Received 7-03-22; Accepted 21-03-22; Published 31-03-22

A Case Report of Primary Infertility with BPES Syndrome with FOXL2 Gene Mutation and PADI6 Gene Mutation

Wu Hongbo wuhongbo20212021@163.com
Zhao zhang zhangzhaogx2018@163.com
Qinzhou maternal and Child
Health Care Hospital China.

***Corresponding Authors:** Zhao zhang.

ABSTRACT

Human forkhead boxl2 (FOXL2) gene is closely related to female ovarian function. Muta- tions in FOXL2 gene often lead to BPES syndrome. BPES is divided into POF (BPES type I) and non POF (BPES type II). This case report shows that when FOXL2 gene is combined with PADI6 gene mutation, the patient may have obstacles to egg maturation in addition to premature ovarian failure, resulting in the inability of the patient to obtain embryos during art, which eventually leads to the inability of the patient to obtain pregnancy.

Keywords: Congenital blepharoplasty syndrome, BPES, Premature ovarian failure, POF, Ovarian insufficiency, POI, FOXL2 gene, PADI6 gene, Gene mutation, Primary infertility, Preimplantation embryo death type 2.

I. INTRODUCTION

The human forkhead boxl2 (FOXL2) gene is closely related to female ovarian function. Mutations in the FOXL2 gene often lead to BPES syndrome [1], BPES is divided into POF (BPES type I) and non-POF (BPES type II). Studies have shown that multiple FOXL2 gene loci are related to this syndrome. Patients with BPES I syndrome usually need assisted reproductive technology to obtain successful pregnancy. However, this case report shows that when the FOXL2 gene is combined with PADI6 gene mutation, patients may have egg maturation disorder in addition to premature ovarian failure. As a result, the patient cannot obtain embryos during art, which eventually leads to the patient's inability to obtain pregnancy.

This report further confirmed the relationship between FOXL2 gene mutation and BPES type I and showed that patients with FOXL2 gene mutation combined with a PADI6 gene mutation might be complicated with egg maturation disorder and POF. Therefore, the purpose of this paper is to provide more basis and help for clinical diagnosis and treatment and prenatal genetic counseling of relevant infertility patients.

Citation: Wu Hongbo and Zhao Zhang. A Case Report of Primary Infertility with BPES Syndrome with FOXL2 Gene Mutation and PADI6 Gene Mutation. Journal of Clinical Medicine & Surgery. 2022;1(1):1.

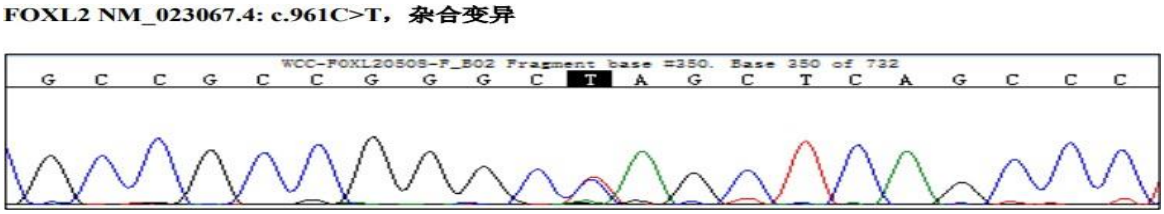

Figure 1: Sanger gene sequencing breakthrough; FOXL2 NM_023067.4:c.961C>T, Heterozygousmutation.

II. CASE DESCRIPTION

The patient, female, 30 years old, went to the reproductive center of Guangxi Qinzhou maternal and child health hospital on August 12, 2019, because of menstrual disorder for 14 years, no contraception after marriage and no pregnancy for more than five years". She complained that her menstruation was 14 years old, menarche, the menstrual period was 4-5, the cycle was 30 days, menstrual disorder occurred at the age of 16 without obvious inducement, the cycle was prolonged to 6-18 months, and progesterone withdrawal bleeding was required, married in March 2014, with normal sexual life after marriage, no contraception, and no pregnancy so far. Ultrasound was used to monitor ovulation for eight menstrual cycles, mature follicles were discharged, and no one in the same room was pregnant. Salpingography showed that both fallopian tubes were unobstructed. Physical examination of the whole body: both eyes have slight ptosis, inverted epicanthus, eyelid fissure length < 20 mm, inner canthus spacing is significantly widened, and nasal dorsum is low and flat. Specialist examination: the vulva develops normally, the vagina is unobstructed, the mucosa is free of flushing, the cervix is smooth, the posterior position of the uterus is normal in size, medium in quality, no tenderness, no tenderness in bilateral accessory areas, and no palpable mass. Laboratory and instrument examination: basic sex hormones: FSH 11.29 miu/ml, E2 34.90 pg/ml, P 1.07 ng/ml, LH 2.69 miu/ml, PRL 9.15 ng/ml, t 0.17 ng/ml, AMH 0.57 ng/ml; Gynecological B-ultrasound: uterine size 42 × forty-one × 35 mm, intimal thickness 5.3 mm, left ovary 22 × 15 mm Right ovary 14 ×12 mm, 11 AFCS. The patient had undergone left eye plastic surgery twice in 1995 and 2006. Combined with the patient's infertility history and considering BPES syndrome, peripheral blood gene detection and analysis were performed after communicating with the patient.

Table 1: FOXL2 NM_023067.4: c.961C>T (p.Q321X), Heterozygous variation.

Mutation results								
Gene	Chromosome position	Transcript	Nucleotide change	Amino acid change	Zygote type	Disease name	Genetic model	Variation rating
FOXL2	Chr3:138664604	NM_023067.4	c.961>T	p.Q321X	Heterozygous	Premature ovarian failure type 3: BPES syndrome	AD:AD/AR	BPES syndrome

The results showed that FOXL2 nm_ 023067.4: c.961c > t (p.q321x), heterozygous variation; PADI6 NM_ 207421.4: c.460 g > A (p.g154 s), heterozygous variation (see Figure 1, Table 1 and Table 2 for the results); Combined with the patient's medical history and auxiliary examination results, the current diagnosis is as follows: 1. Primary infertility 2. BPES syndrome type I, IVF-ET assisted pregnancy for two cycles. The first cycle of GnRHa long-acting case was GN 14 days, the ovarian response

to drugs was poor, and the cycle was canceled. In the second cycle, the PPOS scheme was adopted, the ovarian response to drugs was poor, the cycle was canceled, and the eggs were not taken successfully in the end.

Table 2: PADI6 NM_207421.4: c.460G>A (p.G154S), Heterozygous variation.

Mutation results								
Gene	Chromosome position	Transcript	Nucleotide change	Amino acid change	Zygote type	Disease name	Genetic model	Variation rating
PADI6	Chr1:17707566	NM_20742.4	c.460G>A	p.G154S	Heterozygous	Preimplantation embryo lethal type 2	AR	Clinical signif-icance unknown

III. DISCUSSION

BPES syndrome can be divided into two subtypes: type I, 100% penetrance, passed by father, female patients are infertile; Type II, the penetrance rate was 96.5%. The father and mother had equal passage opportunities. Both male and female patients only involved the eyes without affecting fertility [2]. Studies have shown that the FOXL2 gene is the most common and first pathogenic candidate gene of BPES syndrome. FOXL2 gene mutation has been found in patients with BPES type I and type II. FOXL2 gene is a single exon gene located on chromosome 3q23 (chromosome 3, region two, and band 3). FOXL2 gene is an autosomal gene that plays an essential role in maintaining follicular development and normal ovarian function. It is mainly expressed in granulosa cells in the medium and small follicular stages. It plays an essential role in the proliferation and differentiation of granulosa cells and the production of ovarian steroids by inhibiting the transcriptional activity of promoters such as downstream target genes CYP11A1, cyp1941, and ccnd2 [3]. The low expression level of FOXL2 can cause the mitosis of granulosa cells to be blocked, while FSH receptors only exist on granulosa cells. Therefore, the number of FSH receptors on the surface of granulosa cells with blocked division decreases. Therefore, the ovarian response to ovulation promoting drugs such as FSH and HMG decreases in the process of ovarian hyperstimulation, resulting in the reduction of the number of follicles recruited [4]. Due to the high familial heritability of the disease, according to Mendel's law of heredity, the risk of recurrent disease in the offspring of BPES patients is about 50%. Therefore, when planning pregnancy, coupled with either or both of them suffering from BPES, they should go to a hospital qualified for prenatal diagnosis. It is essential to avoid the birth of children through prenatal screening, diagnosis, and other measures. At the same time, early diagnosis of BPES syndrome from the genetic level can guide women affected by the disease to receive hormone replacement therapy and in vitro fertilization of donated oocytes as soon as possible [1].

According to ESHRE guidelines, the diagnostic criteria for POI are ovarian loss activity before the age of 40, with the menstrual disorder (amenorrhea or menstrual hair) accompanied by elevated gonadotropin and reduced estrogen, with an incidence rate of 1%. [5].

The ovarian function of normal women can be affected by age, gene, endocrine, surgery, drugs, diseases, controlled ovarian stimulation, and other factors. For example, with the increase of age, the ovarian function shows a downward trend, manifested in the decrease of egg reserve, accel- erated depletion, the decline of egg quality, etc. Studies have shown that the FOXL2 gene

plays an essential role in the differentiation of ovarian granulosa cells and the maintenance of ovarian function [6]. This study found that the ovarian granulosa cells of FOXL2 mutant could not complete the transformation from squamous cells to cubic cells, resulting in the loss of secondary follicles and oocyte atresia. The study also found activin in the ovary of FOXL2 mutant-β A, and anti- Mullerian inhibitory hormone were absent or powerfully absent; This study shows that GDF-9 is expressed in most oocytes two weeks after birth (both during oocyte follicular formation, low or no expression in primordial follicles, strong expression in all stages of follicular development, and also in ovulated oocytes), which indicates that almost all primordial follicles have begun follicular genesis at this stage. In the absence of functional granulosa cells, this activation leads to oocyte atresia and progressive follicular depletion. These results provide the molecular mechanism of BPES type I premature ovarian failure and show that the function of granulosa cells is essential not only for the growth of oocytes but also for maintaining follicular quiescence in vivo. In the FOXL2 mutant ovary, once activated follicles apoptosis occurs in the absence of granulosa cells, leading to progressive follicular failure and ovarian atresia. Therefore, for female infants suspected of BPES type I, parents and family doctors should pay close attention to whether the neonatal eyelid development is expected. If there are narrow palpebral fissure, ptosis, reverse epicanthus, widened epicanthus spacing, and other manifestations, they should go to the hospital as soon as possible; If it is diagnosed as BPES type I, measures such as preserving reproductive function shall be taken in time, such as cryopreservation of ovarian tissue.

Combined with the medical history and clinical characteristics, the patient, in this case, can be diagnosed as a primary infertility patient with BPES type I. his ovarian function is incomplete, and his response to drugs is inadequate. He has experienced two IVF-ET assisted pregnancies and adopted GnRHa long-term regimen and PPOs regimen, respectively. After canceling the cycle, he failed to take eggs in the end successfully. The heterozygous variation of FOXL2 gene c.961c > t (p.q321x) was detected in patients so that the glutamine encoded at position 321 was replaced by the stop codon, resulting in the early termination of protein-coding, which is predicted to affect protein function. In addition, frameshift mutations at amino acid 321 of the FOXL2 gene were included as pathogenicity (rs863225453, rs1057516183). The gene contains only one exon. After querying the database, the gene loss of function mutations (LOF) is included as pathogenic or suspected pathogenic. Combined with the existing evidence of ACMG, the mutation is suspected to be pathogenic.

The subject also detected a heterozygous variant of PADI6 gene c.460g > A (p.g154s), resulting in glycine substitution at position 154 by serine. The mutation is located in the central pad domain of the PADI6 gene. Many statistical methods predict that the mutation will cause harmful effects on genes or gene products. PADI6 is a member of the subcortical maternal complex (SCMC), which is very important for forming oocytes. Human embryos produced during in vitro fertilization (IVF) are not 100% viable and used by humans. Studies have confirmed that only about 40-70% of human embryos produced during in vitro fertilization (IVF) are viable embryos, and other embryos will stop growing at different stages. If all embryos of infertile patients have developmental arrest, the patient is in vitro fertilization / intracytoplasmic sperm injection (ICSI) cycle will fail [7]. Some studies have extended the mutation spectrum of PADI6 and proposed for the first time that the preimplantation arrest of PADI6 may be related to abnormal cleavage (mainly DC (direct cleavage)) [8]. This discovery deepens our understanding of the genetic basis of the human early embryonic arrest. It provides a basis for the genetic diagnosis of clinically infertile individuals with this phenotype and lays a foundation for revealing other genetic causes of female infertility caused by the early embryonic arrest.

Normal preimplantation embryo development during ART is critical to a successful pregnancy. Normal oocytes continued to cleavage and produced 6-8 blastomes on day 3 of culture. However, of the human embryos created during ivf, only about 40-70% are viable embryos, and the rest stop at various early stages.So far, only a few genetic factors have been identified as causing early embryonic development stagnation. A multi-protein complex called subcortical maternal complex (SCMC) is located under the cortex of oocytes and embryos, and plays a crucial role in mammalian embryogenesis [4-6]. The gene mutations leading to early embryo stagnation are mainly concentrated in SCMC members. As a member of SCMC, peptidyl arginine deaminase VI (PADI6) is one of the important factors of preimplantation cleavage and early embryo development. Embryonic development of padi6-deficient mice did not exceed the two-cell stage . PADI6 is the first reported pathogenic gene that leads to early embryo stagnation by impinging zygotic genome activation . All patients had a biallelic mutation, which was characterized by fetal development defects, including early or late repeated embryo stagnation, embryo fragmentation, and embryos with implantation potential but unable to become pregnant after implantation [9].

As far as we know, neither of the two variants has been included in the average population in East Asia, nor has it been reported in the literature. Family verification of the FOXL2 gene is helpful to clarify the clinical significance of this locus further. It is suggested that their spouses should monitor the PADI6 gene, which is helpful to evaluate further the correlation between this gene and the phenotype of the subject. It still needs to be comprehensively evaluated in combination with the subject's family history. According to our current findings, the genotype-phenotype correlation of this patient is consistent [10].

The patient underwent HSG in 2014, which showed that both fallopian tubes were unobstructed. The patient had been treated with ovulation induction for eight cycles. Mature follicles were excreted in 8 cycles, and they were not pregnant after guiding the same room; Combined with foxl2nm detected by the patient_ 023067.4: c.961c > t (p.q321x) heterozygous variation and padi6nm_ 207421.4:c.460 g > A (p.g154 s) heterozygous variation, which can not help but lead us to think whether the unsuccessful ovulation promotion is due to the quality problem of the egg itself caused by the simultaneous existence of two gene mutations, or the sudden stop of the embryo after the sperm and egg combine to form a fertilized egg, failing implantation.

In 2019, the patient chose assisted reproductive technology to assist pregnancy and IVF-ET (GnRHa long-acting rectangular case) for gn14 days. The ovarian response to drugs was inadequate, and the cycle was canceled. After returning to the hospital for IVF pregnancy again, the PPOS scheme was adopted. The ovarian response to drugs was poor, the cycle was canceled, and the eggs were not taken successfully in the end. It can not help but arouse our thinking again. It can be seen that the follicles are discharged before the patients choose IVF-assisted pregnancy. Then, when they choose IVF-assisted pregnancy, they have failed to take eggs successfully for two different controlled ovarian stimulation schemes. The interval between before and after several years, whether it can be considered that the ovarian function of BPES type I patients increases with age; BPES type I premature ovarian failure caused by FOXL2 gene mutation has been confirmed, but even in patients with premature ovarian failure, eggs can be obtained during assisted reproductive technology; The patient did not get an egg. Consider whether PADI6 gene mutation will lead to the obstacle of egg maturation to accelerate the premature ovarian failure caused by FOXL2 gene mutation.

The superposition of FOXL2 gene mutation and PADI6 gene mutation leads to premature ovarian failure and abnormal egg cleavage, resulting in embryo arrest, making the patient unable to obtain pregnancy through assisted reproductive technology. The only pregnancy of such patients may be egg donor, which not only avoids unnecessary economic losses but also avoids the psychological and physical pain of patients; However, the limitation of this report is that this conclusion is based on the hypothesis put forward by the patient's clinical manifestation and gene test report, and has not been confirmed by further animal experimental models or ovarian tissue sections. Therefore, it needs to be further studied in the later stage.

Determining the molecular basis of BPES type I and ovarian dysfunction will help us better under- stand the reproductive process involved, guide the diagnosis and treatment of premature ovarian failure from the gene level, and predict the reserve function of the ovary [5]. If we can further improve the understanding of the FOXL2 gene, PADI6 gene, and their mutations, it will provide more basis and help for the clinical diagnosis and treatment and prenatal genetic counseling of relevant infertile patients, so that relevant patients can make reproductive choices in an informed manner, and can also make plans in advance in terms of birth time [11]. Studies have shown that cryopreservation of ovarian tissue may be the first choice in children, while in adults, there are several cryopreservation methods to choose if there are still some ovarian reserves. The most successful method is embryo cryopreservation, with a pregnancy rate of 21 % to 27 %. Oocyte cryopreservation is another option, but the pregnancy rate of this method is between 1 % and 5 % per thawed oocyte. Vitrification of oocytes is a relatively new method of oocyte cryopreservation using a rapid freezing scheme. It has been proved that its fertilization rate is equivalent to the conventional in vitro fertilization cycle of fresh oocytes, which is expected to retain fertility. When the number of mature oocytes is limited, aspiration of immature oocytes for in vitro maturation and subsequent vitrification or fertilization is also a successful choice. Cryopreservation of ovarian tissue is another method to preserve fertility. However, the success rate of this technique is meager and is closely related to the ischemic injury of tissues during thawing and after transplantation. The last option to consider is egg donation, with the highest pregnancy rate in the range of 40 % to 50 % per cycle, so this is considered the priority fertility treatment for patients with POF. For young BPES patients with POF, endocrine and gynecological evaluation should be carried out, and hormone replacement therapy should be carried out for these patients in time as needed, and continue at least until the physiological age of menopause, to reduce postmenopausal symptoms and prevent the long-term impact of estrogen deficiency (such as osteoporosis) [11]. Alternatively, suggest they donate oocytes for in vitro fertilization as soon as possible [1,12], which will have important guiding significance for their diagnosis and treatment process. A detailed understanding of the relevant genetic defects may even provide an opportunity to restore these women's fertility, but its application prospect needs further research.

Ophthalmologists also play an essential role in the diagnosis and treatment of this disease. Ophthal- mologists should focus on patients with narrow palpebral fissure, ptosis, reverse epicanthus, and widened epicanthus spacing, and consider the diagnosis of BPES syndrome early, to carry out gene detection and diagnosis at an earlier stage, and strive for the principle of early detection and early intervention, For patients diagnosed with BPES syndrome type I, because the probability of ovarian dysfunction increases with age, it is recommended that they go to the gynecological or reproductive center as soon as possible to consult fertility problems, to provide more options and possibilities to meet their fertility requirements for patients with BPES syndrome type I in the early stage.

This paper presents a retrospective analysis of the diagnostic process and clinical results, so the ethics committee's approval is not required. The participants mentioned above have agreed in writing to publish these findings and gene sequencing results.

IV. CONCLUSION

1. According to the clinical phenotype of this case, it can be diagnosed as BPES syndrome type I (The FOXL2 gene. 961c > t (p.q321x) heterozygous variation and PADI6 gene c.460 g > A (p.g154 s) heterozygous variation were detected. Both variants were not included in the normal population in East Asia, and there was no relevant literature report. Family verification of the FOXL2 gene is helpful to clarify the clinical significance of this locus further. It is suggested that their spouses should monitor the PADI6 gene to evaluate further the correlation between this gene and the subject's phenotype, which still needs to be comprehensively evaluated in combination with the subject's family history.)

2. If BPES syndrome can be diagnosed early, endocrine and gynaecological evaluation of affected women can be carried out, hormone replacement therapy can be carried out for these patients as needed, or in vitro fertilization of donated oocytes can be recommended as soon as possible [1,8], which will have important guiding significance for their diagnosis and treatment process.

3. Further understanding of FOXL2 gene and PADI6 gene (will help develop genetic diagnosis and provide possible therapeutic targets for infertility treatment.) It will provide more basis and help for clinical diagnosis and treatment and prenatal genetic counselling of relevant infertility patients.

4. The superposition of FOXL2 gene mutation and PADI6 gene mutation leads to premature ovarian failure and abnormal egg cleavage, resulting in embryo arrest so that the patient cannot obtain pregnancy through assisted reproduction. The only pregnancy of such patients may be egg donor- assisted pregnancy [13,14].

V. CONFLICT OF INTEREST

The authors declare no financial or ethical conflicts of interest.

IV. ACKNOWLEDGEMENT

The authors have not received any financial support for the research, authorship and / or publication of this article.

V. REFERENCES

1. Corrêa FJ, Tavares AB, Pereira RW, Abrão MS. A new FOXL2 gene mutation in a woman with premature ovarian failure and sporadic blepharophimosis-ptosis-epicanthus inversus syndrome. Fertil Steril. 2010;93:1006.e3-1006.e6.
2. Huiyan L, Yangshun Gu. Gene mutation detection and functional study in families with congenital blepharoplasty syndrome [a] Ophthalmology branch of Zhejiang Medical Association. Compilation of papers of 2016 Zhejiang ophthalmology academic annual meeting [C] Ophthalmology branch of Zhejiang Medical Association: Zhejiang science and Technology Association.2016:1
3. Hu Jingmei. Mutation detection and function study of FOXL2 gene in families with BPES syndrome and infertility [D] Shandong University.2017.
4. Chen Lan, Cao peixia Clinical application of FOXL2 in evaluating ovarian reserve function

[J] Journal of Hubei University FOor Nationalities (Medical Edition), 2010,27 (01):75-77.
5. European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI, L. Webber, M. Davies, R. Anderson, J. Bartlett, et al. “ESHRE Guideline: management of women with premature ovarian insufficiency.” Human reproduction (Oxford, England). 2016;31:926-937.
6. Schmidt D, Ovitt CE, Anlag K, Fehsenfeld S, Gredsted L, et al. The murine winged-helix transcription factor Foxl2 is required for granulosa cell differentiationand ovary maintenance.
Development. 2004;131:933-942.
7. Gardner DK, Lane M. Culture and selection of viable blastocysts: afeasible proposition for human IVF? Hum Reprod Update.1997;3:367–382.
8. Zheng W, Chen L, Dai J, Dai C, Guo J, et al. New biallelic mutations in PADI6 cause recurrent preimplantation embryonic arrest characterized by direct cleavage. 2020; 37:205-212.
9. Dong J, Fu J, Yan Z, Lin Li, Qiu Y, et al. Novel biallelic mutations in PADI6 in patients with early embryonic arrest. J Hum Genet. 2022.
10. Tang S, Wang X, Lin L, Sun Y, Wang Y, et al. Mutation analysis of the FOXL2 gene in Chinese patients with blepharophimosis-ptosis-epicanthus inversus syndrome. Mutagenesis. 2006;21:35-39.
11. Gersak K, Harris SE, Smale WJ, Shelling AN. A novel 30 bp deletion in the FOXL2 gene in a phenotypically normal woman with primary amenorrhoea: case report. Hum Reprod. 2004;19:2767-2770.
12. Tang S, Wang X, Lin L, Sun Y, Wang Y, et al. Mutation analysis of the FOXL2 gene in Chinese patients with blepharophimosis-ptosis-epicanthus inversus syndrome. Mutagenesis. 2006;21:35-39.
13. Li WX, Wang XK, Sun Y, Wang YL, Lin LX, et al. A novel mutation in the FOXL2 gene in a Chinese family with blepharophimosis, ptosis, and epicanthus inversus syndrome. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2005;22:372-375.
14. Gardner DK, Lane M. Culture and selection of viable blastocysts: afeasible proposition for human IVF? Hum Reprod Update.1997;3:367–382.