Whole-genome sequencing of genetically undiagnosed euploid fetuses with increased nuchal translucency: abridged secondary publication

RKW Choy *, YE Cao, FM Lo, SW Cheung, Y Yang, TY Leung

KEY MESSAGES

1. We studied the genome-wide spectrum and frequency of genetic variants and spatial genomic organisation during early fetal development. We also investigated the variability of genomic variants associated with the increased nuchal translucency–related birth defects.
2. We implemented whole-genome sequencing to screen the candidate pathogenic variants that helped explain the congenital structural abnormalities for 15 trios in Hong Kong.
3. Compared with chromosomal microarray analysis and karyotyping, whole-genome sequencing provided an additional 20% (3/15) diagnostic yield.
4. Applying whole-genome sequencing in fetuses with increased nuchal translucency can comprehensively detect and delineate the various genomic variants that are causative to the diseases.

Hong Kong Med J 2022;28(Suppl 1):S8-10
HMRF project number: 04152666

[1] RKW Choy, [1] YE Cao, [2] FM Lo, [3] SW Cheung, [3] Y Yang, [1] TY Leung

[1] *Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong*
[2] *Department of Health*
[3] *Baylor College of Medicine, USA*

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

Introduction

Detection of fetuses with increased nuchal translucency in routine first-trimester ultrasound screening has been widely used as a sensitive indicator for fetal chromosomal abnormalities and/or fetal structural anomalies such as congenital heart disorders or neurodevelopmental anomalies detected in later gestations. Fetuses with increased nuchal translucency and structural malformations are frequently contributed by genetic abnormalities and have poor prognoses. However, >80% of such cases do not obtain a causative result based on the routine prenatal diagnostic tests and hence genetic counselling and clinical management is challenging. In prenatal diagnosis, since 2010, chromosomal microarray analysis (CMA) has been the first-tier test for high-risk pregnancies to identify microscopic or submicroscopic copy number variations. However, this approach is limited by its resolution and inability to detect single-nucleotide variants and small insertions/deletions. Whole exome sequencing can provide genetic diagnoses for 9.1% to 32% of fetuses with a structural anomaly. Of these fetuses, whole-exome sequencing yielded diagnoses in 3.2% to 21% of the fetuses with increased nuchal translucency with/without structural malformations. Nonetheless, both whole-exome sequencing and CMA are unable to detect apparently balanced structural rearrangements (or structural variants); some of these rearrangements are disease-causing. The wide spectrum of genetic aetiologies in fetuses with increased nuchal translucency (ranging from single-base mutations to those affecting millions of base pairs and numerical disorders) warrants a holistic approach for comprehensive detection of the disease-causing genetic variants. Our previous studies have demonstrated the feasibility and potential diagnostic utility of low-pass whole-genome sequencing for detection of copy number variations and chromosomal structural rearrangements including balanced translocations and inversions in both clinical cohorts and presumably normal populations in the 1000 Genomes Project. By increasing the read depth to a minimum of 30-fold to detect single-nucleotide variants / small insertions/deletions, whole-genome sequencing enables comprehensive detection of various genomic variants, providing a platform for gene discovery and potential clinical application. We aimed to apply whole-genome sequencing to investigate genetic contributions to fetuses with increased nuchal translucency and structural malformations and to evaluate its potential clinical application.

Methods

First, 100 ng of genomic DNA from each sample was sheared to fragment sizes ranging from 300 to 500 bp by the Covaris S2 Focused Ultrasonicator.

Library construction including end repairing, A-tailing, adapter ligation, and PCR amplification was conducted subsequently. The PCR products were then heat-denatured to form single-strand DNAs, followed by circularisation with DNA ligase. After construction of the DNA nanoballs, paired-end sequencing with 100 bp at each end was carried out for each sample with a minimum read depth of 100-fold on the MGISEQ-2000 sequencing platform.

Interpretation of candidate pathogenic variants was based on the classification of potential Mendelian disease-causing variants described previously. According to the American College of Medical Genetics and Genomics recommendations, each variant was classified as benign, likely benign, uncertain significance, likely pathogenic, and pathogenic. Uncertain significance variants are further subclassified into 'favour benign' or 'favour pathogenic'. Variants with allele frequency of <1% or unknown in the database are rare and more likely to be disease causing. Candidate pathogenic variants are supposed to locate on the coding sequence, or splice site. Demonstration of the same mutation in phenotypically normal parents strongly suggests that the observed change in the index patient's genome is without clinical significance.

Results

In 15 trios with increased nuchal translucency (>3.5 mm) and negative results from karyotype and CMA, whole-genome sequencing provided an additional 20% (3/15) diagnostic yield, with two cases with pathogenic point mutations (Fig 1) and one case with cryptic insertions (Fig 2). Follow-up study further demonstrated the potential pathogenicity of an apparently balanced insertion that disrupted an OMIM autosomal dominant disease-causing gene at the insertion site.

Discussion

Applying whole-genome sequencing in fetuses with increased nuchal translucency can comprehensively detect and delineate the various genomic variants that are causative to the diseases. Importantly, prenatal diagnosis by whole-genome sequencing provided additional diagnostic yield, compared with routine protocols. Given a comparable turnaround time and less DNA required, whole-genome sequencing is useful in prenatal diagnosis, particularly in fetuses with increased nuchal translucency.

CMA can serve as first-tier genetic testing for prenatal diagnosis, whereas whole-genome sequencing can serve as a second-tier test in CMA negative patients. For fetuses with high nuchal translucency and structural malformations and at high gestational weeks (>20 weeks), whole-genome sequencing is suggested for a more plausible explanation.

Conclusion

Whole-genome sequencing is powerful to discover the candidate pathogenic variants for fetuses with high nuchal translucency and structural malformations. It has clinical application in Hong Kong.

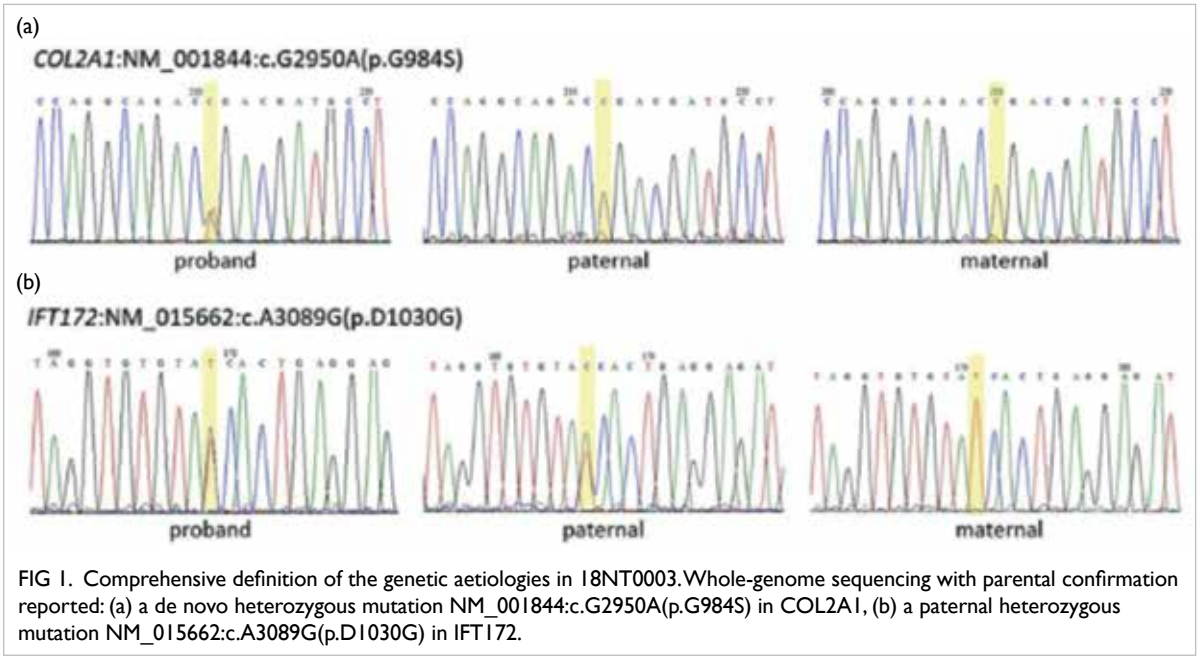

FIG 1. Comprehensive definition of the genetic aetiologies in 18NT0003. Whole-genome sequencing with parental confirmation reported: (a) a de novo heterozygous mutation NM_001844:c.G2950A(p.G984S) in COL2A1, (b) a paternal heterozygous mutation NM_015662:c.A3089G(p.D1030G) in IFT172.

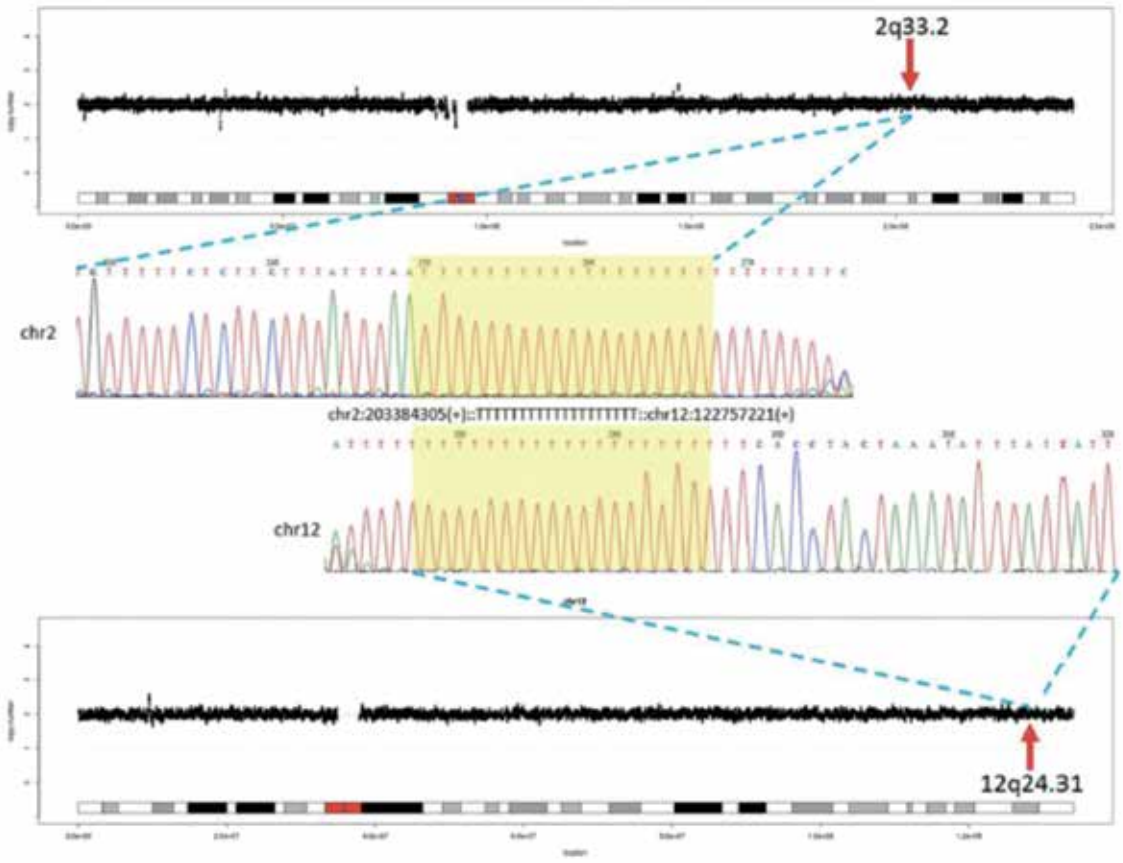

FIG 2. Distributions of copy ratios in chromosome 2 and chromosome 2 (top) and chromosome 12 (bottom). The inserted site in chromosome 2 and the rearranged segments in chromosome 12 are indicated by red arrows with the band numbers; both regions are copy number neutral. Black dots indicate the copy ratio in each window across different chromosomes. The X- and Y-axes represent the genomic coordinates and the copy ratios, respectively. Dotted grey lines indicate the copy numbers 0, 1, 3, and 4. Sanger sequencing results of the breakpoints are shown in the middle. Forward sequencing is shown on the top, whereas the reverse complementary sequencing result of the reverse sequencing is shown at the bottom. The breakpoint coordinates in chromosome 2 and chromosome 12 are shown with the aligned orientation, whereas the inserted sequence is remarked in between and highlighted in yellow in both Sanger sequencing results.

Acknowledgements

We thank the clinical colleagues for their support in recruiting cases. We also thank the patients for their supports and contributions and all other colleagues for their generous help and hard work.

Funding

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#04152666). The full report is available from the Health and Medical Research Fund website (https://rfs1.fhb.gov.hk/index.html).

Disclosure

The results of this research have been previously published in:

1. Choy KW, Wang H, Shi M, et al. Prenatal diagnosis of fetuses with increased nuchal translucency by genome sequencing analysis. Front Genet 2019;10:761.