Birth of a healthy boy following preimplantation genetic diagnosis for congenital adrenal hyperplasia

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ABSTRACT

Classical 3β-HSD deficiency due to mutations in the HSD3B2 gene is responsible for a rare form of congenital adrenal hyperplasia (CAH) and is identified by varying degrees of salt wasting. Preimplantation genetic diagnosis (PGD) was performed in a couple carrying mutation c.690 G>A in the HSD3B2 gene. Four polymorphic short tandem repeat markers closely linked to the HSD3B2 gene were used in embryo genotyping. Two CODIS STRs (VWA and THO1) were also used to confirm embryo zygosity and rule out possible contaminations. Finally, SRY and AMYLOGENIN markers were used for embryo sex determination. PGD was performed by fluorescent multiplex seminested polymerase chain reaction and sequencing. Six embryos were tested and one male carrier embryo was transferred, resulting in the birth of a healthy boy.

Keywords: molecular PGD, monogenic disease, preimplantation genetic diagnosis, congenital adrenal hyperplasia (CAH)

INTRODUCTION

Congenital adrenal hyperplasia (CAH) comprises a group of several autosomal recessive disorders stemmed from the deficiency of one of five enzymes mediating the biochemical steps in steroidogenesis, which include the production of mineralocorticoids, glucocorticoids, or sex steroids from cholesterol by the adrenal glands (Aceto et al., 1966; Speiser & White, 2003). Males and females are equally at risk for these disorders (Aceto et al., 1966).

In humans, there are two forms of 3β-hydroxysteroid dehydrogenase enzyme (3β-HSD): type I and type II with 93.5% homology. 3β-HSD enzyme type II (HSD3B2) is responsible for the conversion of Δ5 (delta 5) to Δ4 (delta 4) steroids, and is almost exclusively expressed in the gonads and adrenal cortex. Type I (HSD3B1) is mainly expressed in the mammary gland, placenta, and skin. Both 3β-HSD genes are located on chromosome 1p13. Enzyme HSD3B2 catalyzes the biosynthesis of progesterone, which is the precursor for aldosterone, and 17α-hydroxyprogesterone, the precursor for cortisol in the adrenal cortex and androstenedione, testosterone, and estrogen in the adrenal cortex and gonads (Rheaume et al., 1991; Simard et al., 1995). 3β-HSD deficiency is categorized into classical and nonclassical forms (Simard et al., 2002).

Classical 3β-HSD deficiency due to mutations in the HSD3B2 gene is responsible for a rare form of CAH identified by varying degrees of salt wasting (SW). Accordingly, the classical form leads to impaired cortisol synthesis and salt-wasting in its most severe form (Bongiovanni et al., 1967; Simard et al., 1995). Preimplantation genetic diagnosis (PGD) has been introduced as an alternative to prenatal diagnosis and termination of pregnancy in couples at high risk of transmitting single gene disorders to their offspring.

The goal of PGD is to detect a particular genetic disease on oocytes or embryos obtained through assisted reproductive technologies (ART) before clinical pregnancy is achieved. This is done by selecting and transferring unaffected embryos to the uterus following direct/indirect mutation analysis. In the present case, linkage analysis was performed using polymorphic short tandem repeat (STR) markers closely linked to the mutated HSD3B2 gene to prepare a further proof of genotyping (indirect analysis) through Sanger sequencing (direct detection) and evaluation for possible allele drop out (ADO). Contamination of exogenous DNA was also evaluated with CODIS-STR markers with different (Fiorentino et al., 2010; Gutiérrez-Mateo et al., 2009).

This case report emphasizes the successful application of PGD for CAH, which resulted in the birth of a healthy boy. To our knowledge, this is the first report of a child born free of CAH after PGD in Iran.

CASE DESCRIPTION

The couple, a 35-year-old woman and a 39-year-old man, was referred to the Royan Institute for genetic counseling. They had had a miscarriage, a dead son to CAH, and a 9-year-old affected girl before coming to our clinic (Fig. 1). Genetic assessment revealed that the couple were heterozygote for the c.690G>A mutation of the HSD3B2 gene and did not manifest the disease. The couple wished to have an unaffected child and underwent ART with PGD. They also expressed the wish to have a baby boy.

MATERIAL AND METHODS

Controlled ovarian stimulation was performed via the long protocol of down-regulation with a gonadotropin-releasing hormone agonist and recombinant FSH for 12 days. Seven oocytes were retrieved and six cleaving embryos were available for biopsy on day 3. Blastomeres were lysed for 10 min at 65°C in lysis buffer (DTT 50mM, KOH 200mM) and then analyzed using our in-house PCR protocol.
Preliminary genetic evaluation (Pre-PGD) was performed in order to identify the pattern of inheritance of the affected alleles by determining the presence of the mutation and related patterns of STR markers in the couple’s parents. The PGD protocol was performed according to the ESHRE PGD consortium best practice guidelines for amplification-based PGD (Harton et al., 2010). In the first step, 4 ml of peripheral EDTA blood was taken from the couple and their parents. Then, the presence of the mutation was detected by PCR followed by Sanger sequencing. In addition, the pattern of three selected STR markers closely linked to the HSD3B2 gene was evaluated in the couples and their parents to enable linkage analysis and reveal the associated mutational inheritance. These markers were chosen based on their polymorphism and distance from the HSD3B2 gene. CODIS STRs and sex selection markers were also designed for further use. In the final step of PGD, semi-nested multiplex PCR was performed using the outer and inner primers listed in Table 1.

The primer sequences of CODIS STRs used in the test are listed in Table 2. First-round multiplex PCR using the external primers was performed in a total volume of 50 µl containing 1.5 µl dNTP (10 mM), 5 µl 10x Buffer (MgCl2, 50 mM), 10 µl QS, 0.3 µl Taq), 10 µl Template, 0.2 µl (20 pmol) of each primer and 22.5 µl DW. PCR condition for the first round was 94°C for 3 min, 35 cycles at 94°C for 30'', 60°C for 30'', 72°C for 30'' and 72 °C for 7 min, carried out in an ABI 9700 machine. Direct mutation was analyzed by Sanger sequencing on an ABI 3130 sequencer. The PCR menu was as follows: total volume 20 µl in 5 µl 5X Buffer, 1 µl Big dye, 0.5 µl Primer, 3 µl PCR product and 12.5 µl DW.

The PCR program for the sequencing reaction was as follows: 96°C for 1 min, 25 cycles of 96°C 10s, 50°C 5s, 60°C 4 min, and final incubation at 4°C for 7min. The PCR products of the first round were used as templates for the second round of PCR with a total volume of 20 µl, while the conditions for the second round were the same as in the first round. Finally, for direct mutation analysis the amplified inner products were electrophoresed in an automated genetic analyzer 3130 (Applied Biosystems). The results were analyzed on the Gene Mapper software (Applied Biosystems).

For linkage analysis and sex selection, the amplified PCR products were electrophoresed on 12% acrylamide gel and stained with silver nitrate.

**RESULTS**

Three STR markers including D1S185, D1S534, D1S514 and two CODIS STRs of VWA and THO1 were used. SRY and AMYLOGENIN were also used as sex selection markers. The couple had six day-3 embryos. The rate of ADO and contamination were 0%. The biopsy specimen from one of the embryos did not yield amplification, possibly due to the absence of cells. Additional tests confirmed wild type homozygosity in two (healthy), heterozygosity in two (carrier status), and mutant homozygosity (affected status) in one of the embryos (Fig. 2). One heterozygote male embryo was transferred to the mother’s uterus and a singleton pregnancy was achieved. Prenatal diagnosis following amniocentesis on the 15th week of gestation confirmed the PGD results and the pregnancy resulted in the birth of a carrier boy.

**DISCUSSION**

Preimplantation genetic diagnosis has allowed the identification of various forms of genetic disorders from single embryo cells. PGD offers an authentic reproductive alternative to prenatal diagnosis (PND) and prevents the termination of pregnancies due to fetal affliction resulting from parental genetic disorders. PGD utilizes two strategies to find single genes abnormalities: direct and indirect mutation diagnosis. Although the direct approach is the gold standard in the identification of affected fetuses in PND, the possibility of losing one allele during PCR makes the indirect approach (linkage analysis) a good alternative to improve the validity of PGD results (Alberola et al., 2009; Fiorentino et al., 2006). For this reason, we used four polymorphic STR markers tied to the mutated gene to provide evidence of ADO (Fiorentino et al., 2010; Gutiérrez-Mateo et al., 2009).

Steroid 3β-HSD defect is a rare cause of CAH, and is usually detected during the first few months of neonatal life. The diagnosis of this defect is based primarily on increased levels of Δ5 steroid hormones before and after an adrenocorticotropic hormone (ACTH) stimulation test subsequently confirmed by urinalysis (Moisan et al., 1999).

Prenatal genetic counseling is recommended for all families affected by CAH. In such an autosomal recessive disorder, one of 6 embryos will be affected when the two parents are carriers (Day et al., 1996; Speiser et al., 1992). We demonstrated a successful application of blastocyst biopsy, Sanger sequencing, and multiplex PCR for PGD of CAH, in conjunction with sex selection.
In conclusion, multiplex semi-nested PCR was successfully used for the preimplantation genetic diagnosis of CAH, resulting in the birth of a disease-free healthy boy.

**Ethics approval and consent to participate**

The Ethic Review Board at Royan Institute approved this study. The study was carried out in compliance with the Helsinki Declaration. The participants gave written consent before joining the study.

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**CONFLICT OF INTERESTS**

The authors have no financial conflict of interest to declare.

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