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APPLICATION OF NEXT-GENERATION SEQUENCING FOR DETECTING BETA THALASSEMIA IN PRE-IMPLANTATION GENETIC TESTING FOR MONOGENIC DISEASE

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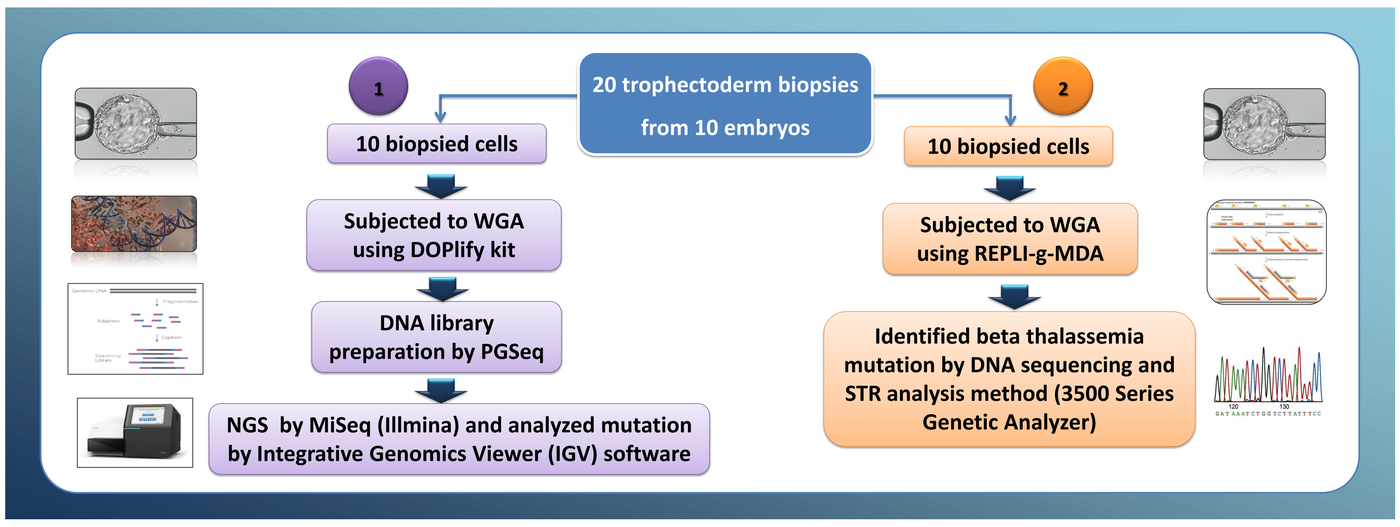
BACKGROUND AND AIMS

Beta thalassemia (HBB gene mutation) is the most common serious monogenic diseases in Southeast Asia. Carrier couple with HBB mutations can transmit their mutations to their child who has a chance to get a severe form of beta thalassemia. Pre-implantation genetic testing for monogenic disease (PGT-M) can assist to screen an unaffected embryo prior to implantation. This study aims to compare the efficiency of a next-generation sequencing-based (NGS) protocol for simultaneous detecting of HBB gene mutations for PGT-M along with low-pass whole genome analysis of chromosome aneuploidies for PGT-A with conventional method (short tandem repeat and DNA sequencing) for screening beta thalassemia mutations in embryos.

METHODS

The embryonic cells at blastocyst stage derived from carrier couples of beta thalassemia (c.7/8 +G and c.41/42 -CTTT) were biopsied into 2 samples per embryo and amplified by DOPlify (PerkinElmer Inc. Waltham, USA) and REPLI-g-MDA (QIAGEN, Germany). DOPlify whole genome amplification (WGA) products were used to identify the beta thalassemia mutation using PG-Seq (PerkinElmer Inc. Waltham, USA) library preparation reagent and analysis by NGS (MiSeq, Illmina, USA). Miseq Reporter (Illmina, USA) and Integrative Genomics Viewer (IGV; UC San Diego and MIT, USA) program were used to analyze beta thalassemia mutation of NGS method. The second biopsied samples amplified with REPLI-g-MDA were used to identify the beta thalassemia mutation by DNA sequencing and STR analysis method (3500 Series Genetic Analyzer, Applied Biosystems, USA).



RESULTS

Table 1 c.7/8+G and c.41/42 (–CTTT) mutation identified by NGS and DNA Sequencing + STR analysis

	c.7/8 +G mutation		c.41/42 (–CTTT) mutation			
Method	**Homozygous mutation**	**Heterozygous mutation**	**Homozygous mutation**	**Heterozygous mutation**	**ADO**	**Concordance**
NGS (N=10)	**4**	**4**	**1**	**1**	**0**	**100%**
DNA Sequencing + STR analysis (N=10)	**4**	**4**	**1**	**1**	**1**	

Total 10 blastocyst stage embryos were included in this study. NGS method identified 4 affected embryos with homozygous mutation of c.7/8+G and 4 embryos that were carried c.7/8 +G mutation (Fig 1). Moreover, it revealed 1 affected and 1 carrier embryos of c.41/42 (–CTTT) mutation (Fig 2). All read depths of NGS investigation are 1,000-4,000 depth. The analysis results of HBB gene mutations detected by NGS showed 100% concordance with the results of conventional method (Table 1). Additionally, NGS method was able to identify HBB gene mutations with no allele drop out (ADO) while the conventional method using DNA target sequencing showed one sample with ADO. For chromosomal aneuploid screening, NGS method identified nine aneuploid embryos and one embryo had normal chromosome numbers.

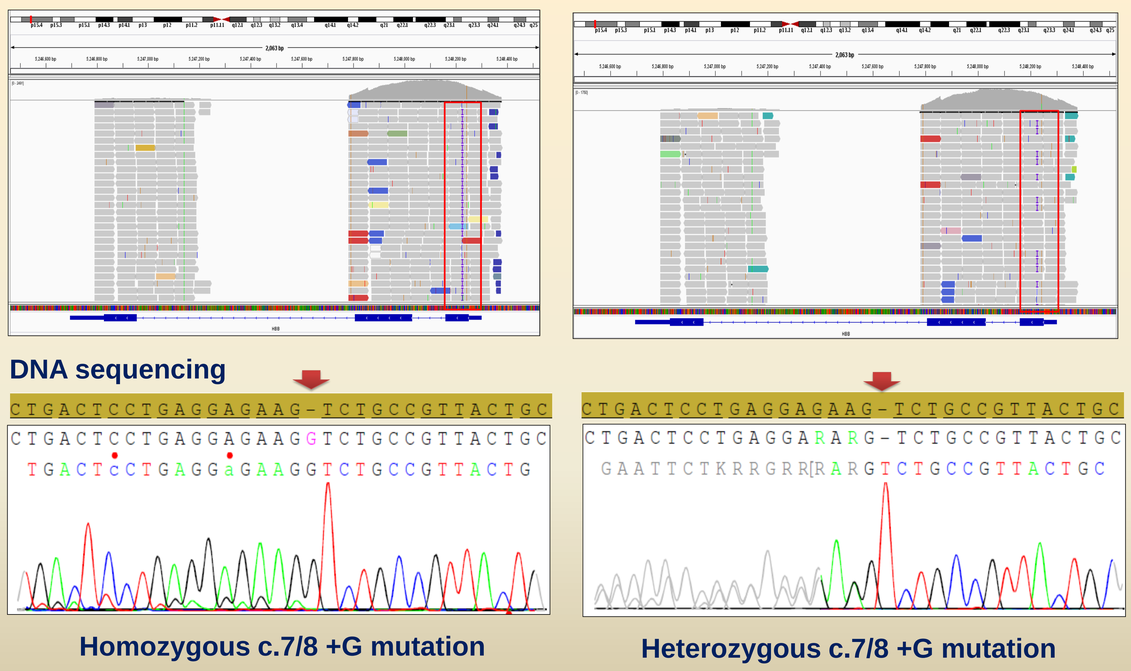


Figure 1 c.7/8+G mutation analyzed by IGV and DNA sequencing program

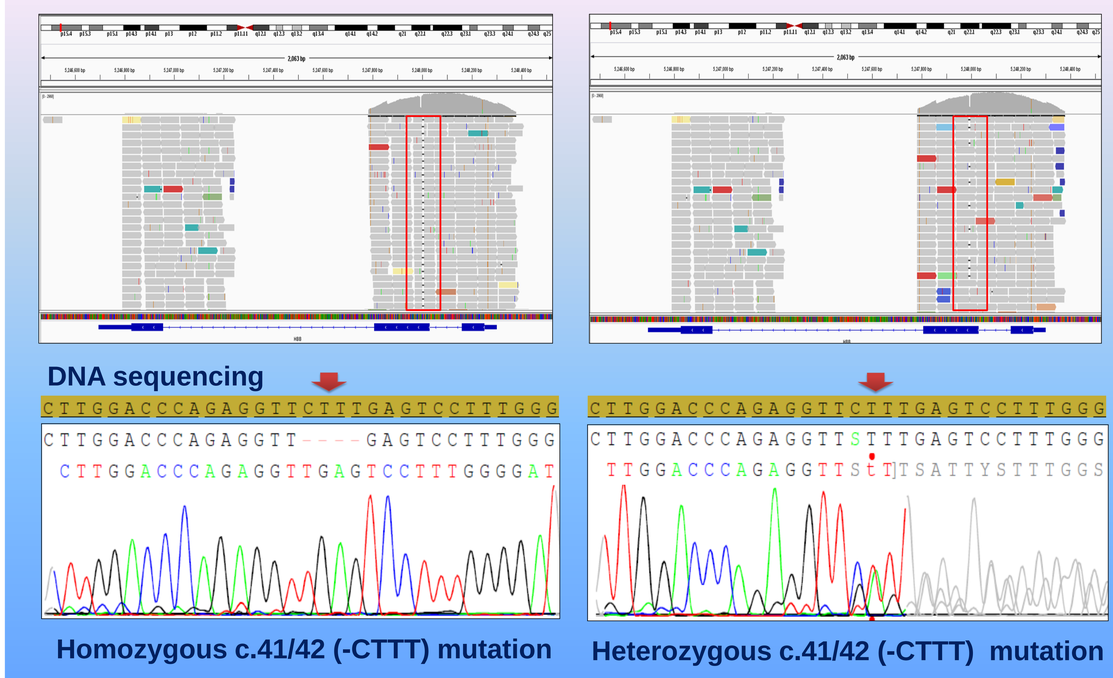


Figure 2 c.41/42 (-CTTT) mutation analyzed by IGV and DNA sequencing program

CONCLUSIONS

Using NGS method reveals an advantage for simultaneously detecting the beta thalassemia for PGT-M along with chromosome aneuploidy screening for PGT-A in a single biopsied sample and it also decreases ADO rate compared to the conventional method. Importantly, the embryos are no need to be biopsied into 2 samples resulting in reduced the worst effect of biopsy on the embryos.

REFERENECES

Jamuar SS and Tan EC. Clinical application of next-generation sequencing for Mendelian diseases. *Hum Genomics*. 2015;9(1):10. https:// doi:10.1186/s40246-015-0031-5

Sun Y, Man J, Wan Y *et al.* Targeted next-generation sequencing as a comprehensive test for Mendelian diseases: a cohort diagnostic study. *Sci Rep.* 2018;8:11646. https://doi.org/10.1038/s41598-018-30151-z

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