

## Clinical Application of Chromosomal Microarray Analysis in Fetuses with Congenital Heart Defects: A Prospective Observational Study

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DOI: [10.36347/sjams.2019.v07i08.032](https://doi.org/10.36347/sjams.2019.v07i08.032)

| Received: 19.08.2019 | Accepted: 26.08.2019 | Published: 29.08.2019

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### Abstract

### Original Research Article

**Background:** Congenital heart disease (CHD) is one of the most common birth defects. Genetic factors have been implicated in its etiology. The aim of the study was to determine the relationship between chromosomal abnormalities and CHD and also to assess the incremental yield of genomic microarray over conventional karyotyping in these fetuses. **Methods:** Prospective observational study was performed in our hospital including fetuses with a nuchal translucency (NT)  $\geq$  99<sup>th</sup> percentile or structural malformations diagnosed by ultrasound between 2013 and 2017. We analyzed the incidence of CHD in both groups and the association of CHD with chromosomal abnormalities, as well as the increase in diagnostic performance of the chromosomal microarray analysis (CMA) compared with conventional karyotype. We performed a descriptive analysis of the mean, the interval and the standard deviation for continuous variables and an analysis of absolute frequency and percentages for the categorical variables. **Results:** Among 225 pregnant women were included, 102 of them had fetuses with NT  $\geq$  99<sup>th</sup> percentile and 123 pregnant had fetuses who exhibited structural malformations. Of the 102 fetuses with increased NT, 36.3% had an abnormal karyotype. Array was performed in 64 of the 102 cases (62.7%). The incidence of CHD in this group was 8.8%. Incremental yield of CMA over karyotyping was 6.5%, but there was no incremental yield in cases of CHD and NT increased. In the second group, fetuses with structural malformation, 14.2% had an abnormal karyotype and the array was performed in 30 cases. The incidence of CHD was 28.4%. Incremental yield of CMA over karyotyping was 14.2% in those fetuses with CHD. **Conclusions:** The use of chromosomal microarray analysis provides good diagnostic performance in the fetal group with structural malformations, higher than in the group with increased NT.

**Keywords:** Chromosomal microarray analysis, array comparative genomic hybridization, copy number variation, congenital heart defect, Chromosome 22q11.2 deletion syndrome.

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## INTRODUCTION

Congenital heart disease (CHD) affects up to 1% of newborns and half of the cases correspond to severe defects. The incidence of moderate and severe forms of CHD is approximately 6 / 1000 live births and increases up to 75 / 1,000 live births in the case of minor defects, especially ventricular septal defect and other trivial lesions [1]. Congenital heart defects (CHDs) can be isolated or associated with other malformations. More recently, the blood flow of ductus venosus (DV), tricuspid regurgitation (RT) and nuchal translucency have been introduced into the first trimester ultrasound as parameters that may have a marker role in aneuploidies and heart defects [2-4].

Congenital heart disease comprises a wide variety of defects with a variety in complexity and

prognosis. In the prenatal setting, the incidence of chromosomal anomalies is reported to be as high as 18–22% of all CHD cases, most being trisomies 21 and 18 and 22q11 microdeletion. The association between CHD and chromosomal abnormalities have been demonstrated [5-8]. Consequently, the first line of management of these pregnancies should be to offer the study of the fetus karyotype. However, the relationship between chromosomal abnormalities, CHD and the incremental diagnostic performance of genomic microarrays compare to the conventional karyotype in these fetuses has yet to be precisely determined.

The aim of this study was to investigate the utility of array-CGH for the prenatal diagnosis of CHD and the improvement of prenatal genetic counseling and to compare this approach to traditional chromosome

analysis. We will analyze separately the fetuses that presented a NT  $\geq$  99<sup>th</sup> percentile and the fetuses that were diagnosed with a structural malformation without association to an increased NT.

## METHODS

### Case Selection

This was a prospective observational study. Among 225 enrolled pregnant women, 102 of them had fetuses with TN > 99<sup>th</sup> percentile and 123 exhibited structural malformations.

A total of 46 fetuses with congenital heart disease were tested by conventional karyotyping / QF-PCR and microarray.

We analyzed: the fetal karyotype (conventional karyotype and chromosomal microarrays), the echocardiography, associated malformations, how did the pregnancy end and the obstetric history.

All the ultrasound examinations were carried out by the authors using an Acuson Antares machine with a 2–3MHz convex transducer. Ultrasonography explorations were performed according to a standardized protocol by specially trained ultrasonographers.

The cases included were those fetuses diagnosed of congenital heart disease, to whom we performed an invasive prenatal diagnosis and an early echocardiography between 14 and 15 weeks of pregnancy. Clinical examination of the neonates was performed by a pediatrician.

The informed consent was signed by all the patients. The exclusion criteria were the loss of gestational follow-up or the refusal to perform the invasive technique.

### Chromosomal Microarray Analysis

Array comparative genomic hybridization compares the genomic content (DNA) of a patient (case) with a normal control and detects not only aneuploidy and major structural changes, but also submicroscopic gains or losses and unbalanced reordering. Previously, we needed to know if the samples came from a male or female fetus, for which the sample was subjected to a QF-PCR (quantitative fluorescence polymerase chain reaction) to diagnose chromosomal abnormalities specific to the chromosomes 13, 18, 21, X and Y. It is a process that can be automated and allows lower costs and a faster diagnosis.

Chorionic villus sampling was performed in these cases at 11–14 gestational week and amniocentesis was performed in gestations of more than 15 weeks, both with informed consent. The analysis was carried out using an oligonucleotides microarray

that compares genomic hybridization of approximately 60,000 probes distributed throughout the genome (qChip Pre v1.1 Complete, qgenomics). The DNA of the patient and internal reference DNA of the same sex with different fluorophores, Cy5 and Cy3 respectively, was marked. Subsequently the samples were hybridized on the array and scanned. The data obtained was analyzed using the Genomic Workbench 7.0 software. The average resolution of the array is 60kb, with higher resolution for areas of microdeletion-microduplication syndromes, telomeric and centromeric regions. The minimum number of consecutive oligonucleotides was established in five to detect an anomaly.

The variants identified were compared to those recorded in the Database of Genomic Variants. These variants were classified as pathogenic, VOUS (Variants of unknown significance, variants of uncertain clinical significance) or benign, following the recommendations of the American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants [9-12]. This report was positively rated by The European Molecular Genetics Quality Network.

### Statistical Analysis

The data was analyzed with SPSS 17.0. A descriptive analysis of the mean, the interval and the standard deviation for continuous variables, and of absolute frequency and percentages for the categorical variables was performed.

Incremental yield was calculated as the proportion of the abnormal results nondetectable by karyotyping divided by the total number of cases with an eventual normal karyotype. This study was approved by the Institutional Ethics Committee of our hospital.

## METHODS

A total of 102 fetuses with a CRL between 45 and 84 mm and an increased NT ( $\geq$  99<sup>th</sup> percentile) were tested by conventional karyotyping or QF-PCR and 123 fetuses diagnosed with a structural malformation without association with NT increased were also tested by conventional karyotyping or QF-PCR. When the results of the cytogenetic analysis were normal, we performed an array-comparative genomic hybridization (aCGH) analysis. Early echocardiography to 14-15 weeks of pregnancy was also realized.

Analyzing both groups separately, we could establish on the one hand the impact of NT on the diagnosis of fetal congenital heart disease and on the other hand the importance of fetal congenital cardiopathy isolated in the diagnosis of chromosomal abnormalities. Along with the contribution of the chromosomal array in both clinical situations. Clinical examination of the neonates was performed by a pediatrician.

## RESULTS

A total of 102 fetuses presented a nuchal translucency  $\geq 99^{\text{th}}$  percentile, an incidence on the first trimester ultrasound in our studied population of 1%. The mean maternal age was 33.4 years (range 21-43 years), 49 % were primiparous and 51% multiparous. We also evaluated other variables (Graphic-1). When we analyzed the obstetric history of the population studied, we observed that 9.8% of them had a prior history of a child with a neurodevelopment disorder or a previous pregnancy with chromosomopathy.

There was a directly proportional relationship between the increase of NT and chromosomal abnormalities (Graphic-2). In our series, the incidence of congenital heart defects was 6% for NT in the 3-3.9 mm range and 20% for NT  $> 5$ mm. Fetal molecular karyotype or QF-PCR were performed in all of the fetuses diagnosed with increase nuchal translucency ( $\geq 99^{\text{th}}$  percentile) and 36.3% of them had an abnormal karyotype: 20 cases of trisomy 21 (54%), 12 cases of trisomy 18 (33%), 1 case of trisomy 13 (3%) and 4 cases of Turner syndrome (10%). All of them requested the legal interruption of the pregnancy (Graphic-2). The array was performed in 65 cases but in one case we did not obtain a valid result due to maternal contamination (64/102, 62,7%). We performed QF-PCR and array-CGH that resulted in 10.9% of abnormal results. Chromosomal microarrays analysis (CMA) was also conducted in all of the cases and allowed us to detect 7 cases of CNV (7/64, 10.9%): 1 not pathogenic (2p22.22p22.1(27,254,626-39,208,926) x3), 2 variants of uncertain clinical significance (VOUS) and 4 pathogenic. One of these CNV was diagnosed in a fetus affected by a CHD (Graphic-3).

Incremental yield of CMA over karyotyping was 6.5%, but there was no incremental yield in cases of CHD and NT increased.

The detailed morphological study among the 18-22 weeks of pregnancy also diagnosed fetal malformations associated with an increased nuchal translucency in 28.1% of the cases, once the chromosomal numerical abnormality was discarded. The fetal malformation most frequently diagnosed was fetal congenital heart disease, which appeared in 9 cases (50%). The association between fetal congenital heart disease and a pathological outcome of array/conventional karyotype was 33%. It is important to point out the relationship between increased nuchal translucency, Noonan syndrome and cardiopathy. The two cases of right-heart hypertrophy were also diagnosed of Noonan's Syndrome using next generation sequencing.

A total of 47 pregnant women (46%) requested the interruption of pregnancy as a result of the findings in the study of the conventional karyotype, QF-PCR, arrays or fetal ultrasound.

We had a neonatal mortality rate of 1.8%. There was a fetus affected with a Noonan Syndrome who died postnatally because of the hypertrophic myocardiopathy that he presented.

123 fetuses were diagnosed prenatally of a fetal structural malformation without association with NT increased, 35 were diagnosed with CHD, which means that 28.4% of the diagnosed fetal malformations were cardiovascular abnormalities. The incidence of congenital malformations in our population was 1.5% and 0.5% for congenital fetal cardiopathy. The mean maternal age was 31.9 years (range 21-43 years), 44% were primiparous and 56% multiparous. When we analyzed the obstetric history of the population studied, we observed that 7.1% of them had a prior history of a child with a neurodevelopment disorder or a previous pregnancy with chromosomopathy.

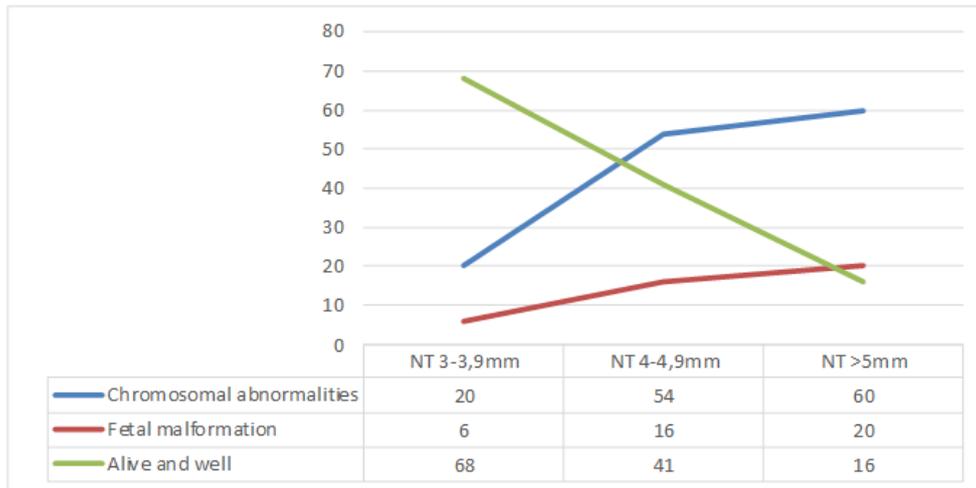
CHD was associated with other fetal structural malformations in 6 cases (6/35, 17.1%). The most frequently diagnosed congenital cardiopathies were: conotruncal anomalies in 20% of cases and ventricular septal defect (VSD) in 17% of cases. VSD (2/3 cases, 66.6%), specifically perimembranous (3/4, 75%) presented a high association with QF-PCR and / or array anomalies (Graphic 4). Of the total 12 VSDs diagnosed during the study period, 6 were isolated VSDs (3 muscular and 3 perimembranous) and the other 6 were perimembranous VSDs associated with major congenital heart disease (4 cases of TGA and 2 cases of Fallot's Tetralogy). 3 cases (3/6, 50%) of isolated VSDs had abnormalities in the karyotype: a trisomy 18, a triploidy (both diagnosed by QF-PCR) and a case of CNV of uncertain significance (VOUS) diagnosed by array. In all three cases the VSD was perimembranous. The perimembranous VSD has worse prognosis than the muscular VSD and with greater association with cardiac and chromosomal anomalies.

In 8 cases (8/35, 22.8%) the fetus affected with a congenital heart disease presented chromosomal anomalies associated with QF-PCR alterations or array-CGH detection: 2 cases of trisomy 21, 2 cases of trisomy 18, 1 triploid fetus, 3 cases of CNV with uncertain significance (VOUS) in 2 fetuses (one fetus presented 2 VOUS) and 2 cases of pathogenic CNV (Graphic-5). The interruption of pregnancy was requested in 11 cases.

Incremental yield of CMA over karyotyping was 14.2 % in those fetuses with congenital heart disease.

**Graphic-1: Pregnancy outcomes**

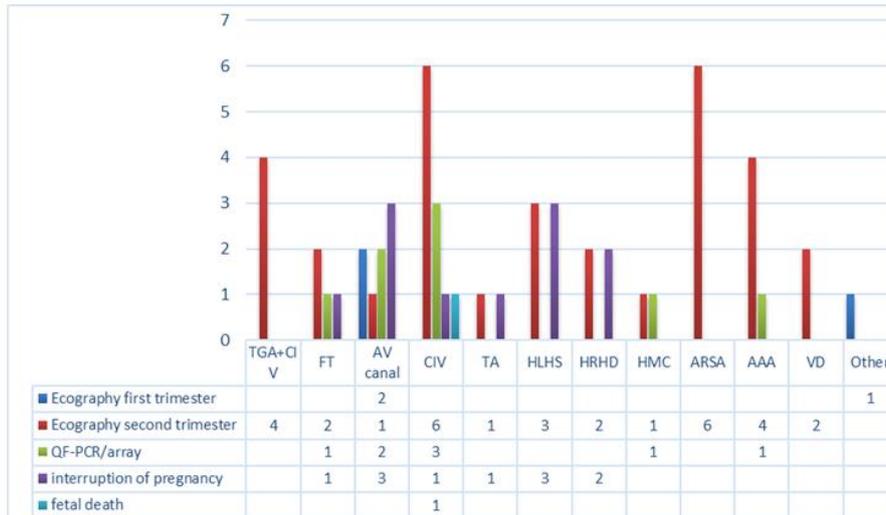
	Fetuses with increase nuchal translucency ( $\geq$ p 99th)
Termination of pregnancy (%)	46 (47/102)
Born alive (%)	50.9(52/102)
Intrauterine demise (%)	1.8 (1/53)
Preterm birth (%)	5.6 (3/53)
Labor induction (%)	32 (17/53)
Cesarean section (%)	33.9 (18/53)
Birth weight (g)	3228
Neonatal unit admission (%)	15 (8/53)



**Graphic-2: Relationship between nuchal translucency thickness, chromosomal defects, miscarriage or fetal death and major fetal abnormalities**

**Graphic-3: Detected CNV in fetus affected NT $\geq$  99th**

	Size	CMA	Detectable by conventional karyotype	Sonographic anomalies	Outcomes Neurodevelopment
Deletion 11(11q24.1q25(121,430,998-134,450,377) x1 Duplication 4q34.11q35.2(176,077,869-191,153,672) x3	13Mb 15Mb	Pathogenic CNV	Yes	NT $\geq$ 99 <sup>th</sup> only	Legal interruption of pregnancy
Arr(2)x2 3 trisomy mosaicism chromosome 2		Pathogenic CNV	Yes	Congenital heart disease	Legal interruption of pregnancy
Deletion 16p11.2(16p11.2(29,256,185-30,098,069) x1	534kb	Pathogenic CNV	No	NT $\geq$ 99 <sup>th</sup> only	Legal Interruption of pregnancy
Duplication 16q24.1(86,533,273-86,743,471) x3	206kb	VOUS	No	NT $\geq$ 99 <sup>th</sup> only	Affected
Duplication 7p21.2(15614415_16222888) x1	608kb	VOUS	No	NT $\geq$ 99 <sup>th</sup> only	Normal
Duplication 2p22.2p22.1(27,254,626-39,208,926) x3	1.6Mb	Benign CNV	No	NT $\geq$ 99 <sup>th</sup> only	Normal
Deletion 8p23.3p22(1573676_16762986) x1	15Mb	Pathogenic CNV	Yes	Diaphragmatic hernia	Legal interruption of pregnancy



**Graphic-4: Congenital cardiopathies in prenatal life, Correlation with anomalies of conventional karyotype and / or prenatal array, Correlation with ILE**

**Graphic-5: Chromosomal abnormalities in fetuses with CHD**

	Size	Detectable by conventional karyotype	CMA	Congenital Heart Disease	Follow-up
<b>Deletion 22q11.21(17,274,835-19,835, 417) x1</b>	2.6Mb	No	CNV Pathogenic	- Fallot Tetralogy - Double outlet right ventricle - VSD	Legal interruption of the pregnancy
<b>Deletion 17q21.32q21.33(46,361,999-48,837,400) x1</b>	2.4Mb	No	CNV Pathogenic	MCH	Baby affected
<b>Duplication 5q23.1q23.2(120,130,212-121,800,740) x3</b> <b>Duplication 9p24.3(607,082-878,209) x3</b>	1.67Mb 271kb	No	VOUS VOUS	Right aortic arch	Healthy baby
<b>Duplication 7p15.3(21,714,059-21,994, 454) x4</b>	280kb	No	VOUS	VSD perimembranous	Dysmorphia

## DISCUSSION

Congenital heart disease comprises a wide variety of defects with diversity in complexity and prognosis, where major CHD typically requires intervention within the first year of life. There is heterogeneity in the conditions associated with the increase of NT, suggesting that there cannot be a unique underlying mechanism for this condition. The mechanisms include cardiac dysfunction associated with anomalies of the heart and major arteries, alteration of the composition of the extracellular matrix, failure of the lymphatic drainage caused by anomalies or delayed development of the lymphatic system, fetal anemia or hypoproteinemia and congenital infection.

Previous reports investigating the potential value of microarray in fetuses with increased NT and normal karyotype showed that microarray study provides clinically valuable additional information about the conventional karyotype by 5.0% (95% CI, 2.0 to 9,1%) of these fetuses, once the most common aneuploidies have been discarded [13, 14]. In this

prospective clinical series of pregnancies with increased NT  $\geq 99^{\text{th}}$  percentile, CMA provided valuable additional information about the conventional karyotype in 6.5% of cases, but there was no incremental yield in cases of CHD and NT increased. Differences in the incremental yield could be explained by the different type (targeted or whole genome oriented) and resolution of the microarrays performed or the small cohort number.

In our series, the rate of congenital heart defects was 6% for NT in the 3-3.9mm range and 20% for NT > 5mm. Ghi [15] described an incidence of major cardiac defects in fetuses with a thickness of nuchal translucency in the 2.5-3.4mm range of 2.5% and in those with a thickness of nuchal translucency  $\geq 3.5$  mm from 7 %. Other authors also reported that this association can be up to 31% of detection of cardiac defects for NT in the 99th percentile or higher. Galindo [16] reported 24% congenital cardiopathies (CC) when the thickness of NT was  $\geq 6$  mm as we have been able to prove, the higher the NT, the higher the risk of aneuploidy and congenital heart disease.

The prevalence of chromosomal abnormalities associated with fetal cardiac defects is also high and can be up to 28-40%. The most described were trisomy 21 (43.6%), 18 (19.1%) and 13 (9.6%), X monosomy (7.4%) and 22q11.2 deletion (7.4%) [17]. However, in those cases, where there are malformations or chromosomal abnormalities associated, these are the ones that mark the prognosis. The fetuses with CC present a risk of additional genetic abnormalities, including microdeletion syndromes, microduplication such as 22q11.2 deletion syndrome or DiGeorge syndrome or monogenic anomalies such as Noonan syndrome [18, 19]. Jansen [20] in his meta-analysis found that there was an incremental diagnostic performance of 7.0% for the detection of CNVs using a-CGH, excluding cases of microdeletion 22q11 and aneuploidy. In general, the array applied to the diagnosis of CC has a diagnostic performance of between 6.6-12% [21]. In our serie, incremental yield of CMA over karyotyping was 14.2 % in those fetuses with congenital heart disease greater than that presented by these authors.

Prenatal diagnosis eliminates the diagnostic delay, permits delivery at a tertiary centre specialized in the management of major CHD, thus enabling early invasive treatment.

## CONCLUSION

There is a strong association between congenital heart defect and increased nuchal translucency in the first trimester of pregnancy and chromosomal anomalies.

When invasive test shows normal chromosome genotype, it is necessary to perform array-CGH to eliminate the possibility of submicroscopic chromosomal abnormalities, especially when the fetus has other structural defects. In all cases, the follow-up of prenatal studies, including molecular fetal karyotype, ultrasound study of fetal morphology, fetal echocardiography, as well as genetic testing and screening of infections was necessary.

## Abbreviations

NT: nuchal translucency, CMA: chromosomal microarray analysis, CNV: copy number variations, VOUS: Variants of unknown significance, TGA: Transposition of the Great Arteries, VSD: ventricular septal defect, AV canal: Atrioventricular canal, TA: truncus arteriosus, HLHS: hypoplastic left heart syndrome, HRHS: hypoplastic right heart syndrome, HMC: hypertrophic cardiomyopathy, ARSA: aberrant right subclavian artery, AAA: aortic arch congenital anomaly, VD: Congenital Valvular Heart Disease.

## DECLARATIONS

### Acknowledgements

The authors thank all women who enrolled in the study. They thank the principal investigators, collaborators and the staff members of the hospital for their contributions to the study.

## Funding

This study was approved by the Institutional Ethics Committee of our center and supported by the Parc Taulí Research and Innovation Institute. The funders had no role in design of the study, data collection, analysis, interpretation of data or writing of the manuscript.

## Availability of data and materials

The dataset supporting the conclusions of this article are available from the corresponding author on reasonable request.

## Authors' Contributions

SP designed the study. JJ, LS, MC and NB acquired the data. JL analyzed the data. SP contributed to the conduct of the study. All reviewed and revised the manuscript and approved the final manuscript as submitted.

## Ethics approval and consent to participate

Written informed consent was obtained from all women prior to enrolment in the study. This study was conducted under the approval of the following institutional review boards or ethics committees: Ethical Committee on Clinical Research, University Hospital Parc Taulí, Sabadell, Barcelona, Spain.

## Consent for publication

Not applicable. The present manuscript does not contain any individual person's data in any form.

**Competing Interests:** All other authors have no conflicts of interest to declare.

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