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Androgen receptor gene CAG repeat polymorphism and risk of isolated hypospadias: results from a meta-analysis

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ABSTRACT. Studies investigating the association between the CAG repeat polymorphism and the risk of isolated hypospadias have reported conflicting results. The aim of this study was to quantitatively summarize the evidence for such a relationship. Two investigators independently searched the Medline, Embase, CNKI, and Wanfang databases. Weighted mean difference and 95% confidence intervals for the CAG repeat polymorphism and isolated hypospadias were calculated using a random-effects model. Subgroup analyses were performed by race, study design, sample for DNA extraction, and hypospadias classifications. This meta-analysis included 6 case-control studies, including 444 isolated hypospadias cases and 727 controls. The results showed that patients with isolated hypospadias had longer CAG repeats in their androgen receptor gene sequence (weighted mean difference = 1.36, 95% confidence interval = 0.60-2.13; P = 0.0005). Similarly, stratified analyses also detected significant associations in all subgroups, excluding the group with severe hypospadias (weighted mean difference = 0.35, 95% confidence interval = -0.42-1.12; P = 0.38). This meta-analysis indicated that longer CAG repeats were

associated with the risk of isolated hypospadias, and that longer CAG polymorphisms may be related to the etiology of isolated hypospadias. Future studies based on Asian and African-American patients should be performed to re-evaluate this association.

Key words: *AR* gene; CAG repeat polymorphism; Isolated hypospadias; Meta-analysis

INTRODUCTION

Hypospadias is one of the most common congenital malformations in males. It is defined as a urethral orifice located along the ventral side of the penis or in the scrotal position, affecting 1 in 250-300 males at birth (Nassar et al., 2007; Ghirri et al., 2009). The occurrence of hypospadias has been linked to genetic, endocrine, and various environmental factors. Although a large number of studies have been conducted to examine hypospadias, the causes are not fully understood. Genetic, endocrine, and environmental factors are thought to contribute to the etiology of isolated hypospadias (Willingham and Baskin, 2007; Kalfa et al., 2011).

The androgen receptor (*AR*) gene is located on chromosome Xq12 and is involved in generating the male phenotype through the actions of testosterone and dihydrotestosterone (Siiteri and Wilson, 1974). During weeks 8-14 of gestation, testosterone is produced by the fetal testes and metabolized in several target tissues (Sajjad, 2010). Then, dihydrotestosterone is generated from testosterone via the 5α-reductase enzyme type II. Dihydrotestosterone is the most important androgen in the development of the male external genitalia, acting through the AR (Kojima et al., 2010). Because of the important role of the AR in male sex differentiation, the *AR* gene has been extensively examined in patients with hypospadias. Some mutations in the *AR* gene have been identified, but do not appear to be a frequent cause of this genital malformation (Sutherland et al., 1996; Albers et al., 1997; Nordenskjöld et al., 1999; Boehmer et al., 2001). Genetic polymorphisms are largely responsible for inter-individual differences in the ability to activate gene transcription, and therefore may influence individual susceptibility to sex differentiation disorders. CAG repeats, a genetic variation in exon 1 of the *AR* gene, may alter the transcriptional activity of the *AR* gene based on the variable number of consecutive repeats. According to previous studies (La Spada et al., 1991; Tut et al., 1997), *AR* gene function is reduced both *in vivo* and *in vitro* as the number of CAG repeats increases.

Over the past two decades, a number of case-control studies have been conducted to investigate the association between the *AR* CAG repeat polymorphism and risk of isolated hypospadias. However, the results of these studies are conflicting. To understand the association between the CAG repeat polymorphism and the risk of isolated hypospadias, we conducted a meta-analysis of available literature.

MATERIAL AND METHODS

Publication search

We searched the PubMed, Embase, China National Knowledge Infrastructure (CNKI), and Wanfang databases to identify all articles regarding the association between CAG repeat polymorphisms and isolated hypospadias. The following key words were used: “hypospadias”

or “isolated hypospadias”, “CAG”, “AR” and “polymorphism”, or “variant”. The search was limited to human subjects regardless of the source language. The reference lists of previous reviews and meta-analyses were manually searched at the same time. If more than one article was published by the same author using the same case series, we selected the study with the largest number of participants.

Inclusion and exclusion criteria

We reviewed the abstracts of all citations and retrieved studies. The following criteria were used to include published studies: i) case-control studies conducted to evaluate the association between the CAG repeat polymorphism and the risk of isolated hypospadias; ii) sufficient data presented to calculate the mean length of CAG repeats and 95% confidence intervals (CIs); and iii) clear descriptions of the sources of cases and controls. Major reasons for excluding studies included: i) lack of controls; ii) duplication; iii) insufficient data.

Data extraction

Two investigators extracted information from all eligible publications independently according to the inclusion criteria listed above. Disagreements were resolved by discussion between the 2 investigators. The following characteristics were collected from each study: first author, year of publication, country of the first or corresponding author, ethnicity, number of cases and controls, study design [population-based case-control (PCC), hospital-based case-control (HCC)], samples for DNA extraction, mean number of CAG repeats, and 95% CIs (Table 1).

Table 1. Characteristics of studies included in the meta-analysis.

First author	Year	Design	Country	Ethnicity	Sample	CAG repeats length					
Reference						Hypospadias			Control		
						N	Mean	SD	N	Mean	SD
Parada-Bustamante A	2012	PCC	Chile	Caucasian	Blood	44	24.8	2.8	79	22.7	3.3
Radpour R	2007	PCC	Iran	Caucasian	Blood	92	22.5	3.2	190	21.8	2.8
Aschim EL	2004	PCC	Sweden	Caucasian	Blood	51	22.2	2.1	210	21.9	3.1
Adamovic T	2012	HCC	Sweden	Caucasian	Tissue/Blood	211	20.07	2.76	208	17.8	3.0
Vottero A	2011	PCC	Italy	Caucasian	Blood	20	22.6	1.6	20	21.6	1.1
Xu Zhe	2002	HCC	China	Asian	Blood	26	24.96	5.0	20	22.1	1.48

PCC = population-based case-control; HCC = hospital-based case-control; N = number of cases; SD = standard deviation

Statistical analysis

The strength of the association between the CAG repeat polymorphism and isolated hypospadias was estimated using weighted mean difference (WMD) with the corresponding 95%CIs. We carried out stratified analyses by ethnicity, the sample for DNA extraction, classifications of hypospadias, and source of controls (HCC/PCC).

Both the Cochran’s Q statistic (Cochran, 1954) to test for heterogeneity and the I^2 statistic to quantify the proportion of total variation due to heterogeneity (Higgins et al., 2003)

were calculated. A P value >0.10 for the Q statistic indicated a lack of heterogeneity across studies, allowing for the use of a fixed-effects model (Mantel and Haenszel, 1959); otherwise, the random-effects model was used (DerSimonian and Laird, 1986). Sensitivity analysis was performed to assess the stability of results.

Visual inspection of funnel plot asymmetry was used to assess potential publication bias. All analyses were conducted using the Review Manager 5.0 software (http://www.cochrane.org). All P-values were 2-sided.

RESULTS

Study characteristics

We identified 23 relevant studies in our database search. Nine publications describing the association between the CAG repeat polymorphism and hypospadias were retrieved. A total of 6 eligible studies involving 444 cases and 727 controls were included in the pooled analyses (Xu et al., 2002; Aschim et al., 2004; Radpour et al., 2007; Vottero et al., 2011; Adamovic and Nordenskjöld, 2012; Parada-Bustamante et al., 2012). The characteristics of selected studies are summarized in Table 1. There were 5 studies of Caucasian patients and 1 study of Asian patients. Studies were carried out in Chile, China, Sweden, Italy, and Iran. Controls were mainly from healthy children and matched for age and gender. Four of the studies were population-based and 2 were hospital-based. DNA was extracted from the peripheral blood in 5 of 6 studies and all studies used polymerase chain reaction-restriction fragment length polymorphism assays. Hypospadias cases were classified in 3 studies. Two studies classified hypospadias as glandular, penile, or penoscrotal, and 1 study classified hypospadias as non-severe (glandular) or severe (penile or penoscrota). We pooled the data of penile and penoscrotal hypospadias into severity classifications using software, and thus all hypospadias cases in the 3 studies were classified as non-severe or severe (Table 2).

Table 2. Characteristics of studies with hypospadias classification included in the meta-analysis.

First author	Non-severe			Severe		
Reference	N	Mean	SD	N	Mean	SD
Parada-Bustamante A	17	24	2	27	24.7	3.1
Radpour R	42	22.6	2.8	50	21.8	1.4
Aschim EL	21	22.3	2.6	30	22.1	3.6

Quantitative synthesis

Table 3 lists the main results of this meta-analysis and Figures 1-4 show the association between isolated hypospadias risk and the CAG repeat polymorphism. The results showed that the variant repeats of the CAG segment are associated with isolated hypospadias risk. A significantly higher mean CAG number was observed in hypospadias cases compared to controls (WMD = 1.36; 95%CIs = 0.6-2.13; P = 0.0005). Between-study heterogeneity was observed (I^2 = 77%).

Table 3. Stratified analyses of CAG repeat polymorphism and risk of isolated hypospadias.

Variable	No. of studies	WMD (95%CIs)	P	I^2
Total	6	1.36 [0.60-2.13]	0.0005	77%
Ethnicity	6	1.32 [0.57-2.07]	0.0005	77%
Caucasian	5	1.22 [0.42-2.02]	0.003	80%
Asian	1	2.40 [0.37-4.43]	0.02	-
Sample for DNA extraction	6	1.36 [0.60-2.13]	0.0005	77%
Blood	5	1.03 [0.42-1.65]	0.001	47%
Tissue/blood	1	2.29 [1.74-2.84]	<0.00001	-
Study design	6	1.36 [0.60-2.13]	0.0005	77%
PCC	4	0.86 [0.36-1.36]	0.0007	23%
HCC	2	2.33 [1.80-2.86]	<0.00001	0%
Hypospadias classification	3	0.54 [0.04-1.04]	0.04	36%
Non-severe	3	0.82 [0.21-1.43]	0.008	0%
Severe	3	0.35 [-0.42-1.12]	0.38	44%

WMD = weighted mean differenc.

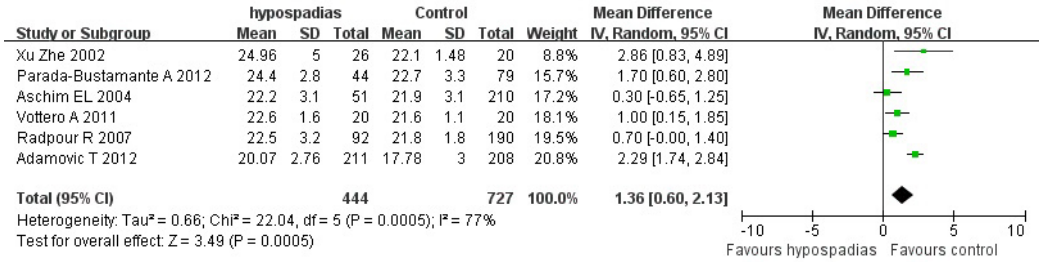

Figure 1. Forest plots of weighted mean difference (WMD) with 95% confidence intervals (CIs) for the CAG repeats polymorphism and isolated hypospadias. The center of each square represents the WMD, the area of the square is the number of samples, and thus the weight used in the meta-analysis, and the horizontal line indicates the 95%CI.

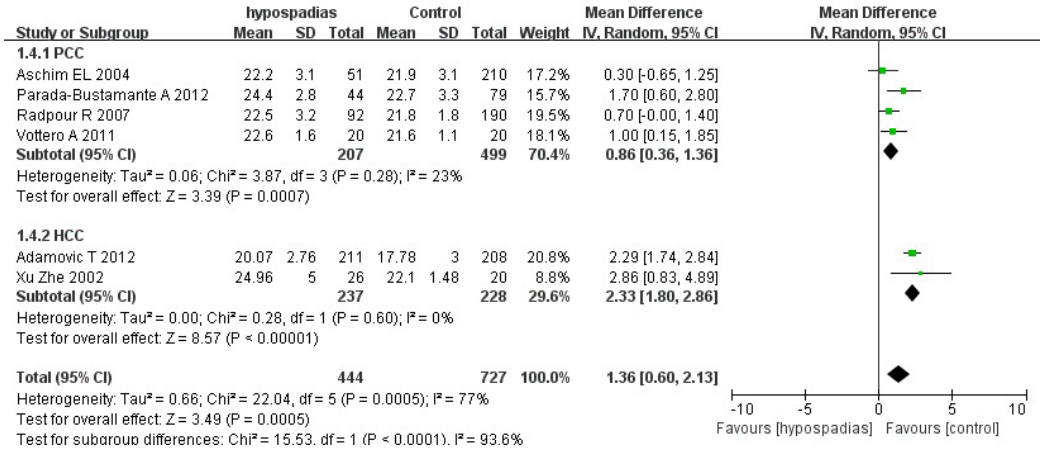

Figure 2. Forest plots for subgroups analyses (study design).

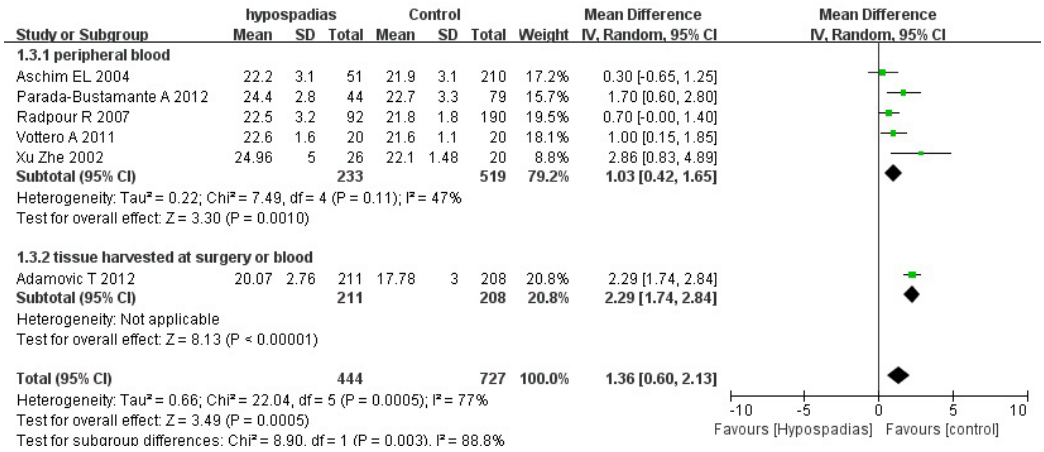

Figure 3. Forest plots for subgroups analyses (sample for DNA extraction).

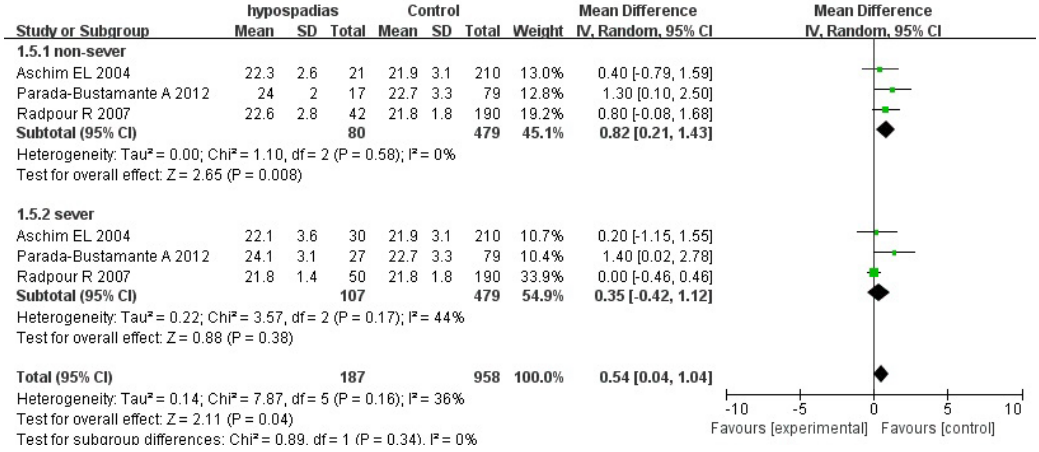

Figure 4. Forest plots for subgroups analyses (hypospadias classifications).

Similarly, associations were observed in the subgroup analyses. We stratified these studies according to ethnicity, source of controls, sample for DNA extraction, and the classification of hypospadias. Different ethnicities were categorized as Asian and Caucasian. Different classifications were categorized as non-severe and severe. In stratified analyses, the longer CAG repeats showed a significant association with isolated hypospadias in all subgroups compared with the control cases, excluding severe type hypospadias (WMD = 0.35-95%= -0.42-1.12; P = 0.38) (Table 3).

Heterogeneity and sensitivity analyses

Heterogeneity between studies was observed in the overall comparative analysis. However, when grouping the studies by source of controls and sample for DNA extraction,

heterogeneity was not observed (PCC group: I^2 =2 3%; HCC group: I^2 = 0%; blood-sample group: I^2= 47%). In the sensitivity analysis, the influence of each study on the pooled WMD was examined by repeating the meta-analysis while omitting each study at a time. This procedure confirmed the stability of our overall results.

Publication bias

A funnel plot was constructed to identify any publication bias of the literature on isolated hypospadias. Figure 5 displays a funnel plot that examines the CAG repeat polymorphism and overall isolated hypospadias risk included in the meta-analysis. The shape of the funnel plot did not reveal any asymmetry.

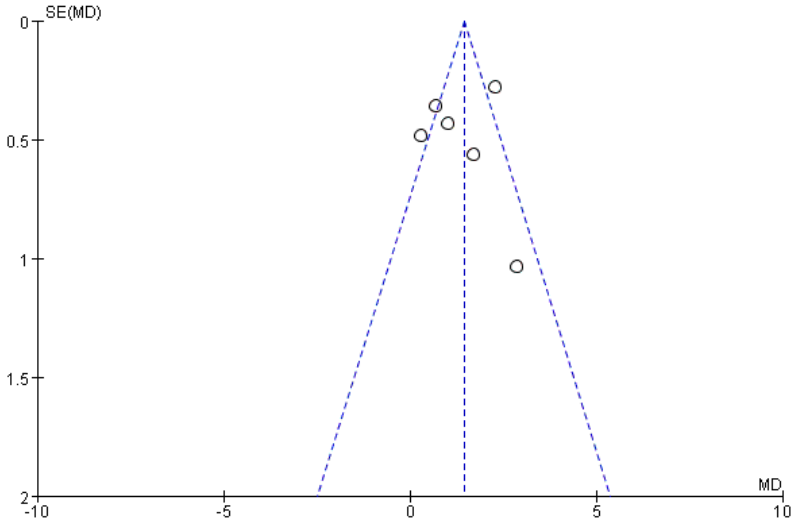

Figure 5. Funnel plot for publication bias test. Each point represents a separate study for the indicated association.

DISCUSSION

Hypospadias is one of the most common congenital malformations in males, affecting 1 in 250-300 individuals at birth (Nassar et al., 1997; Ghirri, et al., 2009). The occurrence of hypospadias has been linked to genetic, endocrine, and several environmental factors. Despite the large number of studies, the causes are not yet fully understood. The *AR* gene plays an important role in sex differentiation. The CAG repeats, locked on exon 1 of *AR*, may alter the transcriptional activity of the *AR* gene by varying the number of consecutive repeats. It has been shown that small changes in the length of the CAG trinucleotide can cause variations in androgen action (Tut et al., 1997). Thus, the CAG repeat polymorphism was studied to determine the susceptibility of isolated hypospadias. Recently, a number of molecular epidemiologic studies have been conducted to evaluate the CAG repeat polymorphism in the *AR* gene on the risk of isolated hypospadias; however, the results remain conflicting (Xu et al., 2002; Aschim et al., 2004; Radpour et al., 2007; Vottero et al., 2011; Adamovic and Nordenskjöld, 2012; Parada-Bustamante et al., 2012). As a powerful statistical method, meta-analysis can provide a quantitative approach for pooling the results of different data on the same topic.

Thus, a systematic review and meta-analysis of association between the CAG repeat polymorphism and hypospadias is important.

This meta-analysis, based on 6 case-control studies with 444 cases and 726 controls, explored the association between the CAG repeat polymorphism and isolated hypospadias. Our analysis indicated that the CAG repeats in the *AR* gene sequence are significantly longer in patients with isolated hypospadias compared to controls. When stratifying for ethnicity, source of controls, sample for DNA extraction, and hypospadias classification, the results were similar, excluding the group of severe hypospadias. Although the reasons for the apparent differences in risk with various classifications of hypospadias are unknown, some possibilities can be considered. First, hypospadias may be the result of multiple factors working together. For severe hypospadias, CAG repeats are just 1 factor but do not appear to be the most important cause. Second, some methodological diversity, such as the quality of original studies, selection bias, type of error, and small sample size, also can contribute to the discrepancy. Third, only 3 studies have reported detailed data of hypospadias classification. Thus, a lack of the information for the data may have affected the results.

The results of meta-analyses often depend on control procedures (Benhamou et al., 2002). Different control sources may be a confounding factor, which may impact the conclusion of our study. For instance, some studies used healthy children as the reference group, whereas others selected hospitalized children without isolated hypospadias as the reference group. To eliminate interference from the confounding factors, we performed subgroup analysis by source of controls. Our results showed that there is a significant association between CAG repeats polymorphism and isolated hypospadias risk in both types of controls (HCC and PCC), confirming the reliability of our overall results.

One of the major concerns in a sound meta-analysis is the degree of heterogeneity that exists between component studies, as non-homogeneous data are likely to give misleading results. In the present study, the Q-test and I^2 statistics test were carried out to evaluate the significance of heterogeneity. Heterogeneity between studies was observed in the overall comparative analysis. However, when stratifying these studies by source of controls and sample for DNA extraction, heterogeneity was not observed. These results demonstrate that the heterogeneity may have been caused by differences in controls and samples for DNA extraction. After stratified analysis, heterogeneity was eliminated and the result was consistent with the overall comparative analysis, suggesting that the results were credible. Another important issue for any meta-analysis is publication bias due to selective publication of reports. In the current study, a funnel plot was constructed to evaluate this problem. The shape of funnel plots revealed no publication bias.

However, there were some limitations to this meta-analysis: 1) only published studies were included in the meta-analysis; therefore, publication bias may have occurred, although the funnel plot did not reveal it; 2) only 3 studies have reported data regarding CAG repeats after classifying cases of hypospadias, and lack of information regarding the data analysis may have caused serious confounding bias; and 3) meta-analysis is a retrospective study that is subject to methodological limitations. In order to minimize bias, we developed a detailed protocol before initiating the study, and performed a meticulous search for published studies using explicit methods for study selection, data extraction, and data analysis. Nevertheless, our results should be interpreted with caution.

In conclusion, this meta-analysis indicated that a longer CAG repeat length is associated with isolated hypospadias. Because few studies have been conducted in the non-Cauca-

sian population, larger and well-designed multicentric studies based on Asian and African-American patients should be performed to re-evaluate the association.

REFERENCES

Adamovic T and Nordenskjöld A (2012). The CAG repeat polymorphism in the androgen receptor gene modifies the risk for hypospadias in Caucasians. *BMC Med. Genet*. 20: 109.

Albers N, Ulrichs C, Glüer S, Hiort O, et al. (1997). Etiologic classification of severe hypospadias: implications for prognosis and management. *J. Pediatr*. shou 386-392.

Aschim EL, Nordenskjöld A, Giwercman A, Lundin KB, et al. (2004). Linkage between cryptorchidism, hypospadias, and GGN repeat length in the androgen receptor gene. *J.J. En. Endocrinol. Metab*. 89: 5105-5109.

Benhamou S, Lee WJ, Alexandrie AK, Boffetta P, et al. (2002). Meta- and pooled analyses of the effects of glutathione S-transferase M1 polymorphisms and smoking on lung cancer risk. *Carcinogenesis* 23:91343-1350.

Boehmer AL, Nijman RJ, Lammers BA, de Coninck SJ, et al. (2001). Etiological studies of severe or familial hypospadias. *J. Urol*. 165: 1246-1254.

Cochran WG (1954). The combination of estimates from different experiments. *Biometrics* 10:101-129.

DerSimonian R and Laird (1986). Meta-analysis in clinical trials. *Control Clin. Trials* 7: 177-188.

Ghirri P, Scaramuzzo RT, Bertelloni S, Pardi D, et al. (2009). Prevalence of hypospadias in Italy according to severity, gestational age and birthweight: an epidemiological study. *Ital. J. Pediatr*. 35: 18.

Higgins JP, Thompson SG, Deeks JJ and Altman DG (2003). Measuring inconsistency in meta-analyses. *BMJ* 327: 557-560.

Kalfa N, Philibert P, Baskin LS and Sultan C (2011). Hypospadias: interactions between environment and genetics. *Mol Cell Endocrinol*. 335: 89-95.

Kojima Y, Kohri K and Hayashi Y (2010). Genetic pathway of external genitalia formation and molecular etiology of hypospadias. *J. Pediatr. Urol*. 6: 346-354

La Spada AR, Wilson EM, Lubahn DB, Harding AE, et al. (1991). Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. 352: 77-79.

Mantel N and Haenszel W (1959). Statistical aspects of the analysis of data from retrospective studies of disease. *J. Natl. Cancer Inst*. 22:22: 719-748.

Nassar N, Bower C and Barker A (1997). Increasing prevalence of hypospadias in Western Australia, 1980-2000. *Arch. Dis. Child*. 92: 580-584.

Nordenskjöld A, Friedman E, Tapper-Persson M, Söderhäll C, et al. (1999). Screening for mutations in candidate genes for hypospadias. *Urol. Res*. 27: 49-55*U*

Parada-Bustamante A, Lardone MC, Madariaga M, Johnson MC, et al. (2012). Androgen receptor CAG and GGN polymorphisms in boys with isolated hypospadias. *J. Pediatr. J. Endocrinol. Metab*. 25 : 157-162.

Radpour R, Rezaee M, Tavasoly A, Solati S, et al. (2007). 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*. 6228: 164-169.

Sajjad Y (2010). Development of the genital ducts and external genitalia in the early human embryo. *J. Obstet. Gynaecol. Res*. 36: 929-937.

Siiteri PK and Wilson JD (1974). Testosterone formation and metabolism during male sexual differentiation in the human embryo. *J. Clin. Endocrinol. Metab*. 38: 113-125.

Sutherland RW, Wiener JS, Hicks JP, Marcelli M, et al. (1996). Androgen receptor gene mutations are rarely associated with isolated penile hypospadias. *J. Urol. J.* 156: 828-831.

Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, et al. (1997). Long polyglutamine tracts in the androgen receptor are associated with reduced transactivation, impaired sperm production, and male infertility. *J.E. Endocrinol. Metab*. 82: 3777-3782.

Vottero A, Minari R, Viani I, Tassi F, et al. (2011). Evidence for epigenetic abnormalities of the androgen receptor gene in foreskin from children with hypospadias. *J. Clin. Endocrinol. Metab*. 96: E1953-E1962.

Willingham E and Baskin LS (2007). Candidate genes and their response to environmental agents in the etiology of hypospadias. *Nat. Clin.Nat. U. Urol*. 4: 270-279.

Xu Z, Zheng KL, Qiao H, et al. (2002). Relationship between CAG polymorphism in androgen receptor gene and congenital hypospadias. *J. Clinical Pediatric. Surgery* 1: 435-437.