Prenatal diagnosis of pathogenic genomic imbalance in fetuses with increased nuchal translucency but normal karyotyping using chromosomal microarray

TY Leung *, KC Au Yeung, WC Leung, KY Leung, TK Lo, WWK To, WL Lau, LW Chan, DS Sahota, RKW Choy

KEY MESSAGES

1. 3.7% of fetuses with increased nuchal translucency (≥3.5 mm) but normal karyotype had pathogenic genomic imbalance.
2. The incidence of pathogenic copy number variants was higher among fetuses with increased nuchal translucency in terms of sonographic anomalies (9.7%), compared with those without (3.0%).
3. Fetuses with increased nuchal translucency (≥3.5 mm) should be considered further testing with chromosomal microarray.

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[1] TY Leung, [2] KC Au Yeung, [3] WC Leung, [4] KY Leung, [5] TK Lo, [6] WWK To, [3] WL Lau, [6] LW Chan, [1] DS Sahota, [1] RKW Choy

[1] *Department of Obstetrics & Gynaecology, The Chinese University of Hong Kong*
[2] *Department of Obstetrics & Gynaecology, Tuen Mun Hospital*
[3] *Department of Obstetrics & Gynaecology, Kwong Wah Hospital*
[4] *Department of Obstetrics & Gynaecology, Queen Elizabeth Hospital*
[5] *Department of Obstetrics & Gynaecology, Princess Margaret Hospital*
[6] *Department of Obstetrics & Gynaecology, United Christian Hospital*

* Principal applicant and corresponding author: tyleung@cuhk.edu.hk

Introduction

In 2010, Hong Kong launched universal first trimester Down syndrome screening using fetal nuchal translucency (NT) and biochemical assay. For screened positive cases, fetal karyotyping by chorionic villus sampling or amniocentesis is recommended. This screening strategy has achieved a high detection of 90%, with a false positive rate of 5%.[1] Although normal fetal karyotyping may be reassuring, these screened positive fetuses, especially if the NT is markedly increased (>95th centile), are at higher risk of developing structural abnormalities and neurodevelopmental delay during childhood. These structural and neurodevelopmental abnormalities may be associated with a wide variety of genetic or genomic diseases that may not be revealed by conventional chromosome analysis.

Prenatal diagnosis for these genetic or genomic diseases is difficult, because some diseases may be associated with morphological abnormalities that are very subtle and non-specific on ultrasonography, and appear in the later gestation or even postnatally. In addition, although over 100 diseases have been reported to be associated with an increased NT, such association has not been fully explored, and more diseases are expected to be discovered in future. Therefore, a more comprehensive genetic test is required to diagnose or exclude these genetic or genomic diseases prenatally.

Chromosomal microarray (CMA) can detect genomic imbalance (microdeletion / microduplication) and offer >40 times the resolution of karyotyping. CMA has dramatically improved the reliability of detecting copy number variants (CNVs) and subtle chromosomal abnormalities, including many disorders not detectable by an optimal karyotype, FISH or DNA direct sequencing. Our preliminary retrospective study has shown that pathogenic genomic imbalance occurred in 4 out of 48 stored DNA samples (8.3%) from fetuses with normal karyotyping but increased NT (≥3.5 mm; 99th centile). A prospective study with larger sample size is required to estimate the prevalence of pathogenic genomic imbalance among this group of patients.

Methods

This was a prospective study by six obstetric units (Kwong Wah Hospital, Prince of Wales Hospital, Princess Margaret Hospital, Queen Elizabeth Hospital, Tuen Mun Hospital, and United Christian Hospital). Women who presented to these units for first trimester combined screening test and had fetal NT ≥3.5 mm despite normal karyotyping (either via chorionic villus sampling or amniocentesis) were invited to participate. Fetal samples were further examined for any genomic CNVs using a custom-designed high-resolution 44K oligonucleotide array.

Parental bloods were also sampled to determine if the fetal CNVs was inherited. Samples were analysed in prenatal diagnostic laboratory, Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong. Chromosomal microarray was analysed via CytoGenomics (Agilent Technologies). Chromosomal structural variant detection was based on our reported method.[2-4] Pathogenic CNVs or variants of uncertain significance (VOUS) were classified in accordance to the American College of Medical Genetics and Genomics guidelines using chromosomal microarray-based databases (ClinVarCNV, DECIPHER) and internal databases from The Chinese University of Hong Kong. Patients detected with pathogenic CNVs and VOUS were contacted for further counselling.

Results

A total of 300 fetuses with NT ≥3.5 mm and normal karyotyping were recruited. Samples included 252 chorionic villus samples, 44 amniotic fluid, and 4 fetal tissue samples (after termination of pregnancy). The NT measurement ranged from 3.5 mm to 14 mm, with a median of 4.3 mm. 31 (10.3%) cases were detected to have additional structure abnormalities by ultrasound in the first trimester (syndromic), whereas the rest (89.7%) only had isolated high NT. The risk of syndromic malformation increased with NT thickness; it was 4% when NT was 3.5-4 mm, 8.6% when 4-5.5 mm, and 25.4% when ≥5.5 mm.

Eleven cases (3.7%) were identified to have pathogenic CNVs. The incidence of pathogenic CNVs in the syndromic group (3/31, 9.7%) was higher than the isolated group (8/269, 3.0%) but not statistically significantly (P=0.09, Fisher's exact test). The prevalence of pathogenic CNVs was 3.7%, 4.5%, and 5.1% when the NT thickness was ≥3.5 mm, ≥4.0 mm, and ≥5.5 mm, respectively. The size of genomic imbalance ranged from 14 kb to 10 Mb. Three cases (27.3%) were detected with two CNVs indicating that complex genomic rearrangement may be the underlying cause for increased NT and other abnormalities. In these 11 cases, one chose to keep pregnancy to live birth, nine chose termination of pregnancy, and one lost to follow-up.

In 14 (4.7%) cases, their CNVs were classified as VOUS, which were about 0.3 Mb to 7 Mb in size. None was detected to have any other fetal structural abnormalities. Twelve cases were live births and two cases lost to follow-up.

Six cases had uncertain significance after karyotyping and required CMA to determine pathogenicity. Three of them were confirmed to be pathogenic. The first case was a 47XY, +mar with a 10 Mb triplication on 15q11.1q13.2, which contained the Prader-Willi/Angelman critical region. After counselling the patient chose medical termination of pregnancy. The abortus did not show any obvious structural abnormality (consistent with ultrasound findings). The second case was 46,XX,del(8)(p23.1) of 6 Mb. The karyotype analysis on this was not confident due to the relative low resolution around 5-10 Mb. CMA helped confirm the karyotype abnormal findings. The third case was 46,XY,add(9)(?::p?22)->qter)dn, which turned out to be an 18 Mb deletion on chromosome 9p22.2-24.2. The other three cases had normal CMA results and were regarded as normal variants (46, XX, 22p-, 46,XY,9p+, 47XY, +mar) inherited from the parents and proceeded to livebirths.

Discussion

In this study, 3.7% of fetuses with significantly increased NT (≥3.5 mm; 99th centile) but normal karyotypes were found to have pathogenic genomic imbalance through CMA. This incidence was lower than the 8% in our retrospective study of 40 cases collected from Demark,[5] but was higher than the 1.4% in our retrospective study of 215 samples collected from the United Kingdom.[6] The Chinese cohort in this study was most representative to our local population.

Our finding was comparable to that reported in a systematic review in 2015.[7] It found that the incidence of pathogenic CNV was 4% (54/1403) in cases with only isolated thick NT and 7% (20/251) when associated with other fetal structural abnormalities. In our cohort, the pathogenic CNV rate of 9.7% in the syndromic NT group was higher than that in the isolated NT group (3.0%) although the difference did not reach statistical significance. Thus, CMA may be indicated in the former group but not the latter group, as recommended by the American College of Obstetricians and Gynecologists.[8]

Whether CMA is indicated in cases with isolated thick NT is debatable. First, 3% to 4% (1/33-1/25) chance of pathogenicity is not low, especially if termination of pregnancy is considered. Second, during the first trimester the fetus is too small to confirm or exclude any fetal malformations and to determine whether it is syndromic or simply isolated thick NT (as shown in our two cases). Hence, a follow-up morphology scan in the second trimester is essential. However, the drawback is that affected parents may have to wait for weeks and this may create anxiety. Furthermore, if abnormal CMA result can be known earlier, first trimester termination of pregnancy is preferred to second trimester in term of maternal physical and psychological health. Third, cystic hygroma is traditionally regarded as a 'soft marker' and not a 'structural' malformation, and hence was not categorised to the syndromic group. However, cystic hygroma is commonly associated with syndromic disorders and should be taken seriously. After considering the potential benefits of early CMA and limitation of ultrasonography,

it is worthwhile to perform CMA for cases with apparently isolated thick NT.

Six cases with karyotyping showing uncertain significant results were not included in our study. Their karyotypes could not be classified as normal or abnormal because of the presence of marker chromosomes or because of the limited banding. However, such conditions are not uncommon in our daily practice (2% in our cohort), and thus CMA has a role in delineating pathogenicity and assisting in clinical management.

In our cohort, the VOUS rate of 4.7% was higher than the generally cited rate of 1% to 3%. One reason may be ethnic group differences. With proper consulting, all VOUS cases kept pregnancy. Twelve of these 14 cases had live birth and were followed up to 6 months old without obvious developmental problem. Long-term follow-up is needed to ensure the developmental outcomes of these babies.

Conclusions

Our study indicated that 3.7% of fetuses with significantly increased NT (>3.5 mm) despite normal karyotype had pathogenic genomic imbalance detected by CMA. The incidence was higher in the syndromic group (9.7%) than the isolated NT group (3.0%). Hence, CMA is warranted to search for any pathogenic CNVs after karyotyping has excluded common chromosomal abnormalities.

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