Journal of Steroid Biochemistry & Molecular Biology 165 (2017) 57–63

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Novel and prevalent *CYP11B1* gene mutations in Turkish patients with 11-β hydroxylase deficiency

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ARTICLE INFO

Article history:
Received 20 January 2016
Received in revised form 29 February 2016
Accepted 3 March 2016
Available online 5 March 2016

Keywords:
Congenital adrenal hyperplasia
CYP11B1
11beta-hydroxylase deficiency
CYP11B1 novel mutations

ABSTRACT

11β-Hydroxylase deficiency is the second most frequent type of congenital adrenal hyperplasia and is more common in those of Turkish descent than in other populations. The purpose of this study is to examine the spectrum of *CYP11B1* gene mutations in Turkish patients with 11β-hydroxylase deficiency.

Twenty-eight patients from 24 families, ages ranging from 0.1 to 7 years, were included in the study. Clinical diagnosis was based on virilization and high levels of 11-deoxycortisol. Twenty-six cases exhibited the classical and 2 cases the non-classical form. Mutation screening of 9 *CYP11B1* exons was performed by direct DNA sequence analysis, specifically amplifying *CYP11B1* gene fragments while avoiding simultaneous amplification of homologous *CYP11B2* gene sequences.

Seventeen different mutations were detected, 6 of which are novel (p.Gln189Hisfs*70, p.Glu198Gly, p.Thr318Lys, p.Gly446Ser, IVS8 + 5G > C and exon 3–5 del). All of the identified mutations resulted in the classical form with severe virilization, except for the p.Gly446Ser mutation, which caused a late-onset type of 11β-hydroxylase deficiency. The c.954G > A;p.Thr318Thr mutation was the most common in our cohort, with an allele frequency of 14.6%.Of the *CYP11B1* gene mutations detected, 75% were found in exons 3, 5 and 7 and the half of the mutations were nonsense, splice site, deletion or insertion mutations, causing severe virilization in female patients. The findings are important for genetic counseling and the prenatal diagnosis of Turkish patients with 11β-hydroxylase deficiency.

1. Introduction

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disease caused by a defect in any of the enzymes involved in cortisol biosynthesis from cholesterol [1]. The most common form is 21-hydroxylase deficiency, responsible for 90–95% of all CAH cases [2], followed by 11β-hydroxylase deficiency, which is reported in 5–8% of cases. In the Caucasian population, CAH occurs in approximately 1:100,000–200,000 live births [3–5].

In the classical form of 11β-hydroxylase deficiency, 11-deoxycortisol and deoxycorticosterone are not hydroxylated to cortisol and corticosterone, respectively, due to enzyme deficiency. In turn, a reduced cortisol level leads to an increase in ACTH secretion owing to a lack of negative feedback, which is followed by overproduction of androgens, resulting in virilization of external genitalia in females and penile enlargement and early pubic hair development in males. Hypertension exists in most of these patients due to increased production of deoxycorticosterone. The milder non-classical form of 11β-hydroxylase deficiency is characterized by normal external genitalia at birth but precocious pseudo-puberty during childhood. In these milder forms, hypertension does not occur, and the diagnosis is based on either high basal or ACTH-stimulated levels of serum 11-deoxycortisol.

The human 11β-hydroxylase (*CYP11B1*) gene is located on chromosome 8q21, approximately 40 kb from the highly homologous *CYP11B2* gene encoding aldosterone synthase. *CYP11B1* and *CYP11B2* display 95% sequence homology in their coding regions and 90% homology in intronic sequences [6]. The human *CYP11B1* gene contains nine exons, spanning approximately 7 kb and encoding 503 amino acids, and more than 100 different mutations distributed throughout the gene have been recorded in Human Gene Mutation Database (www.hgmd.cf.ac.uk).

In Moroccan Jews with 11β-hydroxylase deficiency, nearly all alleles harbor the same mutation, p.R448H [7], which is also

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http://dx.doi.org/10.1016/j.jsbmb.2016.03.006
0960-0760/

reported in other populations [8,9]. Another frequent mutation in the Tunisian population, p.Q356X, is also common in African Americans and North Africans [10]. Nonetheless, the number of patients of a given population in the mutation screening studies performed thus far is rather limited, precluding the identification of common mutations.

CAH is a common disorder in Turkey due to a high frequency of consanguineous marriages [11]. Indeed, a review of 273 Turkish patients with CAH showed 11β-hydroxylase deficiency in 13.5% of cases [12], and the results indicated a higher incidence compared to most other populations. However, the spectrum of mutations responsible for 11β-hydroxylase deficiency in Turkey is not yet known. As mentioned above, few case reports have been published, whereas no large-scale study has been conducted to identify the most prevalent mutation as well as the genotype-phenotype relationship. Accordingly, the purpose of this study is to determine the spectrum of *CYP11B1* gene mutations and to evaluate the relationship between genotype and phenotype in the Turkish population.

1.1. Patients and methods

Clinical data were collected retrospectively based on a review of the medical records of all patients. Twenty-eight patients from 24 families were included. The patients were from throughout the country, with 48% from eastern Turkey, 39% from central Anatolia and the remaining from western Turkey. Diagnosis was based on clinical and hormonal evaluations. Thirteen of the patients with a 46,XX karyotype presented with ambiguous genitalia, and the remaining exhibited male appearance but non-palpable gonads. The patients with a 46,XY karyotype presented with penile enlargement, rapid growth and early pubic hair. Laboratory findings included high levels of 11-deoxycortisol together with other androgens and ACTH. Twenty-six patients had the classical form, and 2 patients had the non-classical form. Eighteen patients had a 46,XX karyotype, all of whom exhibited severe virilization at birth. Blood pressures percentiles for age and gender were evaluated according to nomograms for Turkish children at the time of the diagnosis [13].

Informed consent was obtained from the families and/or patients.

1.2. Serum hormone measurements

Hormone analysis was performed using blood samples drawn at 8.00 a.m. after overnight fasting and prior to drug administration. The samples of serum were stored at −20 °C until assayed.

Plasma ACTH and serum androstenedione levels were measured using chemiluminescence immunoassays (Siemens, Immulite, USA). Analysis of serum testosterone, DHEA-S and cortisol levels were carried out by Access immunoassays (Beckman-Coulter, DXI, USA). Levels of 17-OH progesterone and 11-deoxycortisol were measured by RIA (MP Biomedicals, USA and DIA Source, Belgium respectively). Active renin was measured using IRMA (Beckman-Coulter, USA).

1.3. Genetic analysis

Genomic DNA from the patients was isolated from peripheral blood (10 ml) using a standard salting-out method. Mutation screening of the 9 *CYP11B1* gene exons was performed using direct sequence analysis by the touchdown technique with AccuTaq LA DNA Polymerase (Sigma, Germany). In this approach, the *CYP11B1* gene was specifically amplified in three fragments to avoid simultaneous amplification of homologous *CYP11B2* sequences.

PCR reactions were performed in a total reaction volume of 25 ml (1 × PCR buffer, 200 mM each dNTP, 50 pmol each primer and 0.5 U AccuTaq LA DNA polymerase) with 50 ng of genomic DNA.

Table 1
Clinical characteristics of patients with *CYP11B1* gene mutation.

Case number	Age years	Karyotype	Sex of rearing	External genitalia	11-Deoxycortisol (ng/ml)	Genotype
1	1.4	46,XY	Male	Male	263	**Gln189Hisfs*70/Gln189Hisfs*70**
2	0.4	46,XX	Female	Prader IV	>100	Arg141*/Arg141*
3	2.0	46,XY	Male	Male	>100	**Gln189Hisfs*70/Gln189Hisfs70**
4	1.9	46,XY	Male	Male	552	IVS7 + 1G > A/IVS7 + 1G > A
5	2.7	**46,XX**	**Male**	Prader V	>100	Leu299Pro/Leu299Pro
6*	7.0	46,XY	Male	Male	147	**Gly446Ser/Gly446Ser**
7*	4.9	46,XX	Female	Female	179	**Gly446Ser/Gly446Ser**
8	4.2	**46,XX**	**Male**	Prader V	229	Arg141*/Arg141*
9*	2.1	**46,XX**	**Male**	Prader V	295	Leu299Pro/Leu299Pro
10*	2	46,XY	Male	Male	>100	Leu299Pro/Leu299Pro
11	2.5	46,XX	Female	Prader IV	>100	**Exon 3–5 Deletion?**
12*	0.1	46,XX	Female	Prader IV	289	Arg141*/Arg141*
13*	1.9	46,XY	Male	Male	392	Arg141*/Arg141*
14	0.1	46,XX	Female	Prader IV	320	**IVS8 + 5G > C/IVS8 + 5G > C**
15	0.5	46,XX	Female	Prader V	>100	Asn364Argfs*36/Asn364Argfs*36
16	3.9	46,XY	Male	Male	>100	Arg384Gln/Arg384Gln
17*	0.1	46,XX	Female	Prader IV	268	Leu299Pro/Leu299Pro
18	0.1	46,XX	Female	Prader IV	252	Arg141Gln/**Glu198Gly**
19	1.8	46,XX	Female	Prader IV	>100	Thr318Thr/Thr318Thr
20	4.5	46,XY	Male	Male	>100	Thr318Thr/Thr318Thr
21	0.5	46,XX	Female	Prader IV	>100	**Thr318Lys/Thr318Lys**
22	1.5	46,XX	Female	Prader IV	252	His125Thrfs*8/His125Thrfs*8
23	2.5	46,XY	Male	Male	256	Thr318Thr/Thr318Thr
24	3.5	46,XY	Male	Male	>100	His125Thrfs*8/Thr318Thr
25	0.9	46,XX	Female	Prader IV	>100	Ala386Val/Arg43Gln
26	0.2	46,XX	Female	Prader IV	>100	Ala386Val/Arg43Gln
27	1.1	46,XX	Female	Prader IV	>100	Pro94Leu/Pro94Leu
28	2.1	46,XX	Male	Prader V	>100	**IVS8 + 5G > C/IVS8 + 5G > C**

Patients 6 and 7 are siblings and have a late-onset form of 11β-hydroxylase deficiency; patients 9, 10 and 17 are related; patients 12 and 13 are siblings.
Novel mutations are indicated in bold print.
* Normal level <8 ng/ml.