established the size of the deleted fragment and its genetic content.

3. Our results support existing literature data that array CGH represents a useful tool in pre- and postnatal genetic diagnosis.

4. Conventional karyotyping continues to be an extremely valuable tool in the diagnosis of pre- and postnatal aneuploidies and balanced and unbalanced structural changes.

5. Array CGH represents an important option in prenatal cases with ultrasound anomalies and either a normal karyotype or when the karyotype could not be performed.

6. Array CGH cannot detect all genetic disorders, due to the impossibility to detect balanced genetic abnormalities and the resolution of microarray slide used for performing the testing.

7. Array CGH is a powerful tool in pre- and postnatal diagnosis, being able to identify microduplications and microdeletions in patients with complex and polymorphic clinical traits.

8. Additional analysis using array CGH enables the assessment of distal and proximal breakpoints, of genomic size and genetic content of the microdeletions or the microduplications.

9. Array CGH may not identify any changes in the genetic material in some cases with anomalies detected through ultrasonography, but this does not exclude the genetic background of those changes.

10. Pre- and postnatal large-scale testing through array CGH has improved the capacity to detect genomic imbalances responsible for different clinical features, thus providing a complete and more accurate diagnosis and proper management of both the individual and their family.

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