*Case Report*
# A successful live birth from a 17α-hydroxylase/17,20-lyase deficiency mother by the in vitro fertilization frozen-thawed embryo transfer

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**Abstract:** A 24-year-old Chinese female was manifested by the primary infertility and primary amenorrhea, sexual infantilism, recurrent ovarian cysts and hypertension. Genetic analysis diagnosed this patient as 17α-hydroxylase and 17,20-lyase deficiency (17OHD) as she harbours a homozygous c.707T>G mutation in exon 4 of the *CYP17A1* gene, which resulted in a substitution of amino acid valine (V) at position 236 to glycine (G) (V236G). In vitro fertilization (IVF) frozen-thawed embryo transfer under luteal-phase ovarian stimulation protocol was then recommended due to the complexity of the steroidogenic disorder caused by 17OHD. Other treatment included glucocorticoid administration to suppress the elevation of progesterone, endometrial preparation with exogenous steroids and subsequent transfer of frozen-thawed embryos. At the 29th week of pregnancy, a caesarean section was performed due to the diagnosis of haemolysis elevated liver enzymes low platelets (HELLP) syndrome. A live normal female newborn was delivered with Apgar score 1/4 and 780 g in body weight. The new-born was discharged in a good condition 7 weeks after delivery. This outcome supports that 17OHD females still could have a successful pregnancy and delivery with adequate hormonal control and endometrial preparation. Luteal-phase ovarian stimulation is proved to be a convenient and time-saving approach to retrieve oocytes for these patients. However, 17OHD should be closely monitored when females are pregnant for better management of pregnancy complications and neonatal care.

**Keywords:** 17α-hydroxylase/17,20-lyase deficiency, congenital adrenal hyperplasia, infertility, luteal-phase ovarian stimulation, pregnancy complications

## Introduction

17α-hydroxylase and 17,20-lyase deficiency (17OHD; MIM#202110) is a rare autosomal recessive disease causing congenital adrenal hyperplasia (CAH). These patients have defect in the steroid biosynthetic pathway resulting from the mutations in cytochrome P450 family 17 subfamily A member 1 (CYP17A1) gene [1, 2]. The CYP17A1 gene is located at chromosome 10q24.3 and consists of eight exons, spanning 6.6 kb [3]. It expresses in several steroidogenic tissues, including the adrenal cortex, ovary, and testis [4]. The microsomal enzyme cytochrome P450c17 (17α-hydroxylase/17,20-lyase, OMIM 609300) encoded by the CYP17A1 gene is the key regulator in biosynthesis of glucocorticoids and sex steroids. This enzyme catalyses two distinct reactions in

the steroidogenic pathway: 17α-hydroxylation of steroid and the subsequent cleavage of $C_{17-20}$ carbon bond (**Figure 1**) [5].

The manifestations of 17OHD deficiency include hypertension, hypokalaemia, primary amenorrhea and sexual infantilism. Additionally, female infertility would be the main clinical manifestation of 17OHD deficiency for some patients. However, there are rare investigations into female infertility owing to 17OHD deficiency, and the reports about the fertility treatments and successful pregnancies of these patients are even rarer. Thus far, only three cases of pregnancy in 17OHD females who had live birth following in vitro fertilization (IVF) treatment [6-8] were reported. In this study, we report a case of a live birth from a 24-year-old Chinese female with 17OHD, using her own oocytes with
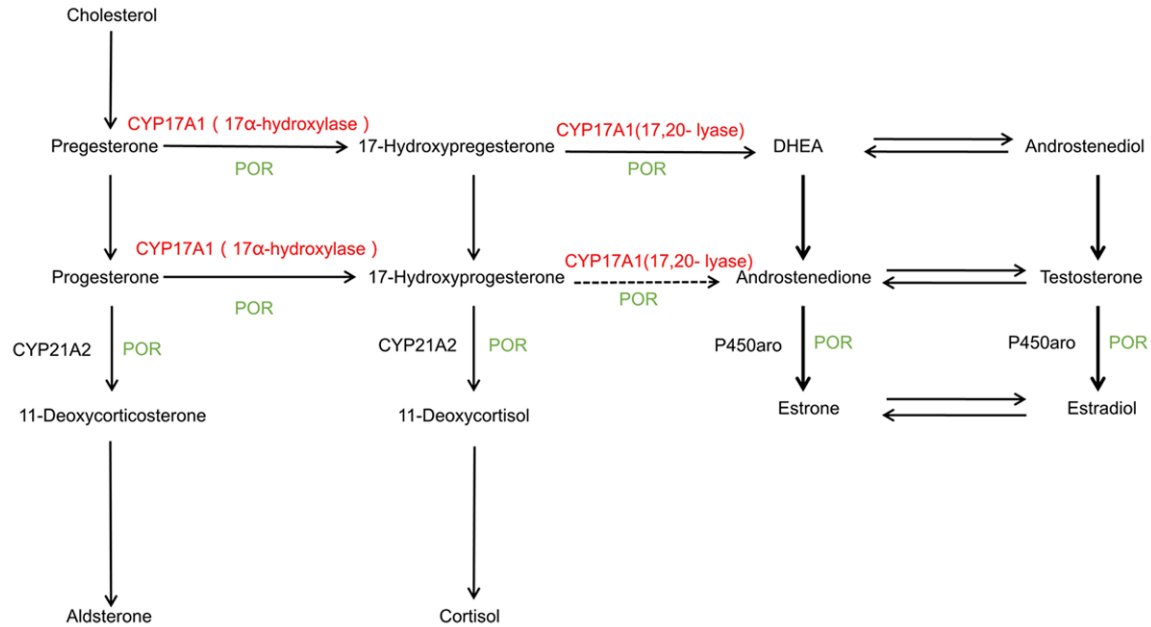
**Figure 1.** Schematic of 17α-hydroxylase/17,20-lyase'role in adrenal steroidogenesis biosynthesis pathway. CYP17A1 gene is of importance in biosynthesis of glucocorticoids and sex steroids, and catalyses two distinct reactions in the steroidogenic pathway: 17α-hydroxylation of steroid and the subsequent cleavage of $C_{17-20}$ carbon bond. Abbreviation: POR = cytochrome P450 oxidoreductase; CYP17A1 = 17α-hydroxylase/17,20 lyase; CYP21A2 = 21-hydroxylase; P450aro = aromatase; DHEA = dehydroepiandrosterone.

**Table 1.** Plasma steroid and pituitary hormone levels in the patient

| Plasma steroid/pituitary hormone | Results | Reference |
|---|---|---|
| FSH (mIU/ml) | 12 | Follicular, 3.85-8.78; Ovulatory, 4.54-22.51; Luteal, 1.79-5.12 |
| LH (mIU/ml) | 11 | Follicular, 2.12-10.89; Ovulatory, 19.18-103.03; Luteal, 1.20-12.86 |
| Estrodiol (pg/ml) | 41 | Follicular, 24-114; Ovulatory, 62-534; Luteal, 80-273 |
| Progesterone (ng/ml) | 9.9 | Follicular, 0.31-1.52; Luteal, 5.16-18.56 |
| Testosterone (ng/ml) | <0.1 | 0.10-0.75 |
| ACTH (pg/ml) | 242 | 25-100 |
| COR (nmol/l) | 139 | 135-650 |
| DHEA-S (nmol/l) | 657 | 3540±1310 |
| AN (nmol/l) | 0.33 | Follicular, 2.7±1; Luteal, 5.2±1.5 |
| 17OH-Pro (nmol/l) | 8.18 | Follicular, 1.3±0.25; Luteal, 7.4±2.0 |

Abbreviation: FSH = follicle stimulating hormone; LH = luteinizing hormone; ACTH = Adrenocorticotropic Hormone; COR = cortisone; DHEA-S = dehydroepiandrosterone sulphate; AN = androstenedione; 17OH-Pro = 17-hydroxyprogesterone.

the help of IVF therapy under the luteal-phase ovarian stimulation (LPS) protocol.

## Case report

### Materials and methods

Genomic DNA was extracted from peripheral blood using the QG-Mini80 workflow with DB-S kit (FUJIFILM Corporation, Tokyo, Japan) according to the manufacturer's instructions. All eight exons of the CYP17A1 gene are amplified by polymerase chain reaction (PCR) using primers as described in Supplementary Table 1 [9]. The PCR products are directly sequenced with BigDye terminator v3.1 on the 3130x1 genetic analyser (Applied Biosystems, Foster City, California, USA). Finally, Chromas and DNAMAN program (Lynnon Biosoft, San Ramon, California, USA) were used to identify mutations by two independent observers.

The study protocol is approved by the Medical Ethics Committee of Tongji Hospital (Wuhan,
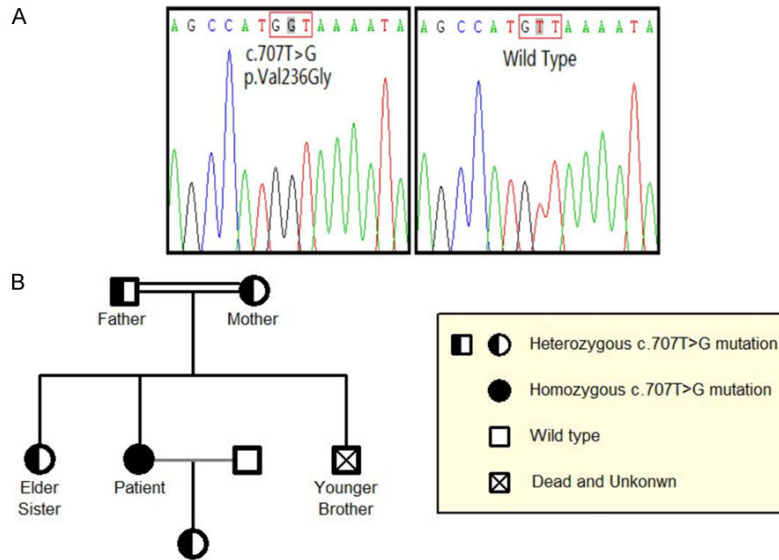
A


B


**Figure 2.** Genetic analysis of CYP17A1 gene and pedigree of the patient and her family. This patient harboured a homozygous mutation in the CYP17A1 gene: c.707T>G in exon 4, resulting in p.Val236Gly; her family members, including her parents, elder sister and her daughter, were all heterozygotes for c.707T>G.

China). Written informed consents are obtained from all participants. Researchers are conducted according to the Declaration of Helsinki for medical research.

## Results

A married 24-year-old Chinese woman visited the Reproduction Medicine Center at the Tongji Hospital, Tongji Medicine College, Huazhong University of Science and Technology, because of primary infertility in 2014 after 3 years of marriage. She manifests with primary amenorrhea and sexual infantilism. Her parents are consanguineous and have no history of hypertension. Additionally, her elder sister and younger brother both appear normal. In 2008, she received a left ovarian cystectomy via laparoscope at a local hospital due to ovarian cysts. A history of high blood pressure was reported, but without report of additional disease. Her body mass index (BMI) was 18.7 kg/m$^2$ and blood pressure was in normal range during the therapy process. The breast development reached Tanner stage III, but absent of axillary hair and stage P1 pubic hair. The external genitalia were of female phenotype but infantile, with a small uterus. The baseline levels of hormone in blood were shown in **Table 1**. Briefly, the serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P), adrenocorticotropic hormone (ACTH) were

increased, whereas testosterone (T), dehydroepiandrosterone sulphate (DHEA-S), and androstenedione (AN) were reduced, with a normal level of cortisol. Pelvic B-type ultrasound showed 34×23×26 mm uterus and bilateral ovarian cysts (52×28 mm on left, 69×32 mm on right) at menstrual day 3. Blood β-hCG, CA-125 and α-fetoprotein (AFP) were in normal ranges. Ultrasound scanning of bilateral adrenal glands was normal. Chromosomal analysis revealed a normal 46, XX female karyotype.

Genetic screening of the CYP17A1 gene was next conducted including the patients and her family members using the peripheral DNA. Remarkably, she was identified to carry a homozygous c.707T>G mutation in exon 4, which resulted in a substitution of amino acid valine (V) at position 236 to glycine (G) (V236G). Her parents and elder sister were noted to be carriers for this mutation (**Figure 2**). Based on the above clinical and biochemical as well as genetic analyses, the patient was diagnosed as 17α-hydroxylase/17,20 lyase deficiency (17OHD).

In vitro fertilization-embryo transfer (IVF-ET) was recommended for this patient because of no pregnancy was achieved without IVF-ET procedure in literatures. The protocol for luteal-phase ovarian stimulation was conducted as previously described [10]. Briefly, pretreatment of oral contraceptive (OC; Marvelon; Organon) was given during the previous menstrual cycle for eliminate ovarian cysts. Ovarian stimulation was carried out with daily administration of 225 IU of HMG (Lebaode; Livzon) from menstrual day 2 without medroxyprogesterone acetate (MPA) addition. The transvaginal ultrasound examination was employed to record the number of developing follicles and serum FSH, LH, E2, and P concentration were monitored. Once at least three follicles reach diameters of 18 mm monitored by transvaginal ultrasound, 10000 IU of hCG (Livzon, China) trigger was administered to induce ovulation. Transvaginal

**Table 2.** Fertility outcomes in patients with 17OHD

| Authors | Year | Genetic deficiency | Outcome | Pregnancy Complication | Newborn Health |
|---|---|---|---|---|---|
| Rabinovici et al. [17] | 1989 | Not reported | Arrest of embryos at cleavage stage | No pregnancy | No newborn |
| Pellicer et al. [16] | 1991 | Not reported | Implantation Failure | No pregnancy | No newborn |
| Ben-Nun et al. [8] | 1995 | Not reported | Two live births following IVF-ET with donated oocytes | Pre-eclampsia, HELLP, premature delivery | One newborn died soon after delivery |
| Neuwinger et al. [18] | 1996 | Not reported | No fertilization | No pregnancy | No newborn |
| Matsuzaki et al. [19] | 2000 | delF53/54+H373L | Implantation Failure | No pregnancy | No newborn |
| Levran et al. [6] | 2003 | Not reported | Triplet live birth from frozen ET | No complication | Healthy newborn |
| Bianchi et al. [7] | 2016 | p.W406R/P428L | One live birth from frozen ET | Pre-eclampsia, gestational diabetes, cholestasis gravidarum, premature delivery | Acute foetal distress |
| This study | 2016 | p.Val236Gly | One live birth from frozen ET | Pre-eclampsia, HELLP, premature delivery | Acute foetal distress |

Note: Matsuzaki et al. reported results of ovulation induction while others report IVF cycle results.

ultrasound-guided oocyte retrieval, sperm preparation, embryo culture, embryos cryopreservation and endometrium preperation was conducted as previously described [11-14]. Additionally, 0.75 mg of dexamethasone q.d. was maintained for the entire FET cycle. For the first cycle, 9 mature oocytes were retrieved, and two grade I embryos were cryopreserved by vitrification, while the others failed to achieve the blastocyst stage. In the following FET, two embryos were frozen-thawed and transferred to the uterus, which only achieved a biochemical pregnancy. For the second cycle, 16 mature oocytes were retrieved and 2 embryos and 1 blastocyst were freezed. In the subsequent FET cycle, one blastocyst was frozen-thawed and transferred to the uterus which achieved clinical pregnancy.

The pregnancy was uneventful until 28.5 weeks of gestation when pre-eclampsia, fetal growth restriction and oligohydramnios were diagnosed. After 29 weeks and 2 days of gestation, the patient developed haemolysis elevated liver enzymes low platelets (HELLP) syndrome, and a Caesarean section was then performed. A true umbilical cord entangled neck was identified, and a live normal female newborn was delivered with Apgar score 1/4 and a weight of 780 g. The newborn is hospitalized for seven weeks at the neonatal intensive care unit (NICU) and discharge home in good condition. Indeed, genetic analysis of the newborn indicated that she is the carrier for the V236G mutation, confirming the normal phenotype observed.

### Discussion

The 17α-hydroxylase/17,20-lyase deficiency (MIM#202110) caused by mutations in the CYP17A1 gene is a rare form of congenital adrenal hyperplasia, leading to the impaired biosynthesis of both cortisol and sex steroids. Thus far, more than 100 mutations have been identified, including missense and nonsense mutations, insertions, deletions, and splice site alterations (www.hgmd.cf.ac.uk/ac/gene.php?gene=CYP17A1). In China, more than 80% of the affected alleles in 26 patients mentioned in a large case series were Y329 frame shifts and D487-F489 deletions [9]. In our case, the patient was identified to carry a novel homozygous c.707T>G mutation in exon 4, which results in the V236G substitution to impair or lose 17α-hydroxylase/17,20-lyase activity.

In general, patients with 17α-hydroxylase/17,20-lyase deficiency fail to produce sex steroids, resulting in pseudohermaphroditism in 46,XY patients and sexual infantilism, primary amenorrhea, lack of secondary sexual characteristics, and infertility in 46,XX patients. Therefore, the affected males manifest ambiguous or female-like external genitalia, while females display a normal genitalia, but immature sexual development and primary or secondary amenorrhea due to oestrogen and testosterone deficiency [5, 15, 16]. In our patient, she displayed decreased testosterone, DHEA and androstenedione (AN) concentration, as well as increased FSH, LH, progesterone and ACTH level, while the cortisol is unaltered. The low level of testosterone and the relatively low level of oestradiol contributed to the sexual development disorder including infantile female genitalia, lack of secondary sexual characteristics and primary amenorrhoea. While high level of FSH and LH as well as the elevation of ACTH were associated with primary gonadal failure and primary adrenal hypocortisolism. However, the level of cortisol remained normal and the ultrasound scan of the adrenal gland revealed negative results, suggesting that this patient may still have a relatively high residual of 17α-hydroxylase activity, which could be high enough for normal cortisol production. Additional functional studies of the V236G mutation coupled with CT scanning of the adrenal gland would be necessary to clarify the question.

To date, there are no published reports on successful spontaneous fertility in 46, XX patients with 17OHD, and we thus summarized the published studies documenting assisted reproduction technology (ART) treatments and outcomes in patients with 17OHD (**Table 2**). The common IVF strategies of medications include glucocorticoid to suppress elevation of progesterone, GnRHa for pituitary-ovarian suppression, endometrial preparation with exogenous steroids and subsequent transfer of frozen-thawed embryos. Luteal-phase ovarian stimulation (LPS) is a novel protocol developed in recent years, and provides a more convenient approach to follicular recruitment compared with current conventional ovulation induction protocols in IVF therapy [10]. In the present case, by using the patient's "natural" elevated progesterone level, there is no need to use GnRH-a or GnRH-A for pituitary suppression in the egg retrieval cycles. In the embryo transfer cycles, glucocorticoid

such as dexamethasone or with GnRH agonist together were added to suppress the elevated progesterone level [6, 7]. In our patient, dexamethasone was not administered until the FET cycles. Therefore, it is more convenient and time-saving for 17OHD females to choose LPS as their IVF strategy.

Pregnant and neonatal complications were observed in most cases of females with 17OHD achieving live births through IVF treatment, suggesting a higher risk of complications including pre-eclampsia, HELLP and premature delivery [7, 8]. Similarly, our patient was complicated by the pre-eclampsia, HELLP and premature delivery. A caesarean section was performed due to HELLP syndrome, and this newborn had severe fetal distress at delivery. Preeclampsia is still one of the main causes of maternal morbidity and adverse perinatal outcomes including prematurity and intrauterine growth restriction [20]. HELLP syndrome is hepatic hemorrhage and rupture, which is one of life-threatening maternal complication [21]. Also, the leading causes of perinatal mortality associated with HELLP syndrome include prematurity, intrauterine growth restriction, asphyxia, placental insufficiency, abruption, and thrombocytopenia related to interventricular hemorrhage and long-term neurologic complications [22, 23]. Therefore, it is important for those 17OHD females achieving pregnancy through IVF treatment to set a series of strategies to reduce risks, including standardised assessment and surveillance of all vulnerable organ systems, avoidance and management of severe hypertension, prevention and treatment of seizures of eclampsia, and the preconception care by obstetricians with experience in management of the disorder. Finally, the subsequent lifestyle education and intervention would be important for the prevention of long-term complications.

In conclusion, we reported a case of live birth from a 24-year-old Chinese female with 17OHD through IVF and FET under the LPS protocol. We also identified a novel mutation (V263G in CYP17A1) for 17OHD, which would provide important information for understanding the molecular mechanisms underlying 17OHD. We demonstrated evidence that females with 17OHD could have a successful pregnancy and delivery with adequate hormonal control and endometrial preparation, and LPS is likely to be a convenient and time-saving approach to retrieve oocytes for this category of patients. However, intensive clinical management of pregnancy complications along with adequate gestational management and neonatal care would be essential to achieve the above goals.

## Acknowledgements

## Disclosure of conflict of interest

None.

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**Supplementary Table 1.** Primers for PCR and sequencing

| Exon | Sequence (5'→3') | Product Size (bp) |
|---|---|---|
| Promoter1-F | GGCGTTAGATCAGGTGGCA | 418 |
| Promoter1-R | GGTCCTGAAGTGACAAACATCCT | |
| Promoter2-F | GGCAGAGGAACATAAATTGTGAAGA | 468 |
| Promoter2-R | AAGATAGACATTCCCAGAGCGG | |
| Promoter3-F | AATGGACATGCAAGTAGGGAAGA | 427 |
| Promoter3-R | GGGGGTGTAAGAACAGGGAGTAG | |
| Promoter4-F | GCCCTTTGTCCTTTCCCTCA | 468 |
| Promoter4-R | GGGAACGAAAGGGGTGCTAA | |
| Promoter5-F | TCCCACCTCTGGCATTCCTA | 506 |
| Promoter5-R | ATCCAGAAGGGAGAGAGGCG | |
| Exon1-F | CTTGTGCCCTAGAGTTGCCA | 401 |
| Exon1-R | GAAGGGGGCAGGGAGGAG | |
| Exon2-F | GAAGGAAAGCAGGGACCAGA | 350 |
| Exon2-R | GGCAGCAGTAGCCAAGAAAA | |
| Exon3-F | CATCTGCTATCTGTCCCCCG | 419 |
| Exon3-R | GGCTGGAGCAGGGAAGTAAA | |
| Exon4-F | GCCCTTTGTCCTTTCCCTCA | 468 |
| Exon4-R | GGGAACGAAAGGGGTGCTAA | |
| Exon5-F | AGTCAGGGACAGAAGTATGGCAG | 389 |
| Exon5-R | TGCACAGAAAGCCTGAGAGAATT | |
| Exon6-F | GGAAGGGACTGGACAGGCTC | 324 |
| Exon6-R | TGAATGCATCATGGGGCTAGA | |
| Exon7-F | AAGGGCATTTTCCTCACGG | 291 |
| Exon7-R | TTGGCAGAGGTGAAGGGGTA | |
| Exon8-F | CTCAACCAGGGCAGAACCAT | 429 |
| Exon8-R | GGTGGGGGGTTGTATCTCTAAA | |

F: Forward; R: Reverse.