Contribution of Chromosomal Microarray Analysis in Fetuses with Increased Nuchal Translucency: A Prospective Observational Study

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Received date: August 14, 2018; Accepted date: August 21, 2018; Published date: August 25, 2018

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Abstract

Background: Multiple factors have been associated with an increased risk of fetuses with increased nuchal translucency. The aim of this study was to determine the relationship between chromosomal abnormalities and increased nuchal translucency and also to assess the incremental yield of genomic microarray analysis compared to conventional karyotyping in these fetuses.

Methods: Prospective observational study of fetuses with increased nuchal translucency (≥ 99th percentile) between 11 and 14 weeks of gestation diagnosed between 2013 and 2017 in our hospital. 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 102 enrolled pregnant women, the incidence of increased nuchal translucency was 1%. We diagnosed 9.3% of pathological results by arrays, but 3 cases (4.6%) were diagnosed by conventional karyotype too. Incremental yield of chromosomal microarray analysis over karyotyping was 6.5%.

Conclusion: The use of chromosomal microarray analysis provided a 6.5% incremental yield of detecting copy number variations in fetuses with increased translucency and normal karyotype. Prenatal array should be part of the usual study in these cases, especially if there is ultrasound malformations associated.

Keywords: Chromosomal microarray analysis; Array comparative genomic hybridization; copy number variation; Increased translucency; Cystic hygroma; First trimester screening; Nuchal septations

Abbreviations: NT: Nuchal Translucency; CMA: Chromosomal Microarray analysis; CNV: Copy Number Variations; VOUS: Variants of Unknown Significance

Introduction

Nuchal translucency (NT) is the ultrasonographic pattern of the accumulation of subcutaneous fluid behind the fetal neck [1]. The increase in fetal nuchal translucency between 11 and 14 weeks of gestation is a common phenotypic expression of chromosomal abnormalities, including trisomy 21 [2,3]. The risk of chromosomal anomalies in these cases is high, approximately 20% for a NT of 4.0 mm, 33% for a NT of 5.0 mm, 50% for a NT of 6.0 mm and 65% for a NT of 6.5 mm or more [4]. Consequently, the first line of management of these pregnancies should be to offer the study of the fetal karyotype.

The measurement technique of NT needs adequate training [5]. The size of the NT thickness through the ultrasound examination between 11 and 14 weeks of gestation has been associated with maternal age and is an effective detection tool for trisomy 21, detecting up to 75-80% of pregnancies. If it is associated with some biochemical markers, such as chorionic gonadotropin (beta-hCG), plasma protein A (PAPP-A), alpha-fetoprotein and/or placental growth factor, it is possible to identify more than 95% of gestations with fetuses affected by trisomy 21 with a false positive rate of 1.2% [6].

More recently, the blood flow of ductus venosus (DV) and tricuspid regurgitation (RT) have been introduced to the first trimester ultrasound as parameters that may have a marker role in aneuploidies and heart defects [7-10]. The detection rates for ultrasonographic markers of trisomy 21 in the first trimester are substantially higher if these three ultrasound markers (NT, DV and RT). Their detection rates are approximately 80%, 87% and 94%, depending on whether one, two or three markers are used, for a false positive rate of 3%. The combination of NT with reverse DV or absence A is a strong indicator of aneuploidy [11].

During pregnancy, we have to be careful with the indications for chromosomal invasive prenatal analysis because of the risk of involuntary abortion associated with fetal sampling. Detection tests, which take into account maternal age, maternal serum biochemical parameters and fetal ultrasonographic markers, are those used nowadays to provide an evaluation of the risk of trisomies, especially T21 and T18. Currently, different screening strategies and diagnostic methods are applied in different countries. The role of fetal DNA in maternal blood within these screening programs is still being discussed by scientific societies [12-14]. However, recommendations by various international medical organizations and societies have been agreed to maximize the detection of fetal defects in the first trimester of gestation by using all the necessary tools [15-18].
The association between increased nuchal translucency, congenital malformations, chromosomal abnormalities and genetic syndromes have been demonstrated [9,19]. However, the relationship between chromosomal abnormalities and the increase in nuchal translucency to evaluate the incremental diagnostic performance of genomic microarrays on the conventional karyotype in these fetuses has yet to be precisely determined. In the present study, we propose a prospective series to evaluate the impact of genomic microarrays on fetuses with increased NT once the trisomy has been ruled out.

Methods

Case selection

This was a prospective observational study that included all the fetuses with increase nuchal translucency (≥ 99th percentile) between 11 and 14 weeks of gestation that were diagnosed in our hospital between March 2013 and May 2017.

We analyzed fetal karyotype (conventional karyotype and chromosomal microarrays), 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 2-3 MHz convex transducer. Ultrasonography to assess nuchal translucency was performed according to a standardized protocol by specially trained ultrasonographers. A minimum of 20 minutes was reserved for the assessment, and transvaginal ultrasonography was used if necessary. The patient could return for a second evaluation if the initial attempt failed.

The cases included were those fetuses diagnosed of an increased fetal nuchal translucency which was defined as a percentile higher than 99th percentile. The indication for invasive prenatal diagnosis was having a fetus with increased nuchal translucency. 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 weeks 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, genomics). The DNA of the patient and internal reference DNA of the same sex with 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 [20-23]. 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 (≥ 99th percentile) were tested by conventional karyotyping or QF-PCR.

When the results of the cytogenetic analysis were normal, the pregnancies were then consulted for array-comparative genomic hybridization (aCGH) analysis and received subsequent morphology scan between 20 and 24 weeks gestation. Early echocardiography to 14-15 weeks of pregnancy was realized too. Submicroscopic chromosomal abnormalities were assessed and compared between the fetuses with and without structural defects. Clinical examination of the neonates was performed by a pediatrician.

Results

A total of 102 cases were detected. The incidence of NT ≥ the 99th percentile on the first trimester ultrasound in our studied population was 1%. The mean maternal age was 33.4 years (range 21-43 years), 49% were primiparous and 51% multiparous and the mean gestational age at diagnosis was 11.8 weeks. Other variables have also been evaluated (Figure 1).

<table>
<thead>
<tr>
<th>Fetus with increased nuchal transluency (%)</th>
<th>46 (47/102)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Born alive (%)</td>
<td>50.9 (52/102)</td>
</tr>
<tr>
<td>Intrauterine demise (%)</td>
<td>1.8 (1/53)</td>
</tr>
<tr>
<td>Preterm birth (%)</td>
<td>5.6 (3/53)</td>
</tr>
<tr>
<td>Labor induction (%)</td>
<td>32 (17/53)</td>
</tr>
<tr>
<td>Cesarean section (%)</td>
<td>33.9 (18/53)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3228</td>
</tr>
<tr>
<td>Neonatal unit admission (%)</td>
<td>15 (8/53)</td>
</tr>
</tbody>
</table>

Figure 1: Pregnancy outcomes.
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. Only one case presented a recurrence fetus with increased NT > 99th and normal array and currently the result of the exome sequencing studied is pending.

We had two cases of twins with only one of the fetuses affected. Both were bichorial twins. However, we should not forget that in monzygotic twins, the discrepancy of NT represents an early sign of feto-fetal transfusion syndrome.

There was a directly proportional relationship between the increase in NT and chromosomal abnormalities (Figure 2).

Fetal molecular karyotype or QF-PCR were performed in all of the fetuses diagnosed with increase nuchal translucency (≥ 99th percentile) and 36.3% (37/102) had an abnormal karyotype: 20 cases of T21 (54%), 12 cases of T18 (33%), 1 case of T13 (3%) and 4 cases of Turner syndrome (10%). All of them requested the legal interruption of the pregnancy (Figure 3).

Of the remaining cases, in one case the array study could not be performed and we only did a conventional karyotype with a normal result, as it was a post-amniocentesis abortion and there were problems with the sample. The array was performed in 64 cases. On the 62.7% (64/102) of the cases with normal QF-PCR we performed array-CGH that result in 10.9% of abnormal results. Microarrays were conducted in all of the cases too and also allowed us to detect 7 cases of CNV (7/64, 10.9%): 1 not pathogenic CVN (2p22.2p22.2(27,254,626-39,208,926) x3), 2 variants of uncertain clinical significance (VOUS) and 4 pathogenic CNV. The cases of VOUS diagnosed were: a duplication of 210 kb in the chromosomal band 16 (16q24.1(86,533,273-86,743,471) x3) and a duplication of 608 kb in the chromosomal band 7 (7p21.2(15614415_16228888) x1) which did not match with polymorphic CNVs and could alter the structure or the reference gens. These alterations can be classified as VOUS since there is not enough literature that classifies them as benign or pathogenic. The pathogenic CNV detected were: a deletion of 13 Mb in the chromosomal band 11 (11q24.1q25(121,430,998-134,450,377) x1) and duplication of 15 Mb in chromosomal band 4 (4q34.11q35.2(176,077,869-191,153,672) x3) to the same fetus, trisomy of chromosome 2 in low degree mosaicism (14%), deletion of 53 kb in the chromosome band 16p11.2 (16p11.2(29,256,185-30,098,069)x1), and deletion of 16Mb in the chromosome band 8 (8p23.3p22(1573676_16762986) x1), three of which were detected by conventional karyotype too, and altered the dose and alter the structure or the reference gens. In all cases, with pathogenic CNV pregnant women requested the legal interruption of the pregnancy. Three cases (42%) were also diagnosed by conventional karyotype (Figure 4).

Incremental yield of CMA over karyotyping was 6.5%

We have diagnosed two cases of mosaicism by conventional karyotype and only one of them has also been diagnosed by array. We were able to detect a case of mosaicism of 14%, however, we missed another case of less frequent mosaicism, of even 5%. As it is already known, the HCG array has its handicap in the low frequency mosaicism.

The detailed morphological study among the 18-22 weeks of pregnancy also diagnosed fetal malformation associated with the increase in nuchal translucency, once the chromosomal numerical abnormality was discarded, in 28.1% of cases. The fetal malformation most frequently diagnosed was fetal congenital heart disease, which appeared in 50% of cases (Figure 5).

The association between fetal congenital heart disease and a pathological outcome of array/conventional karyotype was 33%. In other major fetal malformations, the ratio was 1/1, as it was the case with abdominal wall defects (Figure 6).
In two fetuses (3.1%) we diagnosed malformations in more than two organs or systems: one was a tetralogy of Fallot associated to a malposition of limbs and the other a dominance of right cavities associated to a ventriculomegaly.

In 5 cases (5/64, 7.8%) we detected a persistence of an increased nuchal translucency during the second trimester of pregnancy. In this subgroup a greater index of neurodevelopment problems and facial dysmorphism was observed (2/5 cases, 40%).

Early fetal echocardiography was performed in all cases that did not request legal interruption of pregnancy, showing pathology in 14% of them (9/64). 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.

Fetal MRI was performed in 3.1% of the cases (2/64), all of them affected with sonographic abnormalities, which were confirmed in all the cases.

The study of prenatal infections was negative in all cases, including those cases with mothers who were immune to Cytomegalovirus (CMV). In these cases, the amniotic fluid was studied by PCR-CMV without any pathological results in our series.

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, pathological array or fetal ultrasound anomalies. Five of these pregnant women (7.8%) requested the legal interruption of pregnancy due to fetal structural anomalies with normal array: 2 congenital cardiopathies, 2 hygromas and 1 case of diaphragmatic hernia. There was also a case of a persistent anhydramnios secondary to premature rupture of membranes after performing an amniocentesis that also requested the interruption of pregnancy. Second trimester abortions occurred in 2 cases (3%) attributed to the invasive technique, one of them was an amniocentesis and the other after a chorionic biopsy.

The anatomopathological study of the cases in which legal interruption of pregnancy was requested provided additional information in two cases (4.2%): neural tube defect that was associated with an omphalocele and Trisomy 18 and a second case of malposition of limbs associated with congenital heart disease.

Regarding the perinatal results, 50 pregnancies were carried out and three of them had preterm parts. We had a neonatal mortality rate of 1.8%, once the complementary studies were normal and the pregnant woman decided to continue pregnancy. There was a fetus affected with a Noonan Syndrome who died postnatally as a consequence of the hypertrophic myocardopathy that he presented.

The medium and long-term outcome of the remaining cases was also documented. Of the three pathological cases we had two Noonan syndromes and a case of Pierre-Robin syndrome with a cleft palate. All of them needed neuropediatric follow-up because we detected a development delay.

Discussion

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 [24]. The term “cystic hygroma” in the first period of pregnancy refers to the simultaneous visualization of septations and the increase of NT. However, there is no general consensus on how to precisely define the cystic hygroma, or whether the cystic hygroma is an independent NT factor. Malone [25] defined cystic hygroma as an increase in hypoechoic space on the back of the fetal neck, which extends along the back of the fetus, and where the septation is clearly visible. Kharrat [26] proposed that the term “cystic hygroma” be used to describe the bilateral jugular lymphatic sacs of the fetal neck, an independent entity from NT and its association with increased NT carries a poor prognosis, informing that the prevalence of cystic hygroma was 0.62%. The FASTER study [27] conducted in 15 centers in the United States was designed to determine the visualization of septations in those fetuses with NT increased to ultrasound of the third trimester of pregnancy and the authors concluded that the visualization of the septations in the fetuses with NT increased over the 95th percentile for the fetal crano-caudal length when examined in the suboccipital transverse plane depended on obtaining the correct transverse plane of the head and using high resolution echographs. However, these authors did not specify if all the fetuses were examined with the same configurations and no specific parameters were proposed to be determined in the echographic sequences in the fetuses with increased NT. In later publications, different authors reached similar conclusions,
the septum was observed in all fetuses with increased NT if the scanning occurred in a transverse plane and high resolution echographs were used [28]. At this point, there are discrepancies, in the FASTER study the fetuses initially reported as cystic hygroma were analyzed separately from those with augmented NT and obtained significantly worse results for cases of hygroma compared to fetuses that only had increased the NT. In contrast, a retrospective study of cohorts that included 944 fetuses with a cystic hygroma of the first trimester reported that the thickness of NT increased in the fetuses with cystic hygroma, as well as the possibilities of an abnormal karyotype or congenital anomaly [29]. We agree with this latest author, of course, the cystic hygroma obtained worse results. In our case, 8 cases (8%, 8/102) were reported as cystic hygroma, 62.5% of them with a chromosomal anomaly but undoubtedly the NT in these fetuses was higher. It was to be expected that the results were worse because we already know that the higher the NT, the worse the prognosis is going to be.

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 8.0%) of these fetuses, once the most common aneuploidies have been discarded. In this prospective clinical series of pregnancies with increased NT ≥ 99th percentile, CMA provided valuable additional information about the conventional karyotype by 6.5% of cases. 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.

The choice of CMA platform for prenatal diagnosis requires a balance between achieving a good detection rate while minimizing the detection of VOUS. In our study, we detected VOUS in 2 fetuses (2%), which had not been reported in Database of Genomic Variants (DGV, http://projects.tcg.ca/variation/), DECIPHER database (http://decipher.sanger.ac.uk/) or reported articles. Parental analysis is a useful strategy for deducing the rate of VOUS. We performed parental analysis in each case, one of them was considered to be inherited from their parents and there was no clinical magnificient. The other case was a false positive of the prenatal ultrasound that did not diagnose a cleft palate without an associated cleft lip, so the ultrasound diagnosis we already know is more difficult, which has required surgery to repair the defect. Currently, this child needs follow-up by neuropediatrics due to development delay. VOUS carries an uncertain prognosis and also the possibility of requalifying it as pathogenic CNV if the bibliography so reports it.

In our series, the rate of congenital heart defects was 6% for NT in the 3-3.9 mm range and 20% for NT> 5 mm. Ghi [30] described an incidence of major cardiac defects in fetuses with a thickness of nuchal translucency in the 2.5–3.4 mm range of 2.5% and in those with a thickness of nuchal translucency ≥ 3.5 mm from 7 %. Other authors [9] also reported this association found up to 31% of detection of cardiac defects for NT in the 99th percentile or higher. Galindo reported 24% CC when the thickness of NT was ≥ 6 mm [31]. As we have been able to prove, the higher the NT, the higher the risk of aneuploidy and congenital heart disease.

However, even in the absence of aneuploidy and CC, nuchal thickness was clinically relevant too because it is associated with an increase in adverse perinatal outcome caused by a variety of other fetal malformations, genetic syndromes and intrauterine fetal death [32-34]. More than 50 genetic conditions associated with increased NT have been described. In 3 cases (3/53, 5.6%) a significant neurological involvement was diagnosed. In our series, the prevalence of Noonan syndrome in chromosomally normal fetuses with enlarged nuchal translucency was a 4%, lower than reported by other authors. Prenatally, Noonan syndrome is the most frequently reported genetic syndrome in association with increased nuchal translucency. It is an autosomal dominant disorder and is caused in about 50% of the cases by a missense mutation in the PTPN11 gene on chromosome 12 [35-37].

The proportion of developmental delay in early childhood reported in fetuses with NT ≥ 99th percentile, normal karyotype and normal ultrasound findings during pregnancy had no increased risk of developmental delay at 12 to 72 months of age. These data coincide with those published by other authors [38].

Conclusion

There is a strong association between the increased nuchal translucency in the first trimester of pregnancy and chromosomal anomalies, congenital heart defects, fetal structural anomalies, genetic syndromes and other adverse pregnancy outcomes.

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 have been necessary. The diagnosis of a Noonan syndrome can be suspected prenatally, especially in chromosomally normal fetuses with a large nuchal translucency and hydrops fetalis or cardiac anomalies.

Declarations

Acknowledgement

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, JL and TP acquired the data. JC 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 Tauli, 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.

References


