DEVELOPMENT OF SIMULTANEOUS PRE-IMPLANTATION GENETIC TESTING FOR ANEUPLOIDY SCREENING, SINGLE GENE DISORDERS, AND STRUCTURAL ARRANGEMENT FROM A SINGLE TROPHECTODERM BIOPSY USING TARGETED NEXT GENERATION SEQUENCING IN ONE PROCEDURE

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Abstract Body

Pre-implantation genetic testing (PGT) is used to identify genetic defects in embryos from in vitro fertilization (IVF) before pregnancy. In this study, we developed a targeted next generation sequencing (tNGS) methodology for simultaneous pre-implantation genetic testing for aneuploidy (PGT-A), monogenic/single gene disorders (PGT-M), and structural rearrangements (PGT-SR) from a single trophectoderm (TE) biopsy in a one procedure without whole genome amplification (WGA).

tNGS amplifies~5000 amplicons across the human genome for PGT-A, and preliminary validation was established with 5-cell samples from cell lines with known karyotypes. The patient specifical primers of mutation or informative SNPs for PGT-M or PGT-SR were validated by parental DNA and affected children if available, then were added to PGT-A primer pool. After PCR amplification the samples were pooled and processed for the NextSeq (Illumina). The sequencing reads were filtered for quality and aligned to the human genome, and the copy number of each chromosome is determined. For PGT-A, 147 TEs from 46 aneuploid blastocysts were evaluated for validation. A consistent diagnosis of TEs was made in 98.6% (145/147) of the samples. The two discordant samples were the re-biopsies from the same embryos, suggesting the embryo was mosaic. For PGT-M, sequencing primers were designed for 9 different variants (*CFTR* c.350G>A, *CFTR* c.1521_1523delCTT , *HEXA* c.1421+1G>C, *HEXA* c.1274_1277dupTATC, *PAX6* c.76C>G , *TBX5* c.342C>G, *PHEX* c.1180C>T, *HMGCL* c.122G>A, *HMGCL* c.497+4A>G). 26 TEs of 13 embryos from 7 families, showed 100% concordant PGT-M diagnoses with previous clinical results. For PGT-SR, 38 TEs of 12 embryos with 9 translocation conditions were tested, including t(7;10)(q32;q22), t(4;18)(q21;q21.3), t(3;17)(q21;q21.3), t(9;16)(q21.2;q12.1), t(X;2)(p11.2;q23), t(7;15)(q11.21;q15), t(5;17)(q13;q21.3), t(3;6)(q25;q22.2), and t(12;15)(q24.1;q13). All the samples showed consistent results with previous microarray diagnosis.

This study demonstrated that PGT-A, PGT-M, and PGT-SR can be reliably and consistently performed simultaneously from the same TE biopsy in only one procedure.