Received: 18 October 2017 | Accepted: 17 August 2018

DOI: 10.1002/jcp.27350

REVIEW ARTICLE

WILEY Journal of Cellular Physiology

The genetic factors contributing to hypospadias and their clinical utility in its diagnosis

Marjan Joodi[1,2*] | Forouzan Amerizadeh[3] | Seyed Mahdi Hassanian[3,4*] | Marjan Erfani[5] | Majid Ghayour-Mobarhan[3] | Gordon A. Ferns[6] | Majid Khazaei[3] | Amir Avan[3,7,8*]

[1]Department of Pediatric Surgery, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[2]Sarvar Children's Hospital, Endoscopic and Minimally Invasive Surgery Research Center, Mashhad, Iran

[3]Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

[4]Department of Medical Biochemistry, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[5]Department of Neurology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran

[6]Division of Medical Education, Brighton & Sussex Medical School Falmer, Brighton, UK

[7]Department of Modern Sciences and Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

[8]Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Correspondence
Amir Avan and Majid Khazaei, Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
Email: avana@mums.ac.ir; amir_avan@yahoo.com; khazaeim@mums.ac.ir

Abstract

Hypospadias is among the most common congenital malformations in male neonates. It results from abnormal penile and urethral development, but is a multifactorial disorder that is highly heterogeneous, with several genetic and environmental determinants. Monogenic and chromosomal abnormalities are present in approximately 30% of cases, although the genetic factors contributing to hypospadias remain unknown in 70% of cases. While defects in androgen synthesis can lead to this malformation, mutational analyses have shown several genes, such as sonic hedgehog, fibroblast growth factors, bone morphogenetic proteins, homeobox genes, and the Wnt family, are involved in the normal development of male external genitalia. Mutations in the genes of penile development (e.g., HOX, FGF, Shh) and testicular determination (e.g., WT1, SRY), luteinizing hormone receptor, and androgen receptor have also been proposed to be implicated in hypospadias. Here we review the recent advances in this field and discuss the potential genes that could determine the risk of hypospadias.

KEYWORDS

environmental factors, genetics, hypospadias, polymorphism

1 | INTRODUCTION

Hypospadias is congenital hypoplasia of the penis, with a displacement of the urethral opening along the ventral surface that may occur in different location: Leading to middle (penile) or posterior openings. Hypospadias is often associated with other urogenital anomalies (Akin, Ercan, Telatar, Tarhan, & Comert, 2011) and may occur as a familial clustering, in approximately 7% of cases (Fredell et al., 2002); the chance of another case of hypospadias within a family has been reported to be 9–17% (Schnack et al., 2007). In pedigree and twin studies, the heritability of this condition was reported to be around 57–77% (Schnack et al., 2007). The risks for brothers and sons of hypospadias cases being affected are similar, although it is reported that the majority of subjects have a multifactorial etiology, involving both environmental and genetic factors (Schnack et al., 2007).

There is increasing evidence that defect in the genes involved in penile development (e.g., HOX, FGF, Shh) and testicular determination (e.g., WT1, SRY), luteinizing hormone (LH) receptor, and androgen receptor (e.g., 5α reductase, androgen receptor) may be associated with an increased risk of developing hypospadias (Bouty,

*Marjan Joodi, Seyed M. Hassanian, and Amir Avan have contributed equally to this work.

Ayers, Pask, Heloury, & Sinclair, 2015). Furthermore, hypospadias is often associated with other syndromes, such as the Smith–Lemli–Opitz, Robinow, Klinefelter, Denys–Drash, and Frasier syndromes or other complex conditions, such as disorders of sex development (DSD; Schnack et al., 2007).

2 | MOLECULAR PATHWAYS AND GENES IMPLICATED IN THE ETIOLOGY OF HYPOSPADIAS

There has been increasing interest in evaluating the causal mutations of hypospadias as summarized in Table 1, however, the functional importance of these mutations remains unknown. It appears that posterior cases of hypospadias is more common in monogenic forms of the disease, while anterior cases of hypospadias are more likely to have a polygenic or multifactorial etiology (van der Zanden et al., 2012).

There are three key pathways in the development of male external genitalia: (a) androgen independent, (b) androgen dependent, and (c) pathways dependent on endocrine and environmental factors (Manson & Carr, 2003). In the early stages of the genital tubercle development, an androgen-independent pathway is among the activated pathway, which relies on the function of bone morphogenetic proteins (BMP), wingless-type MMTV integration site family member 5A (Wnt5a), fibroblast growth factor (FGF) proteins, sonic hedgehog (SHH), and homeobox A13 (HoxA13; Blaschko, Cunha, & Baskin, 2012; van der Zanden et al., 2012). It has been reported that SHH is expressed inside the epithelium of the urethral plate, and plays an essential role during normal genital growth and is needed for patterning and cell survival in the developing genitalia. Shh may have a function in the development of the genital tubercle outgrowth and differentiation (van der Zanden et al., 2012). There are other genes which have been reported to be associated with hypospadias including Patched1, MSH homeobox 1, homeobox D13 (van der Zanden et al., 2012), mastermind-like domain containing 1 (MAMLD1, CXorf6), GATA-type zinc finger protein 4 (GATA4), Diacylglycerol kinase κ (DGKK; van der Zanden et al., 2012) and friend of GATA2 (FOG2). Moreover, the process appears to be controlled by the sex-determining region Y (SRY) gene (Sinclair et al., 1990), which leads to the differentiation of Leydig cells to produce testosterone, and Sertoli cells to secrete the anti-Müllerian hormone (AMH; van der Zanden et al., 2012). Sf1 controls AMH secretion, while HCG controls Leydig cell growth and promotes fetal steroidogenesis (van der Zanden et al., 2012). Furthermore, steroid-5-α-reductase (SRD5A) converts testosterone into dihydrotestosterone (DHT), that binds to the androgen receptor (AR), and thereby stimulates the

TABLE 1 Genetic variants in hypospadias

Gene	Chromosome	Genetic variant
Hormone-independent phase		
FGF8	10q24	590C > G, 582-62 G > A (rs3218238 or rs3218233a)
FGFR2	10q26	M186T, 550 + 27C > T, 727 + 180T > G, 2454C > T
BMP4	14q22-q23	H207D: R223H H251Y
BMP7	20q13	R303C
Hormone-dependent phase		
Androgen-related gene		
AR	Xq11.2-q12	CAG/GGN repeat length polymorphism
SRD5A2	2p23	A49T, L113V, H231R V89 allele (rs9282858, rs523349)
HSD17B3	9q22	S232L
MAMLD1/CXorf6	Xq28	E124X, Q197X, R653X, V432A, E109fsX121, CAG10 > CAG13
Estrogen-related gene		
ESR1	6q25.1	rs6932902m, TA repeat, rs1801132, rs2234693, rs9340799
ESR2	14q23.2	CA repeat, rs1887994, rs1256040, rs1256062, rs10483774, rs1271572, rs944050, rs2987983, rs1256049rs4986938
ATF3	1q32.3	Specific "TTC" haplotype in intron 1 missense mutation c.536A > G(R90) in exon 3 817C > T in the 3′-UTR. L23M, C53070T, C53632A, Ins53943A
Other genes		
FKBP4	12p13.33	rs1062478rs3021522
HSD17B3	9q22	rs4743709, rs2066476, rs2066474, rs2066480, rs2066479
DGKK	Xp11.22	rs1934179, rs7063116
MID1	Xp22	rs16986145
CYP1A1	15q24.1	N/A
GSTM1	1p13.3	Gene deletion
GSTT1	22q11.23	Gene deletion

Note. AR: androgen receptor; ATF3: activating transcription factorfactor 3; BMP4: bone morphogenetic protein 4; BMP7: bone morphogenetic protein 7; CYP1A1: cytochrome P450 family 1 subfamily A member 1; DGKK: diacylglycerol kinase κ; ESR1: estrogen receptor 1; FGF8: fibroblast growth factor 8; FGFR2: fibroblast growth factor receptor 2; FKBP4: peptidyl-prolyl *cis–trans* isomerase FKBP4; GSTM1: glutathione S-transferase μ 1; GSTT1: glutathione S-transferase θ 1; HSD17B3: hydroxysteroid (17-β) dehydrogenase 3; MAMLD1: mastermind-like domain containing 1; MID1: midline 1; SRD5A2: steroid-5-α-reductase 2.

formation of the external genitalia. Estrogen receptors (ESR) are also present in the male genital tissue, to allow a balance in the actions of androgens and estrogens (van der Zanden et al., 2012).

Thus genes involved in any of the steps of the formation of male genitalia that malfunction might be associated with hypospadias. Furthermore, the nondifferentiated gonad is depended on the function of WT1 and Sf1 for development of the urogenital apparatus (van der Zanden et al., 2012). Although it has been shown that most of the WT1 mutations were identified in syndromic hypospadia patients and functional mutations in WT1 are reported to be associated with the etiology of hypospadias (Diposarosa et al., 2018; Köhler et al., 2011; Wang et al., 2004). Mutations in the AR gene, and genes involved in testosterone, and DHT metabolism (e.g., SRD5A2, HSD3B2), have also been shown to be related to hypospadias (van der Zanden et al., 2012). Estrogen receptors ESR1 and ESR2 act to keep the balance of the androgen/estrogen and ATF3, CTGF, CYR61 are reported to be implicated in hypospadias (van der Zanden et al., 2012).

3 | GENES DRIVING TO ISOLATED ANDROGEN SYNTHESIS OR ACTION DEFECTS

There is growing evidence showing an association between genes involved in androgen synthesis or its receptor/signaling pathway with the risk of hypospadias. 5α-reductase type 2 is an enzyme that converts testosterone to 5α-DHT (Kim et al., 2002). Mutations in this gene in combination with other genital abnormalities have been implicated in cases of severe hypospadias (Nicoletti et al., 2005). The V89 allele of the SRD5A2 locus has been reported to reduce the risk of hypospadias (Thai et al., 2005).

Mutations of the LH receptor are also associated with hypospadias and micropenis (Huhtaniemi & Alevizaki, 2006). A deficiency in 3β-hydroxysteroid-dehydrogenase leads to a testicular and adrenal block. Diagnosis of this particular syndrome is dependent on the association of hypospadias and adrenal insufficiency and high level of DHEA and 17-hydroxypregnenolone (Codner et al., 2004).

Rare defects in STAR and CYP11A1 have also been reported, leading salt-losing adrenal phenotype and severe underandrogenization. A combined deficiency in 17α-hydroxylase or CYP11A1 oxidoreductase has been shown in cases with hypospadias or micropenis. In summary an abnormality in endocrine activity may confirm the etiology of hypospadias as a defect in androgen synthesis in 20% of cases (Rey et al., 2005).

4 | GENES CODING FOR NONENDOCRINE-RELATED MORPHOGENETIC FACTORS

There are several classes of genes that are involved in the development of the phallus, including Homeobox genes (HOX), FGF, and Shh. HOXA and HOXD are expressed in the fetal urogenital structures (Morgan, Nguyen, Scott, & Stadler, 2003), and mutations of HOXA13 have been shown to be involved in hand–foot–genital syndrome (HFGS). HFGS is associated with small hands, malformed thumbs, small big toe and short first metacarpal, and phalanx (Frisén et al., 2004). HOXA13 plays a key role in the regulation of FGF8, AR expression, and mediates glans vascularization (Mouriquand & Mure, 2006). The FGF family has also been implicated in the development of genital structures (Petiot, Perriton, Dickson, & Cohn, 2005), especially variants of FGF8, FGF10, and FGFR2, that are suggested to increase the risk of hypospadias (Beleza-Meireles et al., 2007). The last group, Shh, is involved in the interactions between mesenchyme and urothelium, which might be related to hypospadias (Beleza-Meireles et al., 2007).

5 | GENE OR CHROMOSOMAL ABERRATIONS LEADING TO TESTICULAR DYSGENESIS

It has been reported that severe abnormalities in the development of testis might lead to gonadal dysgenesis (Swyer syndrome), although this condition can be observed in a wide range of disorders. Thus, genetic alterations in the genes involved in testis determining/promoting factors might also be involved in hypospadias. Moreover, heterozygous mutations in WT1 (Wilms tumour 1 gene) are reported to be linked with severe hypospadias and other genital abnormalities. Mutations in SF1 are reported in isolated hypospadias. SOX9, DMRT1, and GATA4 also appear to be implicated before the differentiation of the gonad into testis and genetic alterations might be correlated with male DSD, including severe hypospadias (Maciel-Guerra et al., 2008).

Furthermore, gonosomal abnormalities (e.g., Klinefelter's syndrome, 48,XXYY as well as other mosaicisms, e.g., 45,X/46,XY, known as mixed gonadal dysgenesis, 45,X/46,XYq-, 45,X/46,X,idic(Yp), 45,X/69,XXY) are found in approximately 7% of cases of hypospadias (Moreno-garcÍa & Miranda, 2002).

6 | OTHER FACTORS THAT INFLUENCE THE DEVELOPMENT OF HYPOSPADIAS

It has been reported that intracytoplasmatic sperm injection (ICSI), might increase the risk of hypospadias (Akre et al., 2008; Ericson & Källén, 2001). Furthermore, in-vitro fertilization may also alter the methylation pattern of the AR gene and thereby increase the risk of hypospadias in the children of women with high body mass index (van der Zanden et al., 2012). Primiparous obese mothers >35 years may have a high risk of children with hypospadias (Haffner, 1996). Being underweight is also reported to increase the risk of having a son with hypospadias (Hughes, Northstone, & Golding, 2002). An early age of onset of menarche, the number of pregnancies, gestational diabetes, and viral infections (North, Golding, & Team, 2000), low sperm motility (Akre et al., 2008) have not found to be associated with an increased risk (van der Zanden et al.,

2012). Placental insufficiency may be related with increased risk of hypospadias, however, the real mechanism is unknown, although maternal hypertension during pregnancy, preeclampsia, and preterm delivery are correlated, probably due to the placental dysfunction (van der Zanden et al., 2012).

7 | CONCLUSION

In most cases, hypospadias has an unknown etiology with a presumed mix of monogenic and/or multifactorial forms, involving both genes and environmental (e.g., low birth weight, maternal hypertension, preeclampsia, antiepileptic drugs, preexisting diabetes, prolonged TTP, and pregnancies resulting from ICSI) factors. Several mutations have been detected that may be implicated in this disease, and it can be classified into two categories: (a) androgen-independent genes which play important role in the early stages of the genital tubercle development (e.g., *WT1*, *SF1*, *BMP4*, *BMP7*, *HOXA4*, *HOXB6*, *FGF8*, *FGFR2*, GLI1, GLI2, GLI3, etc.); (b) mutations in the androgen-dependent genes, specially mutations that affect androgen synthesis or androgen receptors (e.g., *AR*, *HSD3B2*, *SRD5A2*, *ATF3*, CYP11A1, CYP17A1, etc.; Bouty et al., 2015). In addition, several syndromes are associated with hypospadias such as HFGS, which is caused by mutations in *HOXA13*; mutations in zinc finger E-box binding homeobox 2 (*ZEB2*) cause Mowat–Wilson syndrome, which is related with hypospadias in >50% of affected males (Garavelli et al., 2009). Although genome-wide association studies in a large group of cases and controls are needed to validate these markers. Also, exome or even whole-genome sequencing techniques could provide a novel insight on the identification of new genetic variants in combination with studies focusing on gene–gene or gene–environment interactions.

ACKNOWLEDGMENTS

This study was supported by grant awarded (Amir Avan) by the Mashhad University of Medical Sciences.

CONFLICTS OF INTEREST

The authors delcare that there are no conflicts of interest.

ORCID

Seyed M. Hassanian http://orcid.org/0000-0002-5247-4043
Majid Ghayour-Mobarhan http://orcid.org/0000-0001-5947-8904
Majid Khazaei http://orcid.org/0000-0002-7979-5699
Amir Avan http://orcid.org/0000-0002-4968-0962

REFERENCES

Akin, Y., Ercan, O., Telatar, B., Tarhan, F., & Comert, S. (2011). Hypospadias in Istanbul: Incidence and risk factors. *Pediatrics International*, *53*(5), 754–760.

Akre, O., Boyd, H. A., Ahlgren, M., Wilbrand, K., Westergaard, T., Hjalgrim, H., … Melbye, M. (2008). Maternal and gestational risk factors for hypospadias. *Environmental Health Perspectives*, *116*(8), 1071–1076.

Beleza-Meireles, A., Lundberg, F., Lagerstedt, K., Zhou, X., Omrani, D., Frisén, L., & Nordenskjöld, A. (2007). FGFR2, FGF8, FGF10 and BMP7 as candidate genes for hypospadias. *European Journal of Human Genetics*, *15*(4), 405–410.

Blaschko, S. D., Cunha, G. R., & Baskin, L. S. (2012). Molecular mechanisms of external genitalia development. *Differentiation*, *84*(3), 261–268.

Bouty, A., Ayers, K. L., Pask, A., Heloury, Y., & Sinclair, A. H. (2015). The genetic and environmental factors underlying hypospadias. *Sexual Development*, *9*(5), 239–259.

Codner, E., Okuma, C., Iñiguez, G., Boric, M. A. l., Avila, A., Johnson, M. C., & Cassorla, F. G. (2004). Molecular study of the 3β-hydroxysteroid dehydrogenase gene type II in patients with hypospadias. *The Journal of Clinical Endocrinology & Metabolism*, *89*(2), 957–964.

Diposarosa, R., Pamungkas, K. O., Sribudiani, Y., Herman, H., Suciati, L. P., Rahayu, N. S., & Effendy, S. H. (2018). Description of mutation spectrum and polymorphism of Wilms' tumor 1 (WT1) gene in hypospadias patients in the Indonesian population. *Journal of Pediatric Urology*, *14*, 237.e1–237.e7.

Ericson, A., & Kallen, B. (2001). Congenital malformations in infants born after IVF: A population-based study. *Human Reproduction*, *16*(3), 504–509.

Fredell, L., Iselius, L., Collins, A., Hansson, E., Holmner, S., Lundquist, L., … Nordenskjöld, A. (2002). Complex segregation analysis of hypospadias. *Human Genetics*, *111*(3), 231–234.

Frisén, L., Soderhall, C., Tapperpersson, M., Luthman, H., Kockum, I., & Nordenskjold, A. (2004). Genome-wide linkage analysis for hypospadias susceptibility genes. *The Journal of Urology*, *172*(4), 1460–1463.

Garavelli, L., Zollino, M., Mainardi, P. C., Gurrieri, F., Rivieri, F., Soli, F., … Neri, G. (2009). Mowat–Wilson syndrome: Facial phenotype changing with age: Study of 19 Italian patients and review of the literature. *American Journal of Medical Genetics, Part A*, *149*(3), 417–426.

Haffner, S. M. (1996). Sex hormone-binding protein, hyperinsulinemia, insulin resistance and noninsulin-dependent diabetes. *Hormone Research in Paediatrics*, *45*(3-5), 233–237.

Hughes, I., Northstone, K., & Golding, J. (2002). Reduced birth weight in boys with hypospadias: An index of androgen dysfunction? *Archives of Disease in Childhood. Fetal and Neonatal Edition*, *87*(2), F150–F151.

Huhtaniemi, I., & Alevizaki, M. (2006). Gonadotrophin resistance. *Best Practice & Research Clinical Endocrinology & Metabolism*, *20*(4), 561–576.

Kim, K., Liu, W., Cunha, G. R., Russell, D. W., Huang, H., Shapiro, E., & Baskin, L. S. (2002). Expression of the androgen receptor and 5α-reductase type 2 in the developing human fetal penis and urethra. *Cell and Tissue Research*, *307*(2), 145–153.

Köhler, B., Biebermann, H., Friedsam, V., Gellermann, J., Maier, R. F., Pohl, M., … Krude, H. (2011). Analysis of the Wilms' tumor suppressor gene (WT1) in patients 46, XY disorders of sex development. *The Journal of Clinical Endocrinology & Metabolism*, *96*(7), E1131–E1136.

Maciel-Guerra, A. T., de Mello, M. P., Coeli, F. B., Ribeiro, M. L., Miranda, M. L., Marques-de-Faria, A. P., … Guerra-Júnior, G. (2008). XX maleness and XX true hermaphroditism in SRY-negative monozygotic twins: Additional evidence for a common origin. *The Journal of Clinical Endocrinology & Metabolism*, *93*(2), 339–343.

Manson, J. M., & Carr, M. C. (2003). Molecular epidemiology of hypospadias: Review of genetic and environmental risk factors. *Birth Defects Research Part A: Clinical and Molecular Teratology*, *67*(10), 825–836.

Moreno-garcía, M., & Miranda, E. B. (2002). Chromosomal anomalies in cryptorchidism and hypospadias. *The Journal of Urology*, *168*(5), 2170–2172.

Morgan, E. A., Nguyen, S. B., Scott, V., & Stadler, H. S. (2003). Loss of Bmp7 and Fgf8 signaling in Hoxa13-mutant mice causes hypospadia. *Development*, *130*(14), 3095–3109.

Mouriquand, P., & Mure, P.-Y. (2006). *Hypospadias pediatric surgery*. Springer.

Nicoletti, A., Baldazzi, L., Balsamo, A., Barp, L., Pirazzoli, P., Gennari, M., … Cicognani, A. (2005). SRD5A2 gene analysis in an Italian population of under-masculinized 46, XY subjects. *Clinical Endocrinology*, *63*(4), 375–380.

North, K., Golding, J., & Team, A. S. (2000). A maternal vegetarian diet in pregnancy is associated with hypospadias. *BJU International*, *85*(1), 107–113.

Petiot, A., Perriton, C. L., Dickson, C., & Cohn, M. J. (2005). Development of the mammalian urethra is controlled by Fgfr2-IIIb. *Development*, *132*(10), 2441–2450.

Rey, R. A., Codner, E., Iñíguez, G., Bedecarrás, P., Trigo, R., Okuma, C., … Cassorla, F. G. (2005). Low risk of impaired testicular Sertoli and Leydig cell functions in boys with isolated hypospadias. *The Journal of Clinical Endocrinology & Metabolism*, *90*(11), 6035–6040.

Schnack, T. H., Zdravkovic, S., Myrup, C., Westergaard, T., Christensen, K., Wohlfahrt, J., & Melbye, M. (2007). Familial aggregation of hypospadias: A cohort study. *American Journal of Epidemiology*, *167*(3), 251–256.

Sinclair, A. H., Berta, P., Palmer, M. S., Hawkins, J. R., Griffiths, B. L., Smith, M. J., … Goodfellow, P. N. (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*, *346*(6281), 240–244.

Thai, H. T., Kalbasi, M., Lagerstedt, K., Frisén, L., Kockum, I., & Nordenskjöld, A. (2005). The valine allele of the V89L polymorphism in the 5-α-reductase gene confers a reduced risk for hypospadias. *The Journal of Clinical Endocrinology & Metabolism*, *90*(12), 6695–6698.

Wang, Y., Li, Q., Xu, J., Liu, Q., Wang, W., Lin, Y., … Shen, Y. (2004). Mutation analysis of five candidate genes in Chinese patients with hypospadias. *European Journal of Human Genetics*, *12*(9), 706–712.

van der Zanden, L. F. M., van Rooij, I. A. L. M., Feitz, W. F. J., Franke, B., Knoers, N. V. A. M., & Roeleveld, N. (2012). Aetiology of hypospadias: A systematic review of genes and environment. *Human Reproduction Update*, *18*(3), 260–283.

How to cite this article: Joodi M, Amerizadeh F, Hassanian SM, et al. The genetic factors contributing to hypospadias and their clinical utility in its diagnosis. *J Cell Physiol*. 2018;1–5. https://doi.org/10.1002/jcp.27350