CASE REPORT

Conception and pregnancy outcome in a patient with 11-bp deletion of the steroidogenic acute regulatory protein gene

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Objective: To report the pregnancy outcome of a patient with congenital lipoid adrenal hyperplasia (CLAH) due to an 11-bp deletion of the steroidogenic acute regulatory protein (StAR) gene.
Design: Case report.
Setting: University-based pediatric endocrinology unit and private IVF clinic.
Patient(s): A 24-year-old woman homozygous for a StAR gene deletion, married to a man heterozygous for the same molecular defect.
Intervention(s): Ovarian stimulation, oocyte retrieval followed by IVF, blastomere biopsy, preimplantation genetic diagnosis, and additional estrogen support until placental function initiation.
Main Outcome Measure(s): Normal pregnancy outcome and delivery of a healthy newborn.
Result(s): A female patient with CLAH gave birth to a normal newborn after IVF and preimplantation genetic diagnosis.
Conclusion(s): Pregnancy is feasible in patients with StAR gene mutations, provided that extra estrogens are offered until placental function ensues. (Fertil Steril® 2009;91:934.e15–e18. ©2009 by American Society for Reproductive Medicine.)

Key Words: Congenital lipoid adrenal hyperplasia, StAR gene, defective steroidogenesis, Addison's disease, IVF, preimplantation diagnosis, pregnancy

The steroidogenic acute regulatory protein (StAR) gene is mapped to chromosome 8p11.2 (OMIM 600617) and encodes the StAR protein, which enhances the transfer of cholesterol from the outer to the inner mitochondrial membrane, a crucial step for steroidogenesis in the adrenals and gonads (1, 2). Specifically, the StAR protein is necessary for the acute response of aldosterone to angiotensin II, of cortisol to ACTH, and of gonadal steroids to gonadotrophins.

Mutations in the StAR gene lead to severe defects in the synthesis of cortisol and aldosterone by the adrenals and of sex hormones by the gonads (2–4). The latter derangement is responsible for sex reversal in 46,XY individuals with StAR gene mutations, because T is not produced by the fetal testes. Hence, such babies are born with normal female external genitalia but have no uterus because the Sertoli cell function is normal (normal antimüllerian hormone) (5, 6).

The degree of adrenal insufficiency is comparable in both 46,XY and 46,XX individuals with StAR gene mutations. The gonadal defect, however, shows gender differences: testicular failure is antenatally expressed, in most cases leading to sex reversal, whereas E_2 by the ovaries is normally produced at least for some years, and hence affected 46,XX individuals develop breast normally and have menarche at the appropriate age (7, 8). At the peripubertal stage, however, ovarian sonography shows the presence of cysts. Furthermore, the LH/FSH ratio is high as in polycystic ovarian syndrome (PCOS), whereas androgen levels, as expected from a very early defect in steroidogenesis, are low. Progesterone measurements have indicated very low levels. Fertility in female patients with specific defects in gonadal steroidogenesis, like StAR gene mutations, has not been reported to date.

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Here we present the long path to conception and successful pregnancy outcome of one of our patients with mutation in the StAR gene (9). To our knowledge, this case represents the first pregnancy in an individual affected with this molecular defect as well as the first IVF and preimplantation genetic diagnosis in a patient with congenital lipoid adrenal hyperplasia.

CASE REPORT

The patient first presented with vomiting and failure to thrive at age 2 months. However, the diagnosis of adrenal insufficiency was established at the age of 8 months. The patient, administered hydrocortisone and 9a-fludrocortisone substitution therapy, grew up normally and has been in good general health. The patient entered puberty at age 9 years and had menarche at age 12 years. Menses were normal up to age 24 years, but thereafter menstruation occurred every 2 to 3 months. The patient was married at age 16 years to a man originating from the same village of a Greek island.

We initially attributed the adrenal insufficiency to congenital adrenal hypoplasia. However, upon workup for infertility carried out by her gynecologist, "cysts in the ovaries" were detected by sonography, and the LH/FSH ratio was very high, indicative of PCOS. Nevertheless, the androgen levels were very low. With this information we carried out DNA analysis for StAR gene mutations.

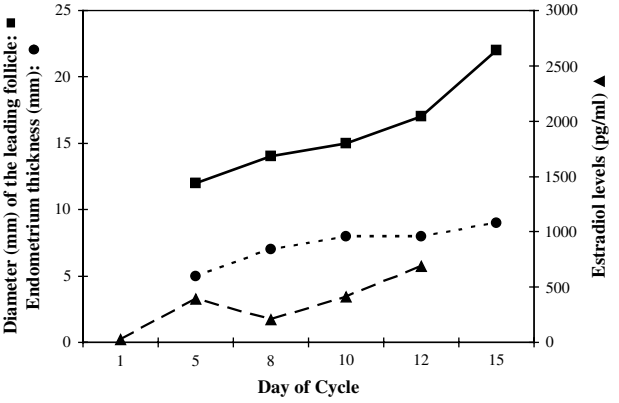
Nine years after her marriage, despite normal menses and good control of the adrenal insufficiency, she could not conceive. After many unsuccessful attempts to conceive and after an operation for ovarian cysts, the patient entered a program of IVF. The first IVF attempt was unsuccessful. During this first attempt, the maximum E_2 value achieved was 115 pg/mL on day 11 of recombinant FSH treatment, and the maximum endometrium thickness was 8 mm. Two embryos (three-cell stage) were transferred, but pregnancy did not progress.

The second IVF attempt was carried out after the DNA analysis showing an 11-bp deletion in exon 6 of the StAR gene (c.834del11bp). Her parents, who are second cousins, were found to be heterozygotes for the same mutation. Her husband, who also originates from the same village, was found to be a heterozygote for the same mutation. On the basis of the molecular data indicating an early defect of steroidogenesis, not only in the adrenal but also in the ovary, the IVF protocol was modified as described below.

The patient underwent pretreatment with an oral contraceptive pill followed by controlled ovarian hyperstimulation with a GnRH agonist (Daronda 3.75 mg; Abbott Laboratories, Abbott Park, IL), initiated in the midluteal phase of the previous cycle, and administration of recombinant FSH (Gonal-F; Merck Serono, Geneva, Switzerland) (225 IU/d on days 5–9, 375 IU/d on days 10–14, and 300 IU on day 15) and recombinant LH (Luveris; Merck Serono) based on serial ultrasonographic measurements of follicular growth and serum E_2 levels (10). Additionally, oral E_2 valerate (Estropause; Faran Laboratories, Athens, Greece) was administered twice daily (4 mg/d) from days 2 to 7 and three times daily (6 mg/d) from day 8. Exogenous administration of estrogens (Es) represented a modification of the standard stimulation protocol to address the E deficiency related to the patient's molecular pathology and achieve optimum endometrium preparation for implantation. The E_2 level before oral administration was <9 pg/mL. On day 12, the last time that the E_2 value was determined, a peak value of 689 pg/mL was achieved. The endometrium stripe thickness was 9 mm on day 15. Follicular growth throughout stimulation, serum E_2 levels, and endometrial thickness in response to exogenous administration of E_2 valerate are depicted in Figure 1. Once the leading follicle of 10 follicles on the right side measured 22 mm in diameter and the leading follicle of 7 follicles on the left side measured 19 mm in diameter (day 16), follicle maturation was initiated by a single injection of 10,000 IU chorionic gonadotrophin (Ovitrelle; Merck Serono). Transvaginal ultrasound–assisted oocyte retrieval was performed 35 hours later. Luteal-phase support included oral administration of E_2 valerate (Estropause; three times daily, 6 mg/d), oral and vaginal administration of P (Utrogestan [Ferring Pharmaceuticals, Berkshire, United Kingdom]; 3 pills vaginally three times daily [900 mg/d], and 1 pill orally three times daily [300 mg/d]), prednisolone (Prezolon [Nycomed,

FIGURE 1

Follicular growth throughout stimulation, serum E_2 levels, and endometrial thickness in response to exogenous administration of E_2 valerate. The solid line represents the diameter of the leading follicle in millimeters, the dotted line represents the endometrial thickness in millimeters, and the hatched line represents the serum E_2 level in picograms per milliliter. Note that serum E_2 levels rose above baseline once exogenous E_2 was initiated on day 2. Serum E_2 levels fell by day 8, so the dosage of exogenous E_2 valerate was increased from 4 mg/d to 6 mg/d.



Sertedaki. Fertility and a StAR gene mutation. Fertil Steril 2009.

Athens, Greece]; 5 mg orally, three times daily for 5 days, and twice daily for an additional 3 days;), doxycycline (100 mg orally twice daily for 8 days; Vibramycin; Pfizer, New York, NY), and aspirin (Salospir [Uni-Pharma, Kato Kifissia, Greece]; 80 mg orally once daily) continuously until the pregnancy test. Estradiol valerate and P were continued to week 11 of pregnancy and were discontinued once placental function was confirmed. Subsequently the pregnancy proceeded uneventfully, and a normal female infant with a birth weight of 3,800 g was delivered by cesarean section. No perinatal problems were encountered, and the baby, 10 months old at the time of manuscript preparation, is healthy. It is understood that the mother, being an addisonian, was supported by appropriate doses of corticosteroids throughout pregnancy and by higher doses during delivery.

MATERIALS AND METHODS

Mutation Detection in Peripheral Blood

Deoxyribonucleic acid was extracted from peripheral blood leucocytes with the QIAmp DNA Blood mini-kit (Qiagen, Hilden, Germany). The seven exons and the splice junctions of the StAR gene were amplified by polymerase chain reaction (PCR) and directly sequenced in an automated sequencer (ABI 3100 Avant; Applied Biosystems, Foster City, CA). The PCR and sequencing primers were designed using the Primer 3 (v. 0.4.0) program.

Embryology and Preimplantation Genetic Diagnosis

Because the prospective father was a heterozygote for the same StAR gene defect, preimplantation diagnosis was considered appropriate.

After controlled ovarian hyperstimulation, 21 oocytes were retrieved from the patient, of which 11 were at the metaphase 2 stage of maturation and were fertilized by intracytoplasmic sperm injection (ICSI), as previously described (11). Eight oocytes were normally fertilized and were cultured until day 3 after insemination. Cleavage-stage biopsy, using a noncontact laser (ZILOS-tk; Hamilton Thorne Biosciences, Beverly, MA), was performed on seven embryos that had developed beyond the six-cell stage (12). Manipulation of cells, cell lysis, and precautions against contamination were as previously described (12, 13). Exon 6 of the StAR gene and another eight loci, including four throughout the Y-chromosome (SRY, Amel Y, and SY127, SY149 from the AZF region), three in the X-chromosome (Amel X, AR exon 6, and STR 45 microsatellite marker), and one autosomal microsatellite marker, D13S314, were amplified. One primer of each pair was fluorescently labeled to facilitate analysis of PCR products. The PCR products were fragmented and analyzed on an automated sequencer (OpenGene System, using Gene Objects software; Visible Genetics, High Wycombe, United Kingdom) (unpublished data). Two embryos were diagnosed as being heterozygotes for the c.834del11bp StAR gene mutation; one developed to the blastocyst stage on postfertilization day 5 and was transferred to the patient's uterus. Positive serum β-hCG was achieved 10 days after transfer. At 6 weeks a singleton pregnancy was confirmed by ultrasound. At 12 weeks, chorionic villus biopsy was performed, a normal XX karyotype was found, and the state of heterozygosity was confirmed by sequencing.

For the DNA analysis and the preimplantation genetic diagnosis, written informed consent was obtained from the patients. This study had the approval of the Ethics Committee of "Aghia Sophia" Children's Hospital. All other procedures were carried out in a private IVF clinic and were part of the routine obstetric management of the case.

DISCUSSION

The exact mechanism of infertility in our patient, and possibly other patients with the same molecular defect, is not readily apparent. The paradigm is unique in that theoretically only the steroidogenic pathway is affected, whereas germ cell migration and evolution is expected to proceed normally. Furthermore, certain analogies to PCOS are thought provoking, in that there is a problem in ovarian follicle evolution and increased LH/FSH ratio in either entity.

It is of considerable interest that females with StAR gene mutations have fewer clinical manifestations than males with regard to gonadal function. Thus, testicular function is already affected in fetal life, leading to low androgen synthesis and consequently to lack of virilization of the external genitalia in a 46,XY individual. On the other hand, 46,XX individuals with StAR gene mutations enter puberty normally, have menarche at the expected age, and menstruate regularly for some years (7, 8, 14). However, ovarian dysfunction is manifested at the peripubertal stage, when the rise of gonadotrophins stimulates the ovaries and leads to cyst formation of undetermined mechanism. This gender dimorphism is attributed to the fact that steroidogenic activity is high in the fetal testis but very low in the fetal ovaries. The increased demand for steroid synthesis in the fetal testes leads to accumulation of lipid droplets in the Leydig cells, which cause secondary "mechanical" damage called "second hit" damage. The ovaries, having low steroidogenic activity in fetal life, possibly escape "second hit" damage and retain some activity for a number of years, producing Es, through a StAR-independent pathway for some time (3).

However, at the peripubertal period and later, the ovaries, under the stimulus of increasing gonadotrophins, possibly undergo progressive damage through either the "second hit" mechanism or the toxic effects of cholesterol auto-oxidation products or both, factors that abolish the StAR-independent steroidogenesis. This theory is supported by relevant findings in StAR knockout mice (15).

Apparently, although a low level of Es, produced by StAR-independent steroidogenesis, is sufficient to support pubertal development, the high demands for E synthesis connected to the process of ovulation and implantation cannot be met. It is

anticipated that extra E administration is necessary until placental function takes over, at which time no further support is required, because for the steroidogenesis in the placenta the StAR gene is not involved. This is expected by the fact that the StAR gene is not expressed in the placenta (16–18).

In the present case, we attributed the successful second IVF outcome to the etiologic diagnosis (molecular defect), which dictated a modified obstetric regimen, namely the additional E administration.

As is always the case in clinical medicine, the uncovering of the pathogenetic mechanism involved in patients with infertility is in certain cases basic to the success of intervention. The present data indicate that pregnancy is feasible in patients with StAR gene mutations, provided that extra E support is offered until placental function ensues. In our patient an additional difficulty was the father's molecular defect, which dictated preimplantation molecular diagnosis, a rather powerful tool in certain disease entities.

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REFERENCES

1. Sugawara T, Holt JA, Driscoll D, Strauss JF III, Lin D, Miller WL, et al. Human steroidogenic acute regulatory protein: functional activity in COS-1 cells, tissue-specific expression, and mapping of the structural gene to 8p11.2 and a pseudogene to chromosome 13. Proc Nat Acad Sci U S A 1995;92:4778–82.
2. Lin D, Sugawara T, Strauss JF III, Clark BJ, Stocco DM, Saenger P, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 1995;267:1828–31.
3. Bose HS, Sugawara T, Strauss JF III, Miller WL. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. New Engl J Med 1996;335:1870–8.
4. Nakae J, Tajima T, Sugawara T, Arakane F, Hanaki K, Hotsubo T, et al. Analysis of the steroidogenic acute regulatory protein (StAR) gene in Japanese patients with congenital lipoid adrenal hyperplasia. Hum Molec Genet 1997;6:571–6.
5. Bhangoo A, Gu WX, Pavlakis S, Anhalt H, Heir L, Ten S, et al. Phenotypic features associated with mutations in steroidogenic acute regulatory protein. J Clin Endocrinol Metabol 2005;90:6303–9.
6. Bose HS, Sato S, Aisenberg J, Shalev SA, Matsuo N, Miller WL. Mutations in the steroidogenic acute regulatory protein (StAR) in six patients with congenital lipoid adrenal hyperplasia. J Clin Endocrinol Metabol 2000;85:3636–9.
7. Bose HS, Pescovitz OH, Miller WL. Spontaneous feminization in a 46,XX female patient with congenital lipoid adrenal hyperplasia due to a homozygous frameshift mutation in the steroidogenic acute regulatory protein (StAR) gene. J Clin Endocrinol Metabol 1997;82:1511–5.
8. Fujieda K, Tajima T, Nakae J, Sageshima S, Tachibana K, Suwa S, et al. Spontaneous puberty in 46,XX subjects with congenital lipoid adrenal hyperplasia. Ovarian steroidogenesis is spared to some extent despite inactivating mutations in the steroidogenic acute regulatory protein (StAR) gene. J Clin Invest 1997;99:1265–71.
9. Sertedaki A, Dracopoulou A, Voutetakis A, Kanaka-Gantenbein C, Magiakou AM, Dacou-Voutetakis C. Mutations in the steroidogenic acute regulatory protein (StAR) in 5 patients of Hellenic origin. Report of certain distinct clinical characteristics. Horm Res 2006;65(Suppl 4): 73.
10. Pantos K, Meimeth-Damianaki T, Vaxevanoglou T, Kapetanakis E. Prospective study of a modified gonadotropin-releasing hormone agonist long protocol in an in vitro fertilization program. Fertil Steril 1994;61: 709–13.
11. Yanagimachi R. Intracytoplasmic injection of spermatozoa and spermatogenic cells: its biology and applications in humans and animals. Reprod Biomed Online 2005;10:247–88.
12. Kokkali G, Traeger-Synodinos J, Vrettou C, Stavrou D, Jones GM, Cram DS, et al. Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation genetic diagnosis of Beta-thalassaemia: a pilot study. Hum Reprod 2007;22:1443–9.
13. Vrettou C, Traeger-Synodinos J, Tzetis M, Palmer G, Sofocleous C, Kanavakis E. Real-time PCR for single-cell genotyping in sickle cell and thalassemia syndromes as a rapid, accurate, reliable and widely applicable protocol for preimplantation genetic diagnosis. Hum Mutat 2004;23:513–21.
14. Bhangoo A, Buyuk E, Oktay K, Ten S. Phenotypic features of 46,XX females with StAR protein mutations. Ped Endocrinol Rev 2007;5: 633–41.
15. Caron KM, Soo SC, Wetsel WC, Stocco DM, Clark BJ, Parker KL. Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipoid adrenal hyperplasia. Proc Nat Acad Sci U S A 1997;94:11540–5.
16. Strauss JF III, Martinez F, Kiriakidou M. Placental steroid hormone synthesis: unique features and unanswered questions. Biol Reprod 1996;54: 303–11.
17. Miller WL. Steroid hormone biosynthesis and actions in the materno-feto-placental unit. Clin Perinatol 1998;25:799–817.
18. Strauss JF III, Christenson LK, Devoto L, Martinez F. Providing progesterone for pregnancy: control of cholesterol flux to the side-chain cleavage system. J Reprod Fertil Suppl 2000;55:3–12.