

NOTE

Homozygous Q258X Mutation in the Steroidogenic Acute Regulatory Gene in a Japanese Patient with Congenital Lipoid Adrenal Hyperplasia

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Abstract. Congenital lipoid adrenal hyperplasia (CLAH) is an autosomal recessive disorder characterized by impaired synthesis of all adrenal and gonadal steroid hormones. It has recently been reported that mutations in the steroidogenic acute regulatory protein (StAR) gene cause CLAH. We analyzed the nucleotide sequences of exon 7 of the StAR gene in a Japanese CLAH patient with a karyotype of 47,XXX, and her parents. The patient was homozygous for a nonsense mutation Q258X, which changed codon 258 (CAG) encoding Gln to the stop codon TAG, and the her parents were heterozygous for the Q258X mutation. Since the Q258X mutation destroys a MvaI site normally present in the StAR gene sequence, we confirmed the Q258X mutation by means of the restriction endonuclease MvaI digestion of the PCR products. Endocrinological examinations of the parents revealed normal responses of adrenal steroid hormones to exogenous adrenocorticotropin administration, confirming the failure to detect the heterozygous carriers of CLAH by hormonal evaluation.

Key words: Congenital lipoid adrenal hyperplasia, Steroidogenic acute regulatory protein, Nonsense mutation, Homozygous, Heterozygous, Restriction endonuclease digestion

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CONGENITAL lipoid adrenal hyperplasia (CLAH), originally described by Prader *et al.* [1], is the most severe form of congenital adrenal hyperplasia (CAH), leading to impaired production of all steroids, including glucocorticoids, mineralocorticoids and sex steroids. This disorder is inherited as an autosomal recessive trait, and the affected individuals are all phenotypically female with a severe salt-losing syndrome that is fatal unless treated with steroid replacement therapy [2]. Because mitochondria from affected

adrenal glands and gonads fail to convert cholesterol to pregnenolone, CLAH had been postulated to be caused by a defect in the cholesterol side chain cleavage enzyme, cytochrome P450_{scc} [3, 4], but no mutations have been revealed in the P450_{scc} gene in affected individuals, suggesting a defect in another undefined factor involving conversion of cholesterol to pregnenolone [5, 6].

Steroidogenic acute regulatory protein (StAR) is a 30-kDa phosphorylated protein that rapidly appears in mitochondria of steroidogenic cells following tropic stimulation, and is required in the acute regulation of steroidogenesis [7]. It has recently been reported that mutations in the StAR gene cause CLAH [8]. To date, only two studies describing two nonsense mutations and one

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splicing mutation of the StAR gene in four families with CLAH have been reported [8, 9]. In the present study, we describe a homozygous Q258X mutation in the StAR gene in a Japanese CLAH patient with a karyotype of 47,XYY, and the lack of detectable hormonal abnormalities in the parents proven to be heterozygotes for the Q258X mutation.

Materials and Methods

Patient

The patient, born in 1974, was observed to have female external genitalia and hyperpigmentation at birth and developed severe salt loss (serum Na 125 mEq/l, K 7.6 mEq/l) during the second week of life. She was successfully treated with hydrocortisone, fludrocortisone and sodium chloride. The karyotype was 47,XYY. Her elder sister had died about 2 months after birth, having shown similar clinical manifestations, and her autopsy had revealed adrenal lipoid hyperplasia. The diagnosis of CLAH was based on classical symptoms and signs during the neonatal period, the clinical course after treatment, and the family history [10]. The patient had only one sister, described above, and two brothers, one of whom was in good health, and the other had died of unknown cause soon after birth. Both her parents were healthy and unrelated. Only the patient and her parents were available for the present study.

Informed consent for the genomic analysis as well as for the endocrinological evaluation was obtained from the patient and her parents.

Selective amplification of fragments of exon 7 of the StAR gene

The genomic DNAs of the patient, her parents and unrelated healthy individuals were isolated from whole blood by proteinase K digestion and phenol/chloroform extraction. Selective amplification of DNA fragments of exon 7 [11] of the human StAR gene was achieved as reported by Lin *et al.* [8]. Briefly, the sequences of the primers were 5'-CCTGGCAGCCTGTTTGTGATAG-3' (sense primer S4), 5'-CCTCATGTCATAGCTAATCAGTG-3' (antisense primer AS4) and 5'-ATGAGCGTGTGTACCAGTGCAG-3' (antisense primer AS1).

Polymerase chain reaction (PCR) [12] was performed in a 100 μ l mixture containing 0.2 μ g genomic DNA, 0.001% gelatin, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 100 μ M of each dNTP, 250 pM of the sense primer and the antisense primer, and 2.5 U Taq DNA polymerase (Takara Shuzo Co., Ltd., Otsu, Japan). After the first step of denaturation at 95 °C for 4 min, 30 cycles of denaturation at 95 °C for 1 min, annealing at 57 °C for 1 min, and extension at 72 °C for 30 sec were followed by an additional extension at 72 °C for 3 min.

Direct sequencing of PCR products

Single-strand DNA was produced from both strands [13], and the PCR DNA fragments were sequenced in both orientations by the dideoxynucleotide chain termination method [14] with a Sequenase kit (Amersham Japan Ltd., Tokyo, Japan).

Restriction endonuclease analysis of PCR products

PCR products amplified with the primer pair S4-AS1 were digested with MvaI (Toyobo Co., Ltd., Osaka, Japan) according to the manufacturer's specifications and electrophoresed in a 2% NuSieve GTG (FMC BioProducts, Rockland, ME)/1% agarose (Bio-Rad Lab., Richmond, CA) gel. Digested fragments were visualized by ethidium bromide staining.

Endocrinological evaluation

Endocrine studies of the patient were performed at the ages of 8 and 15 years. During the endocrinological evaluation, the patient had a replacement dose of hydrocortisone and fludrocortisone. Endocrinological evaluation of the father was carried out at the age of 37 years, and that of the mother was at the age of 35 years.

For the rapid ACTH stimulation tests, 0.25 mg of synthetic ACTH (Cortrosyn; Daiichi Pharmaceuticals Co., Tokyo, Japan) was given as an iv bolus. For the human chorionic gonadotropin (hCG) stimulation test, 4,000 IU/m² of hCG (Gonotropin; Teikoku Zoki Pharmaceuticals Co., Kawasaki, Japan) was injected im daily for 3 days. Serum steroids were measured by specific radioimmunoassays.

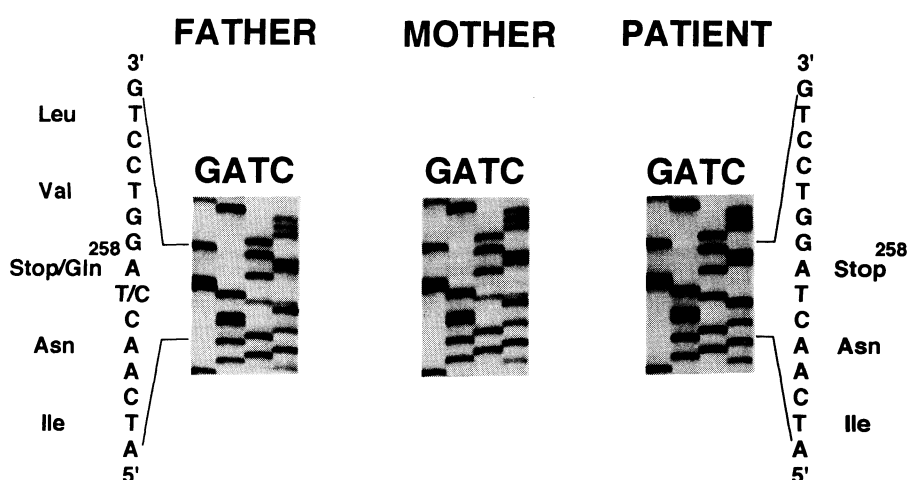


Fig. 1. Partial nucleotide sequence of exon 7 of the StAR gene from the patient and her parents determined by direct sequencing of asymmetric PCR products. The patient has a homozygous nonsense mutation at codon 258. Note that both T and C are present in the first nucleotides at codon 258 in the parents' StAR gene sequence, indicating heterozygosity.

Results

Genetic analysis of the StAR gene

The sequencing analysis revealed that the patient was homozygous for a nonsense mutation Q258X, which changed codon 258 (CAG) encoding Gln to the stop codon TAG, and her parents were heterozygous for the same mutation (Fig. 1).

As shown in Fig. 2, the Q258X mutation destroyed a recognition site for the restriction endonuclease MvaI, a fact that was used to confirm homozygotes and heterozygotes for the mutation. Restriction endonuclease MvaI digestion of the 253-bp PCR products from the normal allele generated by the primer pair S4-AS1 produces six fragments of 2, 112, 18, 33, 26 and 62 bp, whereas MvaI digestion of the PCR product from the Q258X mutant allele creates five fragments of 2, 130, 33, 26 and 62 bp. As shown in Fig. 3, a 112-bp band as well as bands of smaller sizes was demonstrated in normal subjects (lanes 2 and 3), a 130-bp band instead of the 112-bp band in the patient, who was homozygous for the Q258X mutation (lane 5), and both 112- and 130-bp bands in her parents who were heterozygous for the mutation (lanes 4 and 6) on 2% Nusieve GTG/1% agarose gel electrophoresis of the MvaI digested PCR products.

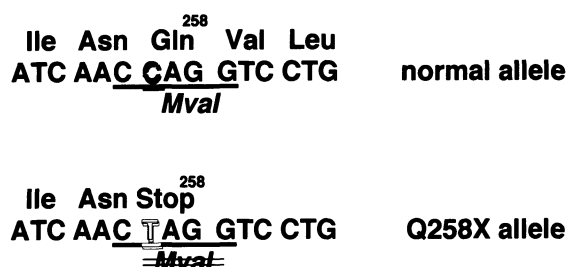


Fig. 2. Nucleotide sequence surrounding codon 258. The C to T conversion in the first nucleotide at codon 258 in the Q258X mutant allele destroys an MvaI site.

Thus the Q258X allele was easily distinguishable from the normal allele on the basis of the fragment sizes.

Hormonal evaluation of patient and parents

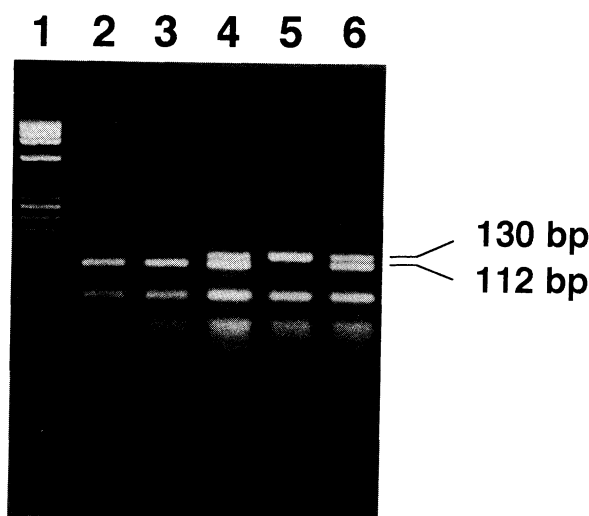
The ACTH stimulation test failed to increase serum progesterone, 17 α -hydroxyprogesterone (17OHP), cortisol and aldosterone levels in the patient at the age of 8 years (Table 1). Basal and ACTH-stimulated adrenal steroid levels proved to be normal in her parents (Table 1).

The hCG stimulation tests performed in the

Table 1. Baseline serum steroid levels and their responses to a 0.25-mg bolus injection of ACTH in the patient and her parents

Serum steroids	Patient ^a (8 yr)		Father (37 yr)		Mother (35 yr)	
	0 min	60 min	0 min	60 min	0 min	60 min
Progesterone (ng/dl)	0.8	1.0	20.2	62.7	420	930
17OHP (ng/dl)	10.0	8.0	102	191	268	388
Cortisol (μ g/dl)	26.3	16.5	16.1	41.5	10.2	30.0
Aldosterone (ng/dl)	2.6	2.7	11.3	23.5	9.8	28.0
Testosterone (ng/dl)	18.0	17.0	710	770	71.2	90.0

^aThe patient had a replacement dose of hydrocortisone and fludrocortisone. 17OHP, 17 α -hydroxyprogesterone.

**Fig. 3.** Electrophoresis analysis of amplified exon 7 genomic DNA from normal subjects, the patient, and her parents after restriction digestion with *Mva*I. Lane 1: *Hae*III digested Φ X174 RF DNA. Lanes 2 and 3: PCR products from normal individuals. Lanes 4 and 6: PCR products from the parents. Lane 5: PCR products from the patient.**Table 2.** Baseline serum steroid levels and their responses to hCG administration (4,000 IU/m², im for 3 days) in the patient

Serum steroids	Age			
	8 yr		15 yr	
	base	post-hCG	base	post-hCG
17OHP (ng/dl)	20	20	<10	<10
DHEA (ng/dl)	–	–	<10	<10
Testosterone (ng/dl)	35.0	37.0	21.8	24.3

17OHP, 17 α -hydroxyprogesterone; DHEA, dehydroepiandrosterone.

patient at 8 and 15 years of age revealed no considerable increase in serum dehydroepiandrosterone, 17OHP and testosterone (Table 2).

Discussion

Mouse StAR has recently been isolated as a protein that is synthesized in response to luteinizing hormone stimulation and is localized to the mitochondria in MA-10 mouse Leydig tumor cells, and mouse complementary DNA (cDNA) has been cloned [7]. StAR was postulated to transfer cholesterol into the mitochondrial inner membrane where the first enzymatic step in the steroidogenesis occurs, and play a key role in the acute regulation of steroidogenesis following tropic stimulation [7]. Soon after, human StAR gene as well as human StAR cDNA has been cloned and mapped 8p11.2 [11, 15], and the importance of StAR in the steroidogenesis has been supported by finding that the StAR gene is mutated in the patients with CLAH in whom all gonadal and adrenal steroidogenesis is impaired [8, 9].

Lin *et al.* [8] reported a nonsense R193X mutation of the StAR gene in a CLAH patient of Caucasian ancestry and the nonsense Q258X mutation of the StAR gene in Korean and Japanese patients with CLAH, and demonstrated that both of the mutations resulted in impairment of StAR activity [8]. In the present study, we also demonstrated that our patient was homozygous for the Q258X mutation and the parents were heterozygous for the same mutation. We therefore conclude that the homozygous Q258X mutation in the StAR gene caused CLAH in our patient.

Since our patient and the Japanese patient reported by Lin *et al.* [8] share the homozygous Q258X mutation in the StAR gene, the Q258X mutation might be predominant in Japanese patients with CLAH. In this context, our restriction analysis method to demonstrate the Q258X mutation is very simple and may be useful in the genetic analysis of the Japanese patients with CLAH, although the analysis of the entire StAR gene is required to confirm the genetic diagnosis of CLAH.

The endocrinological diagnosis of CLAH is inherently indirect, because, in contrast to other forms of CAH, there is no characteristic accumulation of a precursor steroid. Our patient had no increase in serum adrenal and gonadal steroids in response to exogenous ACTH and hCG, which was compatible to the diagnosis of CLAH, but there was no demonstrable hormonal deficiency in the parents who had a proven single allele mutation, confirming the previous finding by Saenger *et al.* [16] that there was no demonstrable hormonal deficiency in heterozygotes for CLAH. The availability of genetic analysis of the StAR gene should make it possible to assess heterozygotes for CLAH accurately.

Prenatal diagnosis of CLAH allows proper identification of the affected fetus and prompt

initiation of hormonal replacement therapy after birth. This is particularly important because the affected individuals can be critically ill during the neonatal period and die from salt loss, hyperkalemic acidosis, and dehydration unless treated with steroid hormone replacement therapy. The prenatal diagnoses of CLAH thus far reported [16, 17] depended on the existence of affected siblings in the family, the appearance of normal female genitalia on ultrasonography in the 46,XY fetuses, and low amniotic fluid steroids. But the possibility that the fetuses might suffer from other disease such as congenital hypopituitarism could not be totally excluded. Furthermore, ultrasonographic findings on the genitalia are not informative in the case of XX fetuses with CLAH. The availability of a genetic diagnosis of CLAH makes prenatal diagnosis more certain not only in XY fetuses, but also in XX fetuses.

In conclusion, 1) we have demonstrated that the homozygous Q258X mutation in the StAR gene caused CLAH in a Japanese patient with a karyotype of 47,XXY; 2) we demonstrated that heterozygotes for the Q258X mutation in the StAR gene had normal adrenocortical function; 3) we have developed a novel restriction endonuclease digestion method to detect the Q258X mutation in the StAR gene.

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