Detection of the Missense Mutation A218V in the Steroidogenic Acute Regulatory Protein Gene of a Japanese Patient with Congenital Lipoid Adrenal Hyperplasia

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Abstract We analyzed the steroidogenic acute regulatory protein (StAR) gene in a Japanese patient with congenital lipoid adrenal hyperplasia (CLAH). The patient was revealed to be a compound heterozygote bearing a missense mutation A218V, which changed codon 218 (GCG) encoding Ala to GTG encoding Val in the StAR gene, and a nonsense mutation Q258X, which changed codon 258 (CAG) encoding Gln to the stop codon (TAG), by polymerase chain reaction amplification and direct sequencing of the StAR gene. The Q258X mutation has been demonstrated to cause CLAH in Japanese and Korean patients. The A218V mutation has been reported to cause CLAH in a Canadian white patient. These findings suggest that the amino acid residue Ala²¹⁸ is important for the normal StAR function, and the codon 218 might be a hotspot for the StAR gene mutation.

Key words: congenital lipoid adrenal hyperplasia, steroidogenic acute regulatory protein gene, nonsense mutation, missense mutation

Introduction

Congenital lipoid adrenal hyperplasia (CLAH), originally described by Prader and Siebenmann (1), is the most severe form of congenital adrenal hyperplasia, 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, it had been postulated to be caused by a defect in the cholesterol side-chain cleavage enzyme, cytochrome P450scc (3, 4). However, no mutations have been revealed in the P450scc gene in affected individuals, suggesting a defect in another undefined factor involving conversion of cholesterol to pregnenolone (5-7).

Steroidogenic acute regulatory protein (StAR) is a 30-kDa phosphorylated protein that rapidly appears in mitochondria of steroidogenic

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cells following tropic stimulation, and is required in the acute regulation of steroidogenesis (8). It has recently been reported that mutations in the StAR gene cause CLAH (9). To date, fifteen different mutations in the StAR gene have been found in patients with CLAH from various ethnic groups (9-11). We now report a compound heterozygous A218V/Q258X mutation in the StAR gene in a Japanese patient with CLAH.

Materials and Methods

Patient

The patient (46, XX), born in 1990, suffered

Table 1 Endocrinological findings in the patient at 12 days of age

ACTH	920 pg/ml
17OHP	0.5 ng/ml
Cortisol	12.9 μ g/dl
Aldosterone	260 pg/ml
DHEA	0.3 ng/ml
DHEA-S	<20 μ g/dl

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

from severe salt loss (serum Na 126 mEq/l, K 7.4 mEq/l) during the second week of life and presented with hyperpigmentation. The patient was diagnosed to have CLAH illustrated by low adrenal steroids in spite of noticeably increased adrenocorticotropin (Table 1) and enlarged adrenal glands demonstrated by abdominal CT scan. Both parents were healthy and unrelated.

Selective amplification of StAR gene fragments

The genomic DNAs of the patient and twenty unrelated healthy individuals were isolated from whole blood by proteinase K digestion and phenol/chloroform extraction after informed consent was obtained from each subject.

Seven pairs of oligonucleotide primers, which were used for selective amplification of DNA fragments that span each of seven exons and the exon-intron boundaries of the human StAR gene, were based on the human StAR gene sequence (9, 12) and are listed in Table 2. Polymerase chain reactions (PCR) (13) were 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 each of the sense primer and the antisense

Table 2 Oligonucleotide primers for PCR amplification of the human StAR gene

Exon	Primer	Direction	Sequence	Annealing
1	S1	Sense	5'-GCAGCAGCAGCGGCGGCAGCAG-3'	65°C
	AS1	Antisense	5'-TCGCCTCCTTCCCAGCAGCGC-3'	
2	S2	Sense	5'-AACAAGGGTTATTCCTTCT-3'	57°C
	AS2	Antisense	5'-GAGCCCAGAAGCCTCAGCAC-3'	
3	S3	Sense	5'-GTCTCTCCTCGGCTGTGTAT-3'	57°C
	AS3	Antisense	5'-ACAGGCTTCTCCCCGACACT-3'	
4	S4	Sense	5'-TCTGGGGGCTCCTTTCTCTG-3'	57°C
	AS4	Antisense	5'-ACCCGCACCTGGACTTTGCT-3'	
5	S5	Sense	5'-GTGAGCAAAGTCCAGGTGCG-3'	57°C
	AS5	Antisense	5'-GGATGCAGTCCACATGCTTGG-3'	
6	S6	Sense	5'-GACAAGGTACCTGAGAAACG-3'	57°C
	AS6	Antisense	5'-CCTGTCTTACAGCCTGTGAT-3'	
7	S7	Sense	5'-CCTGGCAGCCTGTTTGTGATAG-3'	57°C
	AS7	Antisense	5'-CCTCATGTCATAGCTAATCAGTG-3'	

primer, and 2.5 U Taq DNA polymerase (Takara Shuzo Co., Ltd., Otsu). After an initial step of denaturation at 95°C for 4 min, 30 cycles of denaturation at 95°C for 1 min, annealing at an appropriate temperature listed in Table 2 for 1 min, and extension at 72°C for 0.5 min were followed by an additional extension at 72°C for 3 min. The amplified PCR products were fractionated and isolated on a 1% agarose gel (Bio-Rad Laboratories, Richmond, CA), and subjected to direct sequencing.

In order to amplify DNA fragments spanning the last three exons of the human StAR gene, the primer pair of S5-AS7 was used. PCRs were carried out in the same reaction mixture as described above. After an initial step of denaturation at 95°C for 4 min, 30 cycles of denaturation at 95°C for 1 min, annealing at 64°C for 1 min, and extension at 72°C for 1.5 min were followed by an additional extension at 72°C for 3.5 min. The amplified PCR products were subjected to cloning into the pCRII plasmid (Invitrogen, San Diego, CA).

Direct sequencing of PCR products

Single-strand DNA was produced from both strands (14), and the PCR DNA fragments were sequenced in both orientations by the dideoxynucleotide chain termination method (15) with a Sequenase kit (Amersham Japan Ltd., Tokyo).

Cloning and sequencing of PCR products

To confirm the nucleotide sequences of different alleles, the amplified DNA fragments spanning the last three exons were cloned into the plasmid pCRII using a TA cloning kit (Invitrogen, San Diego, CA) and the nucleotide sequences were determined by the dideoxynucleotide chain termination method (15) with the Sequenase kit.

Results

We determined the nucleotide sequences of all seven exons and the exon-intron boundaries

of the patient's StAR gene.

The patient was a compound heterozygote bearing a missense mutation A218V, which changed codon 218 (GCG) encoding Ala to GTG encoding Val in the StAR gene, and a nonsense mutation Q258X, which changed codon 258 (CAG) encoding Gln to the stop codon (TAG) (Fig. 1). The DNA sequences of different alleles were confirmed by cloning the amplified DNA fragments spanning the last three exons into the pCRII plasmid and performing double stranded sequence analysis in the patient (data not shown). The patient was also revealed to be homozygous for a D203A mutation changing codon 203 (GAC) encoding Asp to GCC encoding Ala (data not shown).

No other mutations were found in any of the seven exons or the exon-intron boundaries of the StAR gene from the patient.

The A218V mutation was never identified in the StAR gene from twenty healthy individuals, but all of them were revealed to be homozygous for the D203A mutation.

Discussion

Lin *et al.* (9) first reported a nonsense mutation R193X of the StAR gene in a CLAH patient of Caucasian ancestry, and the nonsense mutation Q258X in Japanese and Korean patients with CLAH. Thereafter, another thirteen different mutations have been identified in patients with CLAH from various ethnic groups (10, 11).

The Q258X mutation has been demonstrated to result in impairment of StAR activity (9). Bose *et al.* (11) reported that the Q258X mutation was found in 80 percent of affected alleles of Japanese and Korean patients. In the present study, we also demonstrated the heterozygous Q258X mutation in our Japanese patient with CLAH, supporting the finding by Bose *et al.* (11).

Bose *et al.* (11) reported the missense mutation A218V of the StAR gene in a Canadian white patient, and demonstrated that the A218V mutant had minimal StAR activity, but they failed to find the A218V mutation in the CLAH patients from the ethnic groups other than the Canadian white (11). In the present study, we detected the A218V mutation in our Japanese patient with CLAH. The A218V mutation is the first one which has been shared by Japanese and Caucasian patients. These findings suggest that codon 218 of the StAR gene might be a mutational hotspot. The importance of the amino acid residue Ala²¹⁸ is further confirmed by the finding that this amino acid residue is conserved in all the StAR molecules so far identified in the mouse, cow and pig and in man (Fig. 2) (8, 16-18).

There seems to be no doubt that both A218V and Q258X mutations are responsible for CLAH in our patient since they have been demonstrated to impair the StAR activity (9, 11). The compound heterozygote carrying the A218V and Q258X mutations in the StAR gene seems to be novel in patients with CLAH. We speculate that the D203A mutation is innocent because all twen-

human StAR	MPEQKG VIR ²¹⁸ AEHGATCMVL
bovine StAR	MPQQKG VIR AEHGPTCMVL
porcine StAR	MPEQKG VIR AEHGPTCMVL
murine StAR	MPEQSG VIR AEHGPTCMVL

Fig. 2 Comparison of the amino acid sequences of mammalian StAR molecules, flanking Ala²¹⁸ in the human StAR. Note that this amino acid is conserved in all the StAR molecules so far identified in the mouse, cow and pig and in man (8, 16-18).

ty healthy individuals as well as the patient were homozygous for the D203A mutation, so that this mutation should not impair the StAR activity.

In patients with CLAH there are no characteristic increases in the concentrations of any circulating steroid hormones, unlike the case with all other forms of adrenal hyperplasia, thus the endocrinological diagnosis of CLAH is inherently one of exclusion (2). The genetic analysis method for the human StAR gene described here should facilitate making a diagnosis of CLAH in phenotypically female patients with signs and symptoms of deficiency of both glucocorticoid and mineralocorticoid.

In conclusion, we have identified a novel compound heterozygous A218V/Q218X mutation in the StAR gene in a Japanese patient with CLAH, and further analysis of the genotype-phenotype relationship in patients with CLAH should provide a clue to understand the importance of the StAR activity in steroidogenesis *in vivo*.

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