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Common CYP21A2 Gene Mutations in South Indian Congenital Adrenal Hyperplasia Patients

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ABSTRACT About 90 percent of Congenital Adrenal Hyperplasia (CAH) patients exhibit defects in the CYP21A2 gene that results in steroid 21-hydroxylase deficiency (21- OHD). As more than 100 mutations prevail in CAH, diagnosis and time responsive therapeutic interventions require knowledge of the common mutations in the regional population. Hence, the present study aims to assess the prevalence of nine common CYP21A2 gene mutations in a South Indian, regional population consisting of a group of CAH patients and a group of CAH patients along with their parents. 6 clinically diagnosed CAH patients, 5 clinically diagnosed CAH patients along with their parents were screened for nine common mutations using allele-specific polymerase chain reaction amplification followed by restriction fragment length polymorphism. Out of 11 patients, 8 were identified to be females and 3 were males. 5 patients were simple virilizers and 6 were salt wasters. The age at presentation varied from 1 day to 24 years. Molecular diagnosis of the CYP21A2 gene revealed that the highest number of patients harbored *P30L* and *Q318X*, followed by *P453S*, *I172N*, *In2 splicing*, *R356W* and *Δ8bp deletion* mutations. The results of the study clearly indicates that allele-specific PCR combined with RFLP is reliable for the molecular diagnosis of 21-OHD and can be easily included in small scale, routine laboratory analysis. Cohesively, the study strongly recommends the initiation of Indian national/regional neonatal screening programs for CAH.

INTRODUCTION

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder caused by the reduced activity or loss of one of the five steroidogenic enzymes necessary for cortisol biosynthesis. More than 90 percent of patients with CAH have CYP21A2 mutations including conversions to the CYP21A1P pseudogene or large deletions (White and Speiser 2000). 21-Hydroxylase-deficient (21- OHD) CAH is manifested in a variety of clinical conditions and is comprised of three subtypes: (1) Classical salt-wasting (SW), (2) Classical simple virilizing (SV) and the (3) Non-classical (NC) form (New and Wilson 1999; New 2006). While the incidence of the classical form of CAH disease is reported to range between 1: 10,000 -1: 18,000 depending on the racial predisposition (White and Speiser 2000; Therrell 2001), the NC form is milder and commonly occurs in the general population at a rate of 1: 1700 (New 2006; Fitness et al. 1999). The SV form is typically characterized by elevated levels in the adrenal androgens that result in precocious pseudopuberty in both males and females, and virilization of the external genitalia in newborn females. In the SW form, severe renal salt loss occurs as a consequence of aldosterone deficiency. The less severe NC form manifests predominantly in female patients with precocious pseudopuberty/ hirsutism and decreased fertility (Speiser et al. 1985).

Clinical diversity of CAH is primarily due to mutations in CYP21A2 gene which encodes the 21-Hydroxylase (21-OH). CYP21A2 is located within the human leukocyte antigen (HLA) class III region on the short arm of the chromosome 6 adjacent to a highly homologous pseudogene (CYP21A1P). Both are 3-4 kb long and each gene consists of 10 exons (Speiser and White 2003; Levine 2000). In most of the CAH cases, inactivating CYP21A2 mutations are generated by unequal crossing-over or gene conversion events. As more than 100 mutations prevail in

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CAH, diagnosis and time responsive therapeutic interventions require knowledge of the common mutations occurring in a regionally specific population. Further, with a wide spectrum of clinical variants, clinical evaluation of CAH also requires inheritance studies that would ascertain parental genotype, and determine the transmission of mutant alleles to the offspring. Therefore, the present study aimed to identify the prevalence of nine common CYP21A2 gene mutations in a south Indian study population, and further evaluate the inheritance pattern from parents to patients, by correlating the genotype with the clinical phenotype.

METHODOLOGY

In order to identify, assess and understand common CYP21A2 gene mutations in a south Indian regional population of CAH patients, 5 clinically diagnosed CAH patients with their family members (parents) and 6 clinically diagnosed CAH patients without their family members were recruited for the present study. Detailed family history in the form of pedigree charts was collected and 5 ml of peripheral blood sample was drawn in ethylenediaminetetraacetic acid (EDTA) for DNA extraction after taking written informed consent. This study was approved by the IEC (Alpha Hospital and Research Centre, Madurai).

Genomic DNA was extracted from peripheral blood samples by salting-out method (Welsh and Bunce 1999). DNA was quantified and subjected to polymerase chain reaction (PCR)/ allele specific PCR (ASPCR) using specific primers to amplify the CYP21A2 gene (Oriola et al. 1997). CYP21A2 gene was amplified in two segments/fragments using specific primers. Using fragment 1 as template, a secondary PCR was performed with the appropriate primers to detect *P30L*, *In2 splicing* and *Δ8 bp* mutations. Fragment 2 was digested with *ApaL1* restriction enzyme to detect both *V281L* and *R339H* mutations at the same time. Fragment 2 was also used as template to perform a secondary PCR with the appropriate primers to detect *I172N*, *Q318X*, *R356W* and *P453S* mutations.

Secondary PCR products were digested with the appropriate restriction enzymes to identify the common mutations using restriction fragment length polymorphism (RFLP) method followed by 1 percent agarose gel electrophoresis or 10 percent polyacrylamide gel electrophoresis so as to separate the restriction fragments. All the samples were analyzed for the presence of at least any one of the above mentioned nine mutations commonly present in the CYP21A2 gene.

RESULTS

The study identified that out of the 8 female patients and 3 male patients (total 11 patients in the study population), six patients were born to parents with consanguineous marriages. The clinical characteristics of the patients are summarized in Table 1. The age of the CAH patients at presentation varied from 1 day to 24 years, and, at the time of sample collection, except for one patient, all the other patients were on treatment. Further, among the 11 CAH patients, 6 patients were identified under the salt-wasting type (54.5%) and 5 patients were identified as simple virilizing type (45.5%).

Table 1: Clinical characteristics of CAH patients

S. No.	*Age at presentation*	*Gender*	*Consanguinity*	*Clinical phenotype*
1.	20 days	M	Yes	SW
2.	3 weeks	F	No	SW
3.	24 years	M	Yes	SV
4.	3 months	F	Yes	SW
5.	11 days	F	No	SW
6.	At birth	F	Yes	SV
7.	16 years	F	Yes	SV
8.	1 year	F	No	SV
9.	20 days	F	No	SW
10.	8 months	F	Yes	SV
11.	5 months	M	No	SW

SW-Salt-Wasting; SV-Simple Virilizing

PCR and RFLP based molecular analysis performed to identify the commonly prevalent CYP21A2 gene mutations (*P30L, In2 splicing, Δ8 bp, V281L and R339H, I172N, Q318X, R356W* and *P453S*), revealed that the *P30L* mutation occurred in all the 11 patients (100%), and was the most prevalent mutation in the study population. While, ten patients (90%) were identified with *Q318X* mutation, nine patients (81.8%) were identified with *P453S* mutation, eight patients (72.7%) were identified with a *I172N* mutation, four patients (36.3%) were identified with *In2* mutation, two patients (18.2 %) were identified with *R356Y* mutation and one patient was identified with *Δ*8 bp mutation (9%), none of the CAH patients presented a

V281L+R339H mutation (Table 2). The study analysis also revealed that all the patients and parents exhibited compound heterozygous genotypes for different mutations. Collectively, the results of this study indicate that in the assessed south Indian study population, the frequency of *P30L* mutation was the highest and that *V281L+R339H* mutation did not occur.

Table 2: Frequency of CYP21A2 mutations in CAH patients

S. No.	*CYP21A2 mutations*	*% frequency*
1.	P30L/I172N/Q318X	9.1
2.	P30L/I172N/Q318X/P453S	45.5
3.	P30L/In2/I172N/P453S	9.1
4.	P30L/In2/Q318X/R356W	9.1
5.	P30L/In2/Q318X/P453S	9.1
6.	P30L/Q318X/P453S/R356W	9.1
7.	P30L/In2/Ä8bp/I172N/Q318X/P453S	9.1

The parental genetic assessment for the mentioned nine common mutations in consenting parents of the patients (5 patients and their family/parents), confirm and indicate that CYP21A2 gene mutations were prevalent and dispersed in the parental population. Parental genetic analysis further revealed the novel occurrence of an In2 mutation in the offspring of family (Table 3), which is absent in the parents. The data obtained from the analysis further identifies that several parents (mother/father) of the patients carried severe mutations but did not present phenotypic correlations/clinical manifestations, and appeared normal. As indicated in Table 2, mothers of family 1 and 2 with heterozygous *I172N* and *P453S* mutations were not affected, but their offspring with the same genotype expressed the CAH phenotype. Similarly, CYP21A2 mutational analysis in family 4, pinpointed that although the mother of the patient presented a *Δ8 bp* mutation, she remained physiologically unaffected. Interestingly, in family 5, both the mother of the patient and the patient, carried the compound heterozygous *P30L/I172N/Q318X/P453S* mutation, yet the mother did not express a CAH phenotype. In a related context, the fathers in families 2, 3 and 5, were identified to carry P30L and Q318X mutations, and were expected to exhibit a severe CAH phenotype, yet they appeared to lack phenotypic correlation. Thus, the genetic analysis of the patients and their parents provided insight on the inheritance of the parental mutant alleles, and the existence of an affected chromosome in patients.

Table 3: Mutational analysis in CAH families by PCR-ACRS method

CAH families	*Observed genotype*
Family 1	
F	-
M	*I172N/P453S*
A	*P30L/I172N/Q318X/P453S*
Family 2	
F	*P30L/Δ8bp/I172N/Q318X/P453S*
M	*I172N/P453S*
A	*P30L/In2/ Δ8bp/I172N/Q318X/P453S*
Family 3	
F	*P30L/I172N/Q318X*
M	*Q318X/P453S*
A	*P30L/Q318X/P453S/R356W*
Family 4	
F	*Q318X*
M	*Δ8bp*
A	*P30L/I172N/Q318X/P453S*
Family 5	
F	*P30L/Q318X*
M	*P30L/I172N/Q318X/P453S*
A	*P30L/I172N/Q318X/P453S*

F - Father, M - Mother, A - Affected child.

DISCUSSION

Investigations on the screening for CYP21A2 gene mutations in CAH patients have gained attention, clinical implementation in order to provide better therapeutic care and prevent the consequences. Since a reportedly large number of CYP21A2 gene mutations are associated with CAH, it is widely acknowledged that identifying, understanding the prevalence of common mutations in a specific/regional population would aid patient recovery and therapy (de Carvalho et al. 2016; Anastasovska et al. 2015; Ma et al. 2014). Hence, the present study objective was to examine the prevalence of nine common CYP21A2 gene mutations in a south Indian population, and evaluate the inheritance pattern. The results from this study indicate that female prevalence was high among the number of children with CAH (8 females : 3 males). While, earlier studies on the CYP21A2 gene mutations report that some of the common CYP21A2 gene mutations observed in CAH could be associated with female preponderance (Fitness et al. 1999), the present study results do not account for a significant association between any of the men-

tioned, nine common mutations on the CYP21A2 allele and sex differences.

Phenotypic variations among CAH patients have been recognized to be associated with the difference in mutations, and the differential effect of the mutations on the activity of the 21-OH enzyme (Choi et al. 2012). Typically, it is observed that the combinatorial presence of any one of the mild mutations on one allele, and the presence of a severe mutation in the other allele attribute for a widely varying clinical and phenotypic expression in CAH (New et al. 2013; Araujo et al 2005). In conjunction with such reports, the present study results also present clinical/phenotypical variations. While most of the present study group patients with I172N mutation present the SV phenotype, a few patients present the SW form of CAH. Similarly, in the present study P30L that is commonly associated with the NC category is also identified in the classic CAH patients, suggesting that the presence of homozygous/compound heterozygous changes with other mutations determines the resulting phenotype of the current CAH study group patients.

The prevalence of CYP21A2 gene mutations differ significantly amidst the global CAH patients (Khan et al. 2011). Analysis of the CYP21A2 mutation is challenging owing to the presence of a highly homologous CYP21A1P pseudogene (98% of homology in exons and 96% of homology in introns), which is known to interfere with targeted CYP21A2 amplification during sequencing. Hence, detection of CAH mutations using polymerase chain reactions requires a robust and targeted approach (Lee et al. 1996) that would enable the amplification of the CYP21A2 rather than the pseudogene. Based on well established protocols (Oriola et al. 1997), the present study utilized a two step amplification that would enable target oriented identification of the proposed CAH mutations. The present study results indicate that a high frequency of *P30L* mutations was present in the south Indian regional population, and this finding is in correlation with published reports (Anastasovska et al. 2015; Marumudi et al. 2012; Yadav et al. 2015). However, a south Indian study assessing the prevalence of CYP21A2 gene mutations in CAH children report that *Δ8 bp* deletion in exon 3 followed by 12g mutation in *In2* were the most prevalent mutations in their study population (Ganesh et al. 2015). The most commonly observed mutations in several other populations are deletion/conversion followed by *In2* splicing, *I172N*, *R356W*, *Q318X* and *P30L* mutations. These contrary studies from the Pakistanian, Slovenian, Iranian, Turkish and Macedonian population, report *P30L* mutation to be the least prevalent in their patient population, and further suggest a correlation between genotype and phenotype (Khan et al. 2011; Dolzan et al. 2003; Ramazani et al. 2008; Tukel et al. 2003; Bas et al. 2009). The occurrence of *P30L* mutations in combination with other severe mutations was also observed in all the eleven CAH patients of this study. A similar pattern was reported in another study on SV CAH patients from Brazil (Araujo et al. 2005), and put together, the present study and other such studies strongly encourage an awareness of the commonly occurring CYP21A2 gene mutations in the specific/regional population. Such an understanding would facilitate screening for CAH in small scale settings and would aid diagnosis, therapy.

The *In2* splice mutation results in the switch of the reading frame due to the changes in the premature mRNA splicing, and is commonly reported in the Malaysian and Indian patient population (Menabo et al. 2012; Khajuria et al. 2016). Sharaf et al. in the year 2015 analyzed *In2* splicing mutation by allele-specific PCR and reported 76 percent and 17.2 percent cases with heterozygous and homozygous mutation respectively in Egyptian patients. In the present study, the patient in family 2 exhibited an additional mutation in In2 not seen in the parents (Table 2). The observed result of the present study is in accordance with the finding that about 1–2 percent of the affected alleles are spontaneous mutations that are not carried by either parent Witchel et al. 1996 and may further account to the mutation spectrum observed in CAH patients.

Similarly, and as narrated earlier in the results section (Table 2) several of the severe mutations present in either of the parents (Mothers: *Δ8 bp* mutation-family 2; compound heterozygous *P30L/I172N/Q318X/P453S* mutation-family 5; Fathers: P30L and Q318X mutations, families-2, 3, 5) were expected to demonstrate CAH manifestations, yet appeared to lack phenotypic correlations. The current study also identified an unusual presentation in some of the CAH families, both in terms of the mutation spectrum, and in terms of the clinical features, thereby indicating genotype/phenotype discrepancies.

It is also interesting to observe that in countries where neonatal screening for CAH is mandatory, the male : female ratio among CAH patients is nearly 1 (Gidlof et al. 2013). Together, several neonatal CAH screening studies caution that in the absence of neonatal screening, clinical diagnosis of CAH is easily accomplishable only in girls, (mostly due the presence of ambiguous genitalia that raises clinical suspicion of CAH), and often results in diagnosis for boys only when they present features of adrenal insufficiency. Supportive to prior neonatal CAH screening studies, our current investigation also recommends screening for CAH in newborns, as it benefits CAH patients by enabling earlier diagnosis, and healthy survival.

CONCLUSION

Based on the study's results it is clearly evident that CAH exhibits a significant prevalence in the south Indian regional population, and that novel mutations in the CAH patients not identified in the parents involve large deletions/ misalignment of parental genes. Molecular diagnosis of the CYP21A2 gene in the south Indian study population revealed that the highest number of patients harbored *P30L* and *Q318X*, followed by *P453S*, *I172N*, *In2 splicing*, *R356W*, *Δ8 bp deletion* mutations and that allele specific PCR and RFLP strategies, serve as a reliable molecular diagnostic tool for 21-OHD.

RECOMMENDATIONS

The study strongly recommends the initiation of Indian national/regional neonatal CAH screening programs, the inclusion of CYP21A2 genotyping as a diagnostic component in the CAH clinical evaluations, and genetic counseling for patients identified with CAH mutations.

REFERENCES

Anastasovska V, Milenkoviæ T, Kocova M 2015. Direct molecular diagnosis of cyp21a2 point mutations in Macedonian and Serbian patients with 21-Hydroxylase deficiency. *J Med Biochem*, 34(1): 52-57.

Araujo RS, Billerbeck AE, Madureira G, Mendonca BB, Bachega TA 2005. Substitutions in the CYP21A2 promoter explain the simple-virilizing form of 21-hydroxylase deficiency in patients harbouring a P30L mutation. *Clin Endocrinol (Oxf)*, 62: 132-136.

Bas F, Kayserili H, Darendeliler F, Uyguner O, Gunoz H, Yuksel Apak M, Atalar F, Bundak R, Wilson RC, New MI, Wollnik B, Saka N 2009. CYP21A2 gene mutations in congenital adrenal hyperplasia: Genotype-phenotype correlation in Turkish children. *J Clin Res Pediatr Endocrinol*, 1: 116-128.

Choi JH, Jin HY, Lee BH, Ko JM, Lee JJ, Kim GH, Jung CW, Lee J, Yoo HW 2012. Clinical phenotype and mutation spectrum of the CYP21A2 gene in patients with steroid 21- hydroxylase deficiency. *Exp Clin Endocrinol Diabetes*, 120(1): 23-27.

de Carvalho DF, Miranda MC, Gomes LG, Madureira G, Marcondes JA, Billerbeck AE, Rodrigues AS, Presti PF, Kuperman H, Damiani D, Mendonca BB, Bachega TA 2016. Molecular CYP21A2 diagnosis in 480 Brazilian patients with congenital adrenal hyperplasia before newborn screening introduction. *Eur J Endocrinol*, 175(2): 107-116.

Dolzan V, Stopar-Obreza M, Zerjav-Tansek M, Breskvar K, Krzisnik C, Battelino T 2003. Mutational spectrum of congenital adrenal hyperplasia in Slovenian patients: A novel Ala15Thr mutation and Pro30Leu within a larger gene conversion associated with a severe form of the disease. *Eur J Endocrinol*, 149: 137-144.

Fitness J, Dixit N, Webster D, Torresani T, Pergolizzi R, Speiser PW, Day DJ 1999. Genotyping of CYP21, linked chromosome 6p markers, and a sex-specific gene in neonatal screening for congenital adrenal hyperplasia. *J Clin Endocrinol Metab*, 84: 960-966.

Ganesh R, Suresh N, Janakiraman L, Ravikumar K 2015. CYP21A2 gene mutation in South Indian children with congenital adrenal hyperplasia. *Indian Pediatr*, 52: 710-711.

Gidlof S, Falhammar H, Thilen A, von Dobeln U, Ritzen M, Wedell A, Nordenstrom A 2013. One hundred years of congenital adrenal hyperplasia in Sweden: a retrospective, population-based cohort study. *Lancet Diabetes Endocrinol*, 1: 35-42.

Khajuria R, Walia R, Bhansali A, Prasad R 2016. The spectrum of CYP21A2 mutations in Congenital Adrenal Hyperplasia in an Indian cohort. *Clin Chim Acta*, 464: 189-194.

Khan AH, Aban M, Raza J, Ul Haq N, Jabbar A, Moatter T 2011. Ethnic disparity in 21-hydroxylase gene mutations identified in Pakistani congenital adrenal hyperplasia patients. *BMC Endocr Disord*, 11: 5.

Lee HH, Chao HT, Ng HT, Choo KB 1996. Direct molecular diagnosis of CYP21 mutations in congenital adrenal hyperplasia. *J Med Genet*, 33(5): 371-375.

Levine LS 2000. Congenital adrenal hyperplasia. *Pediatr Rev*, 21: 159-170; quiz 171.

Ma D, Chen Y, Sun Y, Yang B, Cheng J, Huang M, Zhang J, Zhang J, Hu P, Lin Y, Jiang T, Xu Z 2014. Molecular analysis of the CYP21A2 gene in Chinese patients with steroid 21-hydroxylase deficiency. *Clin Biochem*, 47(6): 455-463.

Marumudi E, Sharma A, Kulshreshtha B, Khadgawat R, Khurana ML, Ammini AC 2012. Molecular genetic analysis of CYP21A2 gene in patients with congenital adrenal hyperplasia. *Indian J Endocrinol Metab*, 16: 384-388.

Menabo S, Balsamo A, Baldazzi L, Barbaro M, Nicoletti A, Conti V, Pirazzoli P, Wedell A, Cicognani A 2012. A sequence variation in 3'UTR of CYP21A2

gene correlates with a mild form of congenital adrenal hyperplasia. *J Endocrinol Invest*, 35: 298-305.
New MI, Wilson RC 1999. Steroid disorders in children: congenital adrenal hyperplasia and apparent mineralocorticoid excess. *Proc Natl Acad Sci USA*, 96: 12790-12797.
New MI 2006: Extensive clinical experience: Nonclassical 21-hydroxylase deficiency. *J Clin Endocrinol Metab*, 91: 4205-4214.
New MI, Abraham M, Gonzalez B, Dumic M, Razzaghy-Azar M, Chitayat D, Sun L, Zaidi M, Wilson RC, Yuen T 2013. Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. *Proc Natl Acad Sci USA*, 110: 2611-2616.
Oriola J, Plensa I, Machuca I, Pavia C, Rivera-Fillat F 1997. Rapid screening method for detecting mutations in the 21-hydroxylase gene. *Clin Chem*, 43: 557-561.
Ramazani A, Kahrizi K, Razaghiazar M, Mahdieh N, Koppens P 2008. The frequency of eight common point mutations in CYP21 gene in Iranian patients with congenital adrenal hyperplasia. *Iran Biomed J*, 12: 49-53.
Sharaf S, Hafez M, ElAbd D, Ismail A, Thabet G, Elsharkawy M 2015. High frequency of splice site mutation in 21-hydroxylase deficiency children. *J Endocrinol Invest*, 38: 505-511.
Speiser PW, Dupont B, Rubinstein P, Piazza A, Kastelan A, New MI 1985. High frequency of nonclassical steroid 21-hydroxylase deficiency. *Am J Hum Genet*, 37: 650-667.
Speiser PW, White PC 2003. Congenital adrenal hyperplasia. *N Engl J Med*, 349: 776-788.
Therrell BL 2001. Newborn screening for congenital adrenal hyperplasia. *Endocrinol Metab Clin North Am*, 30: 15-30.
Tukel T, Uyguner O, Wei JQ, Yuksel-Apak M, Saka N, Song DX, Kayserili H, Bas F, Gunoz H, Wilson RC, New MI, Wollnik B 2003. A novel semiquantitative polymerase chain reaction/enzyme digestion-based method for detection of large scale deletions/conversions of the CYP21 gene and mutation screening in Turkish families with 21-hydroxylase deficiency. *J Clin Endocrinol Metab*, 88: 5893-5897.
Witchel SF, Bhamidipati DK, Hoffman EP, Cohen JB 1996. Phenotypic heterogeneity associated with the splicing mutation in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*, 81: 4081-4088.
White PC, Speiser PW 2000. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Endocr Rev*, 21: 245-291.
Welsh K, Bunce M 1999. Molecular typing for the MHC with PCR-SSP. *Rev Immunogenet*, 1: 157-176.
Yadav S, Birla S, Marumudi E, Sharma A, Khadgawat R, Khurana ML, Ammini AC 2015. Clinical profile and inheritance pattern of CYP21A2 gene mutations in patients with classical congenital adrenal hyperplasia from 10 families. *Indian J Endocrinol Metab*,19: 644-648.

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