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Genetic and other epidemiological risk factors of infants and children with hypospadias: a case control study

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Abstract

Background To study hypospadias as regard epidemiological risk factors and genetic association with mutations in Steroid 5 alpha reductase type 2 genes.

Materials This study was conducted on two groups; the first group included 50 male children with hypospadias and the other group included 50 male healthy children as a matched control. All patients and controls were subjected to detailed history, physical examination and molecular study of 5-alpha-reductase gene polymorphisms (V89L and G34R).

Results Mean age in hypospadias group was 3.28 ± 2.87 years. The most common type of hypospadias was the glanular type in 19 children (38%). Higher maternal and paternal age, consanguinity, rural residence and preterm labor carry significant epidemiological risk factors for hypospadias. According to genetic study, all healthy children carried the wild valine residue (VV) genotype, while only 44% of hypospadias cases carried the wild VV genotype and 56% carried the mutant L allele (homozygote for leucine residue and heterozygote for both valine and leucine (VL)) with high significant *p* value ($p < 0.001$). For Allele Specific—polymerase chain reaction for glycine to arginine (G34R) mutation detection in the 5 alpha reductase type 2 gene, hypospadias children had significantly higher frequency of heterozygous GR genotype than healthy controls. Binary logistic regression analysis showed that mother age and rural residence were the most independent predictors for hypospadias.

Conclusions V89L and G34R Steroid 5 alpha reductase type 2 gene polymorphisms, higher maternal and paternal age, consanguinity, rural residence and preterm labor carry significant risk factors for hypospadias. On multivariate logistic regression, mother age and rural residence are the most independent predictors for hypospadias.

Keywords Hypospadias, Risk factor, Genetic, 5-Alpha-reductase gene

1 Background

Hypospadias is a congenital anomaly in which the urethral opening is not rightly positioned at the tip of the penis as a result of incomplete fusion of urethral folds [1]. Its prevalence varies significantly across different countries ranging from one in 125–250 male live births [2]. It may be syndromic or non-syndromic with an unknown etiology with a supposed mix of monogenic and/or multifactorial forms, including both genes [3] and environmental (e.g., antiepileptic drugs [4], maternal hypertension [5], or preexisting diabetes [6]).

Androgens are essential for the formation of the male urogenital system. Any defect in the androgen synthesis (androgen deficiency) or in androgen receptor (AR) may play a causative role in the development of hypospadias

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[7]. The development of male reproductive tissue, involving the urethra, normally requires testosterone and dihydrotestosterone (DHT) [8]. Testosterone is transformed to the more potent androgen, DHT, by an enzyme known as steroid 5α-reductase type 2 (SRD5A2) which is encoded by the SRD5A2 gene that is located on chromosome 2p23 [9]. Several mutations have been found that may be implicated in hypospadias [10]. So, our work aimed to study hypospadias as regard epidemiological risk factors and genetic association with mutations in Steroid 5 alpha reductase type 2 (SRD5A2) genes.

2 Methods

The present prospective case–control study was carried out in the period from June 2021 to July 2022 after obtaining an informed written consent from all enrolled children family prior to the study. The protocol followed the ethical considerations proposed by our Faculty of Medicine Ethical Committee (IRB approval number 7/2020PED114).

This study was conducted on two groups; the first group included 50 male children with hypospadias and the other group included 50 male healthy children as a matched control who attended to our pediatric unit outpatient clinics. We included children less than 12 years while we excluded children suffering from known genetic or chromosomal abnormalities, children with dysmorphic features and/or multiple congenital anomalies suggestive of genetic syndrome or chromosomal disorder and finally children with parental consent refusal. All patients and controls were subjected to detailed history (demographic data, Pedigree and family history, antenatal history and past history including medical and surgical history), physical examination (thorough clinical examination of all body systems, clinical examination of external genitalia and anthropometric measurements). Scrotal and abdominal ultrasound was undertaken in those with posterior hypospadias to exclude any abnormality. Serum total testosterone level and molecular study of 5-alpha-reductase gene polymorphisms (V89L rs523349 and G34R rs782032018) was carried out before hypospadias correction at our genetic laboratory. According to the standard nomenclature recommendations of the HGVS (http://www.HGVS.org/mutnomen/), genetic variants can be expressed at the level of DNA sequence change or the amino acid change. So, according to NCBI-CLINIVAR, SRD5A2 gene polymorphisms (rs523349 and rs782032018) are expressed as V89L and G34R, respectively, where V89L refers to substitution of valine by leucine and G34R refers to substitution of glycine by arginine. Deoxyribonucleic acid (DNA) was extracted from 2 ml venous blood sample by DNA extraction kit (Gene JET Whole Blood Genomic DNA Purification Mini Kit) according to the manufacturer's instructions. The extracted DNA stored at -20°C.

2.1 PCR- RFLP V89L_SRD5A2 gene

The polymerase chain reaction (PCR) to amplify genomic DNA was performed with the use of forward primer 5′-CGCCTGGTTCCTGCAGGAGCT-3'and reverse primer 5'GTGAAGGCGGCGTCTGTG-3′. The primer sequences were examined for cross homology with repetitive sequences or with other loci somewhere else in the genome using Basic Local Alignment Search Tool (BLAST). The products were then digested with 5 unites of RsaI (Thermo Fisher Scientific Inc. http://www.thermoscientific.com/fermentas) [11].

2.2 Allele-specific polymerase chain reaction (AS-PCR) for G34R mutation detection

The principle of AS-PCR is based on the formation of matched or mismatched primer-target complexes [12]. Two forward primers, i.e., wi-34-f and mut-34-2f for wild and mutated alleles, respectively, as well as two reverse primers, 34-r and 34-2r were used. Primer sequences were: (Wi-34f) 5_AAGCCCTCCGGCTACG3, (Mut-34f) 5_AAGCCCTCCGGCTACA3, (34r) 5_GGAAAAACG CTACCTGTGGA3_, (342r) 5_CAAGGGAAAAACGCT ACCTG3_ [13].

During family counseling, we discussed the clinical aspects, the diagnostic approach and the importance of the genetic study with the family. We reassured patient family, especially about future reproductive function of their children and structured follow-up visits. All hypospadias cases in our study had surgical correction.

2.3 Statistical analysis

Results were statistically analyzed by statistical package SPSS version 20. Two types of statistics were done. Descriptive: e.g. percentage (%), median, mean and standard deviation (SD) and analytical by Mann–Whitney test (a nonparametric test of Student's t-test used to indicate the presence of any significant difference between two groups for a not normally distributed quantitative variable), Chi-Squared (χ2) test (used to compare between two groups or more regarding one qualitative variable), Fisher's exact test (it is a statistical significance test used in the analysis of contingency tables). It is employed when sample sizes are small. Sample was calculated by the following equation $\{n = P_1\ (1-P_1) + P_2(1-P_2)/(P_1-P_2)^{2} * C\}$ where, n = desired sample size, P1, 2:Proportion in each group and C:standard value α and β its equal to 7.85 at power 80% and confidence level 95%. The calculated total sample after adding dropout 10%, was 72 participants. Due to availability of cases during our practical part of the study, sample was increased to 100 participants who

were allocated into two groups. Logistic regression was used, and odds ratio (OR) and confidence interval (CI) were determined for detection of potential predictive factors for hypospadias. The coefficient interval was set to 95%. The level of significance was calculated according to the following probability (p) values: $p<0.05$ was considered statistically significant, $p<0.001$ was highly significant and $p>0.05$ was considered statistically non-significant.

3 Results

In our study, hypospadias children were matched well with the control group as regards age and delivery method. Mean age in hypospadias group was 3.28 ± 2.87 years. Regarding distribution of type of hypospadias among our cases, the most common type of hypospadias was the glanular type in 19 children (38%), coronal in 10 (20%), subcoronal in 7 (14%) and penoscrotal in 9 children (18%) and lastly midshaft in 5 (10%). Chordee was found in penoscrotal cases. All cases had bilateral normal scrotal testes. Stretched penile length was measured by plastic tape measure with no cases had micropenis. After detailed history, we found higher maternal and paternal age, consanguinity; rural residence and preterm labor carry significant risk factors for hypospadias (Table 1).

According to genetic study, all healthy children carried the wild VV genotype, while only 44% of hypospadias cases carried the wild VV genotype and 56% carried the mutant L allele-containing genotype (VL-LL) with high significant p value ($p<0.001$). All healthy children carried the wild allele (100%) versus 69% in hypospadias children with the mutant allele presenting only in hypospadias children (31%) with highly significant p value ($p<0.001$). Hypospadias children had significantly higher frequency of heterozygous GR genotype than healthy controls with odds ratio equal to 2.759. Mutant R allele was present more in hypospadias cases than in controls with odds ratio equal to 2.539. Mutant R containing genotypes (GR + RR) were more significantly associated with hypospadias with odds ratio equal to 2.897 (Table 2).

Comparison of frequency of the genotypes, alleles, and different models with V89L and G34R polymorphisms in the SRD5A2 gene between groups and after adjustment by mother age, father age, consanguinity, residence and gestational age (Tables 2 and 3). The rs523349 and rs782032018 SNP agreed with Hardy–Weinberg Equilibrium (HWE) ($p>0.05$) in all groups. Regarding the SRD5A2 rs523349 genotypes frequencies in different genetic models, dominant, Co-dominant-1, Co-dominant-2, over dominant models showed a significant difference upon comparing cases and controls, while no significant difference in recessive models. While in SRD5A2 rs782032018, dominant, Co-dominant-1, over dominant models showed a significant difference between cases and controls, while no significant difference in recessive and Co-dominant models. Haplotype Association Analysis of SRD5A2 gene polymorphisms (Table 4). There was a significant difference in haplotype frequency between the two studied groups Linkage Disequilibrium Analysis (Table 5). For hypospadias patients, there was a significant linkage disequilibrium between SRD5A2 rs523349 and SRD5A2 rs782032018.

Declaring the association of genotypes and type of hypospadias, the G34R polymorphism showed a significant association with type of hypospadias ($p=0.028$), while V89L polymorphism did not show such association ($p=0.607$) (Table 6).

Univariate and multivariate logistic regression analysis for the parameters affecting hypospadias cases reported that Binary logistic regression analysis showed that mother age and rural residence were the most independent predictors for hypospadias in the studied population

Table 1 Demographic data and clinical characters of hypospadias cases and control group

	Hypospadias cases ($N=50$)		Controls ($N=50$)		Test
	No	%	No	%	
Age in years					
Mean ± SD	3.28 ± 2.87		3.19 ± 2.95		$U=1199.50$
Min.–Max	0.90–12.0		0.70–12.0		$p=0.727$
Mother age					
Mean ± SD	30.74 ± 5.18		25.12 ± 4.15		$U=494.50$*
Min.–Max	20.0–42.0		18.0–36.0		$p<$ **0.001***
Father age					
Mean ± SD	35.66 ± 5.62		32.56 ± 5.37		$U=861.0$*
Min.–Max	25.0–50.0		22.0–49.0		$p=$ **0.007***
Consanguinity					
No	34	68.0	44	88.0	$\chi^2=5.828$*
Yes	16	32.0	6	12.0	$p=$ **0.016***
Residence					
Urban	14	28.0	37	74.0	$\chi^2=21.168$*
Rural	36	72.0	13	26.0	$p<$ **0.001***
Delivery					
Vaginal	25	50.0	28	56.0	$\chi^2=0.361$
Cesarean	25	50.0	22	44.0	$p=0.548$
Birth weight					
Normal	44	88.0	47	94.0	$\chi^2=1.099$
Low	6	12.0	3	6.0	$^{FE}p=0.487$
Gestational age					
Full term	36	72.0	47	94.0	$\chi^2=8.575$*
Preterm	14	28.0	3	6.0	$p=$ **0.003***

Bold indicated that $p<0.05$ was regarded as a significant value

SD: Standard deviation, U: Mann–Whitney test, χ^2: Chi-square test, FE: Fisher Exact, p: p value for comparing between the two studied groups

Table 2 Distribution of V89L and G34R polymorphisms among hypospadias cases and controls

	Cases ($n=50$)		Control® ($n=50$)		χ^2 (^{MC}p)	p_0	OR (LL–UL 95% CI)	p_0	AOR (LL–UL 95% CI)
	No	%	No	%					
V89L									
VV®	22	44.0	50	100.0	44.617* (< 0.001*)				
VL	25	50.0	0	0.0		–	–	–	–
LL	3	6.0	0	0.0		–	–	–	–
HWE	0.233	–							
Allele	($n=100$)	($n=100$)							
V®	69	69.0	100	100.0	36.686* (< 0.001*)				
L	31	31.0	0	0.0		–	–	–	–
G34R									
GG®	29	58.0	40	80.0	5.972* (< 0.031*)	0.027*	2.759 (1.125–6.765)	0.153	2.283 (0.724–7.850)
GR	20	40.0	10	20.0					
RR	1	2.0	0	0.0		–	–	–	–
HWE	0.242	0.432							
GR + RR	21	42.0	10	20.0	5.657* (0.017*)	0.019*	2.897 (1.187–7.067)	0.131	2.490 (0.762–8.140)
Allele	($n=100$)	($n=100$)							
G®	78	78.0	90	90.0	5.357* (0.021*)	0.024*	2.539 (1.133–5.687)	0.144	2.193 (0.765–6.287)
R	22	22.0	10	10.0					

OR: Odds ratio, χ^2: Chi-square test, MC: Monte Carlo, ®: Reference group, AOR: adjust odds ratio by Mother age Father age, Consanguinity, Residence and gestational age, CI: Confidence interval, LL: Lower limit, UL: Upper Limit, p_0: *p* value for univariate regression analysis for comparing with the reference genotype, *p*: *p* value for comparing between the two studied groups

*Statistically significant at $p \leq 0.05$

with odds ratio equal to of 1.305 and 4.833, respectively (Table 7).

4 Discussion

Hypospadias phenotypes are categorized into three groups including distal (glanular, coronal and subcoronal), middle, and posterior/proximal hypospadias (penoscrotal, scrotal, perineal), respectively [14]. This study clinically revealed that 38% of children presented with glanular hypospadias, 20% had coronal hypospadias, 18% had penoscrotal, while 14% had subcoronal hypospadias and 10% had midshaft type. This agrees with Fathi et al. [15] who showed that distal hypospadias which includes glanular and coronal is the most common type (60–70%).

Regarding hypospadias risk factors, we found higher maternal and paternal age, consanguinity; rural residence and preterm labor were significantly more in hypospadias group rather than control group. The mean maternal age of hypospadias cases was (30.74 ± 5.18) years versus (25.21 ± 4.15) years in controls and this is found to be statistically significant ($p < 0.001$). Also, multivariate logistic regression analysis showed that older mother age was an important risk predictor for hypospadias ($p = 0.001$; OR = 1.305; 95% CI 1.114–1.528). This is similar to what is concluded by Sastre et al. [16] and Fisch et al. [17] that increased maternal age is a risk factor for hypospadias and that the frequency of severe cases was more in children of mothers 35 years or older compared to mothers younger than 20 years. Increased frequency of hypospadias with advanced maternal age may be explained based on that older mothers would probably have longer exposure to endocrine disruptors than younger mothers and thus greater risk of hypospadias [18] or may be explained through the underlying genetic defects that associate with aging [19].

The mean paternal age of hypospadias cases was (35.66 ± 5.62) years versus (32.56 ± 5.37) years in controls ($p = 0.007$). Despite the difference in mean paternal age between cases and controls, both groups were not considered to have advanced paternal age. Green et al. [20] who revealed an association between advanced paternal age and risk for hypospadias and explained their finding on the basis of increased DNA mutations and chromosomal aberrations in sperm with advanced paternal age, but this was against Sastre et al. [16] who showed lack of association between paternal age and hypospadias.

The percentage of consanguinity in our hypospadias patients is 32% versus 12% in controls ($p = 0.016$). This is in agreement with Jurat et al. [21] whose study showed that the consanguinity was positive in more than half of his patients. High rates of marriages among

Table 3 Comparison between the two studied groups according to model genotyping

	Cases ($n=50$)		Control® ($n=50$)		χ^2	*p*
	No	%	No	%		
V89L						
Dominant						
VV®	22	44.0	50	100.0	38.889*	< 0.001*
VL + LL	28	56.0	0	0.0		
Recessive						
VV + VL®	47	94.0	50	100.0	3.093	$^{FE}p=0.242$
LL	3	6.0	0	0.0		
Co–dominant – 1						
VV®	22	46.8	50	100.0	35.830*	< 0.001*
VL	25	53.2	0	0.0		
Co–dominant – 2						
VV®	22	44.0	50	100.0	6.250*	$^{FE}p=0.034$*
LL	3	6.0	0	0.0		
Over–dominant						
VL®	25	50.0	0	0.0	33.333	< 0.001*
VV + LL	25	50.0	50	100.0		
G34R						
Dominant						
GG®	29	58.0	40	80.0	5.657*	0.017*
GR + RR	21	42.0	10	20.0		
Recessive						
GG + GR®	49	98.0	50	100.0	1.010	$^{FE}p=1.000$
RR	1	2.0	0	0.0		
Co–dominant – 1						
GG®	29	58.0	40	80.0	5.077*	0.024*
GR	20	40.0	10	20.0		
Co–dominant – 2						
GG®	29	58.0	40	80.0	1.353	$^{FE}p=0.429$
RR	1	2.0	0	0.0		
Over–dominant						
GR®	20	40.0	10	20.0	4.762*	0.029*
GG + RR	30	60.0	40	80.0		

χ^2: Chi-square test, FE: Fisher Exact, *p*: *p* value for comparing between the two studied groups

*Statistically significant at $p \leq 0.05$

Table 4 Comparison between the two studied groups according to Haplotype

Haplotype	Cases ($n=100.0$)		Control ($n=100.0$)		c^2	*p*
	No	%	No	%		
VG	58	58.0	90	90.0	37.967*	< 0.001*
VR	11	11.0	10	10.0		
LG	20	20.0	0	0.0		
LR	11	11.0	0	0.0		

χ^2: Chi-square test, *p*: *p* value for comparing between the two studied groups

*Statistically significant at $p \leq 0.05$

Table 5 Pair-wise linkage disequilibrium of gene polymorphisms

Variant 1	Variant 2	*D*	*D´*	*R*	R^2	χ^2	*p*
Cases							
SNP 1	SNP 2	0.042	0.152	0.218	0.048	4.760	0.029*
Control							
SNP 1	SNP 2	0	0	–	–	–	–

χ^2: Chi-square test, *D*: Linkage disequilibrium, *D´*:Standardization disequilibrium, *R*: coefficient of regression

R^2: Coefficient of determination

SNP1: V89L

SNP2: G34R

Table 6 Association of V89L and G34R polymorphisms with types of hypospadias

Types of hypospadias	V89L polymorphism				*p* value	G34R polymorphism				*p* value
	VV (*N* = 22)		VL + LL (*N* = 28)			GG (*N* = 29)		GR + RR (*N* = 21)		
	No	%	No	%		No	%	No	%	
Glanular	10	45.5	9	32.1	$x^2 = 2.715$	15	51.7	4	19.0	$x^2 = 10.849$
Penoscrotal	3	13.6	6	21.4	$p = 0.607$	4	13.8	5	23.8	**$p = 0.028$***
Coronal	4	18.2	6	21.4		7	24.1	3	14.3	
Sub coronal	4	18.2	3	10.7		1	3.4	6	28.6	
Mid-shaft	1	4.5	4	14.3		2	6.9	3	14.3	

Bold indicated that $p < 0.05$ was regarded as a significant value

x^2: Chi-square test

Table 7 Univariate and multivariate logistic regression analysis for the parameters affecting hypospadias cases (*N* = 50)

	Univariate		#Multivariate	
	p	OR (LL–UL 95% C.I)	*p*	OR (LL–UL 95% C.I)
Mother age	**< 0.001***	1.293 (1.156–1.445)	**0.001***	1.305 (1.114–1.528)
Father age	**0.008***	1.111 (1.027–1.202)	0.196	0.914 (0.798–1.047)
Consanguinity	**0.020***	3.451 (1.220–9.759)	0.325	2.062 (0.488–8.705)
Residence (Rural)	**< 0.001***	7.319 (3.025–17.705)	**0.004***	4.833 (1.639–14.250)
Gestational age (Preterm)	**0.007***	6.093 (1.627–22.815)	0.120	3.508 (0.721–17.063)
G34R (GR + RR)	**0.019***	2.897 (1.187–7.067)	0.131	2.490 (0.762–8.140)

Bold indicated that $p < 0.05$ was regarded as a significant value

OR: Odd's ratio, CI: Confidence interval, LL: Lower limit, UL: Upper Limit

All variables with $p < 0.05$ was included in the multivariate

blood-relatives are well known in my country and other Islamic countries.

Regarding residence, it was found that 72% of cases were from the rural areas versus 26% in controls ($p < 0.001$). Also, multivariate logistic regression showed that rural residence was an important risk predictor for hypospadias ($p = 0.004$; OR = 4.833; 95% CI 1.639–14.250). In another case–control study involving 440 Chinese boys, Huang et al. [22] reported rural residence as a main risk factor predisposing for hypospadias. The most plausible explanation for these finding lies in the fact that mothers in the rural areas are usually engaged in agricultural work which associates with increased exposure to pesticides.

As regard gestational age, our study revealed that there was a statistically significant difference between cases and control, ($p = 0.003$) but not for birth weight ($p = 0.487$). On the other hand, Chong et al. [23] concluded that hypospadias is associated with very low birth weight (VLBW) but not with preterm birth. However,

Ghirri et al. [24] showed that both preterm birth and birth weight seemed to be risk factors for hypospadias.

A great functional polymorphism of the SRD5A2 gene, V89L, is caused by a G to C transversion that results in the replacement of valine for leucine at codon 89. The leucine type of the enzyme is 30% less effective than valine form (decreased DHT levels) which may contribute to hypospadias [25]. Regarding genetic variants of V89L-SRD5A2 polymorphism, our study revealed that hypospadias children had higher frequency of heterozygous genotype VL and homozygous LL genotype than healthy controls ($p < 0.001$). Also, mutant L allele was more predominant in the hypospadias children ($p < 0.001$). This also coincides with Zhang et al. [26] who demonstrated in their study that V89L was a potent determinant of the risk of hypospadias and the risk was further raised in the presence of leucine allele in homozygous form.

Another major functional polymorphism of the SRD5A2 gene, named G34R, which is a point mutation at codon 34 that causes conversion of glycine to arginine leading to decrease in the enzyme activity to less than 5% of its level [27]. G34R mutation also has been reported to decrease the affinity of tissues to testosterone in vitro [28]. Regarding G34R-SRD5A2 distribution in our studied population, hypospadias children had higher frequency of heterozygous GR genotype and homozygous RR genotype than controls ($p < 0.031$). Also, mutant R allele, as the allele suspected to increase the risk, was more predominant in the hypospadias group with significant difference between cases and control ($p = 0.021$) (Table 2). Little studies have been done about association of G34R-SRD5A2 gene polymorphism with hypospadias including Akcay et al. [29] who reported that this mutation was related to severe hypospadias.

In our study, the G34R polymorphism showed a significant association with type of hypospadias, while V89L polymorphism did not show association with the type of hypospadias. Thai et al. [30] stated that SRD5A2 gene mutations are usually found only in severe cases of hypospadias not the more common and less severe variants of glandular or penile forms and they attributed the correlation between phenotype and genotype to the degree of impairment of enzyme function and reduction of the affinity of testosterone so, a stricter approach have advocated for evaluation for differences in sex development (DSD) in all proximal hypospadias associated with micropenis or undescended testes [31].

Table 7 reveals that older age of parents, positive consanguinity, rural residence, preterm delivery and G34R polymorphism of the SRD5A2 gene are strong determinants of the risk of hypospadias among children by univariate analysis. After control of confounding variables, multivariate logistic regression revealed that mother age and rural residence were the most important independent risk predictors of hypospadias so; these factors should be considered when counseling patients and advising about the most important risk factors to avoid.

Hypospadias is an important health problem and can be a significant burden on health care supplies so, it is important to increase the awareness of the community about the risk factors that may lead to the development of hypospadias.

Overall, the study including evaluation of risk factors, study of genetic background and so ability to offer genetic counseling for patients and their families is very important issue to be more extensively studied. Finally, we have some limitations in our study; first, the sample size of our research population did not allow us to differentiate effects of weak or rare risk factors. Larger studies could assist the identification of these risk factors and reserve opportunities for the further in depth investigation of the associations found to date. Second, we did not assess the androgen receptor level so; correlation between androgen receptor level and our results did not evaluate and these is recommended in future studies. Third, the results of genetic polymorphism cannot be generalized over countries due to racial differences and lack of multicenter study.

5 Conclusions

V89L and G34R Steroid 5 alpha reductase type 2 gene polymorphisms, higher maternal and paternal age, consanguinity, rural residence and preterm labor carry significant risk factors for hypospadias. On multivariate logistic regression, mother age and rural residence are the most independent predictors for hypospadias.

Abbreviations

AS-PCR	Allele-specific polymerase chain reaction
BLAST	Basic Local Alignment Search Tool
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
DSD	Differences in sex development
GR	Glycine to arginine
PCR	Polymerase chain reaction
SRD5A2	Steroid 5α-reductase type 2
VLBW	Very low birth weight
VV	Wild valine residue genotype
V89L	Valine to leucine change in codon 89

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Author contributions
WM analyzes the data. SA gave the final approval of this manuscript. MT helped in protocol development. MA collect the data. FZ wrote the manuscript and performed the surgical repair.

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Availability of data and materials
All data generated or analyzed during this study are included in this article. Further enquires can be directed to the corresponding author.

Declarations

Ethics approval and consent to participate
The study was approved by Menoufia University—Faculty of medicine ethics committee with number 7/2020 PED114. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. An informed written consentobtained from all enrolled children family prior to the study.

Consent for publication
The study protocol was approved by our local ethical committee. Written informed consent was obtained from the children family for publication and any accompanying images.

Competing interests
The authors declare that they have no competing interests.

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