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Clinical Research Article

17α-Hydroxylase/17,20-Lyase Deficiency in 46,XY: Our Experience and Review of Literature

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Abstract

Context: There are more than 100 pathogenic variants in *CYP17A1* that have been identified in patients with 17α-hydroxylase/17,20-lyase deficiency (17OHD).

Objective: We aimed to describe 46,XY patients with 17OHD from our center and review the literature.

Methods: We retrospectively analyzed genetically proven index cases of 17OHD from our 46,XY disorders of sex development cohort and reviewed similar cases from the literature (n = 150). Based on the phenotype, 17OHD probands were classified into combined severe deficiency (n = 128) and combined partial deficiency (n = 16). Additionally, patients with the apparent isolated 17,20-lyase deficiency (n = 7, from 6 families) were noted. Residual enzyme activities with the observed mutant enzymes were divided in 2 categories as < 1% and ≥ 1%, each for hydroxylase and lyase.

Results: We present 4 index cases of 46,XY 17OHD with a complete spectrum of undervirilization and 2 novel variants in *CYP17A1*. In the review, the combined severe deficiency was the most common form, with more frequent female sex of rearing, hypertension, hypokalemia, suppressed renin, higher plasma corticotropin, lower serum cortisol, and androgens. Immunoassay-measured serum aldosterone was frequently (68.2%) unsuppressed (>5 ng/dL). Elevated serum progesterone had high sensitivity for diagnosis of combined 17OHD, even in combined partial deficiency (83.3%). Among patients with clinical phenotype of combined severe deficiency, 11.5% had partial 17α-hydroxylase and complete 17,20-lyase deficiency (>1%/<1%) and had significantly higher serum cortisol than those with < 1%/<1% activity.

Conclusion: We report the first monocentric case series of Asian Indian 46,XY patients with 17OHD. We propose that a phenotype of severe undervirilization with milder cortisol deficiency may represent a distinct subtype of combined severe 17OHD with residual 17α-hydroxylase activity but severe 17,20-lyase deficiency (>1%/<1%), which needs further validation.

Key Words: CYP17A1, 17-hydroxylase/17,20-lyase deficiency, 17OH deficiency, 46,XY DSD

Abbreviations: 17OHD, 17-hydroxylase/17,20-lyase deficiency; ACTH, adrenocorticotropic hormone; AMH, anti-Müllerian hormone; AUC, area under the curve; CAH, congenital adrenal hyperplasia; CYP17A1, 17α-hydroxylase/17,20-lyase; DSD, differences/disorders of sexual development; LC-MS/MS, liquid chromatography–tandem mass spectrometry; TART, testicular adrenal rest tumors; WT, wild-type.

Steroid 17α-hydroxylase/17,20-lyase (CYP17A1) catalyzes steroidogenic reactions: 17α-hydroxylation followed by 17,20-lyase [1]. This enzyme is encoded by the *CYP17A1* gene, located on chromosome 10q24.3. Biallelic *CYP17A1* pathogenic variants usually cause combined 17-hydroxylase/17,20-lyase deficiency (17OHD) [2], a rare (~1%) form of congenital adrenal hyperplasia (CAH) [2]. 17OHD causes differences/disorders of sex development (DSD) in 46,XY but not in 46,XX.

More than 100 pathogenic variants in *CYP17A1* have been identified in 46,XX and 46,XY patients with 17OHD. Most variants cause complete 17OHD, while a few cause partial deficiency [3]. Although a small number of studies have attempted to define complete and partial 17OHD based on clinical phenotype, androgens, and cortisol levels [4], there are no universally accepted definitions for the grading of CYP17A1 deficiency. Moreover, a pathogenic variant in *CYP17A1* can have a differential effect on the 2 enzymatic functions of CYP17A1 (17α-hydroxylase and 17,20-lyase) [4, 5]. Hence, there is a need to develop a grading system based on the enzyme activity and validate it against phenotype.

The accuracy of biochemical distinctions is affected by the use of immunoassays. Besides, a few studies have reported hyperaldosteronism in 17OHD, which needs verification by using more specific assays [6-8]. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) may help to resolve these concerns. The data regarding testicular adrenal rest tumors (TART), Leydig cell hyperplasia, gonadal malignancy, and fertility are also limited, requiring a systematic evaluation.

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Table 1. Characteristics of 46,XY patients with genetically proven 17α-hydroxylase deficiency from our center

Patient	Age (years)	Social sex	Consanguinity/ familial	Presentation	Tanner stage	External genitalia/ testes location	Serum FSH/ LH (mIU/mL)	Plasma ACTH (pg/ mL)	Plasma renin (µIU/l)	ACTH-stimulated serum steroid levels (LC-MS/MS assay)									*CYP17A1* nucleotide variant, amino acid change
										Aldosterone (ng/dl)	Progesterone (ng/ml)	Deoxy-corticosterone (ng/ml)	Corticosterone (ng/ml)	17-OH Progesterone (ng/ml)	Cortisol (µg/dl)	Androstenedione (ng/ml)	Testosterone (ng/ml)	DHEAS (µg/dl)	
P1	17.9	F	Y/Y	1°Amenorrhoea Hypertension Hypokalemia	P1,B1	F/Abdomen	54.7/45.6	788	2	2.09	3.7	7.21	108	0.2	0.04	0.08	0.05	14.3	c.715C > T, p.Arg239Ter
P2	19	F	Y/N	1°Amenorrhoea Hypertension Hypokalemia	P1,B1	F/Abdomen	87/38.8	70.48	1.7	4.2[a]	--	--	--	--	8.6[a]	--	0.10[a]	--	c.1432G > T, p.Gly478Cys
P3	5	M	Y/N	Atypical genitalia	P1,B1	Atypical/ Inguinal	-/-	69.14	21.1	22.4	1.46	1.04	157	0.08	4.24	0.03	0.01	6.8	c.160_162delCTT, p.Phe54del
P4a	20	M	Y/Y	Gynecomastia, Infertility	P4,B3	M/Scrotal	14.3/24.8	59.45	3.0	6.8	1.76	0.50	178	2.87	5.92	0.19	2.09	9.99	c.1184A > G, p.Asn395Ser
P4b	19	M		Gynecomastia, Hypertension	P5,B4	M/Scrotal	18.4/20.6	--	4.4	2.94	1.82	0.50	172	1.93	6.33	0.18	0.75	37.2	

To convert values of ACTH to pmol/liter, multiply by 0.2202; aldosterone to nmol/liter, multiply by 0.0277; progesterone to nmol/liter, multiply by 3.179; corticosterone to nmol/liter, multiply by 3.33; 11-deoxycorticosterone to nmol/liter, multiply by 3.02; 17 OH progesterone to nmol/liter, multiply by 3.025; cortisol to nmol/liter, multiply by 27.59; androstenedione to nmol/liter, multiply by 3.491; Testosterone to nmol/liter multiply by 3.467; Estradiol to pmol/liter, multiply by 3.671; DHEAS to µmol/liter, multiply by 0.027.
Normal range: FSH (mIU/ml): 2.1–14.2; LH (mIU/ml): 0.94–7.10; ACTH (pg/ml): 5-46; Plasma renin (µIU/ml): 4.4-46.1; Aldosterone (ng/dl) 0.72-24.12; Progesterone (ng/ml) 0.04-0.22; Deoxycorticosterone ng/ml: 0.02-0.15; Corticosterone ng/ml: 0.07-14; 17-OH Progesterone (ng/ml): 0.08-1.86; Cortisol (µg/dl): 5-29.4; Androstenedione (ng/ml): 0.03-0.6 (6 m-9 y), 0.26-1.26; Testosterone (ng/ml): 0.03-0.43 (6 m-9 y), 2.19-10.7; DHEAS (µg/dl): 33.9-369.2.
Abbreviations: B, breast; F, female; M, male; N, no; P, pubic hair; Y, yes.
[a]Unstimulated levels done with a chemiluminescent assay.

Data on Asian Indian patients with 17OHD are scarce. This study describes our center's experience in managing 46,XY patients with 17OHD. In addition, we performed a review of the literature of genetically proven probands (46,XY with 17OHD).

Methods

Patients From Our Center

This study included case record analysis of 46,XY patients with genetically proven 17OHD, registered between 2010 and 2020, and was approved by the Institutional Ethics Committee of Seth GS Medical College (EC/OA-182/2020). The relevant clinical, biochemical, and genetic data were retrieved. Gender identity was assessed as described previously [9]. Plasma direct renin concentration (DRC) was measured by a solid-phase competitive CLIA (LIASON, DiaSorin Inc). Corticotropin (adrenocorticotropic hormone; ACTH)-stimulated serum steroid levels were measured by LC-MS/MS. Genomic DNA extraction and molecular screening of *CYP17A1* by next-generation sequencing (NGS) was performed as previously described [10]. The NGS covered the gene with 100×. Observed variants were confirmed by Sanger sequencing. The detrimental effect of variants was predicted using in silico tools [Polyphen-2, Sort Intolerant from Tolerant, and MutationTaster]. The minor allele frequency for the variants was checked in 1000 Genomes and gnomAD.

Molecular modeling was done for pathogenic variants found in our patients. CYP17A1 crystal structure (PDB ID: 4NKX), which has a variant Ala105Leu, cofactor heme, and substrate progesterone, was used to model a wild-type (WT) structure by replacing amino acid Leu105Ala using UCSF Chimera (http://www.cgl.ucsf.edu/chimera/). The energy minimization was performed using Gromacs 2018.0 (http://www.gromacs.org/). Progesterone was removed from the prepared WT structure, and substrate pregnenolone was docked using AutoDock4.2 (http://autodock.scripps.edu/). The ligand (heme and pregnenolone) interactions with the CYP17A1 were analyzed using Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download). The variants with p.Gly478Cys, p.Asn395Ser, and p.Phe54del were modeled using UCSF Chimera and new interactions were analyzed using Protein Interactions Calculator (http://pic.mbu.iisc.ernet.in/).

Review of Literature

The PubMed database was searched in May 2021 using the keywords "*CYP17A1* AND *46 XY disorders of sexual development*"; "*17-hydroxylase-17,20-lyase* AND *46 XY DSD*"; "*17-hydroxylase-17,20-lyase* AND *46 XY disorders of sexual development*"; "*17OH deficiency* AND *46 XY disorders of sexual development*." The selection of articles has been summarized in Supplementary data [11]. From the literature, we identified 146 46,XY probands with genetically proven 17OHD. Including 4 probands from our center, a cohort of 150 probands was considered for the final analysis. Per-patient data were tabulated to include demographics, geographical region (https://population.un.org/wpp/DefinitionOfRegions)], clinical findings, hormonal parameters, testicular biopsy findings, genotype, and effects on enzyme activities (in vitro, functional activity as described in the respective study), and treatment details. Patients with the diagnosis of apparent isolated 17,20-lyase deficiency (n = 7, from 6 families) [4, 5, 12, 13] were noted, whereas others were classified into combined severe deficiency (female external genitalia) and combined partial deficiency (atypical or male external genitalia) based on the clinical parameters. Residual enzyme activities with the observed mutant enzymes were arbitrarily divided in 2 categories as < 1% and ≥ 1%, for each hydroxylase and lyase activity. In a patient with compound heterozygous variants, the values for the variant with higher enzyme activities were considered. Variants were described using reference sequences [NM_000102.3(cDNA) and NP_000093.1(protein)].

Statistical Analysis

Statistical analyses were performed using IBM SPSS software version 26.0 (SPSS Inc. software, Chicago, IL, USA). Categorical data were expressed as absolute numbers and percentages, whereas continuous data were expressed as mean ± SD or median and ranges as appropriate. Chi-square and Fisher exact tests were used to compare categorical variables, whereas the *t* test and Mann–Whitney U test were used to compare continuous variables, as appropriate, between the 2 groups. Receiver operating characteristic (ROC) curve was used to test the ability of biochemical parameters to distinguish combined partial deficiency from combined severe deficiency. A 2-sided *P* value of < 0.05 was considered statistically significant.

Results

Patients From Our Center

The characteristics of 46,XY patients with 17OHD from our center are summarized in Table 1. Patients P1 and P2, reared as female, presented for primary amenorrhea and hypertension. Sixteen-year-old sister of P1 had similar concerns and a history of episodic hypokalemic paralysis. Both P1 and P2 had female external genitalia, absent breasts, and pubic hair, and absent Mullerian structures with abdominal gonads on imaging. Both underwent laparoscopic gonadectomy, and histopathology showed seminiferous tubules lined by Sertoli cells and occasional spermatogonia, with hypoplastic Leydig cells in P1, and hyperplastic in P2. They were treated with a daily oral dose of prednisolone (2.5 mg), spironolactone (50 mg), and estradiol valerate (2 mg). On follow-up, they had controlled blood pressure, completed breast development (B5), but no pubic hair.

P3, a 1.2-year-old boy, presented with atypical genitalia (micropenis, penile hypospadias, and inguinal testes). He had serum anti-Müllerian hormone (AMH) of 36.4 (N: 38.8-168.6) ng/ml, and low hCG-stimulated serum androstenedione (< 0.3 ng/mL), and testosterone (0.52 ng/mL). After discussion with parents, male sex of rearing was continued, and orchidopexy was performed. On follow-up, at 5 years of age, his gender identity was male.

P4a, a 20-year-old male, presented with gynecomastia and infertility. He was normotensive with Tanner staging of P4,B3. External genitalia was male, with a stretched penile length of 6.5 cm (< –2.5 SD), and scrotal testes (20 cc each). Serum AMH and inhibin B levels were 7.06 (N: 2-16.5) ng/mL and 198 (N: 169-216) pg/mL, respectively, whereas semen analysis revealed azoospermia. The younger brother (P4b) had a similar phenotype with hypertension (blood pressure, 150/90 mmHg).

P2 (p.Gly478Cys) and P4a (p.Asn395Ser) had novel pathogenic variants. In silico prediction tools unanimously indicated the detrimental effects of these variants. Also,

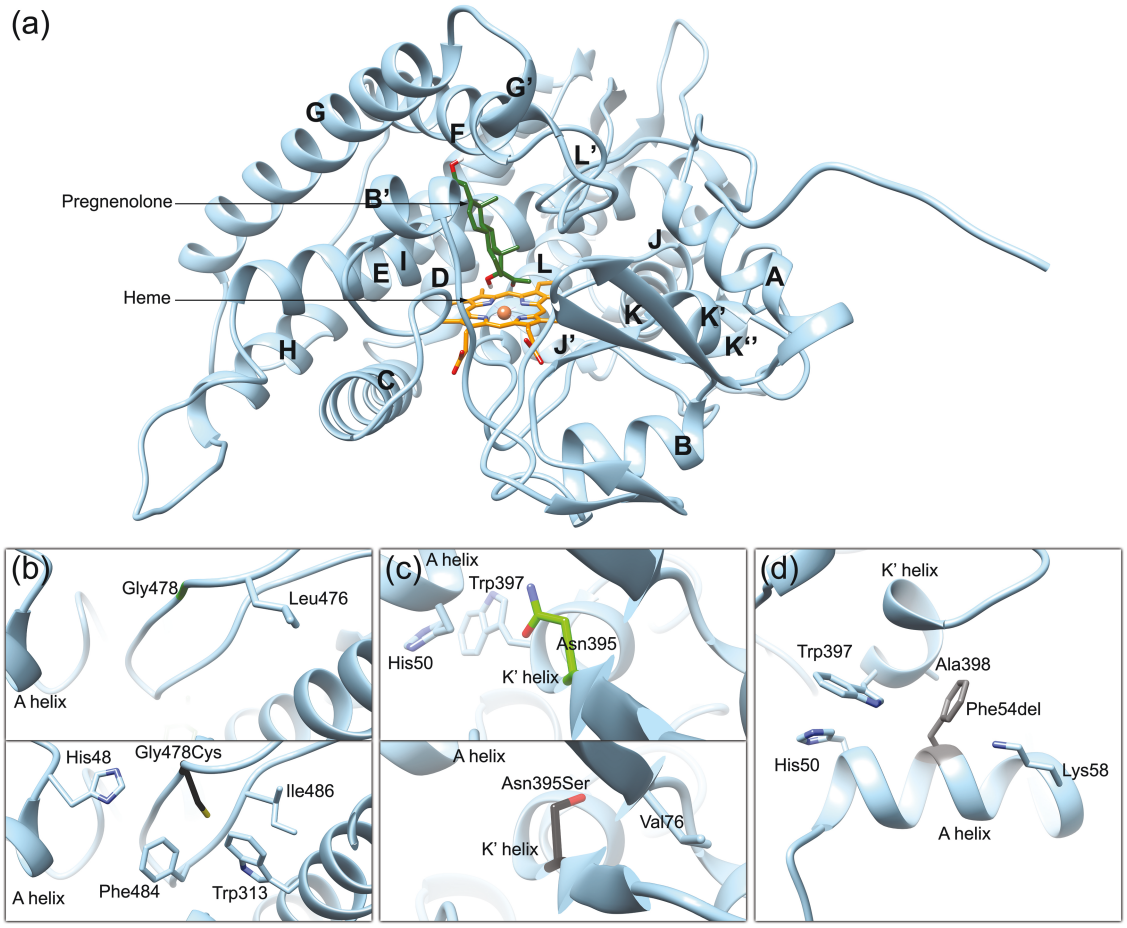

Figure 1. Modeled structure of CYP17A1 and depiction of its pathogenic variants (a) Indexing of helices of CYP17A1 (b) Pathogenic variant p.Gly478Cys: Gly478 has only possible H-bonding interaction with the main chain of Leu476, replacement of Gly with Cys forms new interactions (aromatic-sulfur interaction with Trp313 and Phe484; H-bonding interactions with His48 and Ile486). (c) Pathogenic variant p.Asn395Ser causes the loss of H-bonding interaction with His50 and Trp397, it also leads to a new H-bonding interaction with Val76. (d) Pathogenic variant p.Phe54del causes the loss of hydrophobic interaction of Phe54 with Trp397 and Ala398, it also causes the loss of H-bonding interaction with His50 and cation-pi interaction with Lys58.

these variants have not been reported in 1000 genome and gnomAD databases. P1 (p.Arg239Ter) and P3 (p.Phe54del) had previously described pathogenic variants. Segregation analysis showed parents to be heterozygous carriers for the respective pathogenic variant in all patients. Siblings of P1 and P4a were also homozygous for the pathogenic variant. Modeling of CYP17A1 and its pathogenic variants is shown in Fig. 1.

Review of Literature

A cohort of 144 probands with combined 17OHD (140 from the literature and 4 from our center) were included for analysis. The sex of rearing was female in the majority (n = 135, 93.7%). Characteristics of patients reared as female are shown in Table 2. Patients ≥ 14 years of age (n = 106) presented with primary amenorrhea (100%), hypertension (89.6%), hypokalemia (69.8%), and absent breasts (95.2%) and pubic hair (97.1%). Hypokalemic paresis has explicitly been described in 6 patients. Patients aged < 14 years (n = 25) presented with genital abnormality (n = 6; clitoromegaly in 2 and palpable gonads in 4), hypertension, and/or hypokalemia (n = 19), and poor secondary sexual characters (n = 1). Serum gonadotropins were elevated in all patients aged ≥ 14 years, except in a patient with biallelic *KISS1R* pathogenic variant [14]. Elevated (>10 mIU/mL) gonadotropins were noted as early as 4 years of age. The majority (12/14) in the age group of 4-14 years had elevated FSH and/or LH. Serum aldosterone measured by immunoassay was unsuppressed (> 5 ng/dL) in 68.75% (n = 64). Serum cortisol by immunoassay was < 3 µg/dL in the majority (94/105). Other hormonal characteristics are summarized in Table 2. Gender dysphoria, fertility, gonadal malignancy, TART, and adrenal myelolipoma have not been reported. Nine were reared as male, as summarized in Table 3 [14-20]. Five of 6 patients with atypical genitalia presented in the first 2 years of life, whereas 1 presented at 23 years (hypokalemic paresis). Three patients with male external genitalia presented in the second decade with gynecomastia and hypogonadism. None had hypertension except the 1 patient diagnosed at 23 years. Elevated serum progesterone (above the upper limit of the age-specific reference range for males) was observed in all except a neonate. Serum cortisol by immunoassay was > 3 µg/dL in the majority (6/8).

Combined severe deficiency was seen in 87.1% (n = 128) and combined partial deficiency in 10.9% (n = 16). Hypertension, hypokalemia, and suppressed plasma renin were more frequent, and plasma ACTH level was higher whereas serum cortisol, androstenedione, and testosterone

Table 2. Characteristics of cohort of 46,XY probands with genetically proven 17α-hydroxylase deficiency reared as female: literature review

Parameter	Value
Age at evaluation, years, median (IQR)	18 (14.1-22.5) [n = 129]
Consanguinity	42.9% [n = 84]
Primary amenorrhea for ≥ 14 years	100% [n = 104]
Hypertension	88.9% [n = 126]
Hypokalemia	73.9% [n = 115]
Breast Tanner stage 1 (≥14 years)	95.1% [n = 102]
Pubic hair Tanner stage 1 (≥14 years)	97% [n = 101]
External genitalia: normal/atypical	94.7%/5.3% [n = 133]
Testicular location: abdominal/inguinal/labioscrotal	33.3%/64.7%/2% [n = 51]
Serum FSH (≥14 years) (mIU/mL), median (IQR)	64.2 (40.92-93) [n = 61]
Serum LH (≥14 years) (mIU/mL), median (IQR)	35.2 (24.6-51.8) [n = 61]
Serum androstenedione for ≥ 14 years (ng/mL), median (IQR)	0.2 (0.1-0.3) [n = 35]
Serum testosterone for ≥ 14 years (ng/mL), median (IQR)	0.13 (0.05-0.21) [n = 55]
Serum estradiol ≥ 14 years (pg/mL), median (IQR)	10 (5-13) [n = 47]
Suppressed plasma renin activity (<1 ng/mL/h)	85.7% [n = 49]
Serum aldosterone (ng/dL), median (IQR)	9.97 (4.7-28.8) [n = 59]
Plasma ACTH (pg/mL), median (IQR)	139.1 (80.7-65.5) [n = 69]
Serum progesterone (ng/mL), median (IQR)	4.99 (2.4-7.79) [n = 80]
Serum 11-deoxycorticosterone (ng/mL), median (IQR)	1.44 (0.86-3.78) [n = 24]
Serum corticosterone (ng/mL), median (IQR)	167.8 (92.6-207.4) [n = 20]
Serum 17-OH progesterone (ng/mL), median (IQR)	0.37 (0.18-0.8) [n = 62]
ACTH-stimulated serum 17-OH progesterone (ng/mL), median (IQR)	0.45 (0.25-0.89) [n = 20]
Serum cortisol (µg/dL), median (IQR)	1.26 (0.73-3.19) [n = 83]
ACTH-stimulated serum cortisol (µg/dL), median (IQR)	2.35 (1.14-5.02) [n = 36]
DHEAS-basal (≥ 6 years), median (IQR)	15 (5-15) [n = 45]

were lower in patients with combined severe deficiency than those with combined partial deficiency. However, serum progesterone, 11-deoxycorticosterone, and corticosterone were not different between the 2 forms of combined deficiency. Truncating mutations and variants leading to severe loss of both enzyme activities (<1%/<1%, 93%) were more frequent in patients with combined severe deficiency (Table 4). Baseline serum cortisol measured by immunoassays had the highest area under the curve (AUC) of 0.908 (cutoff: 3.3 µg/dL, sensitivity: 88.2%, specificity: 86.5%) to distinguish combined partial deficiency from combined severe deficiency among all the biochemical parameters (data not shown). Notably, baseline serum cortisol by LC-MS/MS distinguished combined severe deficiency (n = 4, ≤0.4 µg/dL) from combined partial deficiency (n = 2, ≥4.24 µg/dL) with 100% accuracy (Supplementary data) [11]

Among patients with the clinical phenotype of combined severe deficiency, 11.5% had partial 17α-hydroxylase and severe 17,20-lyase deficiency based on enzyme activity (>1%/<1%). Baseline serum cortisol was significantly higher whereas serum progesterone tended to be lower in this subgroup (Supplementary data) [11]. Baseline serum cortisol measured by immunoassay had an AUC of 0.879 (cutoff: 3.35 µg/dL, sensitivity: 77.8%, specificity: 94.2%) to distinguish this subgroup. All patients in this subgroup had nontruncating missense variants compared with only 45% of patients with < 1%/<1% activity. Further Leydig cell hypoplasia was exclusively reported (5/5) in patients with < 1%/<1% activity.

A total of 7 patients (from 6 families) with apparent isolated 17,20-lyase deficiency due to *CYP17A1* pathogenic variants with median age at evaluation of 15 (2.5-20) years were identified from the literature. All these patients presented with atypical genitalia, had normal morning serum cortisol (median: 8.82 µg/dL; range, 6.9-16.9) and missense pathogenic variants in *CYP17A1* (Table 4).

Eighty-eight pathogenic variants in the *CYP17A1* have been reported in 46,XY patients with 17OHD (Supplementary data) [11]. Of these were 49 missense, 15 frameshift, 10 nonsense, 5 in-frame deletions, 4 splice-site variants, 2 large deletions, 1 duplication, 1 Indel, and 1 substitution in 5′UTR. Fewer variants (28 vs 260 mutated alleles) have been reported on exon 3, 4, and 5 (mid-portion of the gene) compared with the exons in the 5′ and 3′ end regions. The recurring variants (≥ 3 patients) specific to geographical locations are p.Pro480HisfsTer27 (Europe); p.Arg362Cys, p.Pro428Leu, and p.Trp406Arg (Latino); p.Tyr329LysfsTer90, p.Asp487_Phe489del, p.His373Leu, p.Ala82Asp, and p.Pro409Arg (East Asia); deletion of exon 1-6(West Asia); p.Phe54del (East and South Asia). Two Arg96 position variants (p.Arg96Gln, p.Arg96Trp) have been reported worldwide.

Discussion

We report 4 Asian Indian 46,XY index cases of 17OHD with a variable spectrum of undervirilization and 2 novel variants. This review of literature on 46,XY patients with 17OHD demonstrates combined severe deficiency as the most

Table 3. Characteristics of XY probands with genetically proven 17-hydroxylase deficiency reared as male: literature review

Sl No	Age at diagnosis (years)	External genitalia/ Tanner stage	Genital description	Hypertension (Y/N)/ hypokalemia (Y/N)	Serum progesterone (ng/mL)	Serum testosterone (ng/mL)	Basal/ACTH-stimulated serum cortisol (µg/dL)	*CYP17A1* variant	17αHydroxlase (%)/17,20lyase (%) activity of the variant	Reference
1	11 days	Atypical	Prader3	N/N	0.13 [0.03-2.13][a]	2.13[c]	9.1/15.5	p.Arg496His	38/33	[11]
2	2 months	Atypical	Enlarged clitoris, Post labial fusion Labial gonads	N/N	7.20 [0.03-0.99][a]	0.32[c]	2.98/--	p.Ile332Thr p.Ala355Thr	15-25/10 0/0	[12]
3	6 months	Atypical	Micropenis Hypospadias Left inguinal testis	N/N	--	1.76[c]	--/5.0[d]	p.Trp121Arg p.Leu36ArgfsTer19	60/16 --	[13]
4 (P3)	1.2	Atypical	Micropenis Penile hypospadias Inguinal testes	N/N	--	0.53[c]	--/4.24[d]	p.Phe54del	10-23/5-12	Our study
5	1.5	Atypical	Micropenis Penoscrotal hypospadias Scrotal testes	N/N	4.75 [0.03-0.18][b]	0.80[c]	8.8/11.1	p.Pro342Thr p.Arg239Ter	<45/<49 0/0	[14]
6	23	Atypical/P2	Micropenis Hypospadias Left cryptorchidism	Y/Y	6.58 [0.16-0.57][b]	1.29	0.69/0.7	p. Thr390Arg	--	[15]
7	15.4	Male/B4,P4	Male genitalia	N/N	3.43 [0.16-0.57][b]	1.6	5.6/7.7	p.Pro35Thr p.Arg239Ter	15-20/8-10 0/0	[16]
8	17	Male/ B > 2,P2	Male genitalia	N/N	6.57 [0.16-0.57][b]	1.98	7.8/15.97	Unidentified p.Pro480Hisfs[c]27	-- 0/0	[17]
9 (P4a)	20	Male/B3,P4	Penile length 6.5 cm, Scrotal testes (20 cc each)	N/N	-	1.2	8.6/5.92[d]	p.Asn395Ser	--	Our study

Abbreviations: B, breast, N, no; P, pubic hair; Y, yes.
Age-adjusted normal reference values:
[a]doi:10.1203/00006450-198001000-00010
[b]:doi:10.1159/000486840
[c]Human chorionic gonadotropin stimulated serum testosterone levels
[d]LC-MS/MS assay.

Table 4. Characteristics of 46,XY 17α-hydroxylase deficiency probands based on phenotype: literature review

External genitalia	Combined severe deficiency (female) n = 128	Combined partial (atypical/male) n = 16	*P* value[a]	Apparent isolated 17,20 lyase deficiency n = 7
Age at evaluation, years, median (IQR)	18 (15-22), n = 124	6.9 (1.27-8.5), n = 16	**0.004**	15 (2.5-20), n = 7
Sex of rearing, female: male	128:0	7:9	**0.000**	2:5 (all 7 had atypical genitalia)
Hypertension, n (%)	111/122 (91)	4/14 (28.6)	**0.000**	0/7 (0)
Hypokalemia, n (%)	84/111 (75.7)	4/12 (33.3)	**0.004**	0/7 (0)
Suppressed plasma renin, n (%)	44/49 (89.8)	2/6 (33.3)	**0.005**	-
Serum progesterone (ng/mL), median (IQR)	5 (2.8-7.85), n = 75	4.09 (1.24-7.05), n = 12	0.445	0.84 (0.66-1.13), n = 4
Serum deoxycorticosterone (ng/mL), median (IQR)	1.52 (1.09-3.47), n = 23	2.04 (0.78-2.04), n = 2	1	-
Serum corticosterone (ng/mL), median (IQR)	168.3 (100.3-208.2), n = 19	88.5 (10.1-88,5), n = 2	0.286	-
Serum 17OH progesterone (ng/mL), median (IQR)	0.37 (0.18-0.8), n = 59	1.1 (0.35-3.66), n = 10	0.06	2.05 (0.43-9.39), n = 7
Plasma ACTH (pg/mL), median (IQR)	150.9 (83.1-296.4), n = 66	73.6 (54.5-128.1), n = 11	**0.006**	32.7 (19.1-46.3), n = 2
Serum cortisol (µg/dL), median (IQR)	1.17 (0.66-2.82), n = 77	6.38 (3.22-8.65), n = 14	**0.000**	8.82 (6.9-16.9), n = 7
Serum androstenedione (ng/mL) (age > 14 years), median (IQR)	0.2 (0.1-0.3), n = 33	0.49 (0.25-0.93), n = 6	**0.005**	-
Serum testosterone (ng/mL) (age > 14 years), median (IQR)	0.13 (0.04-0.2), n = 54	1.2 (0.33-1.6), n = 7	**0.001**	0.79 (0.52-1.41), n = 4
Serum DHEAS (µg/dL), median (IQR)	15 (5-15), n = 45	54.7 (30.6-52.7), n = 3	0.3	36.1 (8.1-121.6), n = 3
Pathogenic variants				
Truncating (deletion with zero activity, nonsense, frameshift), n (%)	51/128 (39.8)	0	**0.001**	-
Non-truncating (missense, deletion with partial activity)	74/128 (57.8)	14/15 (93.3)	**0.009**	7/7 (100)
Splice-site variants, n (%)	3/128 (2.3)	1/15 (6.7)	0.361	-
Enzyme activity				
<1%/<1%, n (%)	**95/113 (84.1)**	1/11 (9)	**<0.05**	-
≥1%/<1%, n (%)	**13/113 (11.5)**	0	0.603	-
≥1%/≥1%, n (%)	5/113 (4.4)	**10/11 (91)**	**<0.05**	-

[a]*P* value has been calculated between combined severe deficiency (female) n = 126 and combined partial (atypical/male) n = 18.

common form with more frequent female sex of rearing, features of mineralocorticoid excess and < 1%/<1% enzyme activity pattern, and lower serum cortisol, and androgens. Baseline serum cortisol was the best biochemical parameter to distinguish combined severe deficiency from combined partial deficiency. The study also found frequent occurrence of unsuppressed aldosterone (immunoassay), high diagnostic sensitivity of elevated progesterone, and rarity of TART, gonadal malignancy and adrenal myelolipoma in 46,XY patients with combined 17OHD. Also, we propose that a phenotype of severe undervirilization with milder cortisol deficiency may represent a distinct subtype of combined severe 17OHD with residual 17α-hydroxylase activity but severe 17,20-lyase deficiency (>1%/<1%), which needs further validation.

Our cohort describes patients across the complete phenotypic spectrum of 17OHD, from female to male external genitalia. Consistent with the review of literature, patients with female and male external genitalia had peripubertal presentation as primary amenorrhea and gynecomastia, respectively. In contrast, those with atypical genitalia presented in early childhood [14]. In addition, hypertension and absent breast and pubic hair development were noted in most of the patients reared as female, whereas in those reared as male, hypertension was less frequent, but the majority developed breast and pubic hair in the peripubertal period, as also noted in our cohort.

17OHD typically leads to low-renin, low-aldosterone hypertension due to the accumulation of excess mineralocorticoid precursors, with consequent transcriptional downregulation of aldosterone synthase [21]. A Japanese study reported biochemical hyperaldosteronism (radioimmunoassay) in 29% of patients with low renin levels [6], which was also noted in a few other studies [22-24]. Similarly, in this review, the median serum aldosterone was 9.97 ng/dL in patients reared as females, of whom 88.9% were hypertensive. It highlights that one should not exclude 17OHD from the differential diagnoses of biochemical hyperaldosteronism [25]. Although aldosterone value can be affected by various factors such as age and severity of mutation, assay-related interference appears to be the major contributor [25]. In a 17OHD hypertensive female with low renin, discordant (elevated on radioimmunoassay but low by high-performance liquid chromatography), serum aldosterone levels have been documented [26]. Notably, none of our patients (P1, P4a, P4b) with low renin, evaluated by LC-MS/MS, had hyperaldosteronism. Although further prospective studies are warranted, LC-MS/MS seems promising to avoid probable diagnostic dilemmas due to falsely elevated immunoassay-measured serum aldosterone in low-renin hypertension.

The biochemical hallmark of 17OHD is elevated serum progesterone, 11-deoxycorticosterone, and corticosterone. Given the limited availability of the latter 2 tests, serum progesterone has been proposed as a simple and reliable test in the diagnosis of complete 17OHD [27]. As noted from this review, most had elevated progesterone, even with the partial deficiency, supporting the proposal. However, it should be noted that elevated progesterone may also be seen in 46,XY DSD patients with P450 oxidoreductase deficiency.

None of our patients had gender dysphoria, which concurs with the literature. Female gender identity in 46,XY 17OHD patients with severe undervirilization can be explained due to a complete lack of fetal brain androgen imprinting, similar to complete androgen insensitivity [28]. Notably, in 17OHD patients with atypical genitalia, gender of rearing was synchronous with gender identity. This observation is similar to partial androgen insensitivity syndrome patients, wherein the sex of rearing predominates in the determination of gender identity when the fetal brain is inadequately imprinted with androgens [28].

Leydig cell hyperplasia has been considered a classic feature in 46,XY DSD with testosterone biosynthetic defects. Notably, all patients with combined severe deficiency and enzyme activity of < 1%/<1% showed few/absent Leydig cells. The Leydig cell hypoplasia in this cohort could be due to the absence of autocrine androgenic action on progenitor cells, essential for differentiation into adult Leydig cells [29]. Leydig cell hyperplasia as seen in P2, and a previously described patient [30], (having basal cortisol of 8.6 and 11 µg/dL, respectively) suggests partial retainment of hydroxylase activity is essential for Leydig cell growth. P4a presented to us for concern of infertility. As per this review, fertility has not been reported with 46,XY 17OHD. Notably, he had normal AMH and inhibin B levels, although he had azoospermia. Hence, testicular sperm extraction has been planned to evaluate his fertility potential.

For patients with female sex of rearing, data on gonadectomy was available for 62 patients, and none reported TART or malignancy. However, the rare occurrence of malignancy, TART, and adrenal myelolipoma have been reported in biochemically, but not genetically, diagnosed 46,XY patients with 17OHD [31-34]. The rarity of malignancy in nondysgenetic gonads, especially those associated with testosterone biosynthetic defects, has been emphasized in recent studies [35]. Although gonadal malignancy was not noted in patients included in our review, a recent study has reported testicular malignancy in 2 genetically diagnosed 17OHD patients with late diagnosis [36]. In CAH patients, the degree of ACTH elevation has been linked to increased prevalence of TART and adrenal myelolipoma [37, 38]. The elevation of ACTH in 17OHD is usually milder than classic 21α-hydroxylase deficiency due to compensatory glucocorticoid activity by the markedly elevated corticosterone [39]. This may explain the rarity of TART and adrenal myelolipoma in 17OHD. Nevertheless, 17OHD is the second most common form of CAH associated with adrenal myelolipoma, which may be due to a longer latency in the diagnosis [40].

Recurring variants specific to geographical locations may constitute an ancestral founder variant. The p.Pro480HisfsTer27 variant in the Friesian population, and p.Tyr329LysfsTer90 and p.Asp487_Phe489del in the Chinese population are the classic examples of the founder effect [20, 41] whereas recurrent variants (p.Trp406Arg, p.Arg362Cys,p.Pro428Leu) from Brazil, due to the heterogeneous nature of the population, are explained based on inbreeding [2, 42]. The review also identified a few other region-specific recurrent variants. Notably, 2 variants (p.Arg96Gln and p.Arg96Trp) on Arg96 position have been reported worldwide. The high mutability of arginine due to deamination of CpG dinucleotides of its codons makes it a mutational hotspot.

Combined severe deficiency was the most common form of 17OHD. Most patients of this group had an enzyme activity pattern < 1%/<1%. However, whether the predominance of combined severe deficiency is due to frequent occurrence of detrimental variants or underdiagnosis of combined partial

deficiency is unclear. The female sex of rearing, and features of mineralocorticoid excess were more frequent whereas serum cortisol, and androgens were lower in combined severe deficiency than combined partial deficiency which is understandable. Among all the biochemical parameters, baseline serum cortisol had the highest AUC (0.908) to distinguish it from combined partial deficiency. The scant LC-MS/MS data suggest that cortisol measurement by this method may further increase the accuracy of serum cortisol to distinguish the 2 forms of combined deficiency.

The subtyping of 17OHD is complex due to dual enzymatic functions of CYP17A1 and continuum nature of phenotypic spectrum and residual enzymatic functions. Interestingly, a small proportion of 17OHD patients with clinical phenotype of combined severe deficiency had some residual 17α-hydroxylase activity but severe 17,20-lyase deficiency (>1%/<1%). Notably, nontruncating missense variants were more frequent and baseline serum cortisol was higher in this subgroup (>1%/<1%). This positive association of retained 17α-hydroxylase activity with greater cortisol production suggests that this subgroup may represent a distinct subtype of 17OHD. However, the clinical relevance of such distinction is not clear. Interestingly, apparent isolated 17,20-lyase deficiency leads to milder undervirilization [5, 43-45], whereas partial hydroxylase and severe 17,20-lyase deficiencies (>1%/<1%), lead to severe undervirilization. This could be due to inability to produce adequate 17OHP as compared to greater production of androgen precursors (17OHP) by the backdoor pathway in the apparent isolated 17,20-lyase deficiency group [46, 47].

The variants in 2 of our patients, P1 (p.Arg239Ter) and P3 (p.Phe54del) have been previously reported. P2 had a novel mutation, p.Gly478Cys, for which enzymatic activity data were not available. Molecular modeling predicted reduced flexibility of the protein with p.Gly478Cys. P4a had the mildest phenotype, with apparent male external genitalia, which has been described only in 2 other patients to date. The variant (p.Asn395Ser) in P4a was also novel and has been predicted in the molecular modeling to alter the orientation of A helix and secondary structure of small K′ helix and increase the stability of parallel β-sheets. However, further studies are warranted to quantify the residual enzyme activity of these novel variants.

The study was limited by the small sample size and retrospective collection of phenotypic data. Nevertheless, patients from our center provide a complete disease spectrum with 2 novel variants. However, functional analysis of the novel missense variants observed in patients from our center could not be performed. In the analysis of data from the literature, the correlation of clinical and biochemical phenotype with the severity and type of enzyme deficiency may be limited by evaluation at varied ages, the varied protocol for stimulation tests, and assays used for hormonal estimations as well as functional studies to estimate the residual enzyme activities. The use of immunoassays with high cross-reactivity and availability of hormonal data in a limited number of patients might have affected the inferences. Most importantly, residual enzyme activities vary greatly based on assay systems employed. Use of similar assay systems in different labs may also yield different results. Performing kinetic analysis to determine Km and Vmax of WT and mutant enzymes and then expressing Vmax/Km of the mutant as a percentage of WT is the most accurate method for measurement of residual catalytic activity. When it is measured by methods other than kinetic analysis, as noted in a few studies included in this review, the intra-assay and inter-assay variability will be even higher. Hence, the proposed novel subtype of combined severe 17OHD with residual 17α-hydroxylase activity but severe 17,20-lyase deficiency (>1%/<1%) needs further validation in prospective cohorts with robust LC-MS/MS serum biochemistry and standardized in vitro characterization of mutant CYP17A1 enzyme activity at a central laboratory. Nonetheless, this review of literature clarifies certain uncertainties in understanding 46,XY patients with 17OHD.

Conclusions

We report 4 Asian Indian index cases of 46,XY 17OHD with a variable spectrum of undervirilization, including 2 novel variants. This review of literature on 46,XY patients with 17OHD demonstrates apparent female external genitalia and female sex of rearing in the majority. The presence of hyperaldosteronism by immunoassay does not rule out 17OHD. Serum progesterone is highly sensitive for the diagnosis of 17OHD, even for those in combined partial deficiency. Fertility, TART, adrenal myelolipoma, gender dysphoria, and gonadal malignancy are rarely reported in 46,XY patients with 17OHD. External genital phenotype and serum cortisol are helpful in subtyping 17OHD, resulting from *CYP17A1* pathogenic variants, based on the residual activities of individual enzyme functions. We propose that a phenotype of severe undervirilization with preserved cortisol production may represent a distinct subtype of combined severe 17OHD with residual 17α-hydroxylase activity but severe 17,20-lyase deficiency (>1%/<1%), which needs further validation.

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Disclosures

The authors have nothing to disclose in relation to the contents of this work.

Data Availability

The data that support the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy or ethical restrictions.

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