Preimplantation genetic diagnosis for Donohue syndrome (Leprechaunism): case report

Donohue sendromunda (Leprechaunism) preimplantasyon genetik tanı: olgu sunumu

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Abstract

Leprechaunism is an inherited insulin resistance syndrome,caused by homozygous or compound -heterozygous mutations in the insulin receptor gene. Preimplantation genetic diagnosis (PGD) is an early form of prenatal diagnosis. Donohue syndrome is a very rare but fatal genetic disorder .A family with four children were diagnosed with Donohue syndrome, three of them were born at term and lost around 5 months of age,and one was diagnosed by amniocentesis and terminated by abortion. By PGT we obtained a healthy baby.Our aim is to report the first case of Donohue syndrome diagnosed after performance of preimplantation genetic diagnosis (PGD) in Turkey.

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Key words: Donohue syndrome, preimplantation genetic diagnosis, pregnancy

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Özet

Leprechaunism insülin reseptör geninin homozigot ya da heterozigot mutasyonuna bağlı gelişen herediter bir insülin rezistans sendromudur. Donohue sendromu, çok nadir görülen ancak öldürücü olan genetik bir hastalıktır. Bu olguyu sunmamızın amacı tek gen hastalıklarında preimplantasyon genetik tanının (PGT) önemini bir kez daha ortaya koymaktır.PGT prenatal tanı koymanın en erken şeklidir. Daha önce dört kez spontan olarak hamile kalan, doğumdan sonraki 5 ay içerisinde ilk üç bebeğini kaybeden ve son gebeliğinde de amniosentezle tanı konarak termine edilen bir gebelik öyküsü olan ailenin preimplantasyon genetik tanı sayesinde sağlıklı bir çocuğu olmuştur. Bu vaka Türkiye'de Donohue Sendromunda PGT ile sağlıklı çocuğun elde edildiği ilk olgudur. (J Turkish-German Gynecol Assoc 2009; 10: 122-3)

Anahtar kelimeler: Donohue sendromu, preimplantasyon genetik tanı, gebelik

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Introduction

Leprechaunism is an inherited insulin resistance syndrome, caused by homozygous or compound -heterozygous mutations in the insulin receptor gene (1, 2). It is a very rare disorder inherited as an autosomal recessive genetic trait (3). It is characterized by intrauterine growth retardation, fasting hypoglycemia, postprandial hyperglycemia, lack of subcutaneus fat, decreased muscle mass and other phenotypic and hormonal changes (4, 5). The physical features most often associated with this condition include hypertrichosis, pachyderma, acanthosis nigricans, prominent genitalia, and elfin-like facial characteristics of prominent eyes, wide nostrils, thick lips, and large, low set ears (6, 7). Preimplantation genetic diagnosis is an early form of prenatal diagnosis. The genetic diagnosis is usually rendered from a single cell biopsied from cleavage -stage embryos with 6-10 cells. In PGD , only unaffected embryos for the targetted disease are transferred to establish pregnancies (8, 9). Preimplantation genetic diagnosis avoids the difficult decision concerning pregnancy termination. For single gene disorders, PGD can be applied to any disease with known mutations.

Case

A Turkish family with known Donohue syndrome was referred from the southern part of Turkey (Kahramanmaraş) for preimplantation genetic diagnosis of the disease. The woman was 28 years old, and the man was 27 years old. They had been married for eight years and were cousins. They had four children diagnosed with Donohue syndrome. Three of them were born at term and died around five months of age. The fourth was diagnosed at the 18 th weeks of gestation by amniocentesis and terminated. The mother and three of the babies were homozygote. One of the babies was compound heterozygote. The couple agreed to undergo a PGD-ICSI procedure to ensure a healthy offspring.

Before IVF, sperm evaluation as well as hormone analyses (FSH,LH,17β -Estradiol,PRL and TSH), hepatitis markers and complete blood test were carried out in the woman . The ovaries were grade III at vaginal ultrasonography. After controlled ovulation induction by multi-dosage flexible antagonist protocol, 16 oocytes were recovered by means of transvaginal follicular punction ,and 15 oocytes were in metaphase II. In total, 12 were fertilized, and all of them were biopsied suc-

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cessfully on day 3 of culture. Only one blastomere was biopsied from each embryo. Two embryos had failure of amplification. Of the remaining embryos, seven were affected. There were only three genotypically normal embryos . Three embryos were given at day 4. 12 days after embryo transfer, the pregnancy test was positive. We examined the INSR gene exon 3 del CAA mutation by single cell PCR and fragmentation analysis (10). Briefly, genomic DNA was extracted from 200μl of peripheral blood in EDTA according to the phenol chloroform procedure. PCR amplification of each region of interest was performed using the outer nucleotide primers. Mutation analysis was carried out by direct sequencing of PCR products using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA), according to the protocol provided by the manufacturer. The cells were lysed by incubation at 65°C for 10 min. The alkaline lysis buffer was then neutralized by the addition of 5 μl of neutralization buffer (900 mmol/l Tris–HCl, 300 mmol/l KCl, 200 mmol/l HCl) before proceeding to PCR. The PCR strategy consisted of initial multiplex external amplification followed by nested PCR specific for each region involving mutations. After cell lysis and neutralization, 1.5 mmol/l $MgCl_2$, 200μmol/l of each dNTP (Roche Diagnostic, Italy), 2.5 IU Ampli Taq Polymerase (Applied Biosystems), 10 pmol of each outer primer, were added to each tube, making a total volume of 50 μl. The first round of PCR involved a 96°C denaturation temperature in the first 10 cycles as a means to reduce ADO (11), followed by a subsequent denaturation temperature of 94°C in 25 remaining cycles. Each round of PCR was preceded by an initial 4 min denaturation step at 94°C and followed by a final extension step of 10 min at 72°C. Specific primers used which were designed by Fiorentino. For the second round of DNA amplification, 2 μl of the primary PCR reaction product were added to another tube containing 5 μl of 10xPCR Buffer II (500 mmol/l KCl, 100 mmol/l Tris HCl, pH 8.3; Applied Biosystems), 1.5 mmol/l MgCl2, 200 μmol/l of each dNTP (Roche Diagnostic), 2.5 IU AmpliTaqPolymerase (Applied Biosystems), 10 pmol of each inner primer, in a total volume of 50 μl, and the tubes were cycled as above on a GeneAmp® PCR System 9700 (Applied Biosystems). To avoid participation in the subsequent primer-extension reaction, primers and unincorporated dNTP were removed from PCR products by performing Microcon 100 (Amicon, USA) purification, according to the manufacturer's protocol. Mutation analysis was carried out by using minisequencing. The minisequencing reaction was performed, starting from 10 ng of the same purified PCR product subjected to sequence analysis, using ABI Prism® SnaPshot Multiplex Kit (Applied Biosystems). The reaction volume was 10 μl, including 5 μl of Ready Reaction Premix and 10 pmol of each minisequencing primer. The reaction conditions were as follows: 25 PCR cycles, denaturation step of 10 s at 96°C, annealing for 10 s at 50°C and extension for 30 s at 60°C.

Discussion:

Preimplantation genetic diagnosis (PGD) is an option for couples who are at risk that enables them to have unaffected progeny without facing the risk of pregnancy termination after prenatal diagnosis as currently practiced. Despite its novelty, PGD has already become an alternative to traditional prenatal diagnosis, allowing establishment of only unaffected pregnancies, avoiding the risk for pregnancy termination. Indications for PGD have currently expanded beyond those practices in prenatal diagnosis, such as late-onset diseases with genetic predisposition, and preimplantation HLA typing with the purpose of establishing potential donor progeny for stem cell treatment of siblings, which makes PGD also an important complement to prenatal diagnosis. The fact that more than 1,000 apparently healthy unaffected children have been born after PGD suggests its accuracy, reliability, and safety(12).We preferred the preventive option by attempting PGD in this family for several reasons: (1) the severe nature of the syndrome, and lack of an effective treatment; (2) family suffering with previously lost children and a strong desire to have a healthy baby. In the present case, PGD was used successfully to help a couple to choose the healthy embryos. To the best of our knowledge, this is the first report of PGD for this devastating syndrome. Preimplantation genetic diagnosis is the best choice for the prevention of the disease in families affected by Donohue syndrome. We consider that the combination of the current ICSI and PGD techniques is a good option for preventing a disease that, up to date, has only experimental or merely palliative treatments. PGD is currently one of the practical options available for couples at-risk in order to avoid the birth of children with genetic and chromosomal disorders.

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