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Molecular and clinical characteristics of 5-alpha reductase type 2 deficiency: A systematic review

Fatinah Shahab[1,2], Nanis S Marzuki[3], Achmad Zulfa Juniarto[4*]

[1]*Doctoral Study Program of Medical and Health Science, Universitas Diponegoro, Semarang, Indonesia.*
[2]*Faculty of Medicine, Universitas Wahid Hasyim, Semarang, Indonesia.*
[3]*Eijkman Research Center for Molecular Biology, National Research and Innovation Agency, Cibinong, West Java, Indonesia.*
[4]*Faculty of Medicine, Diponegoro University, Semarang, Indonesia.*

Corresponding author: Achmad Zulfa Juniarto (*Email: zulfa_juniarto@fk.undip.ac.id*)

Abstract

The 46, XY differences of sex development (46, XY DSD) due to 5-alpha reductase type 2 deficiency (5ARD2) are characterized by extensive phenotypic variability. Molecular analysis of the specific mutated SRD5A2 gene is employed in diagnosing enzymatic defects in the androgen biosynthesis cascade. This review systematically elaborates on the current literature regarding 46, XY DSD caused by 5-alpha reductase type 2 deficiency. Three international databases were searched for literature published from 2014 to 2024. The articles were summarized and reviewed, focusing on molecular causes and clinical presentation. A total of 412 articles were retrieved from the search. Of these, 31 articles met the inclusion criteria and had sufficient power for review. We identified 2,235 patients with 5ARD2 deficiencies from 17 countries, with most cases originating from China (29%), Iran (13%), and India (10%). Sixty-nine percent of patients were assigned female at birth. Regarding genetic variants, 70% were homozygous allelic variants, and 30% were compound heterozygous. Most mutations were missense variants, with homozygous mutations and compound heterozygous mutations being predominant. Additionally, small insertions and deletions (indels), splicing mutations, and large deletions were reported. These mutations were distributed across all exons, with exon 1 accounting for 33% and exon 4 for 25%. Although research remains limited, molecular technology is essential for rapid gene localization in patients, providing valuable clinical information.

Keywords: 46, 5-alpha reductase type 2, SRD5A2, Systematic review, XY DSD.

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1. Introduction

The 46, XY differences in sex development (DSD) arise from various genetic backgrounds. The underlying causes of 46,XY DSD include decreased production of androgens such as testosterone (T) or dihydrotestosterone (DHT) during fetal sex differentiation or impaired androgen action at target tissues throughout life. According to Sandberg et al. [1] and Guerrero-Fernández et al. [2] the most common identifiable cause in the reported series is androgen insensitivity syndrome (AIS), followed by 5-alpha-reductase type 2 deficiency, which is the second most common cause [3]. Male external genitalia virilization relies on the conversion of T into its more potent metabolite DHT during fetal development. Deficiency of 5-alpha-reductase type 2 impairs the ability to convert T into DHT and leads to under-virilization of male external genitalia. The 5-alpha-reductase type 2 enzyme is encoded by the SRD5A2 gene (steroid-5-alpha reductase type 2, MIM 607306) [4] which is located on chromosome 2p23.1. The gene comprises five exons and encodes a 254-amino acid protein containing an androgen-binding domain at the N-terminal and an NADPH cofactor-binding domain at the C-terminal [5]. The 5 alpha-reductase type 2 deficiency is an autosomal recessive disorder, typically identified at birth due to varying degrees of under-virilized external genitalia, such as micropenis, different levels of hypospadias, and/or cryptorchidism [6]. The enzymatic deficiency was first biochemically and clinically reported in isolated clusters in the Dominican Republic in the early 1970s [7].

Severely affected male individuals can be born with female-like external genitalia but develop clinical and psychological virilization at puberty without experiencing gynecomastia [8]. Currently, 5ARD2 is documented with extensive phenotype variability, even in individuals carrying the same SRD5A2 mutation. This systematic review was conducted to compile the existing literature concerning 46, XY DSD due to 5ARD2. This review focused on variations in clinical presentation and types of SRD5A2 gene mutations.

2. Method

2.1. Search Strategy

We conducted a systematic review of published data on 46, XY DSD due to 5ARD2 from 2014 to 2024. The systematic review adheres to the guidelines detailed in the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement [9]. The data were obtained primarily through published articles. The online Web of Knowledge database was used to identify English-language articles, using the search terms: “46, XY DSD” AND “SRD5A2” AND “5-Alpha reductase type 2 OR 5α reductase type 2”. Further articles were traced through Scopus, PubMed, and Wiley-Blackwell Interscience for full-text collection. Additional relevant studies were manually searched, and previous review articles were incorporated.

2.2. Eligibility Criteria

Studies selected included because letter 46, XY DSD are used with various meanings in other disciplines. The search criteria generated many irrelevant papers, but these were readily identified, leaving 31 papers with a focus on 5ARD2. A flow diagram detailing study selection is shown in Figure 1.

2.3. Study Selection and Data Collection Process

Articles were screened in three stages: title, abstract, and full text. In the first instance, articles published between 2013 and 2023 were screened by title (n=412), and duplicates were removed (n=392). All articles were then reviewed by abstract for relevance and excluded at this stage if they did not fulfill the inclusion criteria. The remaining articles (n=31) were then downloaded for full-text review, and the reference lists of these articles were also systematically explored. The final sample consisted of 31 studies meeting all eligibility criteria.

2.4. Inclusion and Exclusion Criteria

Inclusion criteria for this systematic review were as follows: samples that included individuals with 46, XY DSD due to 5ARD2 of any age, accessible full-text articles, from 2014 to 2024. Articles not including original quantitative results published in a peer-reviewed journal (e.g., case reports, unpublished dissertations, editorials, meeting abstracts), not written in English, were excluded. Our data analysis is a systematic narrative and descriptive method, summarized in the text and table. A meta-analysis was not possible due to heterogeneity in study results.

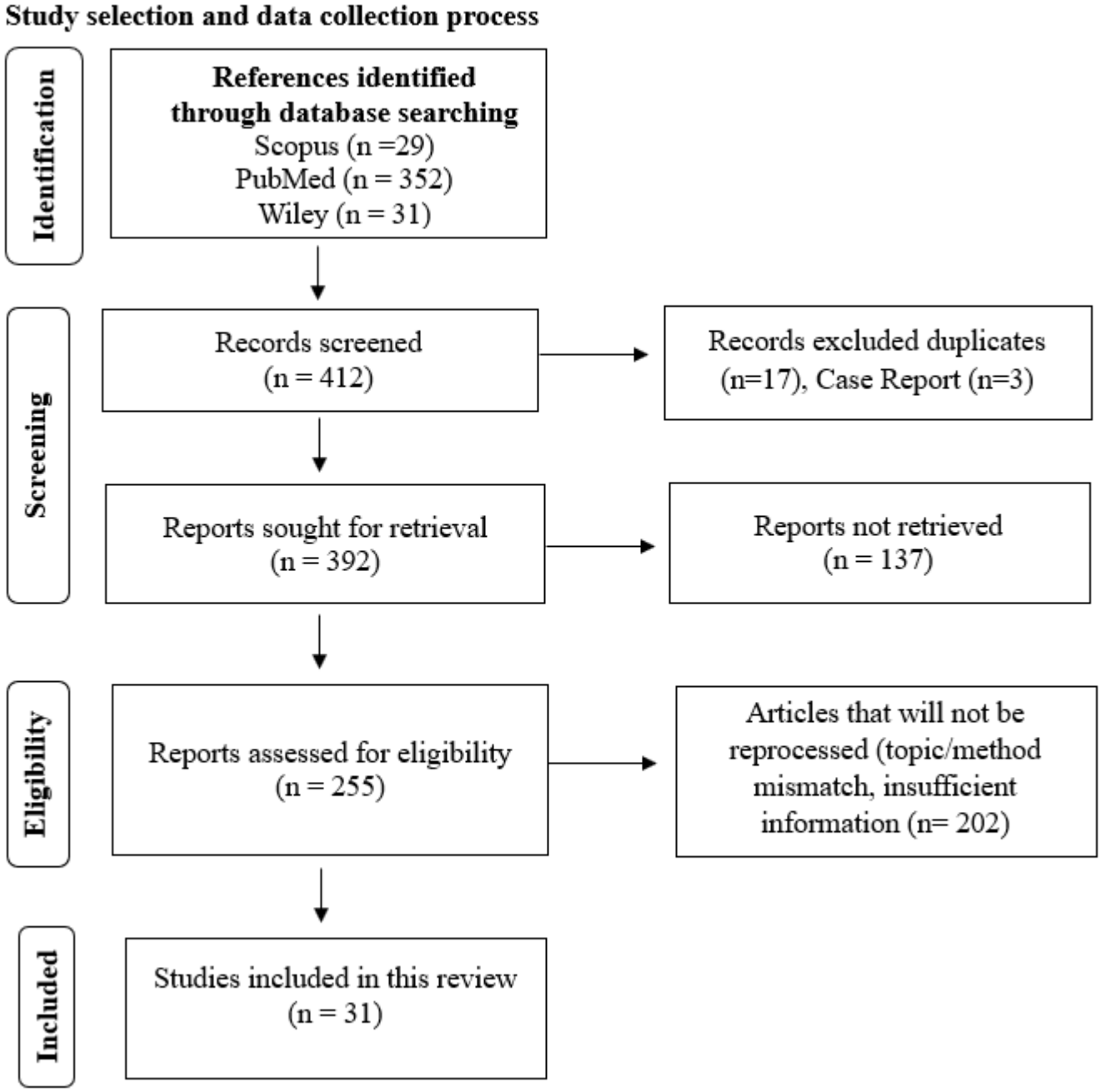

Figure 1.
PRISMA flow diagram of the present study.

3. Results

The database search yielded a total of 412 articles, supplemented by additional abstracts identified through the literature search. Following the removal of duplicates, 392 articles were screened. From these, 255 studies were selected for full-text review based on the eligibility criteria. A total of 202 studies were excluded due to insufficient information regarding the topic, methodological discrepancies, or lack of adequate details. Ultimately, 31 studies were included in the final data extraction. Table 1 provides an overview of these 31 studies, including author details, country, and sample characteristics. Furthermore, the measure employed and a summary of findings from each study are presented in Table 1.

3.1. Sample Characteristics

Table 1 shows that 2235 subjects participated in the reviewed studies. The smallest sample size was 1 participant reported by Ahmadifard et al. [10] and the largest was 451 participants by Batista and Mendonca [11]. Most subjects were under 1 year old, with only three studies involving participants over 35 years of age [8, 12, 13]. Of those detailing the gender, 60.1% were men and 39.9% were women.

Table 1.
Characteristics of included studies.

No	Study and Country	Study Design	Study population	Type of mutation	Exon/ mutation site
1	Marzuki et al. [12] Indonesia	Experimental (Insilco)	0.4-41.8 n = 37 M = 24 F = 13	Point mutation (n =41) Frameshift mutation (n=9) Intronic mutation (n=5)	Exon 1, 2, 4, and 5
2.	Zhu et al. [14] China	Cohort	0-23 n = 9 M = 2 F = 9	Frameshift mutation (n=1) Nonsense mutation (n=1) Missense mutation (n=2) Compound heterozygotes (n=6) Homozygous mutation (n=4) Homozygous splicing mutation (n=1)	Exon 1,4, and 5
3	Mai et al. [15] Germany	Case Control	0,5-13 n = 25 M = 25	Biallelic mutations (n=12)	NA
4	Fan et al. [16] China	Cohort	0.7-3.5 n = 77 M = 93 F = 37	31 variants Missense mutations (77.7%) Nonsense mutations (19.6%) Frameshift/splice mutation (2.3%)	Exon 1,4
5	Gui et al. [5] China	Cohort	0-16 n = 190 M = 154 F =36	42 variants 13 novel variants Homozygous (38.42%) Compound heterozygous mutations (61.58%)	Exon 1, 4 and 5.
6	Akcay et al. [3], Turkey	Experimental	3.9-10 n = 6 M = 3 F = 3	Homozygote mutation (n=3) Compound heterozygous (n=2) Heterozygous (n=1)	Exon 1,3, and 4
7	Han et al. [6] China	Experimental	11-34 n = 16 M = 11 F = 5	Compound heterozygous n=12 Homozygous n=4	NA
8	Guaragna-Filho et al. [13] Brazil	Case Control	0.4-39 n = 8 M = 8	NA	NA
9	Cheng et al. [17] China	Cohort	NA N = 45 M = 41 F = 4	15 different mutations. Homozygous mutation (35·6%) Compound heterozygous (44,4%) patients, Compound heterozygous mutations with the p.Leu89Val polymorphism (20%)	Exons 1 and 4

10	Batista and Mendonca [8] English	Review Literature	1-47 n = 434 M = 134 F = 300	129 variants Missense mutation (76%) Homozygous (70%) Compound heterozygous (30%) Small indels (11%), Splicing mutation (5%) Large deletions (4%)	exon 1 (33%), exon 4 (25%)
11	Khorashad et al. [18] Iran	Retrospective Study	5-32 n = 16 M = 8 F = 8	NA	NA
12	Cheng, et al. [19] China	Experimental (In Vitro)	5-34 n = 14 M = 6 F = 8	Nonsense mutation	NA
13	Batista and Mendonca [11] Brazil	Review Article	1-47 n = 451 M = 139 F = 312	Homozygout 70% Missense mutations (n = 84) Splicing mutations (n = 6) premature stop codons (n =4) Small indels (n=20), Gross deletions (n=7)	Exon 1-5 exon 1 (33%) exon 4 (25%)
14	Samtani et al. [20] India	Meta Analysis	4-10 n = 96 M = 96	NA	NA
15	Khorashad et al. [21] Iran	Case control	5-29 n = 20 F = 20	NA	NA
16	Avendaño et al. [22] Switzerland	Review Article	NA n = 126 NA	Homozygous (60%) Compound heterozygous (40%) missense/nonsense (n=84) Splicing mutation (n=60) Deletion (n=14) Insertion (n=6) Large deletion (n=4)	Exons 1 and 4
17	Abacı et al. [23] Italy	Case Control	0-35.4 n = 85 M = 50 F = 35	NA	NA
18	Misgar et al. [24] Khasmir	Retrospective Study	0-13 n = 17 M = 14 F = 3	NA	NA

19	Zhao et al. [25] China	Cohort Retrospective	0-16 n = 141 M =56 F = 85	NA	NA
20	Nagaraja et al. [26] India	Review Article	NA n = 16 M = 10 F = 6	Missense mutation	Exon 1, 4 and 5
21	Ahmadifard et al. [10]. Iran	Experimental	13 n =1 M= 1	Missense mutation	exon 4
22	Bertelloni et al. [27] Switzerland	Literature Review	NA n = 2 M = 2	Compound heterozygous Missense variants	Exon 2, 4
23	Han et al. [28] China	Experimental	0-34 n = 25 M = 18 F = 17	Polymorphism (n=14)	NA
24	Arya et al. [29] India	Retrospective Study	NA n = 23 M = 19 F = 4	Homozygous mutation (n =9)	NA
25	Avendaño et al. [30] Venezuela	Experimental	NA n = 3 NA	NA	NA
26	Akcan et al. [31] Cyprus	Retrospective review	NA n = 14 M = 3 F = 11	Missense mutation n=11 Frameshift mutation n=2 Deletion n=1 Homozygous Compound heterozygote	NA
27	María Guadalupe et al. [32] Mexico	Experimental	8-50 n = 2 M = 1 F = 1	Compound heterozygote Homozygous	Exon 4
28	Liu et al. [33] China	Retrospective Study	NA n = 103 NA	Compound heterozygote Homozygous	Exon 1 (26%) Exon 4 (44%)
29	Markouli and Michala [34] (England)	Review Article	NA n = 12 M = 10 F = 2	Missense mutation Homozygous Heterozygote	NA
30	Alswailem et al. [35] Saudi Arabia	Experimental	NA	Splice site mutation	NA

			n = 25 NA	Homozygous	
31	Mehrak and Alavi [36]. Iran	Experimental	1-4 n = 109 M = 109	Missense mutation Insertion mutation- (Frameshift) Polymorphism	Exon 1, and 4

Note: M = Male; F = Female; NA = not available;

Table 2.
Clinical, hormonal, molecular, and other findings.

		Outcomes			
No	**Study and Country**	**External Genitalia**	**Hormonal results**	**SRD5A2 gene mutation**	**Other findings**
1	Marzuki et al. [12] Indonesia	Harmful mutations → classic/severe 5ARD2: EMS: 1.5–9 (median = 3) Benign mutations: EMS >6	Diagnostic test sensitivity: T/DHT ratio (cutoff = 10): ▪ 85% sensitivity in severe cases ▪ 64.7% in mild cases Urinary Et/An ratio (cutoff = 0.95): ▪ 90% in severe cases ▪ 47.1% in mild cases ▪ Et/An is slightly more sensitive than T/DHT in severe cases	▪ 12 variants identified, 6 novel: (c.34–38delGinsCCAGC, p.Arg50His, p.Tyr136*, p.Gly191Arg, p.Phe194Ile, p.Ile253Val) ▪ Most frequent variant: p.Val89Leu (58.1% of alleles) ▪ High mutation density in: Exon 1 (72.8%), Exon 4 (13.6%)	Harmful mutations: 95% of those raised female → virilized at puberty → changed gender to male Mostly homozygous or compound heterozygous for harmful variants Late diagnosis with mean age = 15.6 years Benign mutations: All raised as male No abnormal virilization Diagnosed earlier (median = 0.5 years) Mostly p.Val89Leu
2.	Zhu et al. [14] China	EMS median = 3 Ambiguous genitalia in all;micropenis, hypospadias.	T/DHT ratio: 10/11 > 8.5 (cutoff: 10); highest: 61.33; lowest: 2.15 Hormones: T: 0.28–36.4 nmol/L; DHT: 0.02–1.08 nmol/L; LH: up to 19.78; FSH: up to 11.11	9 mutations in *SRD5A2*; 1 novel (IVS4+2 T>C); most common: p.G203S, p.R246Q	9/11 was raised as female, all transitioned to male after puberty Fertility: 1 patient achieved paternity via IVF
3	Mai et al. [15] Germany	NA	5α-THF/THF ratio: all showed markedly low ratios Cut-off ratio 5α-THF/THF at 0,19 showed 100% sensitivity and 100% specificity GC-MS urine profiling: accurate and non-invasive	Genetic testing: 12/25 tested → all had biallelic *SRD5A2* mutations	NA
4	Fan et al. [16] China	EMS: 1-8 (median = 6) Urethral meatus score: 0-1 (median =1) Gonad location score: 2.5-3 (median = 3) Penile length SDS (Mean = −5.08 ± 1.59)	T/DHT ratio (after hCG) = 19.06-37.66 (median = 29.23), T/DHT > 10: 76/77 patients (98.7%) T/DHT > 15: 71/77 patients (92.2%)	Sanger sequencing: 67 patients NGS/WES testing: 63 patients Most common mutation: p.R227Q (39.6%), p.Q6* (16.9%), p.R246Q (13.5%), p.G203S (10.4%) Genotype-phenotype link: p.R227Q → milder/variable; p.Q6*, p.G203S → severe	NA

5	Gui et al. [5] China	Hypospadias was the most common phenotype, found in 66.32% (126/190) of patients, with or without micropenis and/or cryptorchidism.	NA	8 patients tested by targeted Sanger sequencing 103 patients tested by NGS Top 4 most frequent mutations: c.680G>A (p.R227Q) – 52.37% c.16C>T (p.Q6X) – 10.79% c.607G>A (p.G203S) – 9.21% c.737G>A (p.R246Q) – 8.95%	Regional mutation distribution: c.680G>A is more frequent in Southern China (62.62%) than in North (39.16%) *($p < 0.001$)* c.16C>T more frequent in Northern China (16.87%) than South (6.07%) *($p = 0.001$)* Genotype–phenotype correlation: c.680G>A → more often associated with normal meatus or distal hypospadias c.16C>T → more often linked with proximal hypospadias
6	Akcay et al. [3], Turkey	NA	T/DHT ratio in SRD5A2(+) patients: mean 12.0 (range 4.2–23.2). No patients had T/DHT ratio >25.	SRD5A2 mutations found in 6 PAIS patients (12%). 66% of SRD5A2-positive patients had consanguineous parents. 2 novel mutations reported: one in AR (p.F892L), one in SRD5A2 (p.L73WfsX59).	No clear genotype–phenotype correlation observed
7	Han et al. [6] China	NA	T/DHT ratio: Diagnostic cutoff: >27.3, with 93.8% sensitivity and 100% specificity	NA	11-oxygenated adrenal androgens: 11OHA4 and 11KA4 were lower in 5α-RD2 than AIS 11OHT was higher in 5α-RD2 than AIS 11OHT/11OHA4 ratio > 0.048 differentiated 5α-RD2 from AIS
8	Guaragna-Filho et al. [13] Brazil	Median EMS: 5ARD2: 4 (range 2–9)	AMH Levels: Strongest in 5ARD2 ($r = -0.95$; $P < 0.0001$) Inhibin B Levels: Significantly lower in 5ARD2 (median 50.70; $P = 0.045$) T/DHT Ratio: 5ARD2: 14.7 (range 6.6–54.0)	NA	Inhibin B may help distinguish 5ARD2 from PAIS and idiopathic DSD
9	Cheng et al. [17] China	Variable external masculinization ranging from mild Undervirilization to female genitalia with clitoromegaly Hypospadias: 55.6% (25/45) Isolated microphallus: 35.6% (16/45) Clitoromegaly (female sex assignment at birth): 8.9% (4/45) EMS Mean/median not explicitly stated, but varied across phenotypes	hCG-Stimulated T/DHT Ratio: 88.9% (40/45) of children showed a T/DHT ratio > 10 following hCG stimulation – supporting diagnosis of 5α-RD2	p.R227Q – most frequent (associated with milder phenotype) p.Q6*, p.R246Q, p.G203S – associated with more severe undervirilization	p.R227Q homozygous cases showed milder presentations, often hypospadias or microphallus p.Q6*, p.R246Q, p.G203S mutations linked to more severe Undervirilization Clinical variability is still present even among patients with the same mutation, indicating potential modifying factors.
10	Batista and Mendonca	Sinnecker Score: Used to quantify the phenotype (scale	NA	p.Leu55Gly, p.Arg246Gln. Compound heterozygous: 5.02 ± 2.1	Most cases were from Turkey (23%), China (17%), Italy (9%), and Brazil (7%)

	[8] English	1–9). Mean Sinnecker scores by mutation type: Homozygous: 5.44 ± 2.2		Indels: 6.89 ± 1.1 Polymorphisms: p.V89L polymorphism reduces enzymatic activity by ~30%. Associated with isolated hypospadias, common in East Asians.	25% (87/349) underwent gender change from female to male. Overall, no strong genotype–phenotype correlation.
11	Khorashad et al. [18] Iran	5α-RD-2 patients (n = 16): Mean PSAI = 58.76 (SD = 16.13)	NA	NA	NA
12	Cheng et al. [19] China	Microphallus Hypospadias (perineoscrotal type) Cryptorchidism Virilization during/after puberty No gynecomastia reported	Hormone Levels (baseline): T: Mean ± SD: 23.95 ± 8.68 nmol/L DHT: Mean ± SD: 0.40 ± 0.14 nmol/L T/DHT Ratio: Mean ± SD: 72.85 ± 26.22 All except one patient had T/DHT > 8.5 LH: Range among patients: 0.09 – 18.8 IU/L FSH: Range among patients: 0.5 – 32.9 IU/L	Total mutations identified: 9 (in 13 unrelated families) Most common mutations: *p.Q6X*, *p.G203S*, *p.R227Q* Novel mutations: *p.K35N*, *p.Y136X*, *p.H162P* Their enzymatic activities were: p.K35N: 30.0% p.H162P: 37.7% p.Y136X: 1.2% (complete loss of function)	Therapy: Topical DHT was effective in the prepubertal patient (Patient 10), with minimal response in postpubertal cases. Suggested dose: 25–50 mg daily
13	Batista and Mendonca [11] Brazil	NA	The testosterone to DHT ratio (T/DHT) often >18. A diagnostic cut-off T/DHT ratio of >27.3 has 93.8% sensitivity and 100% specificity in differentiating from androgen insensitivity syndrome. The urinary Et/An ratio has a diagnostic cut-off of ≥ 0.95 in affected individuals and ≥0.99 in carriers. The Et/An ratio may have higher diagnostic value than the T/DHT ratio in certain cases.	NA	Gender change to male occurs in 16–70% of cases, depending on cultural and social context.
14	Samtani et al. [20] India	Hypospadias Severity Distribution: Mild (9.9%): Coronal and glandular types Middle (34.6%): Mid-penile and distal penile	NA	NA	Maternal diet: Vegetarian diet associated with hypospadias risk → OR = 2.86 (95% CI: 1.5–5.27, p = 0.001) → likely due to phytoestrogens or agrochemical residues. Environmental exposures (e.g., diet,

		Severe (55.4%): Penoscrotal, proximal penile, scrotal			pesticides) may play a more prominent role than genetics in hypospadias etiology.
15	Khorashad et al. [21] Iran	Extent of virilization at puberty: 9 participants had high virilization (Tanner stages 4–5). 7 had medium virilization (Tanner stages 2–3).	Hormonal treatment: All post-pubertal females received conjugated estrogen. Males received testosterone (250 mg every 10–15 days). Some females also received progesterone due to small breast complaints.	NA	Sexual activity: 55% had a sexual experience. 20% had sex with men, 15% with women, 20% with both. Gonadal removal was performed on all individuals who continued living as female (either with or without genital surgery). No significant association between virilization and gender dysphoria (P = .131).
16	Avendaño et al. [22] Switzerland	Phenotype (n = 256): 66.1%: Clitoromegaly / Microphallus 39.8%: Hypospadias 19.9%: Cryptorchidism 7.0%: Predominantly male phenotype 3.9%: Predominantly female phenotype EMS used for phenotypic scale (0–12): T-binding mutations: EMS ~2.0–3.3 NADPH-binding: EMS ~2.7–4.2 Enzyme activity mutations: EMS ~3.0–8.0 (most variable)	T: DHT ratio post-hCG: cut off >8.5	p.G34R (Egyptian), p.G183S (Brazilian), p.L55Q (Turkish), etc. p.G196S, p.R246Q = found globally	Genotype-Phenotype Correlation: No strong correlation overall
17	Abacı et al. [23] Italy	Phenotypic Findings: Abnormal external genitalia in 92.9% (79/85 patients). Undescended testes in 77.6% (66/85 patients): 63 bilateral, 3 unilateral. External masculinization graded using Sinnecker score: Score 1a–5, showing a wide spectrum of under-virilization.	T/DHT Ratio (Testosterone/Dihydrotestosterone): hCG stimulation increased diagnostic sensitivity. Best cut-off values for stimulated T/DHT ratio by pubertal stage: ≥ 8.5 for mini puberty ≥ 10 for prepuberty ≥ 17 for puberty	Molecular characteristics p.Ala65Pro: 30.6% p.Leu55Gln: 16.5% p.Gly196Ser: 15.3% p.Ala65Pro and p.Leu55Gln were associated with more severe undervirilization than p.Gly196Ser.	Family History and Consanguinity: Parental consanguinity was reported in 66 patients (77.6%). Family history of DSD in 38 patients (44.7%). No consistent genotype–phenotype correlation across all mutations
18	Misgar et al. [24] Khasmir	Common findings: hypospadias, bifid scrotum, micropenis	T/DHT ratio: ranged from 21.2 to 75.4	NA	Consanguinity: documented in 9 patients (52.9%)
19	Zhao et al. [25] China	Clinical Presentation (n = 187): Micropenis only: 64 (34.2%) Hypospadias only: 43 (23.0%) Ambiguous genitalia: 37 (19.8%) Cryptorchidism only: 19 (10.2%)	Hormone Levels after hCG Stimulation (n = 95): Mean T: 362.1 ± 121.6 ng/dL Mean DHT: 123.0 ng/mL T/DHT ratio: 10.67–86.56 (median	NA	Geographical Comparison: Children from northern China had significantly higher THtSDS, birth weight, and WtSDS than those from southern China ($p < 0.05$)

		Mixed genital abnormalities: 24 (12.8%) EMS Group (n = 95): EMS < 7 group had higher HtSDS, WtSDS, T, and significantly higher birth weight (p = 0.028) Pubertal Features: 7 children >10 years in puberty (Tanner stage 2–5) Puberty onset slightly delayed compared to normal boys	32.72) Diagnostic cut-off: T/DHT > 8.5		
20	Nagaraja et al. [26] India	Presented with hypospadias, better virilization	Diagnostic Cut-Off: T/DHT ratio > 30 after hCG stimulation is supportive of 5α-RD2 diagnosis.	p.R246Q – found most frequently among Indian cases; considered a mutational hotspot p.G196S – also considered a hotspot; known to reduce 5α-reductase enzyme activity. Novel Mutation Discovery: 8-nucleotide deletion in exon 1 of SRD5A2 found in a patient with perineal hypospadias.	Lack of strict genotype–phenotype correlation.
21	Ahmadifard et al. [10] 2019. Iran	Clinical signs: micropenis, chordee, perineal hypospadias, bilateral undescended testes	T: 293 ng/dL DHT: 14.3 ng/dL T/DHT ratio = 20.4 → elevated, confirms diagnosis	SRD5A2 gene (Exon 4): p.Asn193Ser (N193S) from homozygous mutation c.578A>G in 1 male patient (age 13)	Affected families involved consanguineous marriages
22	Bertelloni et al. [27] Switzerland	Clinical Features: Ambiguous genitalia at birth Underwent genital reconstructive surgery during childhood Experienced spontaneous virilization at puberty	NA	Compound heterozygous variants: c.308G>C c.689A>C	Fertility Outcomes: Older brother: Achieved spontaneous paternity Younger brother: Achieved paternity via ART
23	Han et al. [28] China	EMS showed a marginal difference in p.Gln6* carriers (P = 0.05), but not in p.Gly203Ser. Cut-off or Key Clinical Data: EMS ranged from 2 to 9.	T/DHT ratio: high values observed but no clear diagnostic cutoff stated; important in distinguishing from androgen insensitivity syndrome. Hormone levels (LH, FSH, T, DHT, T/DHT ratio) were not significantly different between patients with or without the two prevalent mutations (p. Gly203Ser and p.Gln6*).	Most common mutations: p.Gly203Ser in 8 patients (32%) p.Gln6* in 7 patients (28%) p.Val89Leu, a known polymorphism, was found in 14 patients. Haplotype Analysis (Founder Effect): 12 SNPs around the *p. Gln6** locus were analyzed. Three haplotypes were found among 6 patients.	Diagnosis age: 76% (19/25) were diagnosed after age 16.

24	Arya et al. [29] India	Presentation: 82.6% (19/23) presented with atypical genitalia	NA	9 different pathogenic variants, including 2 novel mutations: p. Leu83Pro p. Ala28Leufs*103 Most Common Variant: p. Arg246Gln (n= 12) Homozygous p. Arg246Gln: (n= 9) All homozygous p. Arg246Gln patients were also homozygous for p.Val89Leu Milder undervirilization in p. Arg246Gln homozygotes (Sinnecker score ≤3a in 8/9 patients; $P = 0.04$) Asymptomatic fathers of 2 index cases had homozygous p. Arg246Gln but only heterozygous for p. Val89Leu	NA
25	Avendaño et al. [30] Venezuela	Main features: Hypospadias and cryptorchidism	NA	Genetic Mutation Identified: Homozygous p. Asn193Ser (p.N193S) mutation in *SRD5A2* gene Carrier Frequency: 1 in 80 chromosomes (1.25%) in the studied population Polymorphisms/ Haplotypes Analyzed: p.L89V (rs523349) and 5 intragenic SNPs: rs2300702 rs2268797 rs2268796 rs4952220 rs12470196	Phenotypic variability observed despite identical homozygous mutations → no strict genotype–phenotype correlation
26	Akcan et al. [31] Cyprus	EMS: Range: 1–8 Common presenting symptoms: Ambiguous genitalia (most frequent) Mass in groin, micropenis, undescended testes	T/DHT Ratio for Diagnosis ≥20 → indicative of 5α-RD T/DHT measurement timing: 57% during hCG stimulation, 43% during mini-puberty or puberty	Common SRD5A2 mutations found: p.Leu55Gln (c.164T>A) p.Leu152Tyrfs*8 (c.453delC) p.Ala65Pro (c.193G>C)	Gender of rearing: 75% of 5α-RD patients were female-reared Several patients underwent gender reassignment post-diagnosis Consanguinity: Higher in 5α-RD group (up to 75%)
27	María Guadalupe et al. [32] Mexico	Subject 1 (8 y/o): Ambiguous genitalia, micropenis, hypospadias, pseudovagina, bilateral inguinal hernia, bilateral testes Subject 2 (50 y/o): Raised as female, no	Hormonal Findings: Subject 1: T: 7.6 nmol/L, DHT: 0.4 nmol/L T/DHT ratio = 18.5 Subject 2:	Variant Frequency: p. Glu197Asp: p. Pro212Arg: Identified Variants:	NA

		Müllerian structures, underwent gonadectomy	T: 22.3 nmol/L, DHT: 0.5 nmol/L T/DHT ratio = 43.0 Cut-off: T/DHT >10 indicative of 5α-RD2	○ Subject 1: Compound heterozygous for p. Glu197Asp and p. Pro212Arg ○ Subject 2: Homozygous for p. Glu197Asp	
28	Liu et al. [33] China	Phenotypic Severity: Patients with p.R227Q had milder phenotypes. Patients without p.R227Q had more severe symptoms. Sex Assignment Differences: Female sex assignment: p.R227Q group: 13.89% Non-p. R227Q group: 38.71% (*P = .008*) Cryptorchidism Incidence: p.R227Q: 27.78% Non-p. R227Q: 67.74% (*P = .0003*) Bifid Scrotum Incidence: p.R227Q: 12.5% Non-p. R227Q: 48.39% (*P = .0002*) EMS: Median EMS: Homozygous p.R227Q: 6 Compound heterozygous p.R227Q: 6 Non-p. R227Q: 5 Microphallus Presence: Present in 100% of patients. Isolated microphallus occurred in only 12% of cases.	T: DHT Ratio Findings: All patients had T: DHT > 5.8. >10 in 88.78% >15 in 72.45% >30 is often used as a typical diagnostic benchmark. T: DHT Ratio by Group (median values): Homozygous p.R227Q: 32.62 Compound heterozygous p.R227Q: 21.57 Non-p. R227Q: 23.39 AMH Levels (ng/mL): Homozygous p.R227Q: 250.2 Compound heterozygous p.R227Q: 141.5 Non-p. R227Q: 49.8	Most Common Variant: The p.R227Q variant was the most frequent (43.2% of alleles).	NA
29	Markouli and Michala [34] (England)	NA	NA	NA	All patients had oligospermia or azoospermia, causing fertility problems.
30	Alswailem et al. [35], Saudi Arabia	NA	NA	The splice-site mutation c.282-2A>G in intron 1 was the most common variant in SRD5A2.	Genotype-Phenotype Correlation: a weak genotype-phenotype correlation. Impact of Consanguinity: the high consanguinity rate contributed to the prevalence of homozygous mutations and the discovery of novel variants.

31	Mehrak and Alavi [36] Iran	Hypospadias	NA	L73H mutation: Predicted benign and non-disruptive. 229insA mutation: Causes frameshift and early stop codon, likely deleterious. L73H (c.219T>A): Missense mutation causing Leucine to Histidine substitution. Predicted benign. 229insA (p.Gly77*): Insertion mutation causing a frameshift and early stop codon → predicted to truncate the protein. ymorphisms Studied: V89L (rs523349): Valine to Leucine at codon 89. A49T (rs9282858): Alanine to Threonine at codon 49. ymorphism Associations (with hypospadias): A49T: OR = 10.16 95% CI = 3.94–26.25 p < 0.001 → Strong risk factor V89L: OR = 5.8 95% CI = 3.8–8.8 G196S (c.586G>A) also found in one case.	Phenotype–Genotype Correlations: Mild hypospadias: Most had LL (V89L) and AA (A49T) genotypes. Moderate hypospadias (most common): LL (V89L): 75% AT (A49T): 40.4% Severe hypospadias: AT (A49T): 64% VL (V89L): 56% p < 0.001 → Significant risk factor Exons 1 and 4 may be mutation hotspots in Iranian patients.

Source: EMS = External Masculinization Score; T = Testosterone; DHT = Dihydrotestosterone; FSH = Follicle-stimulating hormone; LH = Luteinizing Hormone; AMH = anti-Mullerian hormone; Et = urinary etiocholanolone; An = androsterone; NGS = Next-Generation Sequencing; DSD = disorders of sex development; AIS = androgen insensitivity syndrome; 5ARD2 = 5α-reductase type 2 deficiency; NA = Not available.

4. Discussion

This review study aimed to provide a comprehensive analysis of 5ARD2 cases. It compiled data from 31 selected studies involving 2,235 patients with 46,XY DSD due to 5ARD2. In humans, the SRD5A2 gene is located on chromosome 2p23, consists of 5 exons and 4 introns, and is responsible for the conversion of testosterone to its active form, DHT. To date, more than 100 mutations of SRD5A2 have been identified, with variations across different geographic and ethnic backgrounds [37]. Mutations in the SRD5A2 gene lead to pseudo-vaginal perineoscrotal hypospadias (OMIM #264600) (Andersson et al., 1991), a rare autosomal recessive disorder.

In a review by Nagaraja et al. [26] a total of 19 mutations were identified in the SRD5A2 gene across various studies involving the Indian population. These mutations were the second most frequently reported among the genes causing 46,XY DSD. Similarly, Yu [38] reported SRD5A2 gene mutations were the second most common mutation (21.7%, 13/60) among 46,XY DSD cases in an Asian population. Our study detected mutations in all five exons of the SRD5A2 gene. Studies in Asia have revealed that the majority of mutations were confined to Exons 1 and 5 of the SRD5A2 gene [39]. This contrasts with findings from other research, which identified Exon 1 and 4 being the primary regions for mutations. Batista and Mendonca [11] reported a predominance of mutations in Exon 1 (33%), Exon 4 (25%), and Exon 3, while Exon 5 was relatively preserved. In previous studies, it was reported that 60% of the patients were homozygotes, and the others were either compound heterozygotes or inferred to be so [14, 40, 41]. Homozygous allelic variants are more frequent than compound heterozygous variants among affected individuals with 5α-reductase type 2 deficiency [35]. Overall, about 70% of allelic variants in the SRD5A2 gene leading to 5α-reductase type 2 deficiency are in a homozygous state, whereas the remaining 30% are compound heterozygous [8, 22]. Other study revealed approximately 60% (150/250) were homozygous and 40% (100/250) were compound heterozygous [22].

Point mutations leading to the formation of premature stop codons have been described in patients with 5ARD2 Marzuki et al. [12]. However, there are limited cases reported with mutations in the coding region of the SRD5A2 gene, leading to a shortened structure of the enzyme. These premature terminations are more frequently observed in frameshift mutations caused by small indels of the gene. Mutations of SRD5A2 have been observed in different ethnic and geographical backgrounds [11] but there are very few reports described with the presence of heterozygous mutations [5, 14].

Clinical diagnosis of 5-alpha reductase type 2 deficiency is usually based on under-masculinization after birth (clitoromegaly, hypospadias with various degrees of micropenis, undescended testes, etc.), virilization after puberty, with normal T levels, and elevated ratios of plasma T to DHT [19]. All the patients with SRD5A2 gene mutations exhibited variable degrees of under-virilization, ranging from hypospadias to different positions of either one or both gonads. Although the ratios of testosterone to DHT were not available for accurate clinical diagnosis in this review due to insufficient data, molecular analysis played a crucial role in confirming the diagnosis of 5ARD2. This is especially relevant in cases presenting early during the neonatal or childhood stages when pubertal virilization has not yet begun, and hormonal profiles are inconclusive. Urinary steroid profiling (USP) has established clinical applications for investigating a broad spectrum of defects in the steroidogenic pathways.

Usually, males with 5ARD2 are characterized by normal germ cell populations but absence of primary spermatocytes on biopsy. They may also have cryptorchidism due to arrest of testicular migration along the inguinal canal. Subsequently, the increased temperature intra-inguinal may harm spermatogenesis and cause decreased germ cell number, suggesting that DHT also affects spermatocyte differentiation and growth. Subfertility may also result from failure of Sertoli cells to become fully functional at puberty [34]. As for other 46,XY DSD conditions, fertility remains a challenge for individuals with 5α-reductase type 2 deficiency [42, 43].

5. Conclusion

This is a systematic review on 46,XY DSD due to 5-alpha reductase type 2, providing insights into the genetics and clinical features caused by a wide spectrum of molecular anomalies. Molecular technology can quickly detect gene locations in patients, offering more valuable clinical information. Finally, this review expands the evidence base for 46,XY DSD due to 5-alpha reductase type 2 in terms of variations in SRD5A2 gene mutations, clinical assessment, therapeutic approaches, and prognosis of the disease. Future studies could validate this genotype-phenotype correlation in 5-alpha reductase type 2 deficiency.

5.1. Limitations

Our study had several limitations, such as restricting the study by not including report cases. Another critical limitation concerns the scarcity of information provided about the mutations. There are practically few sources that have used the same views, and some of the studies lacked data on the type of mutation, exon mutation site, and hormonal results. Furthermore, the studies were heterogeneous, making comparisons difficult. Despite careful selection of keywords and search strategies, it is possible that potentially useful literature may have been excluded from the review.

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