Genetic Aspects of Hypospadias

11

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Abbreviations

AMH	Anti-Müllerian hormone
AR	Androgen receptor
ATF3	Activating transcription factor 3
BMP	Bone morphogenetic proteins
CAH	Congenital adrenal hyperplasia
CAIS	Complete androgen insensitivity syndromes
DGKK	DiacylGlycerol kinase κ
DHT	DiHydroTestosterone
DSD	Disorder of sex development
ESR	EStrogen receptors
FGF	Fibroblast growth factor proteins
GT	Genital tubercle
hCG	Human chorionic gonadotropin
MAMLD1	MAstermind-like domain containing 1
PAIS	Partial androgen insensitivity syndromes
Sf1	Splicing factor 1
SHH	Sonic HedgeHog
SRD5A	Steroid-5-alpha-reductase
SRY	Sex-determining region Y gene
Wt1	Wilms tumor 1

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11.1 Genes Involved in the Embryology of the Male External Genitalia

Development of the male external genitalia can be divided in three stages:

- The indifferent stage.
- The early patterning stage.
- The masculinization stage.

11.1.1 Indifferent Stage

Early development of the external genitalia is similar for males and females. The embryonic cloaca, the far end of the hindgut, is separated from the amniotic cavity by the cloacal membrane. Early in the fifth week of development, a swelling develops on both sides of this membrane, the cloacal folds, which meet in the midline anterior to the cloacal membrane, forming the genital tubercle [1]. At the same time, the genital ridges, the precursors of the gonads, develop. Studies in mice showed that this process requires Wilms tumor 1 (Wt1) activity, which activates splicing factor 1 (Sf1) [2], thus preventing degeneration of the developing gonads [3]. During the seventh week of human development, the urorectal septum fuses with the cloacal membrane, dividing the cloaca into the primitive urogenital sinus and the rectum and dividing the cloacal membrane

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into the urogenital and the anal membrane. The swellings next to the urogenital membrane are then called the urogenital folds, and a new pair of swellings, the labioscrotal swellings, appears on either side of these folds. In addition, the urogenital membrane breaks down [1].

11.1.2 Early Patterning

Early patterning of the genital tubercle (GT) is androgen-independent. The distal urethral plate epithelium is the signaling center regulating GT outgrowth [4]. Fibroblast growth factor proteins (FGF) play a growth-promoting role in this outgrowth [5], whereas bone morphogenetic proteins (BMP) stimulate apoptosis [6, 7]. Sonic hedgehog (SHH) modulates the balance between proliferation and apoptosis by regulating the expression of the genes encoding these and many other proteins [8]. This way, SHH regulates the initiation of GT outgrowth [4]. Immunohistochemical staining of human fetal penises showed expression of *SHH*, its receptor, and several of its downstream genes around the time of urethral closure [9].

11.1.3 Masculinization

Subsequent masculinization relies on hormones produced by the testes. Expression of the sex-determining region Y gene (*SRY*) induces a cascade of gene interactions, involving SRY-box 9 (*SOX9*) [1], resulting in differentiation of the gonads into the testes [10]. SRY leads to the differentiation of Sertoli cells [1], which secrete anti-Müllerian hormone. Anti-Müllerian hormone causes regression of the Müllerian ducts that would otherwise form part of the female genital structures [1]. Human chorionic gonadotropin (hCG), produced by the placenta, controls fetal Leydig cell growth and stimulates fetal testicular steroidogenesis, the generation of steroids from cholesterol [11]. The enzymatic steps in steroidogenesis, mainly taking place in the Leydig cell, are well documented, and expression of key genes in this pathway is dependent on expression of NR5A1 (Fig. 11.1) [13]. Testosterone leaves the Leydig cell and is converted into dihydrotestosterone (DHT) by steroid-5-alpha-reductase (SRD5A). Testosterone promotes formation of the internal reproductive structures from the Wolffian ducts, whereas DHT induces development of the external genitalia [1], both through their effects on the androgen receptor (AR). Expression of estrogen receptors (ESR) in male genital tissue during development suggests that the balance between androgens and estrogens is important as well [14].

11.2 Genes Implicated in the Etiology of Isolated Hypospadias

All genes implicated in one of the three stages mentioned above could play a role in the development of hypospadias. Therefore, much of the genetic research on hypospadias has been focused on these genes.

11.3 Study Types

Different types of studies have been performed to examine whether specific genes have an effect on the occurrence of hypospadias. One example are the mutation analyses, for which researchers sequence candidate genes in hypospadias patients and healthy controls. These studies typically included tens to sometimes a little over a hundred hypospadias patients and healthy controls. Sequencing allows complete coverage of the gene, identifying all genetic variants present in that gene. With this type of studies, researchers were aiming to identify the causal variant for hypospadias in part of their patients. Although several studies identified new and unknown mutations in hypospadias patients that were not present in the healthy controls, it remains unclear whether these mutations truly have functional consequences. Only few studies reported conservation and function of the region in which the mutation is located or predicted a potential influence of the mutation on protein function using

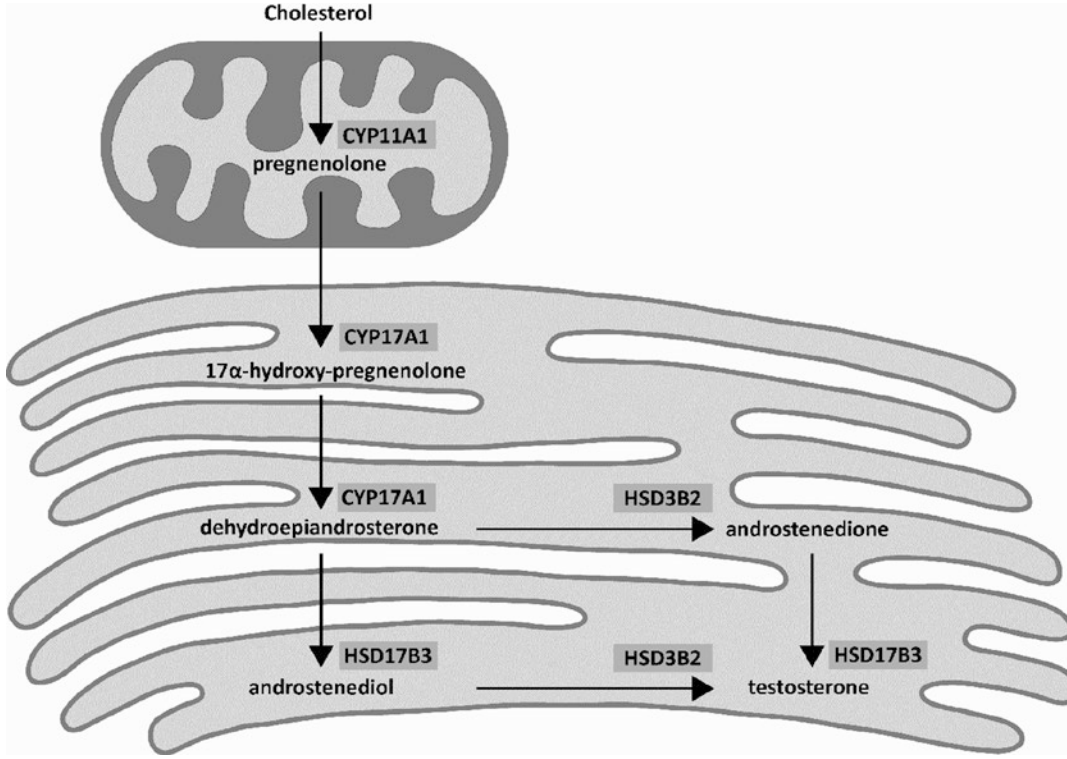

Fig. 11.1 Steroidogenesis in the mitochondrium (top) and smooth endoplasmic reticulum (bottom) of the fetal Leydig cell. Adapted from van der Zanden et al., 2012 [12] (from van der Zanden, L.F.M. and van Rooij, I.A.L.M.: "Aetiology of hypospadias: a systematic review of genes and environment," Human Reproduction Update, 2012, Vol. 18, Issue 3, by permission of Oxford University Press)

bioinformatics. Even if the mutations found are truly causal, they only explain the occurrence of hypospadias in a small part of the patients, and the majority of mutations were found only once and were identified in patients with posterior or penile hypospadias.

Another type of study that has been used to examine whether specific genes have an effect on the occurrence of hypospadias is the association study. In this type of study, known polymorphisms are genotyped in hypospadias patients and healthy controls. Polymorphisms are common genetic variants that occur with a frequency of at least 1%. Due to the haplotype block structure of the human genome, genotyping a specific set of polymorphisms in a gene covers much more of the variation in that gene. Thereby, polymorphisms function as markers for more rare variants with functional consequences. In association studies, typically hundreds to sometimes a thousand hypospadias patients and healthy controls have been genotyped for polymorphisms in candidate genes. If a polymorphism occurs more frequently in the patients compared to the controls, this means that there is an association and the variant may be a marker for a causal variant that increases hypospadias risk. However, due to the large amount of polymorphisms examined, the risk of false-positive results is high. Therefore, replication of results is a major issue in association studies, especially in the genome-wide association studies that aim to cover the whole genome by genotyping hundreds of thousands of polymorphisms.

11.4 Study Results

Table 11.1 shows the candidate genes that were screened for mutations or associations in groups of hypospadias patients and healthy controls.

Table 11.1 Result of genetic association studies and mutation studies screening candidate genes in groups of patients with hypospadias and healthy controls. Genes that were only investigated once and did not show mutations or associations in that study are not shown

	Mutation studies that found mutations	Association studies that found associations	Mutation studies that did not find mutations	Association studies that did not find associations
Indifferent stage				
WT1	Wang et al. [15], Diposarosa et al. [16]	Carmichael et al. [17]	Nordenskjöld et al. [18], Kon et al. [19], Zhang et al. [20]	
WTAP			Utsch et al. [21]	Carmichael et al. [17]
Early patterning				
SHH		Carmichael et al. [17]	Zhang et al. [20]	
FGF8	Beleza-Meireles et al. [22]	Beleza-Meireles et al. [22]	Kon et al. [19], Zhang et al. [20]	Carmichael et al. [17]
FGF10		Carmichael et al. [17]	Beleza-Meireles et al. [22], Zhang et al. [20]	
FGFR2	Beleza-Meireles et al. [22]	Beleza-Meireles et al. [22]	Kon et al. [19], Zhang et al. [20]	Carmichael et al. [17]
BMP4	Chen et al. [23], Zhang et al. [20]		Kon et al. [19]	Carmichael et al. [17]
BMP7	Chen et al. [23], Bouty et al. [24]	Carmichael et al. [17]	Beleza-Meireles et al. [22], Kon et al. [19], Zhang et al. [20]	
HOXA4	Chen et al. [23]	Geller et al. [25]	Kon et al. [19], Zhang et al. [20]	Carmichael et al. [17], Kojima et al. [26], Chen et al. [27]
HOXB6	Chen et al. [23], Kon et al. [19]		Zhang et al. [20]	Carmichael et al. [17]
HOXA13			Utsch et al. [21], Zhang et al. [20]	Carmichael et al. [17]
HOXD13	Zhang et al. [20]			Carmichael et al. [17]
GLI1		Carmichael et al. [17]		
GLI2		Carmichael et al. [17]		
GLI3	Zhang et al. [20]	Carmichael et al. [17]		
ZFPM2	Zhang et al. [20]			
CDH7	Zhang et al. [20]			
Masculinization				
SRY			Wang et al. [15], Zhang et al. [20]	Carmichael et al. [17]
SOX9			Wang et al. [15], Zhang et al. [20]	
NR5A1	Köhler et al. [28], Allali et al. [29], Adamovic et al. [30], Laan et al. [31]		Kalfa et al. [32], Kon et al. [19], Zhang et al. [20]	Adamovic et al. [30]
AR	Hiort et al. [33], Alléra et al. [34], Sutherland et al. [35], Nordenskjöld et al. [18], Wang et al. [15], Thai et al. [36], Borhani et al. [37], Kon et al. [19], Yuan et al. [38], Zhang et al. [20], Chen et al. [39]	Lim et al. [40], Aschim et al. [41], Radpour et al. [42], Parada-Bustamante et al. [43], Adamovic and Nordenskjold [44], Adamovic et al. [45]	Muroya et al. [46], Radpour et al. [42], Kalfa et al. [32]	Muroya et al. [46], Vottero et al. [47], Silva et al. [48]

Table 11.1 (continued)

	Mutation studies that found mutations	Association studies that found associations	Mutation studies that did not find mutations	Association studies that did not find associations
FKBP4			Beleza-Meireles et al. [49], Zhang et al. [20]	Beleza-Meireles et al. [49]
CYP1A1		Kurahashi et al. [50], Carmichael et al. [51], Mao et al. [52]	Kon et al. [19], Zhang et al. [20]	Yadav et al. [53]
CYP1A2		Qin et al. [54]		
CYP3A4		Carmichael et al. [51]		Qin et al. [54]
CYP11A1			Zhang et al. [20]	Carmichael et al. [51]
CYP17A1		Qin et al. [54], Mao et al. [52]		Samtani et al. [55], Yadav et al. [56], Carmichael et al. [51]
CYP19A1				Qin et al. [54], Carmichael et al. [51]
HSD3B1	Chen et al. [39]	Carmichael et al. [51]		
HSD3B2	Codner et al. [57], Kon et al. [19]		Zhang et al. [20]	Carmichael et al. [51]
HSD17B3		Sata et al. [58], Carmichael et al. [51]	Thai et al. [36], Kon et al. [19], Yuan et al. [38], Zhang et al. [20]	
SRD5A1			Tria et al. [59]	Carmichael et al. [51]
SRD5A2	Silver and Russell [60], Wang et al. [15], Thai et al. [36], Kon et al. [19], Rahimi et al. [61], Yuan et al. [38], Zhang et al. [20]	Silver and Russell [60], Wang et al. [15], Thai et al. [36], Sata et al. [58], Samtani et al. [55], Carmichael et al. [51], Samtani et al. [62], Rahimi et al. [61]	Nordenskjöld et al. [18], Kalfa et al. [32]	van der Zanden et al. [63], Adamovic et al. [45]
STAR			Zhang et al. [20]	Carmichael et al. [51]
STARD3		Carmichael et al. [51]		
STS		Carmichael et al. [51]		
Other genes				
ESR1		Watanabe et al. [64], van der Zanden et al. [63], Tang et al. [65] Choudhry et al. [66] Ban et al. [67]	Beleza-Meireles et al. [68], Kon et al. [19], Zhang et al. [20]	Beleza-Meireles et al. [68]
ESR2		Beleza-Meireles et al. [68], Beleza-Meireles et al. [69], Ban et al. [67], Choudhry et al. [66], van der Zanden et al. [63]	Beleza-Meireles et al. [68], Kon et al. [19], Zhang et al. [20]	Aschim et al. [70]
ATF3	Beleza-Meireles et al. [71], Kalfa et al. [72]	Beleza-Meireles et al. [71], van der Zanden et al. [63]	Kon et al. [19], Zhang et al. [20]	
MAMLD1	Fukami et al. [73], Kalfa et al. [74], Chen et al. [75], Kalfa et al. [32], Igarashi et al. [76], Ratan et al. [77]	Chen et al. [75], Kalfa et al. [78], Ratan et al. [77]	Kalfa et al. [78], Kon et al. [19], Zhang et al. [20]	Liu et al. [79]

(continued)

Table 11.1 (continued)

	Mutation studies that found mutations	Association studies that found associations	Mutation studies that did not find mutations	Association studies that did not find associations
DGKK		van der Zanden et al. [80], Carmichael et al. [81], Geller et al. [25], Ma et al. [82], Hozyasz et al. [83], Xie et al. [84], Chen et al. [27]	Kon et al. [19], Zhang et al. [20]	Kojima et al. [26], Richard et al. [85]
TGFBR2		Han et al. [86]		
CTGF			Kon et al. [19], Zhang et al. [20]	
BNC2	Bhoj et al. [87], Kon et al. [19]		Zhang et al. [20]	
MID1	Zhang et al. [88]	Zhang et al. [88]	Kon et al. [19], Zhang et al. [20]	
INSL3			El Houate et al. [89], Zhang et al. [20]	
GSTM1		Yadav et al. [53]	Kon et al. [19], Zhang et al. [20]	Kurahashi et al. [50]
GSTT1		Yadav et al. [53]	Kon et al. [19]	Kurahashi et al. [50]
ARNT2		Qin et al. [54]		
NR1I2		Qin et al. [54]		
AKR1C2			Soderhall et al. [90], Zhang et al [20] Mares et al. [91]	Mares er al. [91]
AKR1C3	Soderhall et al. [90]		Mares et al. [91]	Soderhall et al. [90], Mares et al. [91]
AKR1C4			Soderhall et al. [90], Zhang et al. [20], Mares et al. [91]	Soderhall et al. [90], Mares et al. [91]
KLF6	Soderhall et al. [82]			Soderhall et al. [90]
RYR1		Zhang et al. [92]		
PKDCC		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
HAAO		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
DNAH6		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
EEFSEC		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
PDGFC		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
DAAM2		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
TAX1BP1		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
EYA1		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
CCDC26		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
KCNMA		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]

Table 11.1 (continued)

	Mutation studies that found mutations	Association studies that found associations	Mutation studies that did not find mutations	Association studies that did not find associations
GREM1		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
IRX5		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
IRX6		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
ZFHX3		Geller et al. [25]		Kojima et al. [26], Chen et al. [27]
EYA1		Geller et al. [25]		Kojima et al. [26]
EXOC3		Geller et al. [25]		
PROKR2	Zhang et al. [20]			
TRIM17	Zhang et al. [20]			
SLC25A5	Chen et al. [27]			
SP1		Chen et al. [27]		

Genes that were only investigated once and did not show mutations or associations in that study are not shown. The table shows that only few studies focused on genes involved in the indifferent and early patterning stage. Although some mutations and associations were found in these genes, there is no gene that shows clear evidence of being involved in hypospadias etiology.

Research on genes involved in the masculinization stage has been much more extensive. Notably, although expression of the *SRY* gene, located on the Y chromosome, is crucial for development of the testis from the indifferent gonad ([10, 93]), there is not much evidence that this gene plays a role in the development of hypospadias. Research was especially focused on *AR* and *SRD5A2*. SRD5A2 converts testosterone to the more potent androgen DHT, and both testosterone and DHT exert their effect through the AR. The AR is expressed in the developing human penis and urethra, and SRD5A2 is expressed during male genital development around the ventral part of the remodeling urethra [94]. Multiple studies found mutations and associations with polymorphisms in these genes (Table 11.1). In addition, there are other studies that indicated the involvement of the AR in hypospadias etiology. For example, some studies indicated different expression levels in patients compared to controls [39, 47, 48, 95–98], while others suggested decreased DHT binding capacity of the AR in genital skin fibroblasts of patients with hypospadias [34, 99]. Although not all studies confirmed these results [100–103], evidence that a defect in AR or SRD5A2 may cause or be a risk factor for hypospadias is compelling.

11.5 Other Genes

Not only steroidogenesis but also the balance between androgens and estrogens appears to be important in the development of the male external genitalia. The estrogen receptors *ESR1* and *ESR2* are expressed in the developing human male GT [14], and mRNA expression levels seem to be decreased in foreskin of hypospadias patients compared to controls [95]. Although no mutations were found in *ESR1* or *ESR2*, associations have been reported between hypospadias and several polymorphisms in the genes encoding these receptors (Table 11.1), and evidence for these genes to play a role in hypospadias development is quite strong.

Other genes for which evidence is quite strong are activating transcription factor 3 (*ATF3*), mastermind-like domain containing 1 (*MAMLD1*), and diacylglycerol kinase κ (*DGKK*). *ATF3* is an

estrogen-responsive gene showing strong upregulation in hypospadias [72, 104–107], and several studies found mutations in this gene in hypospadias patients or polymorphisms in this gene to be associated with hypospadias (Table 11.1). *MAMLD1*, previously known as *CXorf6*, contains the NR5A1 target sequence [108] and mutations, and polymorphisms in *MAMLD1* were found in patients with hypospadias (Table 11.1). Ogata et al., concluded that *MAMLD1* mutations exert their effect primarily via compromised testosterone production around the critical period for sex development [109], and Ratan et al., found lower testosterone levels than the mean for their age in 80% of subjects carrying a polymorphism in *MAMLD1* [77]. *DGKK* was identified as a major risk gene for hypospadias in a genome-wide association study [80], a result that was confirmed by several other studies (Table 11.1). A study in mice revealed that differentiated GT epithelial cells are DGKK positive, while undifferentiated preputial lamina epithelial cells are DGKK negative, suggesting that DGKK is a marker or mediator of squamous cell differentiation [110].

11.6 Common Clinical Conditions with Gene Defects

11.6.1 46,XX Disorder of Sex Development (DSD)

The most frequent example of DSD in the 46,XX group is congenital adrenal hyperplasia (CAH), mostly resulting from a deficit of 21-hydoxylase which causes an abnormal growth of the GT and an inappropriate opening of the vagina into the posterior wall of the urethra.

It is important to realize that pubertal and post-pubertal women (46,XX) with CAH develop their GT if they are not compliant to their hormonal substitutive treatment. This shows that GT target tissues remain responsive to steroids until the organ reaches an ultimate size.

In western world, most patients with CAH are raised as female but severely virilized CAH in male-dominant societies or late diagnosis may lead to a male assignment [111].

11.6.2 46,XY DSD

Mutations in genes implied in testosterone biosynthesis (such as 3-beta-hydroxysteroid-dehydrogenase, 17-beta-hydroxysteroid-deshydrogenase and 17-alpha hydroxylase) can lead to testosterone defects which may present as DSD.

11.6.3 The Dysgenetic Gonad

The dysgenetic gonad and more specifically the dysgenetic testis is characterized by insufficient production of testosterone and anti-Müllerian hormone (AMH). Although some etiologies are well identified, the histological definition of the gonadal dysgenesis remains unclear [112] as well as the possible underlying genetic anomalies. It is commonly associated with insufficient development of the GT, impaired testicular descent, and persistence of Müllerian structures. It is most likely that gonadal dysgenesis is a dynamic process leading to a progressive loss of testicular functions. The major concern about dysgenetic or underdeveloped gonads is the development of cancers from immature germinal cells [113].

11.6.4 Partial and Complete Androgen Insensitivity Syndromes

5α-Reductase deficiency is an autosomal recessive syndrome. It is a rare disorder in western countries but frequent in the Dominican Republic, New Guinea, and Gaza strip due to high consanguinity [114]. These 46,XY patients present with quite feminine genitalia at birth which will get virilized after puberty if the testes are left in place. Some newborn babies may present with a slightly more developed hypospadiac micropenis which may grow with topical dihydrotestosterone. These patients represent one of the most difficult situations where gender assignment is an issue as well as the fate of the testes.

Partial and complete androgen insensitivity syndromes (PAIS/CAIS) are related to impaired androgen receptors. CAIS does not raise any gen-

der issue as these individuals have normal female external genitalia and will be raised as females with hormonal substitution from puberty onward. Partial androgen insensitivity syndrome used to raise difficult discussions in terms of gender assignment although most of them are nowadays raised as males [115].

References

1. Schoenwolf GC, Bleyl SB, Brauwer PR, Francis-West PH. Larsen's human embryology. 4th ed. Philadelphia, PA: Churchill Livingstone; 2009.
2. Wilhelm D, Englert C. The Wilms tumor suppressor WT1 regulates early gonad development by activation of Sf1. Genes Dev. 2002; https://doi.org/10.1101/gad.220102.
3. Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell. 1994; https://doi.org/10.1016/0092-8674(94)90211-9.
4. Perriton CL, Powles N, Chiang C, Maconochie MK, Cohn MJ. Sonic hedgehog signaling from the urethral epithelium controls external genital development. Dev Biol. 2002; https://doi.org/10.1006/dbio.2002.0668.
5. Yamaguchi TP, Bradley A, McMahon AP, Jones S. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. Development. 1999;126
6. Morgan EA, Nguyen SB, Scott V, Stadler HS. Loss of Bmp7 and Fgf8 signaling in Hoxa 13-mutant mice causes hypospadia. Development. 2003; https://doi.org/10.1242/dev.00530.
7. Suzuki K, Bachiller D, Chen YPP, et al. Regulation of outgrowth and apoptosis for the terminal appendage: external genitalia: development by concerted actions of BMP signaling. Development. 2003; https://doi.org/10.1242/dev.00846.
8. Haraguchi R, Mo R, Hui CC, Motoyama J, Makino S, Shiroishi T, Gaffield W, Yamada G. Unique functions of sonic hedgehog signaling during external genitalia development. Development. 2001;128
9. Shehata BM, Elmore JM, Bootwala Y, et al. Immunohistochemical characterization of sonic hedgehog and its downstream signaling molecules during human penile development. Fetal Pediatr Pathol. 2011; https://doi.org/10.3109/15513815.2011.555809.
10. Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf AM, Lovell-Badge R, Goodfellow PN. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature. 1990; https://doi.org/10.1038/346240a0.
11. Misrahi M, Beau I, Meduri G, Bouvattier C, Atger M, Loosfelt H, Ghinea N, Hai MV, Bougneres PF, Milgrom E. Gonadotropin receptors and the control of gonadal steroidogenesis: physiology and pathology. Bailliere Clin Endocrinol Metab. 1998; https://doi.org/10.1016/S0950-351X(98)80444-8.
12. van der Zanden LFM, van Rooij IALM, Feitz WFJ, Franke B, Knoers NVAM, Roeleveld N. Aetiology of hypospadias: a systematic review of genes and environment. Hum Reprod Update. 2012; https://doi.org/10.1093/humupd/dms002.
13. Scott HM, Mason JI, Sharpe RM. Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. Endocr Rev. 2009; https://doi.org/10.1210/er.2009-0016.
14. Crescioli C, Maggi M, Vannelli GB, Ferruzzi P, Granchi S, Mancina R, Muratori M, Forti G, Serio M, Luconi M. Expression of functional estrogen receptors in human fetal male external genitalia. J Clin Endocrinol Metab. 2003; https://doi.org/10.1210/jc.2002-021085.
15. Wang Y, Li Q, Xu J, Liu Q, Wang W, Lin Y, Ma F, Chen T, Li S, Shen Y. Mutation analysis of five candidate genes in Chinese patients with hypospadias. Eur J Hum Genet. 2004; https://doi.org/10.1038/sj.ejhg.5201232.
16. Diposarosa R, Pamungkas KO, Sribudiani Y, Herman H, Suciati LP, Rahayu NS, Effendy SH. Description of mutation spectrum and polymorphism of Wilms' tumor 1 (WT1) gene in hypospadias patients in the Indonesian population. J Pediatr Urol. 2018; https://doi.org/10.1016/j.jpurol.2017.11.021.
17. Carmichael SL, Ma C, Choudhry S, Lammer EJ, Witte JS, Shaw GM. Hypospadias and genes related to genital tubercle and early urethral development. J Urol. 2013; https://doi.org/10.1016/j.juro.2013.05.061.
18. Nordenskjöld A, Friedman E, Tapper-Persson M, Söderhäll C, Leviav A, Svensson J, Anvret M. Screening for mutations in candidate genes for hypospadias. Urol Res. 1999; https://doi.org/10.1007/s002400050088.
19. Kon M, Suzuki E, Dung VC, et al. Molecular basis of non-syndromic hypospadias: systematic mutation screening and genome-wide copy-number analysis of 62 patients. Hum Reprod. 2015; https://doi.org/10.1093/humrep/deu364.
20. Zhang W, Shi J, Zhang C, Jiang X, Wang J, Wang W, et al. Identification of gene variants in 130 Han Chinese patients with hypospadias by targeted next-generation sequencing. Mol Genet Genomic Med. 2019;7:e827.
21. Utsch B, Kaya A, Özburun A, Lentze MJ, Albers N, Ludwig M. Exclusion of WTAP and HOXA13 as candidate genes for isolated hypospadias. Scand J Urol Nephrol. 2003; https://doi.org/10.1080/00365590310014517.
22. Beleza-Meireles A, Lundberg F, Lagerstedt K, Zhou X, Omrani D, Frisén L, Nordenskjöld A. FGFR2,

FGF8, FGF10 and BMP7 as candidate genes for hypospadias. Eur J Hum Genet. 2007; https://doi.org/10.1038/sj.ejhg.5201777.
23. Chen T, Li Q, Xu J, Ding K, Wang Y, Wang W, Li S, Shen Y. Mutation screening of BMP4, BMP7, HOXA4 and HOXB6 genes in Chinese patients with hypospadias. Eur J Hum Genet. 2007; https://doi.org/10.1038/sj.ejhg.5201722.
24. Bouty A, Walton K, Listyasari NA, Robevska G, Van den Bergen J, Santosa A, et al. Functional characterization of two new variants in the Bone Morphogenetic Protein 7 Prodomain in two pairs of monozygotic twins with hypospadias. J Endocr Soc. 2019;3:814–24.
25. Geller F, Feenstra B, Carstensen L, et al. Genome-wide association analyses identify variants in developmental genes associated with hypospadias. Nat Genet. 2014; https://doi.org/10.1038/ng.3063.
26. Kojima Y, Koguchi T, Mizuno K, Sato Y, Hoshi S, Hata J, et al. Single Nucleotide Polymorphisms of HAAO and IRX6 Genes as Risk Factors for Hypospadias. J Urol. 2019;201:386–92.
27. Chen Z, Lin X, Lei Y, Chen H, Finnell RH, Wang Y, ET AL. Genome-wide association study in Chinese cohort identifies one novel hypospadias risk associated locus at 12q13.13. BMC Med Genomics. 2019;12:196.
28. Köhler B, Lin L, Mazen I, Cetindag C, Biebermann H, Akkurt I, Rossi R, Hiort O, Grüters A, Achermann JC. The spectrum of phenotypes associated with mutations in steroidogenic factor 1 (SF-1, NR5A1, Ad4BP) includes severe penoscrotal hypospadias in 46,XY males without adrenal insufficiency. Eur J Endocrinol. 2009; https://doi.org/10.1530/EJE-09-0067.
29. Allali S, Muller JB, Brauner R, et al. Mutation analysis of nr5a1 encoding steroidogenic factor 1 in 77 patients with 46, XY disorders of sex development (DSD) including hypospadias. PLoS One. 2011; https://doi.org/10.1371/journal.pone.0024117.
30. Adamovic T, Chen Y, Thai HTT, Zhang X, Markljung E, Zhao S, Nordenskjöld A. The p.G146A and p.P125P polymorphisms in the steroidogenic factor-1 (SF-1) gene do not affect the risk for hypospadias in caucasians. Sex Dev. 2012; https://doi.org/10.1159/000343782.
31. Laan M, Kasak L, Timinskas K, Grigorova M, Venclovas Č, Renaux A, et al. NR5A1 c.991-1G > C splice-site variant causes familial 46,XY partial gonadal dysgenesis with incomplete penetrance. Clin Endocrinol (Oxf). 2020;in press.
32. Kalfa N, Fukami M, Philibert P, et al. Screening of mamld1 mutations in 70 children with 46,xy dsd: identification and functional analysis of two new mutations. PLoS One. 2012; https://doi.org/10.1371/journal.pone.0032505.
33. Hiort O, Klauber G, Cendron M, Sinnecker GHG, Keim L, Schwinger E, Wolfe HJ, Yandell DW. Molecular characterization of the androgen receptor gene in boys with hypospadias. Eur J Pediatr. 1994; https://doi.org/10.1007/BF01956409.
34. Alléra A, Herbst MA, Griffin JE, Wilson JD, Schweikert HU, McPhaul MJ. Mutations of the androgen receptor coding sequence are infrequent in patients with isolated hypospadias. J Clin Endocrinol Metab. 1995; https://doi.org/10.1210/jcem.80.9.7673412.
35. Sutherland RW, Wiener JS, Hicks JP, Marcelli M, Gonzales ET, Roth DR, Lamb DJ. Androgen receptor gene mutations are rarely associated with isolated penile hypospadias. J Urol. 1996; https://doi.org/10.1016/s0022-5347(01)65830-0.
36. Thai HTT, Kalbasi M, Lagerstedt K, Frisén L, Kockum I, Nordenskjöld A. The valine allele of the V89L polymorphism in the 5-α-reductase gene confers a reduced risk for hypospadias. J Clin Endocrinol Metab. 2005; https://doi.org/10.1210/jc.2005-0446.
37. Borhani N, Novin MG, Manoochehri M, Rouzrokh M, Kazemi B, Koochaki A, Hosseini A, Farahani RM, Omrani MD. New single nucleotide variation in the promoter region of androgen receptor (AR) gene in hypospadic patients. Iran J Reprod Med. 2014;12:217.
38. Yuan S, Meng L, Zhang Y, Tu C, Du J, Li W, Liang P, Lu G, Tan YQ. Genotype-phenotype correlation and identification of two novel SRD5A2 mutations in 33 Chinese patients with hypospadias. Steroids. 2017; https://doi.org/10.1016/j.steroids.2017.06.010.
39. Chen Z, Lin X, Wang Y, Xie H, Chen F. Dysregulated expression of androgen metabolism genes and genetic analysis in hypospadias. Mol Genet Genomic Med. 2020;8:e1346.
40. Lim HN, Chen H, McBride S, Dunning AM, Nixon RM, Hughes IA, Hawkins JR. Longer polyglutamine tracts in the androgen receptor are associated with moderate to severe undermasculinized genitalia in XY males. Hum Mol Genet. 2000; https://doi.org/10.1093/hmg/9.5.829.
41. Aschim EL, Nordenskjöld A, Giwercman A, Lundin KB, Ruhayèl Y, Haugen TB, Grotmol T, Giwercman YL. Linkage between cryptorchidism, hypospadias, and GGN repeat length in the androgen receptor gene. J Clin Endocrinol Metab. 2004; https://doi.org/10.1210/jc.2004-0293.
42. Radpour R, Rezaee M, Tavasoly A, Solati S, Saleki A. Association of long polyglycine tracts (GGN Repeats) in exon 1 of the androgen receptor gene with cryptorchidism and penile hypospadias in Iranian patients. J Androl. 2007; https://doi.org/10.2164/jandrol.106.000927.
43. Parada-Bustamante A, Lardone MC, Madariaga M, Johnson MC, Codner E, Cassorla F, Castro A. Androgen receptor CAG and GGN polymorphisms in boys with isolated hypospadias. J Pediatr Endocrinol Metab. 2012; https://doi.org/10.1515/JPEM.2011.379.

44. Adamovic T, Nordenskjöld A. The CAG repeat polymorphism in the Androgen receptor gene modifies the risk for hypospadias in Caucasians. BMC Med Genet. 2012; https://doi.org/10.1186/1471-2350-13-109.
45. Adamovic T, Thai HTT, Liedén A, Nordenskjöld A. Association of a tagging single nucleotide polymorphism in the androgen receptor gene region with susceptibility to severe hypospadias in a Caucasian population. Sex Dev. 2013; https://doi.org/10.1159/000348882.
46. Muroya K, Sasagawa I, Suzuki Y, Nakada T, Ishii T, Ogata T. Hypospadias and the androgen receptor gene: mutation screening and CAG repeat length analysis. Mol Hum Reprod. 2001; https://doi.org/10.1093/molehr/7.5.409.
47. Vottero A, Minari R, Viani I, Tassi F, Bonatti F, Neri TM, Bertolini L, Bernasconi S, Ghizzoni L. Evidence for epigenetic abnormalities of the androgen receptor gene in foreskin from children with hypospadias. J Clin Endocrinol Metab. 2011; https://doi.org/10.1210/jc.2011-0511.
48. Silva TS, Richeti F, Cunha DPPS, Amarante ACM, De Souza Leão JQ, Longui CA. Androgen receptor mRNA measured by quantitative real time PCR is decreased in the urethral mucosa of patients with middle idiopathic hypospadias. Horm Metab Res. 2013; https://doi.org/10.1055/s-0033-1333717.
49. Beleza-Meireles A, Barbaro M, Wedell A, Töhönen V, Nordenskjöld A. Studies of a co-chaperone of the androgen receptor, FKBP52, as candidate for hypospadias. Reprod Biol Endocrinol. 2007; https://doi.org/10.1186/1477-7827-5-8.
50. Kurahashi N, Sata F, Kasai S, Shibata T, Moriya K, Yamada H, Kakizaki H, Minakami H, Nonomura K, Kishi R. Maternal genetic polymorphisms in CYP1A1, GSTM1 and GSTT1 and the risk of hypospadias. Mol Hum Reprod. 2005; https://doi.org/10.1093/molehr/gah134.
51. Carmichael SL, Witte JS, Ma C, Lammer EJ, Shaw GM. Hypospadias and variants in genes related to sex hormone biosynthesis and metabolism. Andrology. 2014; https://doi.org/10.1111/j.2047-2927.2013.00165.x.
52. Mao Y, Zhang K, Ma L, Yun X, Ou F, Liu G, et al. Interaction between CYP1A1/CYP17A1 polymorphisms and parental risk factors in the risk of hypospadias in a Chinese population. Sci Rep. 2019;9:4123.
53. Shekharyadav C, Bajpai M, Kumar V, Ahmed RS, Gupta P, Banerjee BD. Polymorphism in CYP1A1, GSTMI,GSTT1 genes and organochlorine pesticides in the etiology of hypospadias. Hum Exp Toxicol. 2011; https://doi.org/10.1177/0960327110392402.
54. Qin XY, Kojima Y, Mizuno K, et al. Association of variants in genes involved in environmental chemical metabolism and risk of cryptorchidism and hypospadias. J Hum Genet. 2012; https://doi.org/10.1038/jhg.2012.48.
55. Samtani R, Bajpai M, Vashisht K, Ghosh PK, Saraswathy KN. Hypospadias risk and polymorphism in SRD5A2 and CYP17 genes: case-control study among Indian children. J Urol. 2011; https://doi.org/10.1016/j.juro.2011.02.043.
56. Yadav CS, Bajpai M, Kumar V, Datta SK, Gupta P, Ahmed RS, Banerjee BD. Polymorphisms in the P450 c17 (17-hydroxylase/17, 20-lyase) gene: association with estradiol and testosterone concentration in hypospadias. Urology. 2011; https://doi.org/10.1016/j.urology.2011.04.021.
57. Codner E, Okuma C, Iñiguez G, Boric MA, Avila A, Johnson MC, Cassorla FG. Molecular study of the 3β-hydroxysteroid dehydrogenase gene type II in patients with hypospadias. J Clin Endocrinol Metab. 2004;89:957–64.
58. Sata F, Kurahashi N, Ban S, et al. Genetic polymorphisms of 17β-hydroxysteroid dehydrogenase 3 and the risk of hypospadias. J Sex Med. 2010; https://doi.org/10.1111/j.1743-6109.2009.01641.x.
59. Tria A, Hiort O, Sinnecker GHG. Steroid 5α-reductase 1 polymorphisms and testosterone/dihydrotestosterone ratio in male patients with hypospadias. Horm Res Paediatr. 2004;61:180–3.
60. Silver RI, Russell DW. 5 alpha-reductase type 2 mutations are present in some boys with isolated hypospadias. J Urol. 1999; https://doi.org/10.1097/00005392-199909000-00064.
61. Rahimi M, Ghanbari M, Fazeli Z, Rouzrokh M, Omrani S, Mirfakhraie R, Omrani MD. Association of SRD5A2 gene mutations with risk of hypospadias in the Iranian population. J Endocrinol Investig. 2017; https://doi.org/10.1007/s40618-016-0573-y.
62. Samtani R, Bajpai M, Ghosh PK, Saraswathy KN. A49T, R227Q and TA repeat polymorphism of steroid 5 alpha-reductase type II gene and hypospadias risk in North Indian children. Meta Gene. 2015; https://doi.org/10.1016/j.mgene.2014.11.003.
63. Van Der Zanden LFM, Van Rooij IALM, Feitz WFJ, Vermeulen SHHM, Kiemeney LALM, Knoers NVAM, Roeleveld N, Franke B. Genetics of hypospadias: Are single-nucleotide polymorphisms in SRD5A2, ESR1, ESR2, and ATF3 really associated with the malformation? J Clin Endocrinol Metab. 2010; https://doi.org/10.1210/jc.2009-2101.
64. Watanabe M, Yoshida R, Ueoka K, Aoki K, Sasagawa I, Hasegawa T, Sueoka K, Kamatani N, Yoshimura Y, Ogata T. Haplotype analysis of the estrogen receptor 1 gene in male genital and reproductive abnormalities. Hum Reprod. 2007; https://doi.org/10.1093/humrep/del513.
65. Tang KF, Zheng JZ, Xing JP. Molecular analysis of SNP12 in estrogen receptor α Gene in hypospadiac or cryptorchid patients from northwestern China. Urol Int. 2011; https://doi.org/10.1159/000330902.
66. Choudhry S, Baskin LS, Lammer EJ, Witte JS, Dasgupta S, Ma C, Surampalli A, Shen J, Shaw GM, Carmichael SL. Genetic polymorphisms in ESR1

and ESR2 genes, and risk of hypospadias in a multiethnic study population. J Urol. 2015; https://doi.org/10.1016/j.juro.2014.11.087.
67. Ban S, Sata F, Kurahashi N, Kasai S, Moriya K, Kakizaki H, Nonomura K, Kishi R. Genetic polymorphisms of ESR1 and ESR2 that may influence estrogen activity and the risk of hypospadias. Hum Reprod. 2008; https://doi.org/10.1093/humrep/den098.
68. Nordenskjöld A, Beleza Meireles A, Omrani D, Kockum I, Frisén L, Lagerstedt K. Polymorphisms of estrogen receptor beta gene are associated with hypospadias. J Pediatr Urol. 2007; https://doi.org/10.1016/j.jpurol.2007.01.077.
69. Beleza-Meireles A, Kockum I, Lundberg F, Söderhäll C, Nordenskjöld A. Risk factors for hypospadias in the estrogen receptor 2 gene. J Clin Endocrinol Metab. 2007; https://doi.org/10.1210/jc.2007-0543.
70. Aschim EL, Giwercman A, Ståhl O, Eberhard J, Cwikiel M, Nordenskjöld A, Haugen TB, Grotmol T, Giwercman YL. The RsaI polymorphism in the estrogen receptor-beta gene is associated with male infertility. J Clin Endocrinol Metab. 2005;90:5343–8.
71. Beleza-Meireles A, Töhönen V, Söderhäll C, Schwentner C, Radmayr C, Kockum I, Nordenskjöld A. Activating transcription factor 3: a hormone responsive gene in the etiology of hypospadias. Eur J Endocrinol. 2008; https://doi.org/10.1530/EJE-07-0793.
72. Kalfa N, Liu B, Klein O, Wang MH, Cao M, Baskin LS. Genomic variants of ATF3 in patients with hypospadias. J Urol. 2008; https://doi.org/10.1016/j.juro.2008.07.066.
73. Fukami M, Wada Y, Miyabayashi K, et al. CXorf6 is a causative gene for hypospadias. Nat Genet. 2006; https://doi.org/10.1038/ng1900.
74. Kalfa N, Liu B, Ophir K, Audran F, Wang MH, Mei C, Sultan C, Baskin LS. Mutations of CXorf6 are associated with a range of severities of hypospadias. Eur J Endocrinol. 2008; https://doi.org/10.1530/EJE-08-0085.
75. Chen Y, Thai HTT, Lundin J, Lagerstedt-Robinson K, Zhao S, Markljung E, Nordenskjöld A. Mutational study of the MAMLD1-gene in hypospadias. Eur J Med Genet. 2010; https://doi.org/10.1016/j.ejmg.2010.03.005.
76. Igarashi M, Wada Y, Kojima Y, et al. Novel splice site mutation in MAMLD1 in a patient with hypospadias. Sex Dev. 2015; https://doi.org/10.1159/000380842.
77. Ratan SK, Sharma A, Kapoor S, Polipalli SK, Dubey D, Mishra TK, Sinha SK, Agarwal SK. Polymorphism of 3′ UTR of MAMLD1 gene is also associated with increased risk of isolated hypospadias in Indian children: a preliminary report. Pediatr Surg Int. 2016; https://doi.org/10.1007/s00383-016-3856-7.
78. Kalfa N, Cassorla F, Audran F, et al. Polymorphisms of MAMLD1 gene in hypospadias. J Pediatr Urol. 2011; https://doi.org/10.1016/j.jpurol.2011.09.005.
79. Liu Y, Zhuang L, Ye W, Wu M, Huang Y. Association of MAMLD1 single-nucleotide polymorphisms with hypospadias in Chinese Han population. Front Biosci. 2017; https://doi.org/10.2741/4540.
80. Van Der Zanden LFM, Van Rooij IALM, Feitz WFJ, et al. Common variants in DGKK are strongly associated with risk of hypospadias. Nat Genet. 2011; https://doi.org/10.1038/ng.721.
81. Carmichael SL, Mohammed N, Ma C, Iovannisci D, Choudhry S, Baskin LS, Witte JS, Shaw GM, Lammer EJ. Diacylglycerol kinase K variants impact hypospadias in a California study population. J Urol. 2013; https://doi.org/10.1016/j.juro.2012.09.002.
82. Ma Q, Tang Y, Lin H, et al. Diacylglycerol kinase κ (DGKK) variants and hypospadias in Han Chinese: association and meta-analysis. BJU Int. 2015;116:634–40.
83. Hozyasz KK, Mostowska A, Kowal A, Mydlak D, Tsibulski A, Jagodziński PP. Further evidence of the association of the diacylglycerol kinase kappa (DGKK) gene with hypospadias. Urol J. 2018; https://doi.org/10.22037/uj.v0i0.4061.
84. Xie H, Lin XL, Zhang S, Yu L, Li XX, Huang YC, Lyu YQ, Chen HT, Xu J, Chen F. Association between diacylglycerol kinase kappa variants and hypospadias susceptibility in a Han Chinese population. Asian J Androl. 2018; https://doi.org/10.4103/aja.aja_13_17.
85. Richard MA, Sok P, Canon S, Brown AL, Peckham-Gregory EC, Nembhard WN, et al. The role of genetic variation in DGKK on moderate and severe hypospadias. Birth Defects Res. 2019;111:932–7.
86. Han XR, Wen X, Wang S, et al. Associations of TGFBR1 and TGFBR2 gene polymorphisms with the risk of hypospadias: a case–control study in a Chinese population. Biosci Rep. 2017; https://doi.org/10.1042/BSR20170713.
87. Bhoj EJ, Ramos P, Baker LA, et al. Human balanced translocation and mouse gene inactivation implicate Basonuclin 2 in distal urethral development. Eur J Hum Genet. 2011; https://doi.org/10.1038/ejhg.2010.245.
88. Zhang X, Chen Y, Zhao S, Markljung E, Nordenskjöld A. Hypospadias associated with hypertelorism, the mildest phenotype of opitz syndrome. J Hum Genet. 2011; https://doi.org/10.1038/jhg.2011.17.
89. El Houate B, Rouba H, Sibai H, et al. Novel mutations involving the INSL3 gene associated with cryptorchidism. J Urol. 2007; https://doi.org/10.1016/j.juro.2007.01.002.
90. Söderhäll C, Körberg IB, Thai HTT, et al. Fine mapping analysis confirms and strengthens linkage of four chromosomal regions in familial hypospadias. Eur J Hum Genet. 2015; https://doi.org/10.1038/ejhg.2014.129.
91. Mares L, Vilchis F, Chávez B, Ramos L. Molecular genetic analysis of AKR1C2-4 and HSD17B6 genes in subjects 46. XY with hypospadias. J Pediatr Urol. 2020;16:689.e1–689.e12.

92. Zhang H, Zhang Z, Jia L, Ji W, Li H. Genetic polymorphism in the RYR1 C6487T is associated with severity of hypospadias in chinese han children. Biomed Res Int. 2018; https://doi.org/10.1155/2018/7397839.
93. Gubbay J, Collignon J, Koopman P, Capel B, Economou A, Münsterberg A, Vivian N, Goodfellow P, Lovell-Badge R. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. Nature. 1990; https://doi.org/10.1038/346245a0.
94. Kim K, Liu W, Cunha GR, Russell DW, Huang H, Shapiro E, Baskin LS. Expression of the androgen receptor and 5α-reductase type 2 in the developing human fetal penis and urethra. Cell Tissue Res. 2002;307:145–53.
95. Qiao L, Tasian GE, Zhang H, Cao M, Ferretti M, Cunha GR, Baskin LS. Androgen receptor is overexpressed in boys with severe hypospadias, and ZEB1 regulates androgen receptor expression in human foreskin cells. Pediatr Res. 2012; https://doi.org/10.1038/pr.2011.49.
96. Pichler R, Djedovic G, Klocker H, Heidegger I, Strasak A, Loidl W, Bektic J, Skradski V, Horninger W, Oswald J. Quantitative measurement of the androgen receptor in prepuces of boys with and without hypospadias. BJU Int. 2013; https://doi.org/10.1111/j.1464-410X.2012.11731.x.
97. Ohsako S, Aiba T, Miyado M, Fukami M, Ogata T, Hayashi Y, Mizuno K, Kojima Y. Expression of xenobiotic biomarkers CYP1 family in preputial tissue of patients with hypospadias and phimosis and its association with DNA methylation level of SRD5A2 minimal promoter. Arch Environ Contam Toxicol. 2018; https://doi.org/10.1007/s00244-017-0466-x.
98. Balaji DR, Reddy G, Babu R, Paramaswamy B, Ramasundaram M, Agarwal P, et al. Androgen Receptor Expression in Hypospadias. J Indian Assoc Pediatr Surg. 2020;25:6–9.
99. Schweikert HU, Schluter M, Romalo G. Intracellular and nuclear binding of [3H]dihydrotestosterone in cultured genital skin fibroblasts of patients with severe hypospadias. J Clin Invest. 1989; https://doi.org/10.1172/JCI113930.
100. Gearhart JP, Linhard HR, Berkovitz GD, Jeffs RD, Brown TR. Androgen receptor levels and 5α-reductase activities in preputial skin and chordee tissue of boys with isolated hypospadias. J Urol. 1988; https://doi.org/10.1016/s0022-5347(17)42014-3.
101. Terakawa T, Shima H, Yabumoto H, Koyama K, Ikoma F. Androgen receptor levels in patients with isolated hypospadias. Acta Endocrinol. 1990; https://doi.org/10.1530/acta.0.1230024.
102. Bentvelsen FM, Brinkmann AO, van der Linden JETM, Schröder FH, Nijman JM. Decreased immunoreactive androgen receptor levels are not the cause of isolated hypospadias. Br J Urol. 1995; https://doi.org/10.1111/j.1464-410X.1995.tb07719.x.
103. Tack LJW, Praet M, Van Dorpe J, Haid B, Buelens S, Hoebeke P, et al. Androgen receptor expression in preputial dartos tissue correlates with physiological androgen exposure in congenital malformations of the penis and in controls. J Pediatr Urol. 2020;16:43.e1–8.
104. Liu B, Wang Z, Lin G, Agras K, Ebbers M, Willingham E, Baskin LS. Activating transcription factor 3 is up-regulated in patients with hypospadias. Pediatr Res. 2005; https://doi.org/10.1203/01.pdr.0000187796.28007.2d.
105. Wang Z, Liu BC, Lin GT, Lin CS, Lue TF, Willingham E, Baskin LS. Up-regulation of estrogen responsive genes in hypospadias: microarray analysis. J Urol. 2007; https://doi.org/10.1016/j.juro.2007.01.014.
106. Gurbuz C, Demir S, Zemheri E, Canat L, Kilic M, Caskurlu T. Is activating transcription factor 3 upregulated in patients with hypospadias? Korean J Urol. 2010;51:561.
107. Karabulut R, Turkyilmaz Z, Sonmez K, Kumas G, Ergun SG, Ergun MA, Basaklar AC. Twenty-four genes are upregulated in patients with hypospadias. Balk J Med Genet. 2013; https://doi.org/10.2478/bjmg-2013-0030.
108. Fukami M, Wada Y, Okada M, Kato F, Katsumata N, Baba T, Morohashi KI, Laporte J, Kitagawa M, Ogata T. Mastermind-like domain-containing 1 (MAMLD1 or CXorf6) transactivates the Hes3 promoter, augments testosterone production, and contains the SF1 target sequence. J Biol Chem. 2008; https://doi.org/10.1074/jbc.M703289200.
109. Ogata T, Sano S, Nagata E, Kato F, Fukami M. MAMLD1 and 46,XY disorders of sex development. Semin Reprod Med. 2012; https://doi.org/10.1055/s-0032-1324725.
110. Shen J, Liu B, Sinclair A, Cunha G, Baskin LS, Choudhry S. Expression analysis of DGKK during external genitalia formation. J Urol. 2015; https://doi.org/10.1016/j.juro.2015.06.098.
111. Houk CP, Lee PA. Approach to assigning gender in 46,XX congenital adrenal hyperplasia with male external genitalia: Replacing dogmatism with pragmatism. J Clin Endocrinol Metab. 2010; https://doi.org/10.1210/jc.2010-0714.
112. Hughes IA. Disorders of sex development: a new definition and classification. Best Pract Res Clin Endocrinol Metab. 2008; https://doi.org/10.1016/j.beem.2007.11.001.
113. Olesen IA, Sonne SB, Hoei-Hansen CE, Rajpert-DeMeyts E, Skakkebaek NE. Environment, testicular dysgenesis and carcinoma in situ testis. Best Pract Res Clin Endocrinol Metab. 2007; https://doi.org/10.1016/j.beem.2007.04.002.
114. al-Attia HM. Male pseudohermaphroditism due to 5 alpha-reductase-2 deficiency in an Arab kindred. Postgrad Med J. 1997;73:802–7.
115. Mazur T. Gender dysphoria and gender change in androgen insensitivity or micropenis. Arch Sex Behav. 2005; https://doi.org/10.1007/s10508-005-4341-x.