CASE REPORT

Single-gene testing combined with single nucleotide polymorphism microarray preimplantation genetic diagnosis for aneuploidy: a novel approach in optimizing pregnancy outcome

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Objective: To describe a method of amplifying DNA from blastocyst trophectoderm cells (two or three cells) and simultaneously performing 23-chromosome single nucleotide polymorphism microarrays and single-gene preimplantation genetic diagnosis.
Design: Case report.
Setting: IVF clinic and preimplantation genetic diagnostic centers.
Patient(s): A 36-year-old woman, gravida 2, para 1011, and her husband who both were carriers of GM_1 gangliosidosis. The couple wished to proceed with microarray analysis for aneuploidy detection coupled with DNA sequencing for GM_1 gangliosidosis.
Intervention(s): An IVF cycle was performed. Ten blastocyst-stage embryos underwent trophectoderm biopsy. Twenty-three–chromosome microarray analysis for aneuploidy and specific DNA sequencing for GM_1 gangliosidosis mutations were performed.
Main Outcome Measure(s): Viable pregnancy.
Result(s): After testing, elective single embryo transfer was performed followed by an intrauterine pregnancy with documented fetal cardiac activity by ultrasound.
Conclusion(s): Twenty-three–chromosome microarray analysis for aneuploidy detection and single-gene evaluation via specific DNA sequencing and linkage analysis are used for preimplantation diagnosis for single-gene disorders and aneuploidy. Because of the minimal amount of genetic material obtained from the day 3 to 5 embryos (up to 6 pg), these modalities have been used in isolation of each other. The use of preimplantation genetic diagnosis for aneuploidy coupled with testing for single-gene disorders via trophectoderm biopsy is a novel approach to maximize pregnancy outcomes. Although further investigation is warranted, preimplantation genetic diagnosis for aneuploidy and single-gene testing seem destined to be used increasingly to optimize ultimate pregnancy success. (Fertil Steril® 2011;95:1786.e5–e8. ©2011 by American Society for Reproductive Medicine.)

Key Words: PGD, single gene, microarray

In recent years, technology surrounding assisted reproductive technology (ART) has grown exponentially. Two technologies that promise to affect ART significantly are 23-chromosome microarray preimplantation genetic testing and preimplantation genetic diagnosis for single-gene disorders. These methods both rely on removing a cell or cells from the developing embryo and then analyzing genetic information from this sample that influences decisions regarding which embryos would be optimal for subsequent transfer.

In 2007, the first preimplantation genetic diagnosis for aneuploidy cases with use of microarray technology was introduced (1–3). Microarray technology provides the ability to evaluate every chromosome for aneuploidy detection and also can identify subtle chromosome deletions and duplications along with copy number variations (1, 4, 5). Existing genetic studies on spontaneous abortions indicate that aneuploidy can explain most instances of failed pregnancy (6). Emerging data indicate that preimplantation genetic diagnosis for 23 chromosomes with use of comparative genomic hybridization on metaphase chromosomes or 23-chromosome microarray technology could offer a benefit to certain women at high risk for an aneuploid pregnancy (5, 7).

Preimplantation genetic diagnosis for single-gene mutations diagnoses specific genetic mutations that are documented in the parents

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FIGURE 1

Microarray and single gene diagnosis for corresponding embryos.

	Embryo #1	Embryo #2	Embryo #3	Embryo #4	Embryo #5	Embryo #6	Embryo #7	Embryo #8	Embryo #9	Embryo #10
SNP Microarray	46, XX	46, XY	48, XX, +8, +20	46, XX. +16, -18	46, XX	46, XX	46, XX	45, XX, -21	46, XY	46, XY
Single Gene	Carrier R201C	Carrier R59C	Carrier R59C	Normal	Affected	Affected	Carrier R201C	Normal	Carrier R59C	Normal

Brezina. Single-gene testing with microarray. Fertil Steril 2011.

and segregating within their extended family. This is accomplished with use of polymerase chain reaction (PCR) to amplify the DNA of the chromosome where the gene of interest resides. Deoxyribonucleic acid sequencing then identifies the specific gene sequence, and linkage analysis identifies surrounding markers used to determine recombination and whether the DNA of the sperm and oocyte amplified (8, 9). A 2008 survey showed that 74% of IVF clinics in the United States offer some form of preimplantation genetic diagnosis for single-gene aberrations (10).

Preimplantation genetic diagnosis for aneuploidy and preimplantation genetic diagnosis for single-gene disorders, however, have been used in isolation of each other. A Medline literature review did not reveal an example of preimplantation genetic diagnosis for 23-chromosome aneuploidy and single-gene testing being used in conjunction in the same patient. Historically, the principal limitation to the concurrent use of these technologies has been the small amount of DNA that is obtained from a single cell or cells from a day 3 embryo or day 5 or 6 blastocysts. Recent investigation into using the trophectoderm from day 5 embryos, however, is promising. With use of trophectoderm biopsy, two to five cells are obtained that permit sufficient starting DNA material to apply whole genome amplification for 23-chromosome single nucleotide polymorphism (SNP) microarrays and single-gene mutation analysis. This case describes the use of single-gene testing combined with 23-chromosome SNP microarray preimplantation genetic diagnosis for aneuploidy in a carefully selected patient.

CASE REPORT

A 36-year-old woman, G2P1011, and her husband both were documented to be carriers for GM_1 gangliosidosis, an autosomal recessive lysosomal storage disorder, after the diagnosis of the disease in their child (11). Specifically, the patient was a carrier for the R201C mutation and the husband a carrier for the R59C mutation, both housed within the GLB1 gene. The couple also recently had had a first-trimester spontaneous abortion most likely caused by aneuploidy. An exhaustive genetic workup had been performed previously that had found these mutations and documented no other genetic problems that could be detected in either parent. After extensive counseling of available testing options, the couple elected, following signed informed consent, to proceed with preimplantation genetic diagnosis for aneuploidy with use of 23-chromosome SNP microarrays coupled with single-gene testing preimplantation genetic diagnosis for GM_1 gangliosidosis.

MATERIALS AND METHODS

In Vitro Fertilization and Embryo Biopsy

Standard methods of controlled ovarian stimulation with use of FSH coupled with hMGs and GnRH agonist trigger with uncomplicated retrieval were performed. Fourteen oocytes were retrieved, and intracytoplasmic sperm injection then was used to fertilize all of the oocytes. Ten embryos with two pronuclei then were cultured to the blastocyst stage. On the morning of day 5, blastocyst development was assessed. Ten blastocysts underwent biopsy of herniating trophectoderm cells with use of a Zilos laser (Hamilton Thorne Biosciences Inc., Beverly, MA). After this biopsy, all blastocysts were cryopreserved with use of vitrification. Two or three cells were placed in 5 μL of DNA stabilizing buffer and sent to the Center for Preimplantation Genetics for preimplantation genetic diagnosis testing.

Deoxyribonucleic Acid Amplification

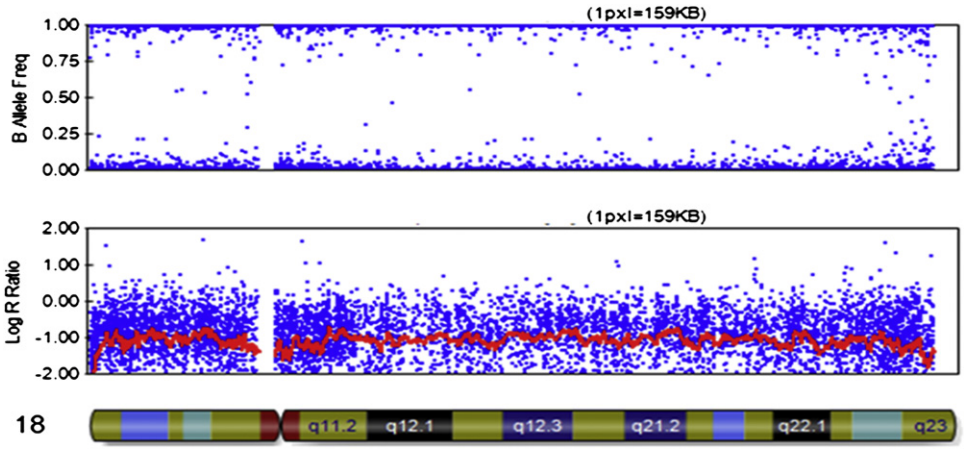
The trophectoderm cells from each embryo then underwent a modified whole genome amplification protocol. To accomplish this, the cells first were lysed with use of a denaturation buffer taking care not to shear the DNA from lysed cells, which could result in breaks in the genomic DNA. In this manner, "allele dropout" errors were minimized. A modified multiple displacement amplification protocol then was used with *phi* 29DNA polymerase to complete the first round of DNA amplification. After the first round of amplification, an aliquot of DNA from each embryo was sent to Reproductive Genetics Institute in Chicago for single-gene testing. Four microliters (200 ng) of the remaining multiple displacement amplified DNA then underwent another round of DNA amplification with use of a modified whole genome amplification protocol. These amplified DNA samples from each embryo then were used for 23-chromosome SNP microarrays.

Twenty-three–chromosome Single Nucleotide Polymorphism Microarray

Single nucleotide polymorphism microarray was performed on the DNA amplified as described earlier. The DNA samples were analyzed with use of the Illumina Infinium high-density HumanCytoSNP-12 DNA analysis BeadChips (Illumina Inc., San Diego, CA). All cytochips were scanned with use of an Illumina iScan BeadArray reader. Bioinformatics was accomplished with use of Illumina GenomeStudio

FIGURE 2

A karyotypic sample reading using 23-chromosome SNP microarray from an embryo that demonstrated monosomy of chromosome 8. Freq = frequency.



Brezina. Single-gene testing with microarray. Fertil Steril 2011.

software. Data from the Illumina system resulted and were interpreted to establish euploidy or aneuploidy for each embryo, and a molecular karyotype was reported out.

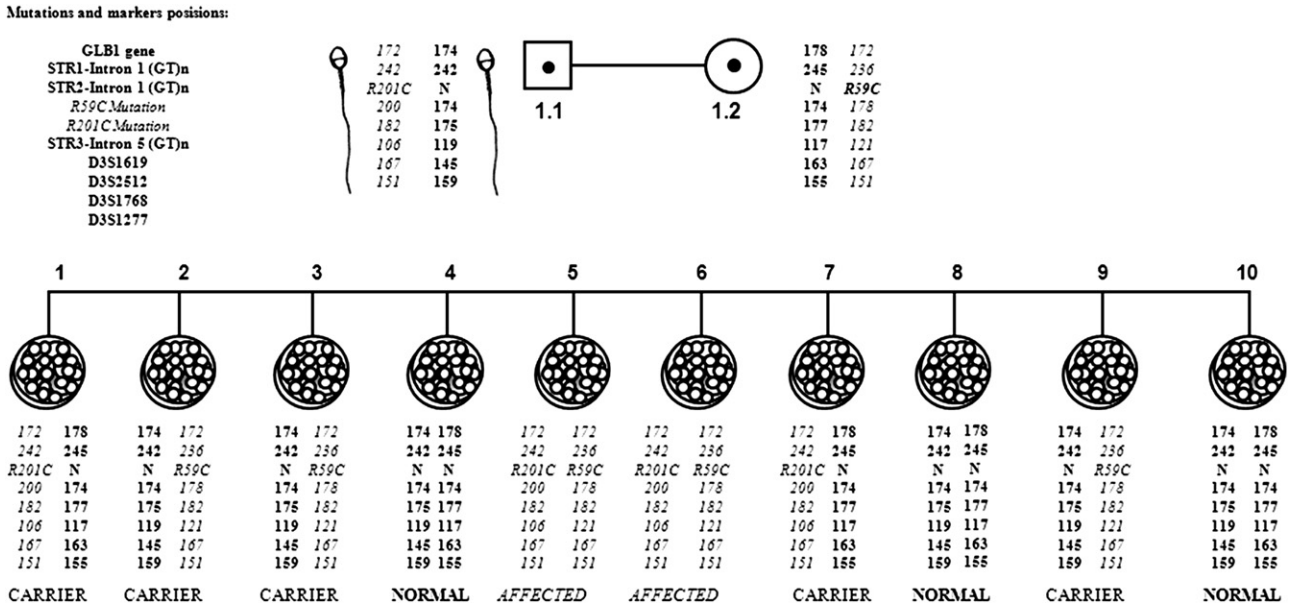
Polymerase Chain Reaction Testing for Single Gene (GM_1 Gangliosidosis)

Specific mutations evaluated were the GM_1 gangliosidosis R201C and R59C within the GLB1 gene along with polymorphic markers along chromosome 3. A total of 20 μL of modifed displacement amplified product was used as a template for mutations and linkage analysis by PCR. Template, 1.5 μL, was transferred in freshly prepared PCR mixture containing either fluorescently labeled primers (seven markers) for linkage study or unlabeled primers for mutation detection (two separate reactions). After 30 cycles of amplification the samples were studied by fluorescent capillary electrophoresis or by restriction digestion with specific enzymes.

The paternal R201C mutation was predicted on the basis of the absence of restriction site for *MwoI* enzyme. Paternal haplotypes were established before the case by single-sperm genotyping. Mismatched primers were designed to detect the maternal R59C

FIGURE 3

Preimplantation genetic diagnosis by blastocyst analysis for mutations *R59C* and *R201C* in GLB1 gene and linked markers.



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mutation by introduction of *MspI* restriction site in normal sequence. Maternal haplotypes were established in the process of embryo testing. After whole genome amplification and multiple displacement amplification, efficiency for both tested mutations in GLB1 gene and linked short tandem repeats was 100%.

RESULTS

After embryo biopsy, the 23-chromosome microarray testing and the single-gene analysis for GM_1 gangliosidosis were completed. Of the 10 embryos evaluated, SNP microarray found seven that were euploid and three aneuploid (Figs. 1 and 2). The DNA sequencing for a single gene (GM_1 gangliosidosis) resulted in the determination that two of the embryos were affected with GM_1 gangliosidosis, five embryos were mutation carriers, and three embryos were normal (Figs. 1 and 3). By combining these sets of data, five embryos were found to be suitable and were recommended for ET. After an elective single ET, an appropriate initial rise in β-hCG was noted followed by ultrasound confirmation of a normal-appearing single intrauterine pregnancy with fetal cardiac activity noted by 6 weeks gestation. Prenatal care then was transferred to an obstetrician who is following the remainder of the pregnancy.

DISCUSSION

The use of 23-chromosome SNP microarrays for preimplantation genetic diagnosis for aneuploidy coupled with DNA sequencing for single-gene disorders is a novel approach to maximizing pregnancy outcomes in selected patients. The use of these techniques concurrently is made possible by the optimal DNA amplification protocol employed. Definitive studies establishing the genetic correlation between the inner cell mass and the trophectoderm are lacking. However, there are promising studies that display excellent preimplantation genetic diagnosis for aneuploidy results with use of trophectoderm samples with microarray technology (7).

This case illustrates the practical applications possible with use of trophectoderm biopsy coupled with the continually improving genetic technologies available within the arena of ART. Although further investigation is warranted, preimplantation genetic diagnosis for aneuploidy and single-gene testing seem destined to be used increasingly as meaningful adjuncts to optimizing ultimate pregnancy success.

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