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Is high fetal nuchal translucency associated with submicroscopic chromosomal abnormalities on array CGH?

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KEYWORDS: array CGH; nuchal translucency; pathogenic submicroscopic CNV; prenatal array; prenatal diagnosis

ABSTRACT

Objective *To examine the possible association between high fetal nuchal translucency thickness (NT) and pathogenic chromosomal copy number variants (CNVs) detected by array comparative genomic hybridization (CGH) in pregnancies with normal fetal karyotype.*

Methods *Array CGH was carried out in stored samples of chorionic villi from 215 singleton pregnancies resulting in live births in which chorionic villus sampling at 11–13 weeks' gestation for high fetal NT (≥ 3.5 mm) had demonstrated normal karyotype.*

Results *Median fetal NT was 4.0 (range, 3.5–9.5) mm. Array CGH detected additional CNVs in 1.4% (95% CI, 0.5–4.0) of the cases, but none of these was a known pathogenic CNV.*

Conclusion *High fetal NT in the absence of sonographically detectable defects may not be associated with pathogenic CNVs.* Copyright © 2014 ISUOG. Published by John Wiley & Sons Ltd.

INTRODUCTION

Fetal nuchal translucency thickness (NT) above the 99th percentile (≥ 3.5 mm) is associated with a high risk for chromosomal abnormalities, major fetal defects and a wide range of genetic syndromes[1–3]. Recent studies have investigated the possible association between high NT thickness with normal karyotype and submicroscopic chromosomal abnormalities detected by comparative genomic hybridization (CGH)[4] or array CGH[5,6], reporting pathogenic copy number variants (CNVs) in 0 of 100 fetuses with NT ≥ 3.5 mm and normal karyotype[4], in four of 48 with NT > 3.5 mm[5] and in one of 41 with NT > 3.5 mm[6].

The objective of this study was to investigate further the possible association between high fetal NT with normal karyotype and pathogenic CNVs.

SUBJECTS AND METHODS

The study population comprised 215 singleton pregnancies resulting in live births, in which chorionic villus sampling (CVS) performed at 11–13 weeks' gestation for high fetal NT and conventional cytogenetic analysis, with G-banding at a resolution of at least 400 bands, had demonstrated a normal karyotype. At the time of CVS, excess villi were collected in RNA*later* RNA stabilization reagent (Qiagen, Dusseldorf, Germany) and stored at −70°C for research purposes. The patients had given written informed consent for storage and subsequent analysis of villi samples, and the study was approved by the research ethics committee of King's College Hospital, London. Approval for the study was also obtained from the ethics committee of the Chinese University of Hong Kong.

Maternal demographic characteristics, ultrasound findings in the first and second trimesters and pregnancy outcome obtained from the hospital records were recorded in a database.

Array CGH analysis of the samples was carried out at the Prenatal Genetic Diagnosis Laboratory, The Chinese University of Hong Kong. DNA was extracted from the samples using established methods (DNeasy Blood & Tissue Kit, Qiagen, Dusseldorf, Germany). A customized 44K Fetal Chip v1.0 (Agilent Technologies, Inc., Santa Clara, CA, USA) was used for the array CGH studies[5] and the data were analyzed with the use of an Agilent Genomics Workbench 7.0, with data aligned to the Human Genome release 19 (hg19). A CGH + SNP array, 8 × 60K format (Fetal Chip v2.0, Agilent Technologies, Inc.) was used to confirm the CNVs reported by array CGH using Fetal Chip v1.0.

Categorization of CNVs as benign, pathogenic or variants of unknown significance (VOUS) was performed as previously described[5], with modifications based on the American College of Medical Genetics standards and guidelines for interpretation and reporting[7,8]. The CNV was benign if its full length was listed in any of three

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databases of apparently unaffected people: the Database of Genomic Variants [http://projects.tcag.ca/variation/downloads/variation.hg18.v10.nov.2010.txt]; the benign database of the International Standards for Cytogenomic Arrays Consortium [https://www.iscaconsortium.org/images/stories/isca/ISCA_Known_benign_regions_hg18.txt]; the DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources)[9]. The CNV was pathogenic when: (a) pathogenicity was confirmed according to published literature, (b) it contained a pathogenic phenotype-genotype-related region listed in the Online Mendelian Inheritance in Man (OMIM) database [http://www.ncbi.nlm.nih.gov/omim], the pathogenic database of the ISCA consortium or the DECIPHER, or (c) there was evidence suggesting that the dosage-sensitivity resulted in clinical phenotype. The CNV was a VOUS when: (a) it contained no gene at all, (b) there was no report of it in probands with a clinical phenotype, (c) dosage sensitivity was unlikely or (d) it did not meet the criteria for classification as pathogenic or benign.

Statistical analysis

Maternal and pregnancy characteristics are presented as median (interquartile range (IQR)) for continuous variables and number (%) for categorical variables. The frequency distribution of fetal NT thickness is presented in a histogram. The statistical software package SPSS 20.0 (IBM SPSS Statistics for Windows, Version 20.0, IBM Corp, Armonk, NY, USA) was used for all data analyses.

RESULTS

The maternal and pregnancy characteristics of the study population are presented in Table 1. The median maternal age was 32.2 (IQR, 28.0–36.0) years, the median gestational age was 12.9 (IQR, 12.3–13.3) weeks and median fetal NT thickness was 4.0 (IQR, 3.7–4.7) mm. The frequency distribution of the fetal NT thickness is illustrated in Figure 1.

In 199 (92.6%; 95% CI, 88.3–95.4) cases there were no fetal defects detected, either prenatally or postnatally. In 12 (5.6%; 95% CI, 3.2–9.5) cases, fetal defects were detected by ultrasound in the first and/or second trimester of pregnancy and these were confirmed postnatally; there were two cases of diaphragmatic hernia, two of exomphalos and one case each of facial cleft, cystic adenomatoid malformation of the lungs, pleural effusion, double outlet right ventricle, ventricular septal defect with coarctation of aorta, pulmonary stenosis, unilateral multicystic dysplastic kidney and bilateral talipes equinovarus (postnatally this was diagnosed as Noonan syndrome). There were four (1.9%; 95% CI, 0.7–4.7) additional cases with abnormalities detected postnatally, including one case each of unilateral hypoplastic kidney, Beckwith–Wiedeman syndrome, Goldenhar syndrome and Gorlin–Chaudhry–Moss syndrome (in this case the father was also affected).

Table 1 Maternal and pregnancy characteristics in the study population of 215 singleton pregnancies in which fetal nuchal translucency was high and karyotype was normal on chorionic villus sampling

Characteristic	*Value*
Crown–rump length (mm)	66.7 (60.1–74.1)
Gestational age (weeks)	12.9 (12.3–13.3)
NT (mm)	4.0 (3.7–4.7)
Age (years)	32.2 (28.0–36.0)
Weight (kg)	63.6 (58.0–73.0)
Height (cm)	165.0 (160.0–170.0)
Racial origin	
Caucasian	192 (89.3)
Afro-Caribbean	10 (4.7)
South Asian	3 (1.4)
East Asian	9 (4.2)
Mixed	1 (0.5)
Smoker	20 (9.3)
Method of conception	
Spontaneous	208 (96.7)
Ovulation drugs	3 (1.4)
In-vitro fertilization	4 (1.9)
Nulliparous	95 (44.2)

Data are given as median (interquartile range) or *n* (%).

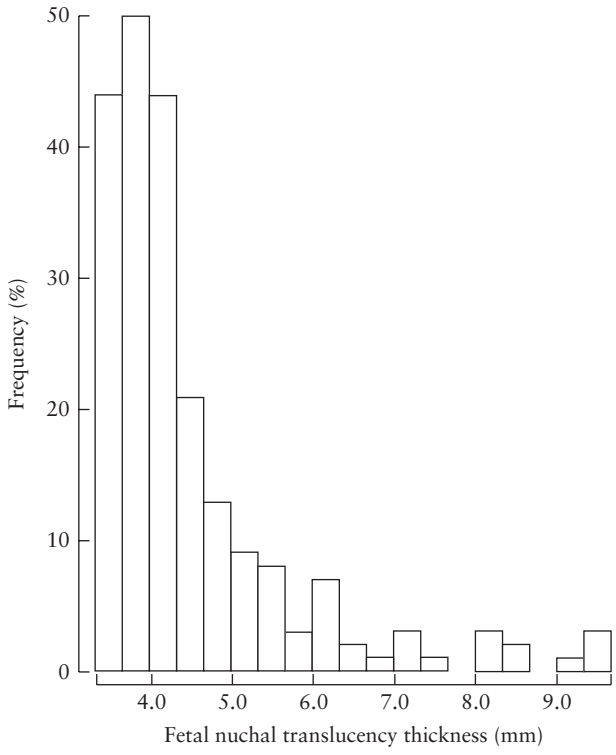

Figure 1 Frequency distribution of fetal nuchal translucency thickness in the study population of 215 singleton pregnancies

Array CGH detected additional CNVs in 1.4% (3/215; 95% CI, 0.5–4.0) of cases. All three CNVs were classified as VOUS and their size ranged from 1.99 kb to 1.32 Mb (Table 2). No cases of known pathogenic or benign CNVs were detected. This was a retrospective study with no parental DNA samples available and therefore the mode of inheritance was not determined.

The findings in our three cases of VOUS are summarized in Table 2. For the first case, the finding of arr[hg19] × p22.31(6452994–7772183) × 3 duplication was originally described as causative of intellectual disability[10], but it was later suggested to be a benign CNV[11]. For the second case, the finding of arr[hg19]17q21(44171342–44208665) × 1 is a partial deletion involving gene *KIAA1267/KANSL1* (*612452). Mutations in this gene have been attributed to 17q21.31 microdeletion syndrome. Although there is evidence that haploinsufficiency of this gene might be a disease-causing mechanism, deletion in our region lacks biological evidence (expression data) and genotype–phenotype correlations[12,13]. For the third case, the finding of arr[hg19]2q31(172950249–172952238) × 1 was a partial deletion of *DLX1* gene (*600029), which is associated with but not causative of autism[14].

DISCUSSION

In 215 pregnancies with high fetal NT and normal karyotype resulting in live births, array CGH detected no cases with pathogenic CNVs, but in 1.4% of cases there were VOUS. High fetal NT was defined by the cut-off of 3.5 mm, which represents the 99th percentile of the normal range[1].

The customized Fetal Chip v1.0 used in our study included telomeric and pericentromeric regions, examining the genome to a resolution of 100 kb (http://www.fetalmedicine.hk/en/Fetal_DNA_Chip.asp)[15]. The chip was specially designed to evaluate over 100 known genomic disorders in the fetus (http://www.fetalmedicine.hk/en/Fetal_DNA_Chip/Fetal_dna_chip_appenidx_I_Eng_v.1.0.pdf) with most of the known common non-pathogenic CNV regions removed; therefore, we did not anticipate missing any clinically significant CNVs.

There have been three previous studies investigating the potential value of array CGH in fetuses with high NT and normal karyotype[4–6]. Leung *et al.*[5] used the same array CGH method as we did to examine 48 fetuses with high NT and normal karyotype, reporting pathogenic CNVs in four (8.3%; 95% CI, 3.3–19.6) cases and VOUS in two (4.2%; 95% CI, 1.2–14.0): in comparison to our study their incidence of sonographically detected major fetal abnormalities was higher (20.8% *vs* 5.6%) and that of live births was lower (75% *vs* 100%). It is therefore possible that the presence of pathogenic CNVs in association with high fetal NT is due to the presence of other abnormalities rather than to the high fetal NT *per se*. This observation requires further investigation. Major fetal abnormalities were detected by Leung *et al.* in two of their four cases of pathogenic CNVs and the other two were liveborn, with no defects detected prenatally or postnatally[5].

Schou *et al.*[4] examined 100 fetuses with high NT and normal karyotype and reported that none had pathogenic CNVs detected by CGH. In this study, 10 cases had fetal abnormalities detected by ultrasound, 80 pregnancies resulted in live births and 20 resulted in spontaneous fetal loss or termination of pregnancy, or were lost to follow-up. Although the findings of this study are compatible with our own, it is possible that some pathogenic CNVs were missed because the CGH they used was only able to detect CNVs of 3 Mb or more.

Scott *et al.*[6] examined 90 fetuses with high NT and reported a pathogenic CNV in one of 41 (2.4%; 95% CI, 0.4–12.6) cases with normal karyotype. There was one case of ventricular septal defect (pers. comm.). The study did not provide data on outcome for the cases with high fetal NT.

Several studies have examined the possible value of array CGH in fetuses with sonographically detected abnormalities and normal karyotype. A recent meta-analysis of 18 studies reported pathogenic CNVs in 104 of 1139 (pooled prevalence, 9.1%; 95% CI, 7.5–10.8) fetuses with multiple defects and in 125 of 2220 (pooled prevalence, 5.6%; 95% CI, 4.7–6.6) fetuses with a defect restricted to one anatomical system[16]. These incidences of pathogenic CNVs in fetuses with sonographically detected abnormalities are higher than are incidences reported in prenatal studies in which array CGH was performed in women undergoing prenatal diagnosis for advanced maternal age or maternal anxiety in the absence of fetal defects. In four such studies, the incidence of pathogenic CNVs was 0.7% (6/852)[17], 0.2% (1/431)[18], 0.7% (3/422)[6] and 0.5% (9/1966)[19].

The incidence of VOUS in our cohort (1.4%) is the same as the reported rate in a meta-analysis evaluating the additional information provided by array CGH over conventional karyotyping in prenatal diagnosis[20]. The clinical relevance of a CNV is determined by certain factors, including size of the deletion or duplication, inheritance, position on the genome and whether it has already been described[21–23].

Array CGH has been applied postnatally as the first-tier diagnostic tool for the evaluation of developmental delay and structural malformations in children[24]. In prenatal diagnosis, there is increasing evidence that in about 5–10% of fetuses with defects detected by ultrasound, array CGH detects clinically significant microdeletions

Table 2 Findings in three cases with variants of unknown significance

Ultrasound findings	*Array CGH results*	*Size*	*OMIM*
NT 8.4 mm, exomphalos	arr[hg19] × p22.31(6452994–7772183) × 3	1.32 Mb	*300747 (STS)
NT 3.5 mm	arr[hg19]17q21(44171342–44208665) × 1	37.3 kb	*612452 (*KANSL1*)
NT 4.0 mm	arr[hg19]2q31(172950249–172952238) × 1	1.99 kb	*600029 (*DLX1*)

CGH, comparative genomic hybridization; NT, nuchal translucency; OMIM, Online Mendelian Inheritance in Man.

and microduplications[16,25]. Consequently, in the investigation of fetuses with detectable anomalies, use of array CGH should be considered, either alone or in combination with traditional cytogenetic karyotyping.

High NT and normal karyotype is associated with many fetal abnormalities and genetic syndromes and their incidence increases exponentially with increasing fetal NT[2,3]. Detection of high NT should prompt a detailed ultrasound examination for fetal abnormalities and, if these are detected, analysis of the samples obtained by invasive testing should include array CGH.

In the combined data of four studies of array CGH in pregnancies undergoing prenatal diagnosis for advanced maternal age or maternal anxiety in the absence of fetal defects, the incidence of pathogenic CNVs was 0.5% (19/3671)[6,17–19]. Similarly, combining the data of our study and those from the three previous studies, in a total of 371 cases with high fetal NT and no other sonographically detected defect, the incidence of pathogenic CNVs was 0.8%, which may therefore not be higher than in the general population. Consequently, the extent to which all pregnancies undergoing invasive prenatal diagnosis should have array CGH, in addition to or instead of traditional karyotyping, will ultimately be determined by health economic, rather than strictly medical, factors.

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