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Short Report

The Frequency of Eight Common Point Mutations in CYP21 Gene in Iranian Patients with Congenital Adrenal Hyperplasia

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ABSTRACT

Background: Congenital Adrenal Hyperplasia (CAH, the inherited inability to synthesize cortisol) is one of the most common (1 in 10000 to 1 in 15000) autosomal recessive disorders. More than 95% of cases of CAH are caused by 21-hydroxylase deficiency (21-OHD). Females with severe, classic 21-OHD are exposed to excess androgens prenatally and are born with virilized external genitalia. Most patients cannot synthesize sufficient aldosterone to maintain sodium balance and may develop potentially fatal salt wasting crisis if not treated. **Methods:** We applied allele specific PCR to detect the eight common mutations in the CYP21 gene in patients. Fifty unrelated patients with symptoms of classical CAH were studied. **Results and Conclusion:** Seventy percent of our subjects had these mutations. The most frequent mutations were found to be I2G and del-8bp (28% and 13%, respectively). The frequencies of other alleles were as following: I172N, 9%; V281L, 3%; exon 6 cluster (I236N, V237E and M239K), 4%; Q318X, 9%; R356W, 5%; and P30L, 0%. The frequency of mutations did not differ substantially from other ethnics, however, a higher rate of del-8 bp (13%) was found in our population. The aim of this study was to detect common mutations for setting up a molecular method for prenatal diagnosis. *Iran. Biomed. J. 12 (1): 49-53, 2008*

Keywords: Congenital adrenal hyperplasia (CAH), CYP21 gene, Mutation

INTRODUCTION

Congenital adrenal hyperplasia (CAH) encompasses a group of genetic disorders of adrenal steroidogenesis characterized by impaired activity of one of the enzymes required for cortisol biosynthesis [1]. Steroid 21-hydroxylase deficiency (21-OHD) is present in more than 95% of the patients with CAH [2]. The incidence of severe form is one in 10,000 to one in 15,000 among Caucasians [2]. The prevalence of non-classic 21-OHD is estimated to be one in 1700 in general population [3]. Based on newborn screening data, the carrier frequency of CAH in the general population has estimated to be 1:55 [4]. Steroid 21-hydroxylase normally converts 17-hydroxy-progestrone into 11-deoxyortisol and progesterone into 11-deoxycorticosterone. These steroids are subsequently converted into cortisol and aldosterone, respectively [5]. Classical 21-OHD is the most common cause of ambiguous genitalia in female [1]. It is also one of the few disorders in which prenatal diagnosis and treatment to prevent a birth defect is feasible and effective [1, 2]. The gene for adrenal 21-hydroxylase, CYP21 is located about 30 kb from an inactive cognate gene, CYP21P, on short arm of chromosome 6 in the area of the HLA genes [6]. This inactive pseudogene is 98% homologous to the active gene. During meiosis, because of high homology between pseudogene and active gene, a gene conversion appears to occur that transfers deleterious point mutations from CYP21P

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Table 1. Annealing temperature and primers used for PCR.

Mutation	Exon 6 cluster	V281L	I172N	8 bp deletion	R356W	Q318X	I2G	P30L
Forward Primer	Ex6na and Ex6ma	Ex7ns and Ex7ms	Ex4ns and Ex4ms	Ex3ns and Ex3ms	Ex82ns and Ex82ms	Ex81ns and Ex81ms	In2ns and In2ms	Ex1ns and Ex1ms
Reverse Primer	Ex3ns	Ex6ns	Ex6na	Ex6na	Ex6ns	Ex6ns	Ex3na	Ex6ns
Annealing Temperature	60°C	63°C	65°C	60°C	63°C	63°C	69°C	67°C
PCR Product (bp)	710	330	420	710	720	650	80	1300

N, normal; m, mutant; s, sense; a, antisense; Ex, exon; I, intron

gene to CYP21 gene and causes either complete or partial inactivation of 21-hydroxylase activity [7].

To date, deletion or conversion of the CYP21 gene, pseudogene-derived point mutations and some other mutations have been reported [1]. The eight common pseudogene-derived point mutations have been studied in most populations [2]. The deleterious mutations in CYP21P include an A to G substitution 13 nucleotides before the end of intron 2 that results in aberrant splicing of pre-mRNA, an 8-nt deletion in exon 3 and a 1-nt insertion in exon 7, each of them shifts the reading frame of translation, and a nonsense mutation in codon 318 of exon 8. There are also 8 missense mutations in *CYP21P*, 7 of them have been observed in patients with 21-OHD. Because particular mutations occur in many unrelated kindred, each mutation, and the degree of enzymatic compromise which it causes may be correlated with the different clinical forms of 21-OHD. Mutation A (or C) to G near the end of intron 2 (12G) accounts for 20 to 25 percent of mutations in most populations. The other point mutations have different frequency in different populations. The objective of this study was to determine the frequency of eight common point mutations in the Iranian population.

MATERIALS AND METHODS

At first, fifty individuals were diagnosed clinically by pediatric endocrinologist and genetic counseling was performed for each family. In this study, informed consent for the molecular tests was provided by those families. DNA samples were extracted according to the standard protocol [8]. The eight most common mutations analyzed in the CYP21 gene were 12G, 1172N, V281L, P30L, exon 6 cluster, Q318X, R356W and 8 bp deletion in exon 3 (G110Δ 8 nt). In this study, we used allele specific PCR for detection of mutations [9]. Different PCR conditions (Table 1) were required to analyze all eight most common mutation sites. For each mutation site in question, two PCR were performed: one reaction detected the normal allele, and other one detected the mutant allele. Each reaction contained either a normal or mutant type primer (Genefanavaran, Tehran, Iran) used with common primer which amplified only the CYP21 gene and not the pseudogene (CYP21P) containing these eight mutations. Primer sequences, mutation names and size of fragments amplified by PCR have been depicted in Table 2. The positive DNA controls were provided from Erasmus University, Netherlands. Finally, PCR products directly were run on 8% polyacrylamide gel electrophoresis, separately for each exon. In normal and homozygote samples, normal and mutant allele are amplified respectively, and in heterozygote samples both normal and mutantalleles are amplified (Fig. 1).

Table 2. Primer sequences and mutations amplified by PCR.

Primer	Sequence	Mutation
Ex1ns	5'-TCCGGAGCCTCCACCTCCC-3'	P30L
Ex1ms	5'-TCCGGAGCCTCCACCTCCT-3'	
In2ns	5'-TTCCCACCCTCCAGCCCCCAA-3'	I2G (656) A/C to G
In2ms	5'-TTCCCACCCTCCAGCCCCCAG-3'	
In2cs	5'-TTCCCACCCTCCAGCCCCCAC-3'	
Ex3ns	5'-CGGACCTGTCCTTGGGAGACTAC-3'	8bp deletion in Exon 3
Ex3ms	5'-ACTACCCGGACCTGTCCTTGGTC-3'	
Ex3na	5'-TCCAGAGCAGGGAGTAGTCTC-3'	
Ex4ns	5'-TCCTCACCTGCAGCATCAT-3'	I172N
Ex4ms	5'-TCCTCACCTGCAGCATCAA-3'	
Ex6ns	5'-GAGGGATCACATCGTGGAGATG-3'	I236N, V237Q, M239K
Ex6ma	5'-TCAGCTGCATCTCCACGATGTGG-3'	
Ex6na	5'-AGCTGCATCTCCACGATGTGA-3'	
Ex7na	5'-TCCACTGCAGCCATGTGCAC-3'	V281L
Ex7ma	5'-TCCACTGCAGCCATGTGCAA-3'	
Ex81na	5'-TTCGTGGTCTAGCTCCTCCTG-3'	Q318X
Ex81ma	5'-AGTTCGTGGTCTAGCTCCTCCTA-3'	
Ex82na	5'-CTAAGGGCACCACGGGCCG-3'	R356W
Ex82ma	5'-CTAAGGGCACCACGGGCCA-3'	

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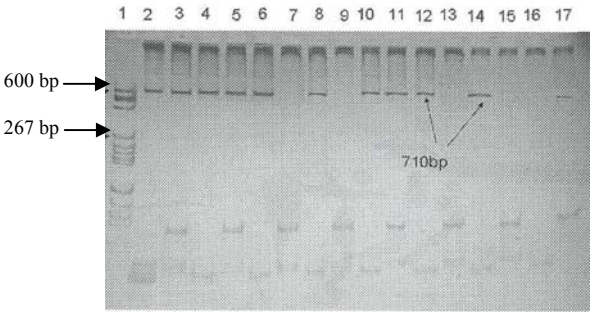

Fig. 1. Polyacrylamide gel electrophoresis for exon 3. Lane 1 is DNA marker, lanes 2 and 3 are related to the positive control for an 8 bp deletion in exon 3 (fragment size is 710 bp), lanes 4 and 5 and lanes 10 and 11 are related to heterozygote samples, lanes 6-9 and 12-15 are normal samples and lanes 16 and 17 are related a homozygote sample.

RESULTS AND DISCUSSION

We totally analyzed 50 unrelated patients by allele specific PCR. Forty patients (80%) had these eight common mutations in the CYP21 gene and 10 patients were normal for these mutations. Thirty cases had mutations in both chromosomes and 10 samples had mutations in one allele. Twenty four patients (48%) were homozygous for each type of mutations (Table 3). From these patients, 20% were homozygote in I2G allele, 10% in 8 bp deletions in exon 3, 8% in I172N allele, 4% in E6 cluster, 4% in R356W and 2% was homozygous in Q318X as well as six cases were compound heterozygote. The most frequent mutations were found to be I2G (26%) and 8 bp deletion in exon 3 (13%). The frequencies of other alleles were: I172N, 9%; V281L, 3%; exon 6 cluster (I236N, V237E, and M239K), 4%; Q318X, 9%; R356W, 5% and P30L, 0%.

Seventy alleles had one of these mutations and twenty patients (48%) were homozygous for each type of mutation. The most frequent mutations were found to be I2G and 8 bp deletion in exon 3 (G110Δ8nt) in our populations (28% and 13%, respectively). Also, our results differ from previous study in northeastern of Iran [10]. Since relative ethnic purity has been maintained by the natural geographic borders within Iran and by ancient culture, we expected these mutations to be homozygous. As we expected, nine genotypes of individuals predominantly were homozygous (Table 3). Comparison of allele frequencies in different populations shows significant similarities with our population (Table 4). The frequency of I2G allele in

Table 3. Genotype of individuals.

Allele 1	Allele 2	Number of individuals
8 bp deletion	8 bp deletion	5
Q318X	Q318X	1
Q318X/8 bp deletion	Q318X	1
Q318X	N	4
R356W	R356W	2
R356W	N	1
Exon 6 cluster	Exon 6 cluster	2
I2G	I2G	10
I2G	N	4
I2G	8 bp deletion	2
I2G	Q318X	1
I172N	I172N	4
I172N	N	1
V281L	I2G	1
V281L	I2G/ V281L	1
Total		40

Exon 6 cluster, (I236N, V237E, M239K); N, normal

Table 4. Allele frequencies in different populations (%).

Nationality	Allele No.	I2G	V281L	I172N	8 bp deletion	Q318X	P30L	R356W	Exon 6 cluster	Ref.
USA	394	31	9	10.0	4.0	4	2	4.0	4.0	11
Sweden	400	27	6	20.0	1.0	2	2	4.0	5.0	12
England	284	40	0	7.0	0.0	0	0	10.0	0.0	13
French	258	21	17	9.0	4.0	4			6.0	14
Italy	146	20	11	6.0		8	4		12.0	15
Japan	102	29	1	14.0	0.0	0	0	14.0	4.0	16
China	40	25		28.0		8		10.0	5.0	17
Turkey	31	22	0	11.4	3.2	8	0	9.6	3.2	18
Spain	58	26	17	2.0	5.0	4	2	4.0	5.0	19
Chilly	126	19		7.0		9		11.0	10.0	20
Mexico	94	48	9	12.0	2.0	4	9	7.0	1.0	21
Brazil	74	25	4	19.0	1.0	11		8.0	5.0	22
Argentina	72	18		15.0	4.0	14		6.0		23
Fenland	102	12	3	29.0		2			10.0	24
Iran	100	28	3	9.0	13.0	9	0	5.0	3.0	Our study

In blank sites, mutations have not been studied.

our population had not different from other populations and this mutation has high frequency in all over the world that may be a hotspot. But the frequency of 8 bp deletion in Exon 3 (G110Δ8nt) had high frequency (13%) in our population in comparison with other populations that may be arise from an ancient mutation (founder effect) requiring to further investigations. The frequency of other mutations (P30L, Q318X, R356W, I172N, exon 6 clusters) did not differ substantially from frequencies of other countries (Table 4).

In cases that were homozygous for any type of mutations, they may be as a heterozygous for this mutation; therefore the frequency of these mutations may be higher than actual frequency because we did not detect the allele frequency for large deletion and conversions. Since the treatment and prenatal diagnosis of this disorder are feasible, setting up a molecular method for detection of these mutations with genetic counseling will help in prevention policies. We suggest that four mutations (I2G, G110Δ8nt, I172N, and Q318X) should be screened by ASP method in our country at first and then other mutations can be analyzed. Further investigations are required to detect frequencies of other mutations, including deletion and large conversions, in our country.

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