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REVIEW ARTICLE

5-α-Reductase type 2 deficiency: is there a genotype-phenotype correlation? A review

Andrea Avendaño[1] · Irene Paradisi[2] · Francisco Cammarata-Scalisi[1] · Michele Callea[3]

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Abstract

5-α-Reductase type 2 enzyme catalyzes the conversion of testosterone into dihydrotestosterone, a potent androgen responsible for male sexual development during the fetal period and later during puberty. Its deficiency causes an autosomal recessive disorder of sex development characterized by a wide range of under-virilization of external genitalia in patients with a 46,XY karyotype. Mutations in the *SRD5A2* gene cause 5-α-Reductase deficiency; although it is an infrequent disorder, it has been reported worldwide, with mutational heterogeneity. Furthermore, it has been proposed that there is no genotype-phenotype correlation, even in patients carrying the same mutation. The aim of this review was to perform an extensive search in various databases and to select those articles with a comprehensive genotype and phenotype description of the patients, classifying their phenotypes using the external masculinization score (EMS). Thus, it was possible to objectively compare the eventual genotype-phenotype correlation between them. The analysis showed that for most of the studied mutations no correlation can be established, although the specific location of the mutation in the protein has an effect on the severity of the phenotype. Nevertheless, even in patients carrying the same homozygous mutation, a variable phenotype was observed, suggesting that additional genetic factors might be influencing it. Due to the clinical variability of the disorder, an accurate diagnosis and adequate medical management might be difficult to carry out, as is highlighted in the review.

Keywords 5-α-Reductase deficiency · *SRD5A2* gene · Disorders of sex development (DSD) · Genotype-phenotype correlation · External masculinization score (EMS)

Introduction

Gender assignment at birth based on the appearance of the external genitalia is a routine protocol in medical practice. The pathway for the development of the external and internal genitalia acts during fetal life, being a continuum with a hormonal independent phase followed by a hormonal dependent one. Mutations affecting any component of these genes and perturbation of the hormonal pathways could impair the normal development of the genitalia structure, causing different grades of masculinization or under-virilization in newborns with 46,XX or 46,XY karyotypes, respectively [1, 2]. When this situation occurs, it is not possible to arbitrarily assign a sex to the newborn.

These disorders, formerly called male pseudohermaphroditism, group a wide variety of conditions now termed "disorders of sex development" (DSD), the definition of which was established by the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrinology Society in a document known as the Chicago Consensus. It was defined as a congenital condition in which there is no concordance between chromosomal, gonadal, or anatomical sex [3, 4].

Among the 46,XY DSD group, 5-α-Reductase type 2 deficiency (OMIM 264600) is infrequent; it is an autosomal recessive sex-limited condition resulting in the inability to convert testosterone (T) to the more physiologically active dihydrotestosterone (DHT), thus producing a wide

✉ Irene Paradisi
ireneparadisi@hotmail.com; iparadis@ivic.gob.ve

[1] Medicine Faculty, Medical Genetics Unit, Los Andes University, Mérida, Venezuela

[2] Human Genetics Laboratory, Venezuelan Institute for Scientific Research (IVIC), Caracas, Venezuela

[3] Unit of Dentistry, Bambino Gesù Children's Hospital, Rome, Italy

range of genital ambiguity at birth and pronounced masculinization at puberty.

This disorder was first reported in 1974 in two siblings from Dallas and in a large kindred from the Dominican Republic, all of whom had pseudovaginal perineoscrotal hypospadias, ambiguous external genitalia and marked masculinization at puberty [5, 6]. From that time to the present, the etiology of the disease has been elucidated, as well as its molecular basis.

The disorder, although infrequent, has been reported in many populations worldwide (Caucasian, Arabic, Asian, and South American). To date, more than 60 mutations have been described, some of these shared by patients of different ethnic origin and others associated with a unique origin [7].

The aim of this review was to collect the main information generated to date assessing the genotype-phenotype correlation in patients worldwide. The need to upgrade our knowledge about the pathways involved in sex differentiation and thereby to improve the medical management of patients is discussed.

5-α-Reductase type 2 deficiency

5-α-Reductase type 2 enzymatic activity

The microsomal enzyme steroid 5-α-Reductase catalyzes testosterone (T) to dihydrotestosterone (DHT) conversion by double-bounded reduction, using NADPH as a cofactor. Both hormones (T and DHT) bind to a unique receptor, the androgen receptor (AR). T-AR or DHT-AR complexes participate in several physiological reactions, acting as transcription factors of genes involved in sexual differentiation [8, 9]. The initial data about its mode of action were obtained from fibroblast cultured studies in which two enzyme activities were observed in two pH ranges with a markedly different cellular distribution. A 5.5 optimum pH was found in fibroblast derived from genital skin and a second enzyme activity with an alkaline optimum pH was widely located in fibroblast from non-genital skin. Furthermore, patients with 46,XY DSD presented no activity of 5-α-Reductase at pH 5.5, but the activity at pH 9 was unaltered. This finding suggested the existence of at least two different 5-α-Reductase activities that represent two enzymes, or one enzyme with post-translational modifications [10–12]. The cloning and expression of the two different cDNA (from rat liver and prostate) showed different biochemical features, demonstrating that both genes codify for two different proteins which catalyze the same reaction. Thus, two isozymes were described, type 1 the first cloned and type 2 the second one, which was associated with 5-α-Reductase deficiency. Type 1 isozyme comprises 259 amino acids and has a molecular weight of 29.5 KDa and an optimum pH between 6 and 8.5. On the other hand, type 2 isozyme is formed of 254 amino acids and has a molecular weight of 28.4 KDa and an acidic optimum pH near to 5.0. Both isozymes also differ in T affinity, type 2 showing higher affinity (apparent $Km = 0.1–1.0$ μM). Conversely, apparent *Km* between 1.0 and 5.0 was detected for type 1 isozyme. A third characteristic is that both enzymes differ in their inhibitor susceptibility: type 1 5-α-Reductase is insensitive to the inhibitor finasteride [8, 13–15].

The *SRD5A2* gene

SRD5A2 belongs to a family of isozyme genes. As mentioned, two members were identified, *SRD5A1* and *SRD5A2*, named in the order of their cloning. Both genes share 50% identity and both have five exons and four introns, but *SRD5A1* is located on chromosome 5 at 5p15 and *SRD5A2* on chromosome 2 at 2p23, being differentially expressed [13, 15–17]. Subsequently, other members of this family were identified, belonging to two different subfamilies. *SRD5A3* is located on chromosome 4 at 4q12 and does not show 5-α-Reductase activity; recently its function was established, being mainly involved in protein N-glycosylation [18, 19]. The third subfamily is composed of two genes: *GPSN2* and *GPSN2-like* located on chromosomes 19 and 4, respectively, both participating in de novo synthesis of fatty acids [20]. Despite the fact that each subfamily has been associated with different substrates, its biochemical characteristic of double-bounded reduction is preserved.

Phenotype

The first patients presenting male pseudohermaphroditism, identified as having 5-α-Reductase deficiency, were described by Imperato-McGinley and Walsh in 1974. They reported that at birth the patients presented genital ambiguity, with a clitoris-like phallus, a blind vagina pouch, palpable testes in the scrotum that resembled mayor labia or cryptorchidism and no Müllerian structures. A relevant characteristic was that at puberty they virilized and male physiognomies appeared, i.e., deep voice, phallus enlargement, with no breast development [5, 21]. Since then several cases have been described with a wide phenotypic range. In an extensive cohort studying 55 patients of different ethnic origins, the main characteristics shared by them were female external genitalia with clitoromegaly or microphallus and variable grades of hypospadias [22]. In the current review, an extensive search for all published articles regarding the 5-α-Reductase deficiency was undertaken using the PubMed resource. Unfortunately, specific clinical phenotype descriptions are scarce; thus, only articles providing ample phenotype and genotype details were selected.

A total of 256 patients were included in the review. The main phenotypic findings were grouped as follows: clitoromegaly or microphalus was reported in 66.1% (169/256) of patients; different grades of hypospadias was the second most common feature presented in 39.84% (102/256) of cases; unilateral and bilateral cryptorchidism were found to be reported in 19.92% (51/256) of cases. Those phenotypes considered mostly male or female were infrequent, 7.03% (18/256) and 3.9% (10/256) of cases, respectively. Among all cases, virilization at puberty has been extensively reported in SRD5A2 deficiency and gender change from female to male has been described frequently, the latter attributed to brain exposition to T in fetal, neonatal, and puberty stages [23–25].

Genotype

To date, more than 100 mutations have been described; 84 missense and nonsense mutations are scattered through the *SRD5A2* gene, 10 alter splicing, 1 localized in a regulatory region, 14 small deletions, 6 small insertions, 3 small indels, and 4 large deletions (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/ac/index.php). After an extensive search for reported cases with *SRD5A2* mutations, it was found that approximately 60% (150/250) were homozygous and 40% (100/250) were compound heterozygous; these percentages are slightly lower than those reported by Maimoun et al. in 55 patients of whom 69.1% were homozygous and 29.9% compound heterozygous, including the V89 L polymorphism [22]. Exons 1 and 4 are considered hot spots in the gene [22, 26, 27]. All of these mutations can produce either complete loss of activity, affecting the binding domain to testosterone, or diminished NADPH *Km*, producing a poor assembly of a functional protein or decreasing its half-life [9, 28–30]. As a consequence, a wide phenotypic range has been described attributed to the residual enzymatic activity and probably to the individual genetic background. The most studied *SRD5A2* gene polymorphism is V89L, produced by a change of guanine to cytosine in exon 1 that results in a substitution of valine for leucine at position 89. In vitro studies showed that V89L decreases enzymatic activity by around 30% [31]. Leucine in codon 89 has been associated with an increased hypospadias risk among Chinese and Indian children [32–34]. Another less frequently studied polymorphism is A49T; in vitro studies revealed that it produces increased enzymatic activity and has been associated with a higher risk for prostate cancer, mainly in African-American and Latino-American populations [31]. Conversely, a modest association has been proposed for this polymorphism with less severe forms of hypospadias [33, 35].

Genotype-phenotype correlation

Many reports about SRD5A2 deficiency have been published in the last 20 years, and it is very commonly mentioned that there is no genotype-phenotype correlation among patients carrying the same genotype. In our study, in order to methodically assess this topic, all patients reported in the literature with a detailed description of their phenotype from 1992 to the present were phenotypically classified using the external masculinization score (EMS), which constitutes a very useful method to standardize the clinical features observed. The use of EMS was first proposed by Ahmed et al., who scored specific features of the external genitalia, assigning a score range between 0 to 12, with those nearer to 12 composing a normal male phenotype [36]. Of the nearly 250 patients reported in the literature, 126 were included in this analysis. Only cases with homozygous genotypes were selected to avoid variability of the phenotype produced by two different mutations in the compound heterozygotes.

Tables 1, 2, and 3 show the main mutations reported at least three times in the literature in the homozygous condition and the average EMS with its standard deviation, which indicates how variable the phenotype is depending on the mutation. The most frequently reported mutations were those that increase testosterone and NADPH *Km* or those that decrease enzymatic activity.

Table 1 shows the most cited mutations that diminish testosterone affinity. Comparing the phenotypes of p.G34R, p.H231R, and p.G115D mutation carriers, it was observed that the EMS varied between 2.0 to 3.3, indicating a predominantly female phenotype. It is interesting to note that the average EMS for mutation p.G115D had a low standard deviation, despite having few observed patients; thus, when in homozygosis, this genotype seems to produce less variability as compared to the other two depicted in Table 1.

Table 2 summarizes mutations that interfere with the NADPH binding domain. EMS varied between 2.67 to 4.17, being slightly higher than the above-mentioned EMS averages (Table 1). Although there was no statistical difference between the EMS for mutations that affect testosterone and those affecting NADPH *Km* (Tables 1 and 2), a great variability in the EMS was observed in the second instance, with five out of the six described mutations showing ample standard deviation values (Table 2). Only the p.G196S mutation, with many reported cases ($n = 12$), seems to produce a less variable phenotype. Several other known mutations affecting NADPH domain, such as R171S found frequently in different populations (Mexican, Turkish, Spanish, Mediterranean), had very few homozygous reported cases, being found more frequently in compound heterozygotes.

Table 3 shows these mutations decreasing enzymatic activity. It is interesting to note that this patient group had less severe phenotypes, with EMS values ranging from 3.0 to

Table 1 External masculinization scores (EMS) for mutations affecting testosterone (T) *Km*

Genotype	Number of reported patients	EMS average	Standard deviation	References
p.G34R/p.G34R	12	3.33	2.06	[37, 38]
p.H231R/p.H231R	4	2.00	1.15	[25, 39, 40]
p.G115D/p.G115D	5	2.60	0.55	[22, 39, 41, 42]

8.0, attributable to different residual enzymatic activities caused by different mutations. It is important to highlight that the standard deviations of the average EMS values for these mutations were the highest among the three groups (Tables 1, 2, 3); thus, in patients carrying mutations belonging to this group, a genotype-phenotype correlation seems to be the most difficult to establish, masculinized external genitalia being the most predominant phenotype. The frequent Mediterranean IVS1-2A > G mutation, which is thought to abolish enzymatic activity, had a low EMS value (average 4) nearer to a female phenotype, as is expected for mutations that severely impair enzymatic activity [62, 63].

The statistical Student *t* test was performed to compare each group according to the protein mutation effect (i.e., T binding, NADPH cofactor binding or enzymatic activity failure). When mutations affecting T *Km* were compared with those that affect NADPH *Km*, no statistic difference was found between EMS values; conversely, when these two groups were compared independently with mutations affecting protein activity (Table 3), significant statistic differences were obtained in both instances with $p < 0.001$ ($p = 1.48 \times 10^{-5}$ and $p = 6.89 \times 10^{-6}$, respectively). According to these analyses, although there is no strong genotype-phenotype correlation, the effect of the location of the mutation in the protein is an important variable to consider because it has influence on the patient's phenotype.

Regarding the possible effect of the genetic background on the phenotype, the population-specific prevalence of mutations has been studied, showing that many mutations are ethnic-specific, whereas certain others are found among different populations [34]. Among the mutations shown in Tables 1 to 3, p.G34R and p.N160D are only found in Egyptians, p.L55Q has only been described in Turkish patients, p.G183S in Brazilian patients, and IVS1-2A>G with a 0.98% carrier frequency in the Cyprus population [63]. Other mutations such as p.G196S, p.Q126R, and p.H231R are widely distributed among Caucasians. p.Y91H and p.R227Q have been reported in patients from the middle East and Asia, respectively. Conversely, p.G115D, p.G196S, p.R246Q, and p.G246W have been reported in patients of different ethnic origins including American, European, Asian, and North Indian, mutations that are considered hot spots. Thus, ethnic origin does not seem to have a relevant effect on the phenotype-genotype correlation according to the EMS (Tables 1 to 3).

Diagnosis

Several reports have been published in which patients diagnosed as suffering from a partial insensitivity androgen syndrome (PAIS) are indeed carriers of mutations in the *SRD5A2* gene. A correct diagnosis of these cases is essential for appropriate patient management. Biochemical diagnosis was the first diagnostic approach to the disease. Serum T and DHT concentration post hCG (human chorionic gonadotropin) stimulation was measured in many cases and T:DHT ratio was calculated [64] and almost always a normal to high T concentration was found along with a low concentration of DHT and an increased T:DHT ratio. Establishing a cutoff value for T:DHT ratio has been controversial. Though the first proposed value was 20, Walter et al. suggested 8.5 after hCG stimulation as a much more reliable cutoff value [24, 64, 65]. However, in many instances, this method has failed as a good predictor and various other approaches have been proposed [22, 39, 41, 45, 49], urinary steroid profiling (UPS) and gene mutation analysis being two alternatives. Chan et al. suggested that UPS results can be misinterpreted; thus, molecular

Table 2 External masculinization scores (EMS) for mutations affecting NADPH *Km*

Genotype	Number of reported patients	EMS average	Standard deviation	References
p.P181L/p.181L	2	4.0	2.83	[43]
p.G183S/p.G183S	6	4.17	2.48	[44]
p.G196S/p.G196S	12	3.30	0.95	[38, 42, 44–48]
p.Y235F/p.Y235F	5	4.00	3.46	[22, 37, 43, 45]
p.R246Q/p.R246Q	14	3.68	1.67	[22, 25, 40, 43, 49–53]
p.R246W/p.R246W	6	2.67	1.21	[5, 22, 44, 53]

Table 3 External masculinization scores (EMS) for mutations affecting enzymatic activity

Genotype	Number of reported patients	EMS average	Standard deviation	References
p.L55Q/p.L55Q	8	3.00	3.00	[22, 47, 54]
p.Q126R/p.Q126R	12	4.17	1.47	[22, 40, 47, 55, 56]
p.Y91H/p.Y91H	8	6.12	2.53	[22, 38, 57, 58]
p.N160D/p.N160D	4	4.75	2.98	[8, 37, 39]
p.R227Q/p.R227Q	24	8.00	1.79	[22, 50, 59–61]
IVS1-2A>G/IVS1-2A>G *	4	4.00	1.83	[62, 63]

*Mutation affecting splicing site

analysis was considered the most effective diagnostic method also taking into account the small size of the gene makes the diagnostic procedure easier [50].

Treatment

A psychological evaluation must be performed in individuals prior to 27 months of age before any hormonal or surgical treatment can be undertaken. Frequently, pre- or postnatal brain exposure to androgens in 46,XY individuals raised as girls causes a later development of male gender identity in adolescence or early adulthood [66]. A prevalence of around 60% of gender changes has been reported in individuals who were raised as female [67]. Thus, it is recommended that the sex reassignment should be done before 27 months of age to avoid identity conflicts [24].

Hormonal therapy

Testosterone replacement is not usually required in male patients given that most of them have retained testicular function during puberty. However, high doses of intramuscular testosterone (e.g., testosterone cipionate 200–500 mg twice a week) or dihydrotestosterone gel (e.g., 5–10 mg/day) can be used to improve body hair and penile length. Maximum penis enlargement is obtained after 6 months of high-dose treatment, but without reaching a normal length. Treatment with dihydrotestosterone gel has the advantages of being more active than testosterone, promoting faster increase of penis size and glans before any eventual surgery [57, 66]. In addition, since dihydrotestosterone is not an aromatizable molecule, it would not be expected to promote bone maturation or cause gynecomastia, while it would enable the use of higher doses than testosterone and, consequently, attaining a higher degree of virilization [66].

For those patients raised as females, the rationale of hormonal therapy is the development of female sexual characteristics. The treatment must simulate normal puberty. Low estrogen doses (0.07–0.3 mg of conjugated estrogen) should be administered at 10 to 11 years of age to avoid excessive bone maturation except in tall girls, in whom adult estrogen doses are indicated. After breast development is completed, adult estrogen doses (0.625–1.25 mg/day of conjugated estrogen) are maintained continuously. Progesterone replacement is not necessary due to the absence of the uterus. Vaginal dilation with acrylic molds has been shown to be an excellent management choice. It should be started when these patients express a desire to initiate sexual intercourse [66].

Surgical treatment

Genital surgery is widely performed in children with genital ambiguity. Penile construction remains a challenging task for surgeons. However, new techniques are available in males with severe micropenis and aphalia [24]. The aim is to build adequate external genitalia and remove internal structures incompatible with the assigned sex. For children assigned as female, laparoscopy is the ideal technique to perform gonadectomy and resection of internal organs if appropriate [66].

Feminizing genitoplasty should provide an adequate vaginal opening into the perineum, create a normal-looking vaginal introitus, fully separate the urethra from the vaginal orifice, remove phallic erectile tissue preserving glandular innervation and blood supply, and prevent urinary tract complications. The most reasonable technique to perform clitoroplasty is based on the concept of maintaining the clitoral glands and sensory input which facilitates orgasm. The use of an adequate size of tissue flap is mandatory in the Y-V vaginoplasty technique. Failure to interpose an adequate flap will result in persistent introital stenosis, requiring a later surgical procedure [66].

For those raised as males, surgery consists of orthophaloplasty, scrotumplasty with vaginal removal, proximal and distal urethroplasty, and orchidopexy when necessary [57, 66]. In patients with perineal hypospadias, surgeries can be performed in 2 or 3 steps (masculinizing genitoplasty using a modified Denis Browne technique).

The most frequent surgical complications are urethral fistula in the penoscrotal angle and urethral stenosis that can occur several years after surgery [66].

Conclusions

5-α-Reductase type 2 deficiency causes pronounced ambiguity of the external genitalia with a wide phenotypic spectrum. It is an infrequent disorder, reported in many different populations worldwide, that is caused by diverse mutations scattered throughout the *SRD5A2* gene. Although it is well known that there is no strong genotype-phenotype correlation, the herein described analysis performed in 126 patients showed that the specific location of the mutation in the protein has an effect on the severity of the phenotype, the loss of enzymatic activity being the most relevant variable of statistical significance ($p < 0.001$). Nevertheless, even in patients carrying the same homozygous mutation, a variable phenotype can be observed, suggesting that genetic factors other than 5-α-Reductase enzyme activity contribute to the phenotype. Extensive studies on different pathways affecting sexual development should be undertaken to identify genetic modulators of the external genitalia differentiation.

Parents of patients with DSD must be offered appropriate medical guidance, receiving all necessary information regarding diagnosis and gender assignment, as well as the benefits, risks, and complications of the different treatment options. A better understanding of the genetic factors influencing the condition could improve the decision as to the assignment of the correct adult gender identity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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