Original Article

Clinical profile and inheritance pattern of *CYP21A2* gene mutations in patients with classical congenital adrenal hyperplasia from 10 families

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ABSTRACT

Context: Congenital adrenal hyperplasia (CAH) is an autosomal recessive metabolic disorder caused by mutations in the *CYP21A2* gene. Genetic diagnosis of 21-OH deficiency causing CAH is more complicated than any other monogenic disorder due to high variability of the locus. The disease has a wide spectrum of clinical variants making it difficult to establish a genotype–phenotype correlation. Therefore, family studies are necessary to ascertain parental genotype and segregation of the mutant allele among the offspring. **Aim:** The present study aimed to identify *CYP21A2* gene mutations and analyze the segregation pattern in CAH trios (patients and their parents). **Materials and Methods:** A total of ten families having at least one CAH child were recruited. **Results:** Out of 31 children from ten families, 15 were affected with CAH and 13 of /them (12 females and 1 male) were available for genetic testing. One family had all the children affected with CAH. Compound heterozygous mutations were identified in seven patients (53.8%) whereas p.P30L, In2 and Δ8 bp mutations were present in homozygous state in three (23.1%), two (15.3 %) and one (7.6%) patient respectively. **Conclusions:** In majority of the families, mutant alleles observed in the patients were inherited from the parents whereas three families showed sporadic mutations without any paternal or maternal origin. This indicated their novel occurrence due to misalignment of the parental genes and /or large deletion of the gene. Female preponderance was noted in the CAH families and also among the patients raising the possibility of survival advantage among females.

Key words: Adrenal crisis, ambiguous genitalia, *CYP21A2* gene mutations, female preponderance, precocious puberty

Introduction

Congenital adrenal hyperplasia (CAH) is one of the hereditary diseases with autosomal recessive inheritance resulting from a deficiency of steroid 21-hydroxylase (21-OH). It is characterized by cortisol deficiency, with or without aldosterone deficiency, and androgen excess.[1] The incidence varies according to ethnicity and geographical area.[2-10]

The 21-OH (*CYP21A2*/*CYP21B*) active gene, (OMIM #201910), is located on chromosome 6p21·3 within the histocompatibility complex and shares >95% homology with an inactive pseudogene (*CYP21A1P*/*CYP21A*) which is a result of an ancestral gene duplication. Genetic diagnosis of 21-OH deficiency is more complicated than any other monogenic disorder due to the high variability of the locus.

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In most of the CAH cases, inactivating *CYP21A2* mutations are generated by unequal crossing-over or gene conversion events. The disease has a wide spectrum of clinical variants making it difficult to establish genotype–phenotype correlation. Therefore, family studies are necessary to ascertain parental genotype and transmission of mutant alleles to the offspring. The present study was planned to identify *CYP21A2* gene mutations and to identify the inheritance pattern from parents to patients.

Materials and Methods

The study was approved by the Institute ethics committee. A total of 13 patients from ten families clinically diagnosed with classical CAH along with their available family members were recruited from the outpatient Department of Endocrinology Clinic between 2007 and 2012. All patients underwent a physical examination, and the diagnosis was based on the hormonal profile.[2] Detailed family history in the form of pedigree charts was collected, and 5 ml of peripheral blood sample was drawn in ethylenediaminetetraacetic acid for genetic screening from the available family members after taking informed consent.

Methods

Hormonal analysis

Levels of cortisol, ACTH, testosterone, and DHEAS were estimated using electrochemiluminescence immunoassay using commercial kits (Roche, Germany). Radioimmunoassay kit based method was used for estimating 17 OHP levels. (Diagnostic Systems Laboratories, Inc., Webster, TX, USA).

Cytogenetic analysis

Conventional cytogenetic analysis was carried out on peripheral blood using standard techniques.[11] Karyotyping was carried out on G-banded metaphases obtained from 72-h cultures.

Molecular analysis

Genomic DNA was extracted from peripheral blood leukocytes using a standard protocol.[12] Polymerase chain reaction (PCR) amplification was done for analyzing eight most common mutations in *CYP21A2* gene. The gene was amplified in two segments, S1 and S2 with primers that selectively amplify *CYP21A2*. S1 and S2 segments were used as templates for nested PCR to amplify the desired region harboring the known mutations as described previously.[2] Amplification was done using 70–100 ng DNA, 1.5 mM $MgCl_2$, 0.25 mM of each of the dNTPs, 10 pM of each primer, and 0.5 units of Taq Polymerase (Invitrogen, Carlsbad, CA, USA) in a 25 µl volume mixture using thermocycler (ABI 9700; AB, Foster City, CA).

The PCR products were subjected to digestion using respective restriction enzymes as described previously[2] for rapid genotyping. The digested products were subjected to electrophoresis in 10% polyacrylamide gels, at 100 V for 3 h at room temperature. Gels were stained with ethidium bromide and visualized under UV light. All samples were analyzed to check for the presence of any of the eight mutations commonly present in *CYP21A2* gene namely p.P30L, In2, Δ8 bp, p.I172N, p.V281L, p.Q318X, p. R356W, and p.P453S.

Results

Molecular analysis identified mutant alleles in the parents and their transmittance to the offspring [Figures 1 and 2]. Of 31 children from 10 CAH families, 15 children (12 girls and 3 boys) were diagnosed to have CAH [Table 1]. The age at presentation varied from 0.6 to 21 years. Parental consanguinity was documented in one family. Three families had a history of infant/fetal death. All the children were affected with CAH in one family whereas four families had more than 1 affected child. Two of the female patients were reared as males and five of the 13 (38.5%) patients had a history of hospitalization due to adrenal crisis. Of these 15 CAH children, 13 were available for genetic testing. Normal genotype was observed in two of the parents from different families, rest all the individuals had one or more mutant alleles present either in heterozygous or homozygous state. The most prevalent mutation was p.P30L found in three patients (23.1%), followed by In2 in two patients (15.4%), and Δ8 bp in one (7.6%) patient. Remaining seven patients were compound heterozygous (53.8%) for different mutations. Genetic analysis showed that the patients inherited the parental mutant alleles. However, in 3 families (2, 5, and 6), mutations In2, p.I172N, p.Q318X, and p.R356W were identified in the offspring, which were not present in the

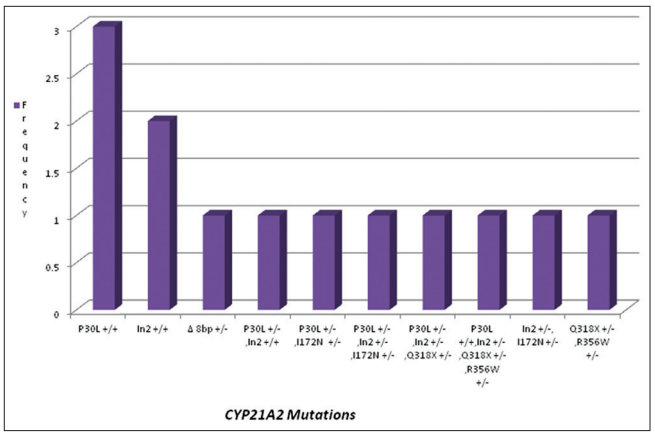

Figure 1: Frequency of *CYP21A2* mutations in congenital adrenal hyperplasia patients

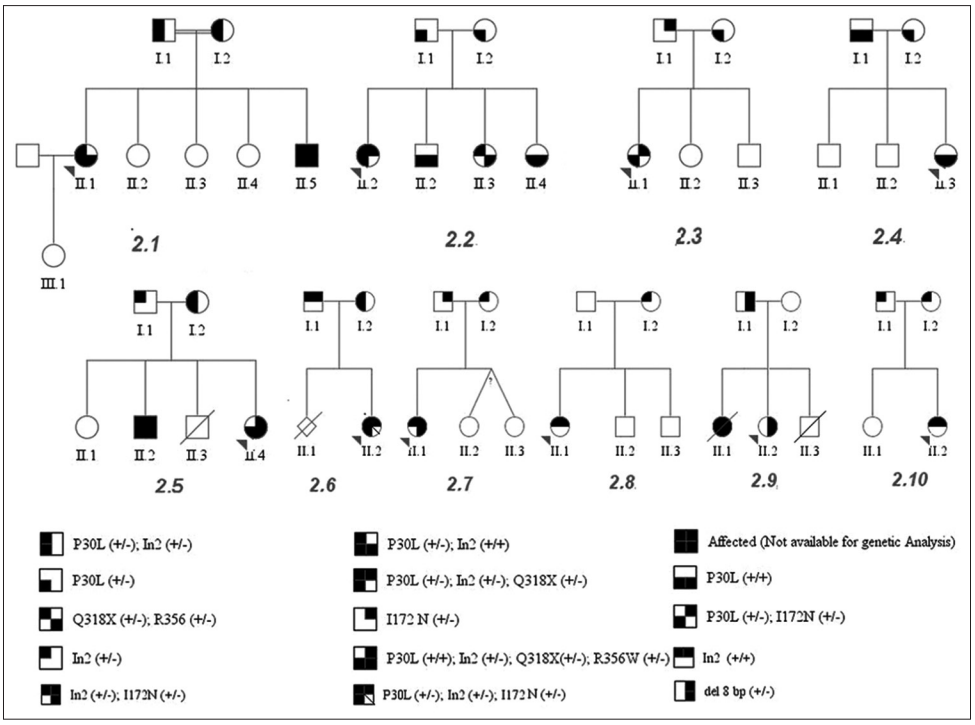

Figure 2: Pedigree charts of the patients showing the segregation of mutations

Table 1: Clinical presentation and genotype of the congenital adrenal hyperplasia patients and family members

Family number	Type	Age/sex	Karyotype	Total number of children/deaths/ affected	Clinical presentation	Imaging findings	Genotype		
							Father	Mother	Affected siblings
2.1#	SV	21/female	46, XX	5/0/2	AG, C, PSSC	Hypoplastic uterus	P30L/-, In2/-	P30L/-, In2/-	(2.1.II.1) P30L/-, In2/In2
	SV	14/male	46, XY		PP	NA			(2.1.II.5) NA
2.2	SV	15/female	46, XX	4/0/4	AG, C	NA	P30L/-	P30L/-	(2.2.II.1) P30L/-, In2/-, Q318X/-
	SV	4/male	46, XY		PP	NA			(2.2.II.2) P30L/P30L
	SV	8/female	46, XX		AG, C	NA			(2.2.II.3) P30L/P30L
	SW	6/female	46, XX		AG, C	NA			(2.2.II.4) R356W/-, Q318X/-
2.3	SV	12/female	46, XX	3/0/1	AG, C, HP, H	Bilateral adrenal hyperplasia	I172N/-	P30L/-	(2.3.II.1) P30L/-, I172N/-
2.4	SV	14/female	46, XX	3/0/1	AG, C	NA	P30L/P30L	P30L/-	(2.4.II.3) P30L/P30L
2.5	SV	9/female	46, XX	4/1 (male)/2	AG, C, H	Enlarged adrenals	In2/-	P30L/-, In2/-	(2.5.II.2) NA
	SV	13/male	46, XY		PP	NA			(2.5.II.4) P30L/P30L, In2/-, R356W/-, Q318X/-
2.6	SV	1.5/female	46, XX	1/1 (female)/1	AG, C	Infantile uterus	In2/In2	P30L/-, In2/-	(2.6.II.2) P30L/-, In2/-, I172N/-
2.7	SW	7/female	46, XX	3/0/1	AG, C	NA	I172N/-	In2/-	(2.7.II.1) In2/-, I172N/-
2.8	SW	4/female	46, XX (reared as male)	3/0/1	AG, C	Uterus like structure seen, ovaries or testes not identified	Negative	In2/-	(2.8.II.1) In2/In2
2.9	SW	3/female	46, XX	3/2(1 male, 1 female)/2	AG, C	Common urogenital sinus, bilateral adrenal hyperplasia	Δ 8 bp/-	Negative	(2.9.II.2) Δ 8 bp/-
2.10	SW	0.6/female	46, XX (reared as male)	2/0/1	AG, C	NA	In2/-	In2/-	(2.10.II.2) In2/In2

#Consanguinity. SV: Simple virilizing, SW: Salt wasting, AG: Ambiguous genitalia, C: Clitoromegaly, HP: Hyperpigmentation, H: Hirsutism, PSSC: Poor secondary sexual characteristics, PP: Precocious puberty, NA: Not available

parents. This indicated their novel occurrence, which may be due to misalignment of the parental genes or the large deletion of the gene.

Discussion

In the present study, a segregation analysis of the eight common *CYP21A2* mutations in ten CAH families was performed. All the recruited families had at least one CAH affected individual. The parents were genotyped, and the pattern of segregation of mutations was analyzed.

In the absence of CAH neonatal screening, several studies have shown female preponderance as presence of ambiguous genitalia raises clinical suspicion of CAH among girls whereas in boys the diagnosis may be missed till they present with features of adrenal insufficiency leading to preventable deaths among them.

In the present study also, female prevalence was observed among the number of children born in these ten families (10 males: 21 females) and the number of children with CAH (1 male: 12 females). The findings highlight two important observations; first, lack of neonatal screening in our set up, which could enable earlier diagnosis and reduce the number of deaths. This speculation is further strengthened by the observation of a report from Sweden[13] where neonatal screening for CAH is mandatory, the male: female ratio among CAH patients is nearly 1. Second, female prevalence in the number of children born raises the question of advantage for girls even prenatally.

Different mutations confer different effect on the activity of the enzyme, and thus severity of the disease is directly proportional to the degree of enzyme activity compromised by the mutation.[14] In2 mutation is known to affect 98–99% of the activity whereas p.I172N mutation leads to the residual enzyme activity <10%. The other three mild point mutations (p.V281L, p.P453S, and p.P30L) cause 7–75% of residual enzyme activity. Thus, the presence of homozygous and/or compound heterozygous changes with other mutations governs the resulting phenotype.

Clinical expression of the disease is determined by the presence of any one of the mild mutations on one allele despite the presence of a severe mutation in the other allele leading to varied phenotype among the affected. Mutation p.P30L has a special status in the group of mild mutations as its *in vitro* expression is high, but it does not translate in corresponding *in vivo* activity.[8] It was evident by studies that the mild p.P30L mutation along with an additional severe mutation or a gene conversion could explain the salt wasting or simple virilizing (SV) phenotype in patients.[15] The finding is replicated in the present report where p.P30L, considered to be commonly found in NC CAH was identified in classic CAH patients.

The high frequency of p.P30L mutations in this study is similar to previous reports on a larger sample size.[16] This mutation was identified in combination with severe mutations in five patients. Similar pattern was reported in another study on SV CAH patients from Brazil.[17]

On the contrary, several studies found p.P30L mutation to be less prevalent in their patient populations[18-26] and also suggested a correlation between genotype and phenotype.[8,20,27,28]

In contrast, the present study identified unusual presentation in some of the classic CAH families, both in terms of the mutation spectrum and clinical features indicating genotype/phenotype discrepancy.

An interesting pattern of disease expression was observed on genetic and pedigree analysis. Analysis of eight common *CYP21A2* mutations in the CAH families confirmed transmission from parents. Four probands exhibited additional mutations not seen in the parents, which is in accordance with literature as about 1–2% of affected alleles are known to be spontaneous mutations not carried by either parent. Mothers (2.1, 2.5, 2.6) with heterozygous p.P30L and In2 mutations were not affected, but their offspring with the same genotype expressed the CAH phenotype. In families (2.4, 2.6), the fathers with homozygous p.P30L and In2 mutations, respectively, were expected to show a severe phenotype, but they presented as normal individuals. Molecular analysis of these families confirmed the variability of presentation in carriers of different mutations thereby posing difficulties in decision making regarding therapy and genetic counseling.

Conclusions

India has a diverse population with people belonging to different ethnic groups. Novel mutations in the offspring not identified in the parents indicated large deletions, and/or misalignment of parental genes. The present study also showed female preponderance in terms of number of children born and the number of children diagnosed with CAH raising the possibility of survival advantage for girls, even prenatally. There is a need for more genetic studies to substantiate these observations and delineate the pathophysiology of the genotype and the resulting phenotype.

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Conflicts of interest

There are no conflicts of interest.

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