NON-SYNDROMIC 46,XY DISORDERS OF SEX DEVELOPMENT

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Abstract

Non-syndromic 46,XY DSD (disorders of sex development) represent a phenotypically diversiform group of disorders. We focus on the association between gene variants and the most frequent types of non-syndromic 46,XY DSD, options of molecular genetic testing which has surely taken its place in diagnostics of DSD in the past couple of years. We emphasize the need of molecular genetic testing in individuals with non-syndromic 46,XY DSD in Slovak Republic.

Keywords: 46,XY DSD, non-syndromic, gene

INTRODUCTION

The aim of this article is to bring closer possibilities of molecular genetic testing in patients with non-syndromic 46,XY DSD. The point of our interest are the most frequent DSD, those we get in touch with the most, hypospadias, cryptorchidism etc., and the existing options of their molecular genetic testing based on studies published in recent years. Despite the fact that the etiology of mild, non-syndromic 46,XY DSD is usually referred to as multifactorial, with the influence of environmental factors during intrauterine life, there is an undeniable association between multiple gene disruptions and DSD. Although these mild DSD are often described as only "cosmetic" problems and are surgically corrected, proven genetic defect necessarily brings further diagnostic, therapeutic, and preventive actions in order to assure full-valued and healthy life of an affected individual and his/her family.

DISORDERS OF SEX DEVELOPMENT (DSD)

Disorders of sex development are a group of conditions involving atypical chromosomal, gonadal, anatomical, or psycho-social sex development. The prevalence of DSD was estimated to be 1:4500 live births by some authors [1] but it varies in frequency depending on their etiology. For example, the prevalence of hypospadias per 10 000 live births was reported from 5,2 in South America to 19,9 in Europe and 34,2 in North America [2].

According to present DSD classification, there are three major groups of DSD. *Sex chromosome DSD*: a group represented by Turner´s syndrome (45,X), Klinefelter´s syndrome (47,XXY), mixed gonadal dysgenesis (45,X/46,XY), and ovotesticular DSD (45,X/46,XY and 46,XX/46,XY). *46,XX DSD:* a group represented by ovotesticular DSD, testicular DSD,

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and gonadal dysgenesis based on disorder of ovaries development; over-expression of androgens (CAH) and other DSD such as vaginal atresia or cloacal extrophy. The third and the biggest group is *46,XY DSD* [3].

46,XY DSD (CLASSIFIED INTO FIVE MAJOR GROUPS) [4].

1. 46,XY DSD due to abnormalities of gonadal development (complete and partial forms of gonadal dysgenesis): 46,XY DSD due to under-expression of several genes such as *WT1* gene (Denys-Drash syndrome), *NR5A1/SF1*, *SRY*, *DMRT1*. Dysgenetic 46,XY DSD due to under-expression of several genes such as *GATA4*, *FOG2*/ZFPM2, *CBX2*, *FGF9/FGFR2*, and *MAP3K1*. Dysgenetic 46,XY DSD associated with campomelic dysplasia (under-expression of *SOX9*). Dysgenetic 46,XY DSD due to disruption in the Hedgehog signaling – *DHH* or *HHAT* gene. ATR-X syndrome (X-linked a-thalassemia and mental retardation). 46,XY DSD due to the over-expression of *DAX1/NR0B1* or *WNT4* gene.

2. 46,XY DSD associated with cholesterol synthesis defects: Smith-Lemli-Opitz syndrome. 46,XY DSD due to testosterone production defects such as complete or partial forms of impaired Leydig cell differentiation (*LHCGR* defects). Enzymatic defects in testosterone synthesis, either in adrenal and testicular steroidogenesis (STAR, P450scc, 3-b-hydroxysteroid dehydrogenase II, 17a-hydroxylase and 17,20-lyase, P450 oxidoreductase defficiency) or in testicular steroidogenesis (isolated 17,20-lyase deficiency, cytochrome b5 defect or 17b-hydroxysteroid dehydrogenase III deficiency). Alternative pathway to DHT – 3a-hydroxysteroid dehydrogenase deficiency due to *AKR1C2* and *AKR1C4* defects.

3. 46,XY DSD due to defects in testosterone metabolism: 5a-reductase type 2 deficiency (*SRD5A2* gene).

4. 46,XY DSD due to defects in androgen action: Complete or partial forms of androgen insensitivity syndrome. 46,XY DSD due to persistent Müllerian ducts (defect in AMH synthesis). Defect in AMH receptor.

5. Other forms include congenital non-genetic 46,XY DSD such as maternal intake of endocrine disruptors or disorders associated with impaired prenatal growth. 46,XY ovotesticular DSD and non-classified forms (hypospadias, 46,XY gender dysphoria).

The 46,XY DSD represent a wide spectrum of abnormalities, in which 46,XY karyotype is present. Phenotypically they are characterized by atypical, ambiguous, or female external genitalia, with or without the presence of Müllerian structures. 46,XY DSD can result either from decreased synthesis of testosterone or dihydrotestosterone, or from impaired androgen action [4]. The development of phenotypic male has two steps. Determination and differentiation. Determination takes place in bipotential gonad. Expression of several genes is present (*WT1*, *NR5A1*, *M33*, *Lhx9*, *Lim1*, *GATA4, FOG2*, *DMRT1*, *EMX2*). Their interaction with *SRY* down-regulates the female pathway and up-regulates *SOX9* in Sertoli cells, which loops the sex determination into male pathway. One of the most powerful genes to positively affect *SRY* is *NR5A1* (gene for steroidogenic factor 1), which induces *CBX2* expression necessary for *SRY* expression. If *SRY* is not present, bipotential gonad takes the female pathway, regulated by *DAX1*, *RSPO1,* and *WNT4*. Testis and its hormonal production induce sex differentiation of internal and external genitalia. In Sertoli cells genes such as *SOX9*, *NR5A1*, *WT1*, *GATA4,* and *HSP70* interact with *AMH* promoter and up-regulate *AMH* gene expression. Expression of *DHH* in Sertoli cell all together with *NR5A1* and *MAMLD1* is needed for Leydig cell development. Leydig cell produces *INSL3* which is necessary for correct migration and descent of testes. *NR5A1* regulates steroidogenesis (Fig. 1) [4, 5].

As we can see, there are many different types of 46,XY DSD and genes regulating their development. We focus on those that are non-syndromic (only urogenital system is affected) and seen the most often in our daily praxis. The most frequent male DSD is hypospadias.

Other non-syndromic DSD include epispadias, cryptorchidism, and micropenis. In many cases they combine. The etiology is usually unknown and is thought to have environmental background, although gene variants linked to non-syndromic DSD have been identified. Micropenis is defined as penile length smaller than 2,5 standard deviations below

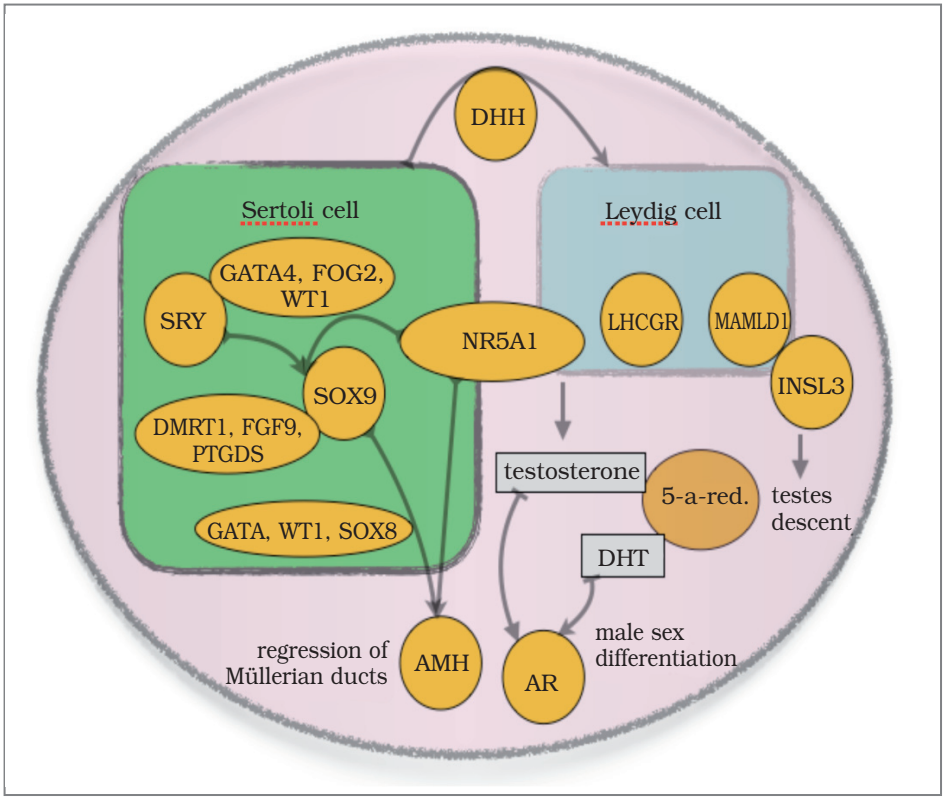

Fig.1 Male sex differentiation in testis

mean [6]. It can be isolated or as a part of many congenital syndromes. Cryptorchidism is defined as hidden testis and can be divided into several groups: retention of testis (abdominal or inguinal), sliding testis, testis migrans, retractile testis or pseudo-cryptorchidism, ectopic testis (perineal, femoral, pubo-penile, supra-pubic, or transversal), or anorchism. The most frequent is inguinal retention (72 %), pre-scrotal (20 %), and abdominal cryptorchidism (8 %). Bilateral retention is present in 10–30 % [7, 8]. Epispadias is a rare congenital penile malformation, usually a part of BEEC (bladder-extrophy-epispadias complex), but sometimes also found in its isolated forms [9]. Hypospadias is a malformation of external genitalia characterized by insufficient fusion of urethral folds, incorrect urethra ending, and different level of penis angulation. It is usually isolated, but sometimes associated with other malformations such as cryptorchidism or micropenis. It is also a part of many genetic syndromes. Hypospadias can be divided into several groups based on the position of urethra ending. In 60–70 % it is anterior hypospadias (glandular, coronal or penile). The incidence in Europe is approximately 0,35 % of live born boys [2]. Many forms stay unrecognized during the whole life, because they are mild and do not cause any major discomfort. Non-syndromic DSD such as hypospadias or cryptorchidism are associated with small birth weight and there is a six-fold higher risk of hypospadias in boys short for gestational age (SGA) [10].

It is already known that even though non-syndromic DSD are of multifactorial etiology, there are several genes that should be taken into account while searching for genetic cause. *NR5A1/SF1*, *MAMLD1*, *AR*, and *SRD5A2* are the most discussed among many others. *MAMLD1* was the first gene to be linked with isolated hypospadias [11]. *AR*, *SRD5A2* were

linked to hypospadias long time ago, although mutations of *AR* and *SRD5A2* are rarely associated with isolated forms [12]. Many researches proved that hypospadias have genetic background [13].

Until today there have been several studies describing genetic basis of DSD. There are more than 200 DSD-associated genes. 219 genes were sequenced (using targeted next-generation sequencing) in a cohort of 21 DSD patients. A total of 11 mutations in *SRY*, *NR0B1*, *AR*, *CYP17A1*, *GK*, *CHD7,* and *SRD5A2* genes were identified, including five single nucleotide variants, three InDls, one in-frame duplication, one *SRY*-positive 46,XX, and one gross duplication with an estimated size of more than 427,038 bp containing *NR0B1* and *GK*. Six novel mutations in *AR* gene were also identified. The assay was able to make genetic diagnosis for 38,1 % of DSD patients [14]. In a study of 40 patients with 46,XY DSD they used exome sequencing and were able to achieve genetic diagnosis in 35 % of cases. The authors identified four cases of *MAP3K1* gene variants. One in a patient with female phenotype and complete gonadal dysgenesis, one with bilateral streak gonads, and two with ambiguous genitalia and severe hypospadias, one of which was prematurely born with IUGR. They also identified cases of *AR*, *NR5A1*, *HSD17B3,* and *MAMLD1* gene variants [15]. In an international cohort study of 326 patients with DSD (278 were 46,XY), using MPS (massively parallel sequencing) as a diagnostic method, 43 % of 46,XY DSD cases confirmed a likely genetic diagnosis. 159 of 278 patients (57 %) had a variant in a clinically relevant DSD gene. 48 % of these had pathogenic variant, 26 % likely pathogenic, and 26% had variant of unknown significance (VUS). The highest diagnostic rate of 60 % was for 46,XY patients who had disorders of androgen synthesis and action. This study showed association of multiple genes with the development of all types of DSD. There are at least 28 diagnostic genes causative for 46,XY DSD. As for our point of interest, the most frequent, non-syndromic DSD are linked to several genes. The authors found many previously reported pathogenic variations in *AR* gene, four of which were associated with hypospadias. Two previously unreported VUS in *CHD7*, one VUS in *DHH,* two VUS and one likely pathogenic variant in *GATA4*, three likely pathogenic variants and one VUS in *HSD17B3*, two pathogenic variants in *HSD3B2*, one previously unreported VUS in *MAMLD1*, one likely pathogenic variant and one VUS in *MAP3K1*, both previously unreported, one pathogenic, previously unreported variant in *NR5A1*, one likely pathogenic variant in *POR*, one pathogenic and one VUS in *SRD5A2*, three previously unreported VUS in *WDR11* gene, and three likely pathogenic, previously unreported variants in *ZFPM2* gene – all in patients with hypospadias [16].

GENES LINKED TO NON-SYNDROMIC 46,XY DSD

CHD7 (8q12.2) mutations cause CHARGE syndrome, a complex multi-organ disorder including genital abnormality [17]. There were cases reported with atypical CHARGE syndrome with only genital abnormality (hypospadias) [15]. *DHH* (12q13.12) produces a protein believed to be involved in male sexual development. Its biallelic pathogenic variants are responsible for 46,XY DSD [18]. VUS was identified in patient with hypospadias [16]. *GATA4* (8p23.1) is necessary for normal testicular development. Its variants were linked to non-syndromic DSD [16]. Males with uncommon defects in 17β-HSD/17-ketoreductase type 3, or 3β-HSD type 2, resulting from mutations in the *HSD17B3* (9q22.32), *HSD3B2* (1p12) usually present at birth as phenotypic females with partial virilisation or with ambiguous genitalia. However, patients with incomplete defects in these enzymes may present as phenotypic males with hypospadias, gynecomastia, and primary hypogonadism with androgen deficiency manifested by delayed puberty [19]. *MAP3K1* (5q1.2) helps to regulate signaling pathways that control various processes in the body, including the processes of determining sexual characteristics before birth. Mutations in *MAP3K1* cause 46,XY disorders of sex development and implicate a common signal transduction pathway in human testis determination [20]. *WDR11*(10q26.12) VUS were identified in twin patients with hypospadias [16]. *ZFPM2* (8q23.1) belongs into *FOG* family of transcription factors and interacts with *GATA* family affecting gonadal differentiation via *SRY* expression regulation. Mutations in

ZFPM2 gene are associated with anomalies of human testis determination [21]. *MAMLD1* (Xq28) is expressed in fetal Sertoli and Leydig cells. Most patient with impaired function of this gene have ambiguous genitalia. *MAMLD1* supports testosterone production via *NR5A1/SF1* regulation [22]. Different studies identified mutations in *MAMLD1* gene in patients with hypospadias [16, 23]. There are authors who suggest that *MAMLD1* gene should be routinely sequenced in all 46,XY patients with severe under-virilisation and normal function of *AR*, *SRDA2* and *NR5A1/SF1* genes [24]. *AR* (Xq12) defects cause 46,XY DSD with signs of under-virilisation despite the presence of bilateral testes and serum levels of testosterone are within or above normal levels. Mutations in *AR* gene lead to androgen insensitivity syndrome [25]. *SRD5A2* (2p23.1) mutation leads to deficit of 5-alfa-reductase, characterized by minimum virilisation of external genitalia with hypospadias of different severity. Syndrome of 5-alfa reductase deficiency is inherited in autosomal recessive manner. Most of the men are infertile. Phenotype is usually with normal looking female external genitalia, sometimes ambiguous genitalia, and sometimes with male genitalia with micropenis and hypospadias. Puberty brings signs of virilisation due to direct testosterone action to external genitalia. Approximately 50% of affected individuals choose to become males as adults [26]. *NR5A1* (9q33.3) is expressed in adrenal gland and bipotential gonad during intrauterine development. It plays a major role in testis determination and the expression of *SF1* continues in cells of early testis, and together with *SRY*, it plays a crucial role in induction of *SOX9* expression. In Sertoli cells, around seventh week of gestation, SF1 activates *AMH* expression, which leads to dissolution of Müllerian structures. In Leydig cells, around eight week of gestation, it activates expression of steroidogenic enzymatic systems, which leads to external genitalia differentiation [27]. *SF1* expression was detected in an early stage of ovaries development as well as adult ovaries [28]. Mutations in *NR5A1* are a quite frequent cause of 46,XY DSD [29] which represent a wide phenotypic spectrum consisting of complete testicular dysgenesis with persistent Müllerian structures, individuals with light clitoromegaly or ambiguous genitalia, and severe penoscrotal hypospadias and cryptorchidism [30]. Heterozygous mutations are present in premature ovarian failure [31]. Some authors recommend genetic screening for *NR5A1* mutations in all patients with hypospadias or gonadal dysgenesis [32]. Recently there is an opinion that some mutations in NR5A1 gene are also responsible for 46,XX ovotesticular DSD [33] and it has also shown to induce spermatogenic failure in 46,XY individuals [34].

CONCLUSION

Many non-syndromic 46,XY DSD certainly have genetic background. We should search for their genetic basis and, if a gene defect is present, prepare for further possible complications and optimize the patient´s medical management. We shall not overlook even the least severe, isolated non-syndromic DSD such as hypospadias, micropenis, or cryptorchidism corrected surgically. Surgery may be the only visible treatment but could be insufficient. By definition, hypospadias is a form of 46,XY DSD and although most of the patients present fertility and masculinisation at puberty, their testicular function should be assessed to rule out causes such as defects in testosterone synthesis and action, which require hormonal treatment and genetic counselling in addition to surgical treatment. Yet still, many DSD patients in Slovakia are diagnosed through a combination of endocrinology and phenotypic examination, with genetic testing being a secondary option. However, known genetic diagnosis can shape future endocrine, imaging, and potentially unnecessary invasive testing. Many researchers proved that more than a third of 46,XY DSD do have known genetic background. Therefore, we point out the need for genetic testing, not only in patients with severe forms of DSD but also in non-syndromic, and often mild disorders, which if have genetic background, may result in male infertility, if passed on to our daughters in premature ovarian failure or represent a higher risk of malignant diseases development.

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