CASE REPORT

Successful application of preimplantation genetic diagnosis for Leigh syndrome

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Objective: To perform preimplantation genetic diagnosis (PGD) for a *SURF1* gene mutation of the Leigh syndrome to transfer unaffected or carrier embryo/embryos.
Design: Case report.
Setting: Clinical IVF laboratory.
Patient(s): A couple carrying an nt769 G/A mutation that is associated with Leigh syndrome.
Intervention(s): Oocytes were fertilized by means of intracytoplasmic sperm injection. The resulting embryos were biopsied 3 days after fertilization. One blastomere was taken and whole-genome amplification was performed. Amplification of the mutation site was achieved by polymerase chain reaction (PCR) and restriction digestion was completed. Gel Imager was used to measure the digests of normal and mutant load.
Main Outcome Measure(s): Embryo testing by means of PGD-PCR and pregnancy. Successful preimplantation genetic diagnosis for a *SURF1* gene mutation and transfer of healthy or carrier embryos.
Result(s): Successful singleton pregnancy resulting in the delivery of healthy baby girl.
Conclusion(s): We report the first case of successful PGD for Leigh syndrome resulting in delivery of a healthy newborn. (Fertil Steril® 2008;90:2017.e11–e13. ©2008 by American Society for Reproductive Medicine.)

Key Words: PGD, Leigh syndrome, IVF, PCR

Leigh syndrome, also known as subacute necrotising encephalomyelopathy, is the most common form of cytochrome c oxidase (COX) disorders and is one of the most frequently occurring respiratory chain defects during infancy and childhood (1). Affected patients experience delayed onset of symptoms such as hypotonia, feeding difficulties, motor regression, failure to thrive, and other neuromuscular symptoms involving independent organ systems (2, 3). The main laboratory findings are raised lactate levels in the blood and cerebrospinal fluid (hyperlactatemia).

Leigh syndrome is genetically a heterogenous disease, which may occur due to a number of different defects in mitochondrial energy metabolism, especially due to the defects in the enzymes normally involved in the respiratory chain. Cytochrome c oxidase deficiency is an autosomal recessive trait, and most patients belong to a single genetic complementation group.

There are at least five known enzymes associated with Leigh syndrome, including pyruvate dehydrogenase complex (PDHC), respiratory chain complex I, complex II, complex IV (COX), and complex V (adenosine triphosphatase) deficiencies. One of the most common enzymatic defect is the COX deficiency (4). Leigh syndrome can result from the inheritance of mutations in either nuclear or mitochondrial DNA (2, 3). The genetic defect responsible for the Leigh syndrome and COX deficiency is usually a mutation in the *SURF1* gene, which encodes a cytochrome oxidase assembly factor (5–7). This gene maps to chromosome 9q34, consists of nine exons, and encodes a protein of 300 amino acids. Most of the mutations on *SURF1* gene were characterized as nonsense mutations, including the formation of a premature stop codon. Missense and splicing site mutations are also, but less frequently, detected (6, 8).

Preimplantation genetic diagnosis (PGD) is an early form of prenatal diagnosis in which genetic testing is performed on one blastomere from 3-day-old embryos obtained after IVF (9). Only those embryos proving to be unaffected at the end of genetic investigation are transfered back to the mother.

Couples opting for PGD to avoid the transmission of a genetic disease have to undergo an IVF treatment with ovarian stimulation and intracytoplasmic sperm injection (ICSI). As for monogenic diseases, polymerase chain reaction (PCR) protocols are applied at the single-cell level.

Received February 7, 2008; revised June 9, 2008; accepted July 9, 2008.
E.Ü. has nothing to disclose. Y.A. has nothing to disclose. Ö.Ü. has nothing to disclose. A.B. has nothing to disclose. S.Ö. has nothing to disclose. F.T. has nothing to disclose. V.B. has nothing to disclose.
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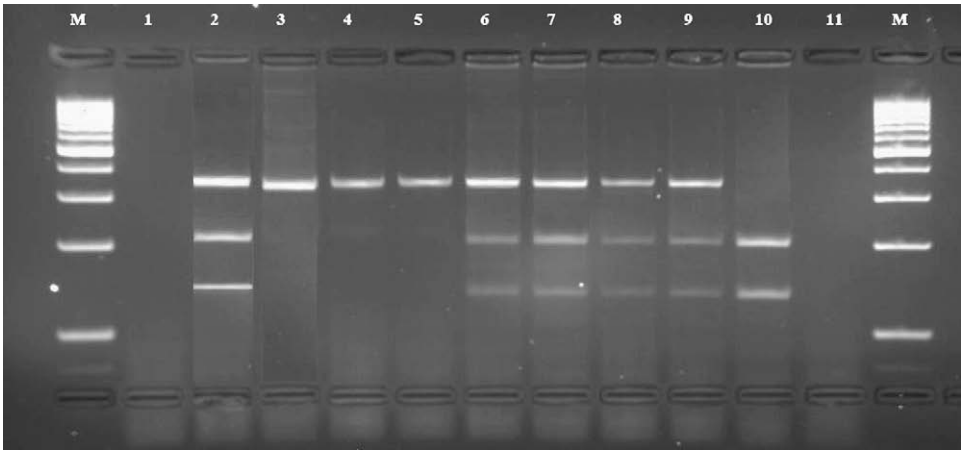
doi:10.1016/j.fertnstert.2008.07.023

FIGURE 1

PCR results. nt769 G/A mutation in the *SURF1* gene was tested.
M = marker; 1 = unevaluated; 2 = heterozygous; 3 = mutant; 4 = mutant; 5 = mutant; 6 = heterozygous; 7 = heterozygous embryos; 8 = heterozygous mother; 9 = heterozygous father; 10 = normal DNA; 11 = negative control.



Ünsal. PGD for Leigh syndrome. Fertil Steril 2008.

This case report presents the first clinical experience demonstrating the preimplantation genetic diagnosis of Leigh syndrome, which resulted from a point mutation in *SURF1* gene.

CASE REPORT

We report a case of a 42-year-old woman with a history of loss of two children who died at the ages of 5 and 7 years because of Leigh syndrome. Being unaffected carriers of nt769 G/A mutation in the *SURF1* gene, the parents requested IVF treatment together with PGD for Leigh syndrome. The woman was administrered an ovulation stimulation protocol (10). In her IVF cycle, 12 cumulus-oocyte complexes were retrieved, seven M2 oocytes were microinjected, and seven embryos were obtained. Preimplantation genetic diagnosis was performed by means of following micromanipulation procedure to biopsy single blastomeres from seven embryos on their third cleaving day (11).

Blastomeres were tested for nt769 G/A mutation in the *SURF1* gene by means of polyacrylamide gel analysis of PCR product digested with *PpuMI* (NEB, Beverly, Massachusetts) restriction enzyme.

Whole-genome amplification was applied for each biopsied single cell by means of using Repli-G mini kit (Qiagen). Amplification of the mutation site in exon 8 was achieved using 5′-GCAGCAACTCAGCAAAGAAC-3′ for the forward primer and 5′-AAGCAAGCCAGCATTAGCAG-3′ for the reverse primer. The resulting 325-bp PCR product was digested with *PpuMI* (NEB). The digest for each PCR product was then analyzed on %2 w/v agarose gel. *PpuMI* site was not observed and was considered to be lost in mutated (nt769 G/A) DNA. The normal and mutant load were measured by the Gel Imager (ImageMaster VDS, Pharmacia, U.K.).

According to gel results, three embryos were found to be heterozygous and three mutant (Fig. 1). One embryo could not be evaluated. The couple signed a consent form approving replacement of 3 carrier embryos.

A singleton pregnancy was confirmed at 7-week gestation by ultrasonography. After an uneventful pregnancy period, a carrier baby girl was born.

DISCUSSION

More and more single-gene disorders have been diagnosed by means of PGD. The number of diseases diagnosed is expanding rapidly, as revealed by the incoming information on the positive correlation between gene sequencing and corresponding disorders in cases of mutations.

This report describes a case where PGD was performed for a couple who were carriers of nt769 G/A mutation associated with Leigh syndrome. This is the first successful PGD for Leigh syndrome caused by mutation in the SURF1 gene, with the subsequent delivery of a heterozygous baby; the literature we searched revealed not one single such result. The analysis of single-gene disorders has always required PCR, despite the complexity of the amplifications. Working on single cells to detect gene disorders introduces some restrictions, such as amplification failure and allele dropout. The development of PGD-PCR protocols can technically be very demanding, because a single blastomere has a very small DNA content. This fact necessitates a large number of PCR cycles to pinpoint and observe the mutation. In

this case, we preferred to perform whole genome amplification before PCR in an effort to increase the minute amounts of genetic material. As a result of PCR procedures, 6 of 7 embryos could be evaluated and diagnosed for Leigh syndrome.

To date, more than 30 different pathogenic mutations have been described in SURF1 (12). Having scanned the literature, we found no reference to the topic of PGD for Leigh syndrome or nt769G/A mutation. Therefore, we strongly believe that the present case report will contribute significantly to prenatal and preimplantation analysis of Leigh syndrome.

CONCLUSIONS

Preimplantation genetic diagnosis for a nt769 G/A mutation in *SURF1* gene was successfully carried out for this couple. This case provides hope to other couples at a similar status to have healthy-born heterozygous babies, with no single-gene disorders being transmitted. It can be concluded that more carrier couples could be detected who may benefit from PGD for a *SURF1* gene mutation.

Leigh syndrome is one of these genetically heritable diseases that can be diagnosed in the preimplantation period. Approximately 200 single-cell diseases have been diagnosed via PGD-PCR (D. Wells, personal communication). Preimplantation genetic diagnosis could detect single gene disorder even for most rare genetic conditions, if the gene and/or gene changes are known in advance. Therefore, the number of diagnosed genetic disorders which constitute rare disorders will also increase.

As moleculer genetics and associated technologies advance, the PGD-PCR strategies will grow simpler and more accurate. This will lead to a significant increase in the number of disorders diagnosed. Effectively, PGD will be more widely and frequently administered and will offer benefits to many couples who are at risk of transmitting an inherited disease to their offspring.

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