Development and validation of concurrent preimplantation genetic diagnosis for single gene disorders and comprehensive chromosomal aneuploidy screening without whole genome amplification

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Objective: To develop a novel and robust protocol for multifactorial preimplantation genetic testing of trophectoderm biopsies using quantitative polymerase chain reaction (qPCR).
Design: Prospective and blinded.
Setting: Not applicable.
Patient(s): Couples indicated for preimplantation genetic diagnosis (PGD).
Intervention(s): None.
Main Outcome Measure(s): Allele dropout (ADO) and failed amplification rate, genotyping consistency, chromosome screening success rate, and clinical outcomes of qPCR-based screening.
Result(s): The ADO frequency on a single cell from a fibroblast cell line was 1.64% (18/1,096). When two or more cells were tested, the ADO frequency dropped to 0.02% (1/4,426). The rate of amplification failure was 1.38% (55/4,000) overall, with 2.5% (20/800) for single cells and 1.09% (35/3,200) for samples that had two or more cells. Among 152 embryos tested in 17 cases by qPCR-based PGD and CCS, 100% were successfully given a diagnosis, with 0% ADO or amplification failure. Genotyping consistency with reference laboratory results was >99%. Another 304 embryos from 43 cases were included in the clinical application of qPCR-based PGD and CCS, for which 99.7% (303/304) of the embryos were given a definitive diagnosis, with only 0.3% (1/304) having an inconclusive result owing to recombination. In patients receiving a transfer with follow-up, the pregnancy rate was 82% (27/33).
Conclusion(s): This study demonstrates that the use of qPCR for PGD testing delivers consistent and more reliable results than existing methods and that single gene disorder PGD can be run concurrently with CCS without the need for additional embryo biopsy or whole genome amplification. (Fertil Steril® 2016;105:286–94. ©2016 by American Society for Reproductive Medicine.)
Key Words: Single gene disorder, monogenic disorder, preimplantation genetic diagnosis, trophectoderm

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Preimplantation genetic diagnosis (PGD) has been in clinical practice for more than 20 years to help prevent the transmission of single gene disorders (SGDs) to the offspring of high-risk couples (1). The European Society for Human Reproduction and Embryology PGD Consortium has collected detailed information on more than 6,000 polymerase chain reaction– (PCR-) based SGD treatment cycles (2). Many advances, including the use of multiple markers linked to the mutation by identifying family-specific informative polymorphisms, have helped to improve the accuracy and reliability of SGD PGD (3). More recently, investigators have begun to develop strategies that allow simultaneous diagnosis of multiple genetic factors such as a monogenic disorder in combination with comprehensive chromosome screening (CCS) for aneuploidy (4–9).

While there are already many strategies to perform multifactor preimplantation genetic testing, all methods suffer important limitations. For example, some studies have involved performing two separate biopsies on the same embryo to perform two independent tests (4, 5, 9). The advantage of this strategy is that existing methods can be used for CCS on one biopsy and PGD on the other, thus avoiding the need for novel methods of assessment. However, one important limitation is the negative impact that performing two biopsies is known to introduce (10). Another strategy involves whole genome amplification (WGA) and genome-wide single nucleotide polymorphism (SNP) microarray-based haplotype analysis (11). In this methodology, both aneuploidy and SGDs are diagnosed using linkage-based analysis of parental and embryonic genotypes. While this strategy has the obvious advantage of a single biopsy and the potential to eliminate the need for preclinical workups, it remains time consuming, costly, and extremely complex and has yet to demonstrate the ability to accurately detect all types of aneuploidy. Other array-based strategies using a single biopsy have been reported (6, 8, 9) but also suffer from high cost and extensive times to complete. Therefore, the present study develops a new, simple, low-cost, and universally applicable strategy, which allows simultaneous PGD of SGDs or small duplications or deletions and CCS from the same biopsy within 4–6 hours.

MATERIALS AND METHODS

Experimental Design

To demonstrate the validity of simultaneous quantitative PCR- (qPCR-) based PGD and CCS, this study was conducted in multiple phases. To determine the allele dropout (ADO) and amplification failure (AF) rate, cell lines with known genotypes were evaluated. To demonstrate the consistency of qPCR with conventional methods of PGD (Sanger sequencing and short tandem repeat PCR), PGD results from qPCR were compared with results from one of three commercial SGD reference laboratories (Genesis Genetics Institute, Reproductive Genetics Institute, or Reprogenetics). To establish the use of TaqMan allelic discrimination assays for direct mutation and indirect linked marker PGD analysis, results were compared to one of the above listed reference laboratories. Clinical applicability for a variety of disorders was demonstrated by using qPCR for simultaneous CCS and PGD with multiple markers for SGDs, duplications, or deletions.

Genotyping Performance on Single or Multiple Cells

To evaluate the performance of TaqMan SNP genotyping on single and multiple cells, 1- to 5-cell samples were prepared from a cell line where known genotypes could be evaluated. Fibroblasts were isolated as a single cell or a 2-, 3-, 4-, or 5-cell sample. Twenty replicates of each cell number–specific sample were prepared. Cells were derived from a publically available cell line (GM002498) from the Coriell Cell Repository. The impact of cell number was determined by assessing 40 previously described SNPs (12) in duplicate by TaqMan qPCR and based upon consistency of genotypes with those determined from DNA purified from large quantities of cells from the same cell line. ADO was defined as a homozygous genotype result in single or multiple cells when heterozygosity was expected. AF was defined as no amplification at a given locus.

Embryonic PGD and CCS

SGDs involved obtaining TaqMan allelic discrimination assays from Life Technologies Inc. All final concentrations were used according to the manufacturer's instructions. Assays allowing direct interrogation of the mutations were designed, using public human genome sequence data and prior clinical reports. Duplications and deletions involved a combination of TaqMan copy number and informative allelic discrimination assays. In cases involving deletions, four informative SNPs (hemizygous for one allele in the carrier partner and homozygous for the other allele in the other partner) were identified within the deletion region using SNP array analysis of the parental DNA. In cases involving duplications, two linked informative SNPs (heterozygous in the carrier and homozygous in the unaffected partner) on each side outside of the duplication region were identified using SNP array analysis of the parental DNA and used on a linkage basis only, not as a quantitative measure of the duplication. When indicated, informative linked SNPs were established through SNP microarray-based analysis (Affymetrix NspI, Affymetrix Inc.) of the patient, partner, and appropriate relatives. TaqMan assays were validated on the original family members' DNA and 5-cell lymphocyte aliquot samples and used to confirm the SNP array-based genotypes and mutation clinical reports before clinical use.

Trophectoderm biopsy was performed, and embryos were transferred after vitrification as described elsewhere (13, 14). Negative controls included tubes loaded with leftover or unexposed wash buffer for each case. Samples were processed by alkaline lysis, and a 50-μL multiplex preamplification reaction was performed for CCS as described elsewhere (15), with the addition of TaqMan allelic discrimination or copy number assays. In clinical cases where a linkage-only approach was used, two SNPs on each side of the mutation locus were used (four total). In clinical cases where the mutation could be directly interrogated, one