A healthy delivery of twins by assisted reproduction followed by preimplantation genetic screening in a woman with X-linked dominant incontinentia pigmenti

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The purpose of this study is to report a successful twin pregnancy and delivery in a female patient with X-linked dominant incontinentia pigmenti (IP) who underwent assisted reproductive technology followed by preimplantation genetic screening (PGS). A 29-year-old female with IP had a previous history of recurrent spontaneous abortion. A molecular analysis revealed the patient had a de novo mutation, 1308_1309insCCCCTTG(p.Ala438ProfsTer26), in the inhibitor of the kappa B kinase gamma gene located in the Xq28 region. IVF/ICSI and PGS was performed, in which male embryos were sexed using array-based comparative genomic hybridization (aCGH). After IVF/ICSI and PGS using aCGH on seven embryos, two euploid male blastocysts were transferred with a 50% probability of a viable male pregnancy. The dizygotic twin pregnancy was confirmed and the amniocentesis results of each twin were normal with regard to the mutation found in the mother. The patient delivered healthy twin babies during the 37th week of gestation. This case shows the beneficial role of PGS in achieving a successful pregnancy through euploid male embryo gender selection in a woman with X-linked dominant IP with a history of multiple male miscarriages.

Keywords: Assisted reproductive technology; Gene mutation; Preimplantation screening; Recurrent miscarriage; X chromosome

Introduction

Incontinentia pigmenti (IP), also known as Bloch-Sulzberger disease, is a rare X-linked dominant genodermatosis that affects varying parts of the body, including the dermatological, ocular, nervous, and immune systems, and is generally lethal in male fetuses [1,2]. IP is caused by mutations in the inhibitor of kappa B kinase gamma (IKB-KG) gene, also known as the nuclear factor-kappa B essential modulator gene, in the Xq28 region. The most common mutations (60%–80% of cases) involve a large-scale deletion of IKBKG exons 4 through 10 [3-5].

Though IP is related to recurrent spontaneous abortions in male fetal pregnancies, there have been few reports of attempts to achieve successful pregnancies in patients with this condition through assisted reproductive technology and genetic testing techniques. In the
past, patients with IP underwent in vitro fertilization and male embryo selection using preimplantation genetic diagnosis (PGD) with fluorescent in situ hybridization (FISH), resulting in either a normal male pregnancy or early miscarriage in the case of affected male fetuses while excluding possible female carriers [6-8]. However, no cases of successful childbirth resulting from this approach have yet been reported. Recent case reports of PGD in patients with IP have included molecular analyses of the IKBKG gene through polar body biopsies, but this method does not involve a comprehensive genomic analysis [9,10].

We report a successful pregnancy and delivery in a female patient with IP who underwent in vitro fertilization/intracytoplasmic sperm injection and preimplantation genetic screening (PGS) using array-based comparative genomic hybridization (aCGH) followed by a prenatal diagnosis.

Case report

A 29-year-old patient with known IP was referred to Fertility Center of CHA Gangnam Medical Center for fertility therapy. She had manifested skin erythema followed by vesicles as a neonate. Patchy hyperpigmented skin lesions subsequently appeared in multiple sites. At the time of her first visit to our institution, she had only mild skin lesions involving scattered hyperpigmented spots in the abdomen and upper legs. She had experienced three early spontaneous pregnancy losses during three years of marriage. No anatomical, immunological, thrombophilic or endocrinological factors contributed to recurrent spontaneous abortions in this patient. She had been clinically diagnosed with IP by Landy and Donnai’s criteria [11] and by skin biopsy at ten years of age in the dermatology department of the referring hospital, with no family history. A cytogenetic analysis of this couple revealed normal karyotypes, but confirmatory molecular genetic analysis of IP had not been completed when assisted reproductive technology was applied. After genetic counseling, she was scheduled for an IVF cycle with PGS using aCGH to select euploid male embryos. Simultaneously, mutation screening was performed using polymerase chain reaction-direct sequencing to identify her pathogenic mutation, which is crucial for further genetic counseling and prenatal diagnosis. Genomic DNA was extracted from a blood sample. All exons and intron boundaries of the IKBKG gene were analyzed and a pathogenic mutation was identified in IKBKG exon 9 (1308_1309insCCCCTTG(p.Al438ProfsTer26)). A seven-base insertion of CCCCTTG at position c.1309 was identified, which had resulted in a frameshift in which the 438th amino acid was changed from alanine to proline and a premature stop codon occurred at the 464th codon (Figure 1).

"Figure 1. Polymerase chain reaction-direct sequencing in the IKBKG gene of the patient: a pathogenic mutation is present in IKBKG exon 9(1308_1309insCCCCTTG(p.Al438ProfsTer26)), in which the seven-base sequence CCCCTTG was inserted at position c.1309, resulting in a frameshift (the 438th amino acid changed from alanine to proline) and a premature stop codon at the 464th codon. NEMO, nuclear factor-kappa B (NF-κB) essential modulator; IKBKG, inhibitor of κB kinase gamma; PB1, first polar body."
A total of nine oocytes were retrieved after controlled ovarian hyperstimulation with gonadotropin-releasing hormone antagonist protocol (cetrorelix, Cetrotide, Merck Serono Europe Ltd., London, UK; 0.25 mg) using recombinant follicle-stimulating hormone (recombinant follitropin alfa; GONAL-f, Merck Serono S.p.A, Modugno, Italy, 225 IU daily), of which seven were fertilized using the intracytoplasmic sperm injection procedure. In each of the seven eight-cell stage embryos, a single blastomere was biopsied on day three and subjected to aCGH analysis by a commercial laboratory (MGMED Co., Seoul, Korea). The laboratory reported that there were two euploid male embryos, one euploid female embryo, and four aneuploid male embryos (–16, –8/+15, +20, +2/+3/+6/+11/–20) (Figure 2). Both of the euploid male embryos, which were grade two mid-blastocysts, were transferred on day five. After 12 days, serum beta human chorionic gonadotropin was 442.17 mIU/mL, increasing to 4,676 mIU/mL six days later. A clinical pregnancy was confirmed with two gestational sacs and twin fetuses showing viable heartbeats by ultrasonography at the sixth week of gestation. The patient underwent amniocentesis for a confirmatory prenatal diagnosis during the seventeenth week of pregnancy and both fetuses showed normal male karyotypes. They were shown to be normal for the mutation found in the mother through polymerase chain reaction-direct sequencing of the cultured amniocytes. This patient delivered healthy live twin babies during the 37th week of gestation with no obstetric or neonatal complications.

**Figure 2.** Array-based comparative genomic hybridization results of the seven embryos.

### Discussion

IP is a rare X-linked dominant genodermatosis with its incidence of 0.7–2/100,000 newborns [12-14], which is lethal in males in utero in 97% of cases [15]. As a result, many women with IP have recurrent early miscarriages [16]. Although IP is usually lethal in males, approximately 72 cases of surviving male fetuses with IP have so far been reported [17]. The IP Consortium has proposed three mechanisms resulting in the survival of males carrying a mutation in the **IKBKG** gene: hypomorphic alleles, the 47,XXY karyotype (Klinefelter syndrome), and somatic mosaicism [16]. IP presents multystemically but especially dermatologically, involving four typical stages of vesiculo-bulbous, verrucous, hyperpigmented, and hypopigmented skin. Diagnostic criteria for IP have traditionally been based on the clinical features established by Landy and Donnai [11]. Several researchers have recently proposed that these criteria should be updated to reflect a firmer molecular understanding of how the nuclear factor-κB pathway is affected by the **IKBKG** gene mutation [18,19]. Approximately 65% of those mutations occur de novo and 69 different mutations have been reported [5,20,21].

The patient in this study had a phenotypically very mild form of IP that only manifested dermatologically. Her family history, including her parents and one brother, showed no evidence of IP. She had a de novo insertion/deletion (indel) mutation causing a frameshift and a premature stop codon, which occurs in 10% of IP cases. Her mutation in **IKBKG** exon 9, 1308_1309insCCCCTTG(p.Ala438ProfsTer26), is the first reported genetic aberration affecting the **IKBKG** gene. This finding is analogous to other mutations, which have also been reported just once [21].

Many cases of recurrent spontaneous abortions in patients with IP have been reported, reflecting its X-linked dominant nature and lethality to male fetuses, resulting in the suggestion that PGD should be used for patients with IP [22]. However, only a few cases have been reported of PGD used in embryos from IP-affected females in infertile couples who underwent IVF procedures (Table 1) [6,8-10]. FISH methods have conventionally been used to sex male embryos in the course of PGD in patients with IP, which entails the possibility of either viable male fetal pregnancies or miscarriages if affected male embryos are transferred [6,8]. However, in all of these case reports using FISH methods with fewer than six chromosome probes, the fetuses were spontaneously aborted before confirmatory prenatal diagnosis resulting from chromosomal aneuploidies which were not tested.

In two recent case reports, the PGD for patients with IP was performed through polar body analysis strategies using polymerase chain reaction primers of the **IKBKG** gene to focus on the molecular basis of IP. The authors reported the delivery of a healthy boy and an ongoing pregnancy with one male fetus after 32 weeks of gestation.
### Table 1. Summary of published PGD cases in patients with incontinentia pigmenti

| Study                  | Age (yr) | Gravidity       | Clinical manifestation                                      | Mutation type                  | IVF protocols                      | PGD method                                                                 | Result of PGD                                                                 | Pregnancy outcome                                    |
|------------------------|----------|-----------------|------------------------------------------------------------|--------------------------------|-----------------------------------|----------------------------------------------------------------------------|--------------------------------------------------------------------------------|------------------------------------------------------|
| Munne et al. [6]       | 28       | Parous; having one affected daughter | Mild                                                        | De novo; Xq27→xq28 Distal to f8c in xq28 | Not mentioned                   | FISH of single blastomeres from seven 8-cell stage embryos on day three using five probes of chromosome X,Y,18/21/21 | Of total seven embryos - Two normal male, one normal female - Two trisomy 18 - Two trisomy 13 or 21 | Two normal male ET on day three → pregnant but spontaneously aborted (trisomy 9) |
| Pettigrew et al. [8]   | 29       | Nulliparous     | Mild                                                        | De novo; specific mutation site not mentioned               | Luteal phase regulation with or without ICSI | FISH of single blastomeres from four 8-cell stage embryos on day three using three probes of chromosome X,Y,18 | Of total four embryos - Two male and two female | One normal male ET on day three → pregnant but spontaneously aborted at 7th week of gestation (trisomy 16) |
| Griesinger et al. [9]  | 36       | Parous          | Mild; skin changes with dental defects                     | A mutation in NEMO gene (type of mutation?)                 | Protocol not mentioned; ICSI       | Polar body biopsy; electrophoretic demonstration of heterozygous genetic markers that are closely linked with the mutation | Of total nine PBI and five PB2 - Two PB1 carried the mutation, and the PB2 contained wild-type alleles → a high probability of both oocytes carrying the wild-type allele | Two 4-cell stage ET → pregnant and no mutation in CVS → a healthy boy delivered after 39 weeks and 2 days of gestation |
| Altarescu et al. [10]  | 31       | Nulliparous     | Not mentioned                                              | De novo; deletion G. (exon4→exon10) del in the ikbkg gene (6mim *300248) | Long down-regulation protocol with ICSI | Polar body biopsy; real-time reverse linkage with multiplex PCR using primers not detecting pseudogenic sequences located adjacent to the IKBKG gene blastomere biopsy | In the first cycle - of total ten PB1; Two homozygous, six heterozygous, two unclear - all wild-type PB2 - blastomere biopsy of four 6-8 cell stage embryos; IKBKG gene in three (linkage with the PB1 samples), one unclear → No ET In the second cycle - blastomere biopsy; one of eight embryos wild-type male → ET | One wild-type male ET → Ongoing pregnancy (32 weeks) |
| Present study          | 29       | Nulliparous; previous three spontaneous abortion           | Mild; skin lesions only                                      | De novo; mutation in ikbkg exon 9, 1308_1309inscccccttg (p.Ala438Pro/e28) | GnRH antagonist protocol with ICSI | aCGH of single blastomeres from 8-cell stage embryos on day three           | Of total seven embryos - Two euploid male - One euploid female - Four aneuploid male embryos: -16, -8/+15, +20, +2/+3/+6/+11/+20 | Two euploid male blastocysts transferred on day five → pregnant and normal results of amniocentesis → healthy twin boys after 36 weeks and 6 days of gestation |

ET, embryo transfer; PGD, preimplantation genetic diagnosis; CVS, chorionic villus sampling; NEMO, nuclear factor-kappa B (NF-κB) essential modulator; IKBKG, inhibitor of κB kinase gamma; PB1, first polar body; PB2, second polar body; aCGH, array-based comparative genomic hybridization.
In a more recent study evaluating 151 PGD cycles using polar body analysis for de novo mutations in 38 different genetic disorders, the researchers applied eight PGD cycles to five patients with IP. They reported four births from eight embryos, which were transferred after sequential polar body analysis and embryo karyotyping of blastomeres for several chromosomes by polymerase chain reaction [23].

In this case, we also selected male embryos with a 50% probability of a viable male pregnancy. However, we performed PGD as part of the embryo transfer process followed by amniocentesis with a molecular analysis of possible IP after the clinical confirmation of pregnancy. The patient delivered healthy dizygotic twin babies at the 37th week of gestation. We were able to prevent a miscarriage arising from an aneuploid pregnancy involving other autosomal mutations through a whole chromosome analysis using aCGH instead of only sexing the embryo, which was a significant difference from previous studies.

Although successful pregnancy outcomes have been presented in recent case reports in which polar body analysis was used, these reports have mainly focused on the molecular analysis of possible mutations inherited from the mother, without performing whole genome analysis. It is well known that aneuploidies are common in early human embryos [24,25]. According to a study that used FISH probes for chromosomes X, Y, 13, 15, 16, 17, 18, 21, and 22 in 6054 cleavage-stage embryos [26], 70% of embryos showed chromosomal abnormalities. In another study analyzing aCGH of 70 single blastomeres, the authors reported that 55.7% of the blastomeres were diploid, 44.3% contained chromosomal abnormalities, and 29% were abnormal cells with structural aberrations [25]. The relatively high aneuploidy rate of 57% previously reported in IP cases [6] corresponds to the aneuploidy found in four of the seven embryos in our case. In light of this, embryo selection in IP patients through comprehensive genomic analytical techniques like aCGH, as performed in this study, is a promising approach.

Recently, next-generation sequencing has emerged as a PGD strategy both for mutation target diagnosis and simultaneous whole genome analysis [27,28]. Another very promising study has been published about single-gene disorders like IP, but the clinical applicability of the relevant technology remains to be confirmed [29].

In conclusion, this case shows the beneficial role of PGS in achieving successful pregnancy through sexing euploid embryos in a woman with X-linked dominant IP who had experienced multiple male miscarriages.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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