

The Spectrum of *CYP21A2* Gene Mutations from 16 Families of Congenital Adrenal Hyperplasia: Genotype-Phenotype Correlation

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Abstract

Aim: Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder of the adrenal steroidogenic pathway. The most common form of CAH is due to 21-hydroxylase deficiency resulting from mutations in *CYP21A2* gene. The present study aimed to identify *CYP21A2* common gene mutations, phenotype correlation, and to analyze the segregation pattern in CAH patients, parents, and siblings. **Materials and Methods:** Sixteen families having at least one classic CAH child in each family, a total of 58 subjects were recruited. The presence of six most common gene mutations, namely, Intron 2 (c.293-13A/C>G), c.844G>T (p.Val282Leu), c.1019G>A (p.Arg340His), c.92C>T (p.Pro31Leu), c.955C>T (p.Gln319*), and c.518T>A (p.Ile173Asn) in *CYP21A2* gene were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using specific primers. **Results:** Out of 16 classic CAH females analyzed, salt-wasting (SW) form was present in 12 (75%) and simple virilizing form in four (25%) children. Isolated clitoromegaly was the most common clinical presentation followed by ambiguous genitalia. The most common mutation observed in CAH patient population was Intron 2 (c.293-13A/C>G) (100%) followed by p.Pro31Leu (98%), p.Gln319* (93%), p.Val282Leu (91.4%), and p.Ile173Asn (19%). Although p.Arg340His mutation was not observed in this study. Interestingly, Intron 2 (c.293-13A/C>G) homozygous was observed in 31.3% of the entire study cohort and p.Ile173Asn mutation was found to be associated with SW form. **Conclusions:** Our results suggested a high prevalence of *CYP21A2* gene mutations among CAH patients and heterogeneous mutation spectrum in their families of south Indian cohort. The outcomes afford valuable evidence for premarital and prenatal screening as well as planning suitable programs to prevent the development of CAH in Indian population.

Keywords: 21-hydroxylase deficiency, ambiguous genitalia, clitoromegaly, congenital adrenal hyperplasia, *CYP21A2* gene mutations, India

INTRODUCTION

Congenital adrenal hyperplasia (CAH), OMIM No. 201910 is a group of autosomal recessive disorders, characterized by cortisol deficiency and androgen excess, mainly due to deficiency of steroidogenic enzyme 21-hydroxylase.^[1] The worldwide incidence of classic CAH in most studies ranges from 1:14,000 to 1:18,000 live births, whereas nonclassic CAH is more frequent and occurs in one in 200 Caucasian populations.^[2]

Mutations in 21-hydroxylase gene (*CYP21A2*) account for over 95% of cases of CAH.^[3] Depending on the extent of enzyme impairment, there are two recognized clinical

forms of CAH, classic, and nonclassic. Classic CAH may be subclassified as salt-wasting (SW) and simple virilizing (SV). Females with classic CAH are born with ambiguous genitalia, whereas nonclassic presents with hyperandrogenism at the 2nd decade of life.^[1]

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CYP21A2 gene is located in chromosome 6p21.3 and consists of 10 exons. The active gene *CYP21A2* and its pseudogene *CYP21AP* present 98% sequence identity.^[4] The spectrum of mutations in *CYP21A2* gene in 21-hydroxylase deficiency patients had been established from previously published studies around the world. Despite more than 300 *CYP21A2* mutations being known to occur in CAH, 50% of classic CAH alleles are due to deletions, conversions, point, and splicing mutations that ablate enzyme activity.^[5] There are only six studies that had reported the spectrum and frequency of the *CYP21A2* gene mutations in the Indian population.^[6-11] Except for the recently published one,^[11] there is a lack of data on the clinical profile and spectrum of mutations in the south Indian CAH population. In addition, there was a wide spectrum of clinical presentation among different ethnicities and a lack of phenotype-genotype correlation. Therefore, family studies may be important to assess the parental genotype and transmission of mutation to the offspring. Henceforth, the present study was designed to study the clinical profile of classic CAH and to identify the spectrum of common mutation in CAH patients and their family members.

MATERIAL AND METHODS

The study was performed at pediatric endocrine unit of 3,000-bedded tertiary level care hospital, funded by the Department of Health Research/Indian Council of Medical Research through the Multidisciplinary Research Unit (MRU), Madurai Medical College, Madurai. Written informed consent

from parents and assent from children (as appropriate) was obtained. The study protocol was approved by the Institute Ethical Committee and registered in the Indian Clinical Trial Registry (CTRI/2020/05/025128).

Subjects

Clinical diagnosis of CAH was suspected in female newborns, presented with ambiguous or atypical genitalia, clitoromegaly, and hyperpigmentation with or without salt crises and premature pubarche and clitoromegaly in childhood period. Sixteen CAH females, and their parents, along with their available siblings, a total of 58 subjects were included in the study over a period of 3 years (2019–2021). Clinical diagnosis of CAH was made based on the physical examination and hormonal profile. Detailed family history was collected and pedigree chart was made.

Methods

Cortisol, total testosterone, Luteinizing Hormone (LH), and Follicle Stimulating Hormone (FSH), were estimated using electrochemiluminescence immunoassay (ECLIA) using kits (Roche Cobas e411, Germany). 17-hydroxyprogesterone (17-[OH] P) was measured in serum by radioimmunoassay (RIA) (Diagnostic system laboratories, Inc., Webster, USA). Karyotype was carried out on peripheral blood, by using standard G-banded metaphase techniques from 72 h culture.

Molecular analysis of *CYP21A2* gene

DNA was isolated from anticoagulated blood samples using QiagenDNeasy kit as per the manufacturer's instructions. The presence of six most common gene mutations like Intron 2 (c.293-13A/C>G) (NM_000500.9), c.844G>T (p.Val282Leu) (NM_000500.9), c.1019G>A (p.Arg340His) (NM_000127.2), c.92C>T (p.Pro31Leu) (NM_000500.9), c.955C>T (p.Gln319*) (NM_005650.3), and c.518T>A (p.Ile173Asn) (NM_000500.9)*in *CYP21A2* were analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using specific primers [Table 1]. The specific enzymes used for mutational analysis and the fragment size before and after digestions were mentioned in Table 2. At least one symptomatic classic CAH affected individual was included in every family. The parents and available siblings were genotyped and the pattern of segregation of mutation was analyzed.

Table 1: Primer Sequence used *CYP21A2* gene mutation analysis

Primer	Sequence
P2	5'-GCATCTCCACGATGTGA-3'
P3	5'-TTGTCTTGGGAGACTACTCC-3'
P4	5'-ACCTCTCGCACCCCAGTATGACT-3'
P5	5'-GCTCCGGAGCCTCCACCTCG-3'
P6	5'-TTGGAGTTCAGCACCAC-3'
P7	5'-GTGGTGCTGAACTCCAA-3'
P8	5'-ACACCAGCTGTCTGCAGGAGGCG-3'
P11	5'-TCTCTCTCCTCACCTGCAGCATCG-3'
P12	5'-ACCGGCCACTCAGGCTCACTGGG-3'
P13	5'-GCCCAGTTCGTGGTCTAGC-3'

Table 2: Characteristics of mutation analysis in the *CYP21* gene

Mutation	Primers	PCR product (bp)	Restriction Enzyme	Fragment Sizes, bp	
				Normal	Mutation
Val-281-Leu	P3 P4	2219	Alw 441	376, 853, 990	853, 1366
Arg-339-His	P3 P4	2219	Alw 441	376, 853, 990	990, 1229
Pro-30-Leu	P5 P6	249	HhaI	21, 228	249
Intron 2 (A, C-G)	P7 P8	378	HhaI	378	24, 354
Gln-318-Stop	P12 P13	136	PstI	25, 111	136
Ile-172-Asn	P11 P2	416	TaqI	416	22, 394

RESULTS

The age at presentation varied from birth to 4 years. All CAH patients were female and confirmed by blood karyotype (46 XX). Consanguinity history was present in 13 families. Based on the clinical manifestations and hormonal evaluation, all the classic CAH patients were classified into SW form ($n = 12$, 75%) and SV form ($n = 4$, 25%). Among the varied clinical manifestations, the most common were clitoromegaly (100%), ambiguous genitalia (75%), hyperpigmentation (75%), and premature pubarche with heterosexual precocious pseudo puberty (25%) [Figure 1a-c]. We found elevated level of 17(OH) P in the range of $>10,000$ ng/dL (>250 nmol/L) in all classic SW CAH, whereas in simple virilizing CAH 17(OH) P level was between 1,500 and 9,400 ng/dL, (37.5–272.5 nmol/L). There was a good correlation between the 17(OH) P levels and the type of classic CAH presentation, SW, and SV form. In addition to hyperpigmentation, most SW CAH patients also had hyponatremia, hyperkalemia, and elevated total testosterone with low cortisol.

In genetic analysis of 58 individuals including 16 symptomatic CAH patients, the most common mutation observed in CAH patient was Intron 2 (c.293-13A/C>G) (100%) followed by p.Pro31Leu (98%), p.Gln319* (93%), p.Val282Leu (91.4%), and p.Ile173Asn (19%) [Figure 2] while p.Arg340His mutation was not observed in this study. Moreover, Intron 2 (c. 293-13A/C>G) homozygous was observed in 31.3% cases, p.Ile173Asn homozygous and p.Pro31Leu homozygous were observed in 18.8% and 12.5% cases, respectively. Interestingly, p.Ile173Asn homozygous was found to be associated with SW form and completely absent in SV form.

Table 3 and Figure 3 highlight the frequency and type of mutations reported in the present study among patients and their families. Family 9 and 11 probands, had simultaneous presence of two homozygous mutations of p.Ile173Asn and Intron 2 (c.293-13A/C>G), with clinical presentation of severe SW CAH phenotype and biochemical 17(OH) P levels of more than 100,000 ng/dL. First sibling of family 11, male child, died 1 week after birth, probable of SW CAH. Mother of family 11 proband, had homozygous mutation p.Ile173Asn, had regular cycles, no ambiguity, and normal fertility. Family 13 proband also had two homozygous mutations of p.Pro31Leu, p.Ile173Asn, and Intron 2 (c.293-13A/C>G) with SW CAH and 17(OH) P levels of 60,200 ng/dL with good phenogenotype correlation.

DISCUSSION

To our knowledge, the present cohort study is the largest single-center experience of CAH patients due to 21-hydroxylase deficiency and their family members from southern India. In the present study, all the affected newborn and children were female. It is because all female classic CAH patients are symptomatic at birth with either ambiguous genitalia or clitoromegaly that makes early recognition. In India, absence of neonatal screening, CAH diagnosis is missed in male gender

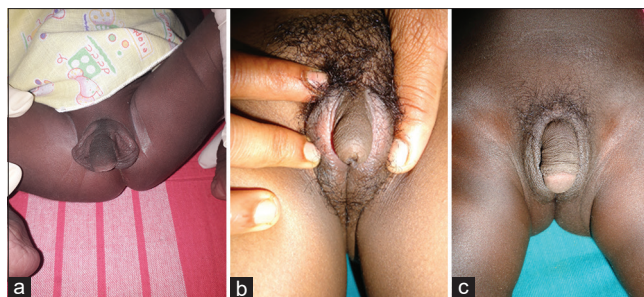


Figure 1: (a) Ambiguous genitalia with clitoromegaly and hyperpigmentation in classic SW CAH (b and c) Severe form of clitoromegaly in SV CAH

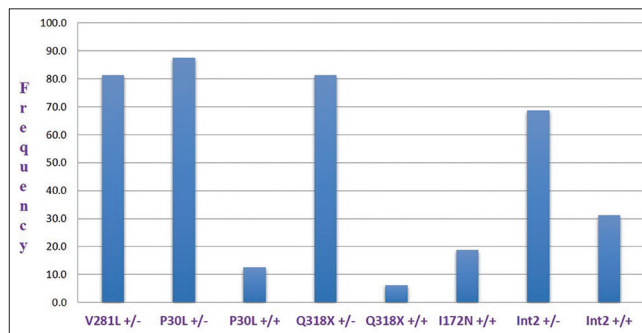


Figure 2: Frequency distribution of *CYP21* gene mutations in CAH patients

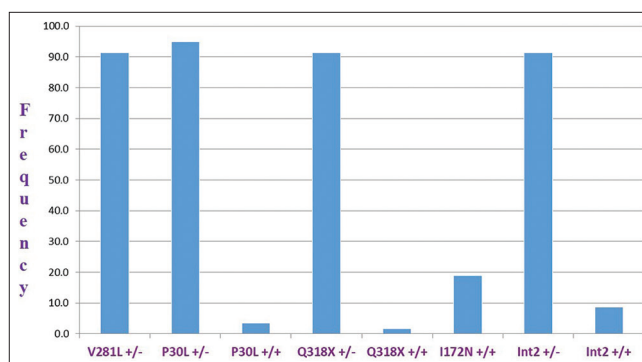


Figure 3: Frequency distribution of *CYP21* gene mutations in CAH families

and recognized only in the later part of life when signs of adrenal insufficiency like hyperpigmentation, hypotension, or precocious pseudo puberty appear.^[12,13]

Serum cortisol and 17(OH) P are the most important biochemical investigations in all suspected cases of CAH due to 21-hydroxylase deficiency. Rarely 17(OH) P may be elevated in CAH due to 11 β hydroxylase deficiency, 3 β hydroxysteroid dehydrogenase deficiency, and P450 oxidoreductase deficiency.^[3] From the Endocrine Society Clinical Practice guidelines, 17(OH) P level of greater than 1,000 ng/dL is more in favor of classic CAH.^[1] Similarly, in the present study, 17(OH) P levels of more than 1,000 ng/dL were noted on SW CAH patients and levels were seen in between 1,500 and 9,400 ng/dL in SV CAH. Hence without corticotropin (ACTH) stimulation test, baseline 17(OH) P levels correlated well with severity of CAH and the clinical presentation. The percentage

Table 3: Segregation of CYP21 gene mutation among CAH families

Family	CYP21 gene mutation	Family	CYP21 gene mutation
Family 1		Family 9	
F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	F	V281L ^b /P30L ^b /Q318X ^b /I172N ^a /In2 ^b
M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	P	V281L ^b /P30L ^b /Q318X ^b /I172N ^a /In2 ^a
S	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	S	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
Family 2		Family 10	
F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	M	V281L ^b /P30L ^b /Q318X ^b /I172N ^a /In2 ^b
P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
S	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	S	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
Family 3		Family 11	
F	V281L ^b /P30L ^b /Q318X ^b /I172N ^a /In2 ^b	F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	M	V281L ^b /P30L ^b /Q318X ^b /I172N ^a /In2 ^b
P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	P	P30L ^a /Q318X ^a /In2 ^a
S	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	S	V281L ^b /P30L ^b /Q318X ^b /I172N ^a /In2 ^b
Family 4		Family 12	
F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	F	V281L ^b /P30L ^b /In2 ^b
M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	P	P30L ^a /In2 ^a
Family 5		S	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
F	P30L ^b /Q318X ^b /In2 ^b	Family 13	
M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	F	V281L ^b /P30L ^b /Q318X ^b /I172N ^a /In2 ^b
P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	M	V281L ^b /P30L ^b /I172N ^a /In2 ^b
S	P30L ^b /Q318X ^b /In2 ^b	P	P30L ^a /I172N ^a /In2 ^a
Family 6		Family 14	
F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	M	V281L ^b /P30L ^b /Q318X ^b /I172N ^a /In2 ^b
P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	P	V281L ^b /P30L ^b /Q318X ^b /I172N ^a /In2 ^b
S	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	Family 15	
Family 7		F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	S	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
Family 8		Family 16	
F	V281L ^b /Q318X ^b /In2 ^b	F	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	M	V281L ^b /P30L ^b /Q318X ^b /In2 ^b
P	V281L ^b /P30L ^b /Q318X ^b /In2 ^b	P	V281L ^b /P30L ^b /Q318X ^b /In2 ^a

F, Father; M, Mother; P, Patient; S, Sibling; a: +/+; b: +/- . V281L, p.Val282Leu; P30L, p.Pro31Leu; Q318X, p.Gln319*; In2, Intron 2 (c. 293-13A/C>G); I172N, p.Ile173Asn

of 21-hydroxylase enzyme activity is correlated with different clinical presentations, complete inactivation is associated with SW CAH, 2% enzyme activity is with SV CAH, and 20%–60% residual enzymatic activity is associated with nonclassic CAH.^[14] Other laboratory features more in favor of SW CAH are hyponatremia, hyperkalemia variable levels of elevated total testosterone with or without cortisol deficiency. Severe clitoromegaly, premature pubarche, and heterosexual precocious pseudo puberty is the most common clinical presentation of SV CAH in our cohort, due to late presentation and referral.

Mutations in 21-hydroxylase gene (*CYP21A2*) account for over 95% of cases of CAH.^[5] Due to the high variability of active gene *CYP21A2* and its pseudogene *CYP21AP*, genetic diagnosis of 21-hydroxylase deficiency is more complicated than any other monogenic disorder.^[4,5] Among the six analyzed mutations, the most common homozygous

was Intron 2G (31.3%) followed by p.Ile173Asn (18.7%), among CAH patients in the present cohort. According to the reported literature worldwide, p.Ile173Asn as well as Intron 2 (c.293-13A/C>G) mutations lead to residual enzyme activity less than 5% and all are expressed in homozygous state, leading to SW severe CAH phenotype. The presence of homozygous p.Ile173Asn and Intron 2 (c.293-13A/C>G) mutations has a good phenotype-genotype correlation in the present familial cohort study. Moreover, the prevalence of the above mutations is similar to the previously published studies from India.^[8-11]

All the analyzed CAH patients, families, siblings had some form of compound heterozygous mutations in the present study. The most common heterozygous mutation noted in the present study was p.Pro31Leu found in 98% of study cohorts. Unexpectedly in two of the families, both parents had homozygous. Ile173Asn mutations were expected to

show a severe phenotype, but they were asymptomatic with normal fertility. In all four SV CAH patients of present cohort, no homozygous mutation was detected. The compound heterozygous mutations of p.Val282Leu and p.Gln319* were detected in all SV CAH. From the reported literature from other series, p.Val282Leu was the most common mutation in SV CAH and nonclassic CAH.^[15] In contrast, in a few of the series SV form of CAH is most often caused by the p.Ile173Asn followed by p.Val282Leu. Although comparing phenogenotype among CAH, SW form has good correlation than SV CAH. None of the 58 individuals showed p.Arg340His mutation. Significantly 17(OH) *P* levels correlated with classic CAH. All homozygous mutations are associated with severe CAH phenotype and very high 17(OH) *P* levels. Previous history of early neonatal death was noticed in one of the homozygous mutation-positive families.

The large majority of mutations detected in the present study were found in compound heterozygous, whereas homozygous mutations were observed in 22.4% (13 out of 58 analyzed, including patients and parents) of the entire study cohort. The importance of genetic analysis in CAH is different mutations confer different effects on 21-hydroxylase enzyme activity. For a better understanding of pathophysiology, to predict the severity, and its implications in diagnosis, premarital and genetic counseling, implementation of National Preventive Health Program, mutation analysis will be helpful. Among the analyzed mutations, the point mutation like p.Val282Leu, p.Pro31Leu, are associated with >50% of residual enzyme activity and mild disease.

Compared with previous northern Indian published studies, none of our present cohorts had p.Arg340His mutation.^[9,10] The high frequency of p.Pro31Leu mutations in this study is similar to the other published literature from India. The most common mutations identified in the other published Indian studies were Intron 2 (c.293-13A/C>G) mutation, p.Pro31Leu, and p.Ile173Asn.^[8-11] More than 90% of mutant alleles carry one or more of a discrete number of mutations, if no mutations are detected, we may assume that the individual is unaffected. The reasons for difference in mutation prevalence among different studies were due to the degree of consanguinity, ethnicity, availability of newborn screening, varied time of presentation as SW/SV, and nonclassic nature of disease.^[16-18] The segregation analysis of present cohort confirmed the variability of presentation in carriers of different mutations, and genetic analysis alone is insufficient to initiate treatment in CAH when there are no clinical manifestations. The strength of the present study is that patients and family members were analyzed from a single center in south India.

Study limitations

Due to the absence of newborn screening in India, in the present cohort, males are unreported and untreated. This is a preliminary study aimed at targeted gene sequencing by PCR-RFLP rather than extensive screening of Exons of *CYP21A2* gene by next-generation sequencing (NGS) or

multiplex ligation-dependent probe amplification (MLPA), hence novel mutations were not reported.

CONCLUSION

We analyzed the spectrum of *CYP21A2* gene mutations of 16 patients and their 42 related family members of SW and SV CAH with varied clinical manifestations. Our results suggested a high prevalence *CYP21A2* gene mutations in CAH patients and a heterogeneous spectrum of mutations among their family members. The outcomes afford valuable evidence for premarital screening as well as planning suitable programs to prevent the development of CAH in the south Indian population. There is a need for more genetic analysis in India, due to the complicated nature of *CYP21A2* gene and pseudo gene, diverse population, and different ethnicity.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, *et al.* Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: An Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2018;103:4043-88.
- Hannah-Shmouni F, Morissette R, Sinaii N, Elman M, Prezant TR, Chen W, *et al.* Revisiting the prevalence of nonclassic congenital adrenal hyperplasia in US Ashkenazi Jews and Caucasians. *Genet Med* 2017;19:1276-9.
- Krone N, Dhir V, Ivison HE, Arlt W. Congenital adrenal hyperplasia and P450 oxidoreductase deficiency. *Clin Endocrinol (Oxf)* 2007;66:162-72.
- Yang Z, Mendoza AR, Welch TR, Zipf WB, Yu CY. Modular variations of the human major histocompatibility complex class III genes for serine/threonine kinase RP, complement component C4, steroid 21-hydroxylase CYP21, and tenascin TNX (the RCCX module). A mechanism for gene deletions and disease associations. *J Biol Chem* 1999;274:12147-56.
- Simonetti L, Bruque CD, Fernández CS, Benavides-Mori B, Delea M, Kolomenski JE, *et al.* *CYP21A2* mutation update: Comprehensive analysis of databases and published genetic variants. *Hum Mutat* 2018;39:5-22.

6. Mathur R, Menon PS, Kabra M, Goyal RK, Verma IC. Molecular characterization of mutations in indian children with congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. *J Pediatr Endocrinol Metab* 2001;14:27-35.
7. Marumudi E, Sharma A, Kulshreshtha B, Khadgawat R, Khurana ML, Ammini AC. Molecular genetic analysis of *CYP21A2* gene in patients with congenital adrenal hyperplasia. *Indian J Endocrinol Metab* 2012;16:384-8.
8. Yadav S, Birla S, Marumudi E, Sharma A, Khadgawat R, Khurana ML, *et al.* Clinical profile and inheritance pattern of *CYP21A2* gene mutations in patients with classical congenital adrenal hyperplasia from 10 families. *Indian J Endocr Metab* 2015;19:644-8.
9. Gangodkar P, Khadilkar V, Raghupathy P, Kumar R, Dayal AA, Dayal D, *et al.* Clinical application of a novel next generation sequencing assay for *CYP21A2* gene in 310 cases of 21- hydroxylase congenital adrenal hyperplasia from India. *Endocrine* 2021;71:189-98.
10. Khajuria R, Walia R, Bhansali A, Prasad R. The spectrum of *CYP21A2* mutations in Congenital Adrenal Hyperplasia in an Indian cohort. *Clin Chim Acta* 2017;464:189-94.
11. Ravichandran L, Korula S, Asha HS, Varghese D, Parthiban R, Johnson J, *et al.* Allele-specific PCR and Next-generation sequencing based genetic screening for Congenital Adrenal Hyperplasia in India. *Eur J Med Genet* 2021;64:104369.
12. Kishore Kumar R, Das RH, Kini P. Newborn screening for congenitaladrenal hyperplasia in India: What do we need to watch outfor? *J Obstet Gynaecol India* 2016;66:415-9.
13. Kaur G, Thakur K, Kataria S, Singh TR, Chavan BS, Kaur G, Atwal R. Current and future perspective of newborn screening: An Indian scenario. *J Pediatr Endocrinol Metab* 2016;29:5-13.
14. Speiser PW, Dupont J, Zhu D, Serrat J, Buegeleisen M, Tusie-Luna MT, *et al.* Disease expression and molecular genotype in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Invest* 1992;90:584-95.
15. Nordenström A, Falhammar H. Diagnosis and management of the patient with non-classic CAH due to 21-hydroxylase deficiency. *Eur J Endocrinol* 2019;180:R127-45.
16. Tardy V, Menassa R, Sulmont V, Lienhardt-Roussie A, Lecointre C, Brauner R, *et al.* Phenotypegenotype correlations of 13 rare *CYP21A2* mutations detected in 46 patients affected with 21alfa hydroxylase deficiency and in one carrier. *J Clin Endocrinol Metab* 2010;95:1288-300.
17. New MI, Abraham M, Gonzalez B, Dumic M, Razzaghy-Azar M, Chitayat D, *et al.* Genotype-phenotype correlation in 1,507 families with congenitaladrenal hyperplasia owing to 21-hydroxylase deficiency. *Proc Natl Acad Sci USA* 2013;110:2611-6.
18. Baş F, Kayserili H, Darendeliler F, Uyguner O, Günöz H, Yüksel Apak M, *et al.* *CYP21A2* gene mutations in congenital adrenal hyperplasia: Genotype-phenotype correlation in Turkish children. *J Clin Res Pediatr Endocrinol* 2009;1:116-28.