Genotyping of congenital adrenal hyperplasia due to 21-hydroxylase deficiency presenting as male infertility: Case report and literature review

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Abstract We describe here two infertile male patients who were referred to our hospital with azoospermia at the ages of 33 and 30 years, respectively. Hormonal examinations led to a diagnosis of congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency in both patients. Genotyping revealed that the patients had a homozygous I172N and a heterozygous compound I172N/IVS2-13A/C>G mutation, respectively. Glucocorticoid replacement therapy succeeded in improving the seminal status of one patient, but not the other. For the latter patient and his wife, a pregnancy was achieved by testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) following genetic counseling. It is important to investigate genotyping and to classify patients on the basis of genotypic information in order to

arrive at better treatment strategies for male infertility; especially in counseling of TESE-ICSI.

Keywords 21-hydroxylase deficiency · Congenital adrenal hyperplasia · Genotyping · TESE-ICSI

Introduction

Congenital adrenal hyperplasia (CAH) with 21-hydroxylase deficiency (21-OHD) is one of the most common inherited metabolic diseases [1]. Cases of CAH can be clinically classified into three categories: the classic salt-wasting type that causes life threatening symptoms during the perinatal period, the classic type with simple virilization, and the nonclassic type with late onset of symptoms during puberty or adulthood. Recently, genotyping has been used for the definite diagnosis of CAH and has revealed that different types of mutations are correlated with various clinical features. Screening studies indicate that the worldwide (including Japan) incidence of classic 21-OHD is about 1 in 15,000–20,000 live births, and that the allelic variant of nonclassic 21-OHD is estimated to occur in 1 in 100 persons based on population genetic studies in the general white population [2].

Male infertility is one of the clinical features of CAH in male patients. In patients with classical CAH, if glucocorticoid replacement therapy is started in the perinatal period or childhood, normal spermatogenesis can occur, although, rarely, CAH is also diagnosed in adulthood because of associated male infertility.

To our knowledge, genotyping of a patient with congenital adrenal hyperplasia due to 21-OHD presenting as male infertility has been reported in only one previous instance. Here we present genotyping data for two such CAH patients and discuss about how we take counsel with the couples about

Genotypic information of CAH patients is important in the treatment for male infertility, especially in counseling of TESE-ICSI.

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genetic problem. Here we presented two cases of CAH presenting male infertility and short review of the literature.

Case report

Case 1: A 33-year-old man was referred to our hospital with the chief complaint of infertility in March 2000. He had had a growth spurt at age 9 with accompanying voice changes, growth of hair of pubis, and had ended his growth period by age 12. The patient was 157 cm tall, normotensive, and normally virilized. The testicular size was 4 and 5 cc on the right and left sides (normal > 14cc), respectively. There were no nodules around the testes, and the vas deferens and epididymis were palpable and normal. Two separate semen analyses demonstrated azoospermia, and semen volumes were 4.2 mL and 3.1 mL for each of the analyses. Hormonal examination revealed that the follicle-stimulating hormone (FSH) concentration was <0.5 mIU/mL (normal 1.3–10.4 mIU/mL), luteinizing hormone (LH) was <0.5 mIU/mL (normal 1.1–8.8 mIU/mL), serum testosterone was 420 ng/dL (normal 71.8–623 ng/dL), adrenocorticotrophic hormone (ACTH) was 115 ng/mL (normal 10–70 ng/mL), and serum 17 α hydroxy-progesterone (17 α OHP) was 137 ng/mL (normal 0.1–3.7 ng/mL). Chromosomal analysis revealed a normal 46XY male karyotype. The laboratory results and the patient's history of early pubertal growth (started at nine-year-old) and short stature (stand 157cm tall) were consistent with the diagnosis of CAH due to 21-OHD. Genotyping revealed a homozygous I172N mutation in the *CYP21* gene. In April 2000, the patient started dexamethasone therapy (0.5 mg/day), and improvements in hormonal concentration and semen analysis parameters (sperm count was 6×10^6 /mL and motility was 65%) were apparent after six months. The patient and his wife tried conception by intrauterine insemination (IUI), but pregnancy wasn't achieved. The couple didn't undergo further treatment for infertility. After the treatment, the patient stopped taking dexamethasone.

Case 2: A 30-year-old man was referred to our hospital with the chief complaint of infertility in May 2002. He had had a growth spurt at age 9 with accompanying voice changes, growth of hair of pubis, and had ended his growth period by age 12. The patient was 155 cm tall, normotensive at 119/77 mmHg, normally virilized, and had dark skin. The testicular size was 5 cc on both sides (normal > 14cc). A semen analysis demonstrated azoospermia. Semen volume was 4.0 mL. Hormonal examination revealed that the FSH concentration was <0.5 mIU/mL (normal 1.3–10.4 mIU/mL), LH was <0.5 mIU/mL (normal 1.1–8.8 mIU/mL), serum testosterone was 480 ng/dL (normal 71.8–623 ng/dL), ACTH was 670 ng/mL (normal 10–70 ng/mL), and serum 17 α OHP was 221.4 ng/mL (normal 0.1–3.7 ng/mL). Chro-

mosomal analysis revealed a normal 46XY male karyotype. The laboratory results and the patient's history of early pubertal growth (started at nine-year-old) and short stature (stand 157 cm tall) were consistent with a diagnosis of nonclassic late-onset CAH due to 21-OHD. Genotyping revealed a heterozygous compound I172N/IVS2-13A/C>G mutation in the *CYP21* gene. The patient started dexamethasone therapy (0.5 mg/day) and improved with respect to hormonal concentration parameters, although azoospermia was again found on semen analysis after 12 months of therapy. The patient's FSH and LH concentrations increased to 32 mIU/mL and 16.4 mIU/mL, respectively, and his serum testosterone concentration was 230 ng/dL. Genotyping revealed that his wife had no mutations in the *CYP21* gene. After genetic counseling, the couple elected to undergo testicular sperm extraction-intracytoplasmic sperm injection (TESE-ICSI) treatment. Sperm was extracted from the patient's testis by microdissection TESE and a pregnancy was achieved, although Johnsen's score was found to be 3 during pathological studies of biopsy specimens. A normal child was delivered. After the treatment, the patient continued to take dexamethasone.

Discussion

In CAH patients, hypogonadism is thought to occur because decreased synthesis of cortisol leads to excessive secretion of ACTH via feedback regulation, which results in overstimulation of the adrenal gland. Upregulated adrenal androgen affects the pituitary gland and downregulates FSH and LH, which causes downregulation of testosterone and inhibition of spermatogenesis, although some reports have said that adrenal androgens alone have no effect on gonadotropin secretion via a negative feedback mechanism in male patients [2]. To our knowledge, 11 patients, including the two presented here, have been diagnosed with 21-OHD presenting with male infertility as the chief complaint (Table 1). Six were azoospermic and the other five oligozoospermic [3–10]. Regarding treatment, in most patients hormone replacement therapy successfully improved semen analysis parameters and led to pregnancies within 4 to 27 months, although this treatment did not improve seminal findings in two azoospermic patients, including one patient described here [4]. In these two patients, TESE-ICSI was carried out, and pregnancy was successfully achieved.

Genotyping for CAH is commonly carried out for definite diagnosis, and there is generally some correlation between the clinical disease severity and the discrete mutations observed [2]. For example, deletions or large conversions in the *CYP21* gene have been found in patients with the classic salt-wasting type of CAH, whereas mutations at I172N or IVS2-13A/C>G have been found in patients with

Table 1 Treatment results for patients with CAH due to 21-OHD presenting as male infertility

Case	Age (years)	Classification	Mutation	Sperm count: pretreatment	Sperm count: post-treatment	Treatment	Result ^a	Reference
1	28	Nonclassic	N/A	$9 \times 10^6/\text{mL}$	$14 \times 10^6/\text{mL}$	Dexa	pregnancy at 27 months; unassisted	(3)
2	22	Nonclassic	N/A	$0.5 \times 10^6/\text{mL}$	$54.8 \times 10^6/\text{mL}$	Dexa	pregnancy at 9 months; unassisted	(3)
3	29	Nonclassic	N/A	azoospermia	azoospermia	Dexa. + TESE	pregnancy by TESE-ICSI	(4)
4	45	Nonclassic	Combination of 6 mutations (I172N, Cluster E6, V281L, L307insT, Q318X, R356W)	$1.8 \times 10^6/\text{mL}$	$35 \times 10^6/\text{mL}$	Dexa	pregnancy at 5 months; unassisted	(5)
5	29	Nonclassic	N/A	azoospermia	$20 \times 10^6/\text{mL}$	Dexa	pregnancy at 8 months; unassisted	(6)
6	27	Nonclassic	N/A	$10.8 \times 10^6/\text{mL}$	$140.8 \times 10^6/\text{mL}$	Dexa	pregnancy at 9 months; unassisted	(7)
7	N/A	Nonclassic	N/A	azoospermia	N/A	N/A	pregnancy at 4 months; unassisted	(8)
8	32	Nonclassic	N/A	azoospermia	$2 \times 10^4/\text{mL}$	Dexa	pregnancy at 5 months; unassisted	(9)
9	35	Nonclassic	N/A	$4.5 \times 10^6/\text{mL}$	$25 \times 10^6/\text{mL}$	Dexa	pregnancy at 4 months; unassisted	(10)
10	33	Nonclassic	Homozygous I172N	azoospermia	azoospermia	Dexa	no pregnancy	Present report
11	30	Nonclassic	Heterozygous compound I172N/IVS2-13A/C>G	azoospermia	azoospermia	Dexa. + TESE	pregnancy by TESE-ICSI	Present report

Note. N/A = not available, Dexa. = Dexamethazone replacement.

^aThe number of months indicate the time between initiation of therapy and pregnancy.

the classic type with simple virilization. Mutations such as V281L, P30L, etc. tend to be reported in patients with the nonclassic type of CAH. In the present study, we reported two patients with 21-OHD who were diagnosed with male infertility in adulthood and possessed a homozygous I172N and a heterozygous compound I172N/IVS2-13A/C>G mutation, respectively. Another patient with 21-OHD described in the literature, who was diagnosed with male infertility, had a heterozygous compound I172N mutation together with six mutations (I172N, Cluster E6, V281L, L307insT, Q318X, R356W) in the other allele [5]. Clinically, these three CAH patients diagnosed with male infertility were classified with the non-classic or late-onset type of CAH, but all possessed the I172N mutation, which is linked to the classic simple virilization type of CAH on genotyping. These male patients may well have been underdiagnosed in childhood because of the lack of obvious symptoms, such as the virilizing of external genital organs that occurs in female patients.

Generally, genetic counseling is important when performing TESE-ICSI in patients with congenital diseases. A screening study found that the allelic variant of nonclassic 21-OHD occurs in 1 in every 100 persons [11]. Because 21-OHD is an autosomal recessive disease, a child has a 50% chance of having 21-OHD if his or her father has 21-OHD and his or her mother possesses the allelic variant of 21-hydroxylase. More investigations will be needed to determine what genotypic features influence the phenotype of male infertility.

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