ORIGINAL ARTICLE: GENETICS

First systematic experience of preimplantation genetic diagnosis for single-gene disorders, and/or preimplantation human leukocyte antigen typing, combined with 24-chromosome aneuploidy testing

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Objective: To study the feasibility, accuracy, and reproductive outcome of 24-chromosome aneuploidy testing (24-AT), combined with preimplantation genetic diagnosis (PGD) for single-gene disorders (SGDs) or human leukocyte antigen (HLA) typing in the same biopsy sample.
Design: Retrospective study.
Setting: Preimplantation genetic diagnosis center.
Patient(s): A total of 238 PGD patients, average age 36.8 years, for whom 317 combined PGD cycles were performed, involving 105 different conditions, with or without HLA typing.
Intervention(s): Whole-genome amplification product, obtained in 24-AT, was used for PGD and/or HLA typing in the same blastomere or blastocyst biopsy samples.
Main Outcome Measure(s): Proportion of the embryos suitable for transfer detected in these blastomere or blastocyst samples, and the resulting pregnancy and spontaneous abortion rates.
Result(s): Embryos suitable for transfer were detected in 42% blastocyst and 25.1% blastomere samples, with a total of 280 unaffected, HLA-matched euploid embryos detected for transfer in 212 cycles (1.3 embryos per transfer), resulting in 145 (68.4%) unaffected pregnancies and birth of 149 healthy, HLA-matched children. This outcome is significantly different from that of our 2,064 PGD cycle series without concomitant 24-AT, including improved pregnancy (68.4% vs. 45.4%) and 3-fold spontaneous abortion reduction (5.5% vs. 15%) rates.
Conclusion(s): The introduced combined approach is a potential universal PGD test, which in addition to achieving extremely high diagnostic accuracy, significantly improves reproductive outcomes of PGD for SGDs and HLA typing in patients of advanced reproductive age. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Microarray-based 24-chromosome aneuploidy testing, PGD for single gene disorders, preimplantation HLA typing, allele drop out (ADO) in whole genome amplification, blastocyst biopsy, reproductive outcome

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Development of a universal preimplantation genetic diagnosis (PGD) test for various indications in the same biopsied single cell is of practical importance, as a significant number of at-risk PGD patients are of advanced reproductive age,

especially those who require preimplantation human leukocyte antigen (HLA) typing, who may also benefit from preselection of euploid embryos. The current development and application of microarray-based technology or next-generation sequencing (NGS) for preimplantation, 24-chromosome, aneuploidy testing (24-AT) in a single biopsied cell greatly improves detection of chromosomally abnormal oocytes and embryos (1–9). As one of the major steps of these procedures is whole-genome amplification (WGA), or multiple-displacement amplification (MDA), they provide a valuable opportunity for combining 24-AT with PGD for single-gene disorders (SGDs) or HLA typing in the same biopsy material.

However, using this combination still requires development of a PGD test design for a particular family, to avoid misdiagnosis in deoxyribonucleic acid (DNA) analysis of WGA or MDA products for SGDs, using the multiplex nested approach described elsewhere (10). Attempts have been made to detect multiple conditions in the same reaction, using NGS; however, this process is highly dependent on equipment-assisted technology and may be prone to error at the present time (11, 12). Next-generation sequencing provides base-pair resolution data, with the unique opportunity to evaluate multiple customized genomic loci and multiple samples on the same run and on 1 chip (11). Thus, DNA from polar body, blastomere, or throphectoderm samples from different couples can be analyzed simultaneously, which may reduce cost, provided that many samples are processed at the same time.

Although the applicability of this technique for PGD has yet to be validated, the principal possibility of using NGS to test single cells has been demonstrated (13–15). In addition, the first attempt to evaluate the feasibility of NGS for PGD (11), using a specific protocol to test DNA from trophectoderm biopsy by semiconductor-based NGS, demonstrated perfect consistency of NGS results with those from 2 independent, conventional PGD methodologies. The major concern relating to this technique is that it is prone to allele drop-out (ADO), owing to the fact that WGA is the first step, needed to generate an adequate amount of DNA for analysis, but still extremely inefficient for recovering all genomic sequences. So, without simultaneous testing of a sufficient number of relevant linked markers, false-negative results cannot be excluded, especially in PGD of dominant diseases. Therefore, the technique likely will need to be upgraded, by performing NGS using single-nucleotide polymorphism analysis for this purpose, or by adjusting the level of deep sequencing to overcome the ADO problem, or by developing more-efficient WGA (11, 12).

The other approach, which is currently being validated, is karyomapping (16). However, this approach has its own limitations and cannot be applied in a number of circumstances, described later. In the meantime, the first attempt at combining 24-AT with traditional PGD for SGD was successfully accomplished (17). In this study, 10 embryos were tested via single-nucleotide polymorphism–based microarray analysis on biopsied blastocysts. Seven embryos were euploid, of which 2 were determined to be affected with GM1-gangliosidosis; 5 were mutation carriers; and 3 were normal. By combining the results of the 2 tests, 5 of 10 embryos tested were found suitable for transfer. This approach has now been extended to a variety of genetic conditions, applied along with preimplantation HLA typing. The first systematic experience of using this combined test in 317 PGD cycles for 105 different conditions is presented here.

MATERIALS AND METHODS

A combined approach was performed for 238 patients (317 PGD cycles) of average maternal age of 36.8 years, who were at risk for producing offspring with genetic disorders, or had requested preimplantation HLA typing, and required 24-AT owing to advanced reproductive age. The combined testing involved 105 genetic disorders, and 31 cycles for preimplantation HLA typing.

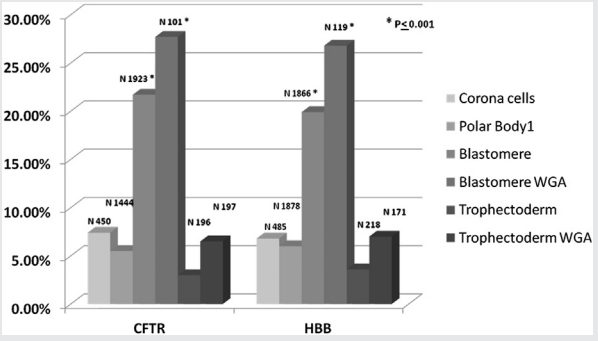
The procedure was performed according to the protocol of microarray-based 24-AT testing, using a BlueGnome (BlueGnome, Ltd.) platform for array-comparative genome hybridization (CGH), according to the manufacturer's instructions. The first step of the procedure was WGA, performed with a SurePlex Single Cell WGA Kit (Rubicon Genomics), so the PGD for SGDs and preimplantation HLA typing was performed on a portion of WGA product, using a specific custom-made PGD design, developed in advance for each couple, using a multiplex nested polymerase chain reaction system, described elsewhere (10). To evaluate the impact of WGA on the ADO rate in various types of biopsied cells, 2 mutations were used—cystic fibrosis (CFTR) and beta-globin gene mutation—comparing the ADO rates to that in single-cell analysis without WGA (10, 18).

Statistical analysis was performed using chi-squared analysis, and differences at $P<.05$ were considered significant. The work was approved by the institutional review board of the Reproductive Genetics Institute.

RESULTS

As shown in Figure 1, the highest ADO rate was observed after WGA of blastomeres, which was 27.7% for CFTR and 26.8%

FIGURE 1



Allele dropout rates in different types of cells heterozygous for the CFTR gene and beta-globin gene mutations, with or without WGA (see description in the text). In both mutations, a significantly higher ADO rate is seen after WGA. This rate is much lower in blastocyst samples than in blastomere samples. HBB = beta-globin gene mutation.

Rechitsky. Combined PGD with 24-chromosome aneuploidy testing. Fertil Steril 2014.