Department of Pediatric Surgery, Jinan Children's Hospital, Jinan, Shandong, China

Association between *SRD5A2* polymorphism and hypospadias: a meta-analysis

Liquan Sun[#,*], Mei Zhou[#], Tao Liu

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**Corresponding author: Liquan Sun, Department of Pediatric Surgery, Jinan Children's Hospital, NO.23976, Jingshi Road, Jinan, Shandong 250022, China*
liquansun15@126.com
[#]These authors contributed equally to this article.

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Steroid 5a-reductase type-2 (*SRD5A2*) has been reported to be associated with hypospadias, but the available results are not consistent across studies. Therefore, we conducted a comprehensive meta-analysis to systematically review the association between *SRD5A2* gene polymorphisms and hypospadias. Public databases PubMed and Embase were searched before March 1, 2016. Hardy-Weinberg equilibrium (HWE) tests were performed to identify genotypic distribution of single-nucleotide polymorphisms (SNPs) in samples. Among the distribution, we only performed analyses for one SNP (rs523349). The stability of the results was evaluated by sensitivity analysis. Additionally, Egger's test was performed to test a potential publication bias. A total of 6 studies were enrolled. The genotype frequencies were almost consistent with HWE. There were significant differences among *SRD5A2* gene polymorphisms and the gene models of rs523349. The results of sensitivity analysis revealed that the results were not unduly influenced by any one study. There was a publication bias in mutational homozygote vs. Wild type homozygote (t = 5.4207, P = 0.0002) or wild type homozygote vs. heterozygote + wild type homozygote (t = 5.2649, P = 0.0002), but no evidence of publication bias was found in other models. *SRD5A2* is associated with hypospadias, and *SRD5A2* gene polymorphisms might be one of risk factors of hypospadias.

1. Introduction

Hypospadias is a male congenital malformation in which the urethral meatus is found on the ventral surface of the penis (Blaschko et al. 2012). The incidence of hypospadias is estimated to average 1 per 200-300 boys worldwide (Blaschko et al. 2012) and 5.8 per 1000 in Chinese populations (Wang et al. 2013). Recently, the incidence has shown a tendency to increase. It is the most common congenital anomaly of the penis and the second most prevalent urogenital malformations in boys following cryptorchidism (Sagodi et al. 2014). Hypospadias may be classified as simple or severe according to the anatomical location of the urethral meatus. Surgery is often necessary for treatment of hypospadias (Snodgrass et al. 2011). Additionally, patients with hypospadias may experience cosmetic or functional impairment that cause urinary and sexual dysfunction (Aulagne et al. 2010). Hypospadias has become a public health issue and imposes a substantial burden on health-care system (Bouty et al. 2015). However, the etiology of hypospadias is complex and incompletely understood.

It has been reported that environmental influences and genetic susceptibility contribute to the disease, either alone or in combination (Kalfa et al. 2011; Marrocco et al. 2015; Thai et al. 2005). However, only 30% of cases have a clear genetic cause (Sagodi et al. 2014). The development of male urethra and external genital system is an androgen-dependent process and several related genes have been identified to play a critical role in the pathogenesis of hypospadias (Wang et al. 2013). Steroid 5a-reductase type-2 (*SRD5A2*) is located on chromosome 2 and encodes the steroid 5α-reductase type 2 enzyme that is of great importance to convert testosterone to the more potent dihydrotestosterone (DHT) (Yamada et al. 2003). The variants of *SRD5A2* have been suggested to be associated with hypospadias in several studies (Makridakis et al. 2000; Samtani et al. 2010; Sata et al. 2010; Silver and Russell 1999; Thai et al. 2005), but other studies have shown inconsistent results (van der Zanden et al. 2010). Meta-analysis is a powerful and useful tool to summary inconsistent findings, which can address the problem of basing conclusions on individual studies (Egger et al. 1997b). Therefore, we systematically reviewed the literature and conducted a meta-analysis to better explore the association between variants of *SRD5A2* and hypospadias.

2. Investigations and results

2.1. Characteristics of the eligible studies

The process of selecting studies for the meta-analysis is shown in Fig. 1. Based on the search criteria, we identified a total of 160 articles that were relevant to the search terms. Among the enrolled articles, 89 articles were from the PubMed and 71 articles were

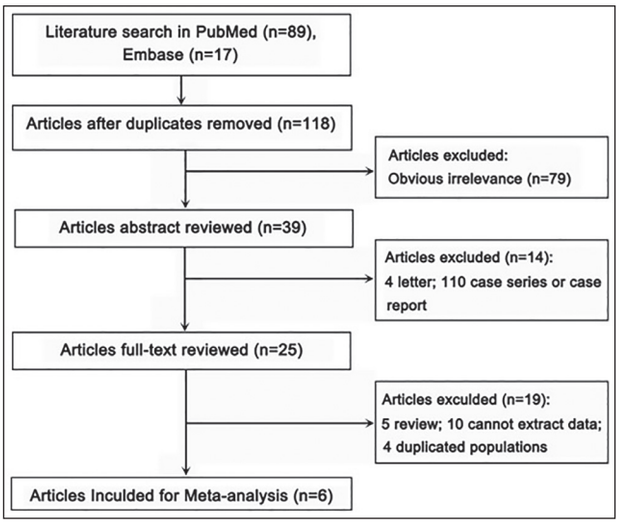

Fig. 1: Literature search and study selection

Table 1: Main characteristics of the selected studies

Author	Public year	Study location	Study Year	NOS scores	SNP	Wild type type	Hypospadias				Control				HWE X^{2*}	*HWE P-value*
							N	GG	CG	CC	N	GG	CG	CC		
Samtani R	2011	India	2008-2010	6	rs523349	G	80	19	40	21	100	43	44	13	0.108	0.7421
Wang YP	2004	China	NA	5	rs523349	G	90	16	52	22	87	31	44	12	0.338	0.5611
Zanden LFMVD	2010	Netherlands	NA	7	rs523349	G	609	55	263	291	596	51	255	290	0.232	0.6301
Sata F	2010	Japan	1999-2005	7	rs523349	G	89	23	49	17	291	89	148	54	0.300	0.5837
Thai HT	2005	Sweden	NA	5	rs523349	G	158	44	64	50	96	59	18	19	27.580	<0.001

SNP: Single Nucleotide Polymorphism; *: likelihood-ratio.; NOS: Newcastle-Ottawa Scale; N: The total number of including.

from the Embase database. After removing the duplicate documents (42 articles), 118 studies remained. Seventy nine studies that were obviously irrelevant were then excluded. The remaining 39 documents were screened by browsing titles and abstracts and 14 articles were excluded (4 letters and 10 case series or case reports). After reviewing the full text of the remaining articles, 19 articles were excluded (including 5 reviews, 10 articles in which the data could not be extracted and 4 articles with duplicated populations). Manual search of references from retrieved articles showed no additional articles. Finally, a total of six relevant studies were included in this meta-analysis (Carmichael et al. 2014; Samtani et al. 2011; Sata et al. 2010; Thai et al. 2005; van der Zanden et al. 2010; Wang et al. 2004).

2.2. Characteristics of the enrolled studies

The main characteristics of the selected studies were listed in Table 1. The publication year of these studies ranged from 2004 to 2014, and the study areas were mainly in China, USA, Japan, Netherlands, Sweden and India. The NOS scores of the included studies were all ≥ 5, indicating that the studies were medium- and high -quality. The results of HWE showed that the genotype frequencies were almost consistent with HWE. In the present study, all SNPs were examined only once, therefore, we only performed analyses for rs523349.

dominant model (wild type homozygote + heterozygote vs. wild type homozygote).

A random-effects model was performed in our meta-analysis. We found that there were significant differences among different genetic models (Table 2), indicating that *SRD5A2* was involved in the etiology of hypospadias, and *SRD5A2* was one of risk factors of hypospadias. Additionally, Egger's test was performed to ensure whether there was a publication bias. The results showed that there was a publication bias in mutational homozygote vs. wild type homozygote (t = 5.4207, $P = 0.0002$) or wild type homozygote vs. heterozygote + wild type homozygote (t = 5.2649, $P = 0.0002$), but no evidence of publication bias was found in other models. Sensitivity analysis was carried out to evaluate the stability of the results. The results of sensitivity analysis of rs523349 demonstrated that the pooled analysis did not vary substantially after exclusion of any of the included studies (Fig. 2), indicating the stability of the results. Moreover, we found that significant differences were also found in rs523349 among different gene models (Fig. 3).

3. Discussion

To our knowledge, this meta-analysis is the first one to explore the association between variants of *SRD5A2* and hypospadias. This meta-analysis investigated the associations from six studies and found evidence that rs523349 is associated with hypospadias.

Table 2: Association of rs523349 in *SRD5A2* with hypospadias

Gene	Gene model	Sample size		Test of association	Model	Egger's test for publication bias	
		Cases	Control	*OR(95%CI)*			
rs523349	C vs G	2052	2340	1.5465 [1.0357; 2.3094]	Random	2.6496	0.0770
	CG vs. GG	625	782	2.0913 [1.0773; 4.0598]	Random	1.7562	0.1773
	CC vs. GG	558	661	1.8801 [1.0606; 3.3328]	Random	2.1034	0.1262
	CC vs. GG+CG	1026	1170	1.4253 [0.9579; 2.1206]	Random	2.9974	0.0580
	CC+CG vs. GG	1026	1170	1.9390 [1.0839; 3.4687]	Random	1.5039	0.2296

Random-effects model was used when the p for heterogeneity test<0.05, otherwise the fixed-effect model was used. Egger's test to evaluate publication bias, P< 0.05 is considered statistically significant. OR: Odds ratio; CI: confidence interval.

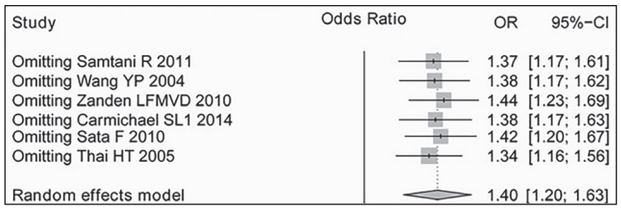

Fig. 2: Sensitivity analyses of different gene models of rs523349 OR, odds ratio; CI, confidence interval

2.3. Meta-analysis of quality assessment

In the present study, different models of *SRD5A2* were analyzed, including allele model (wild type vs. mutational), codominant model (heterozygote vs. wild type homozygote and mutational homozygote vs. wild type homozygote), recessive model (wild type homozygote vs. heterozygote + wild type homozygote), and

It has been reported that male urethra formation is fully androgen dependent during the first trimester of gestation (Chen et al. 2007). Therefore, the formation of testosterone and then its translation to DHT are important in the development and process of the internal/external genitalia, urethra and prostate. The *SRD5A2* gene has been reported to be associated with circulating sex steroid concentrations (Jiang et al. 2010). This gene consists of five exons and four introns, which is located on the short arm of chromosome 2 (2p23). It is critical to convert testosterone to DHT. More than 50 mutations have been identified, including heterozygotic mutations and homozygotic mutations (Maimoun et al. 2011). However, there appears to be no consensus in the literature about the interactions between *SRD5A2* gene polymorphisms and hypospadias. We, therefore, systematically reviewed the published articles and carried out a meta-analysis to better understand the association between variants of *SRD5A2* and hypospadias.

After reviewing the literature according to the inclusion criteria, a total of six studies were enrolled. Several SNPs were associ-

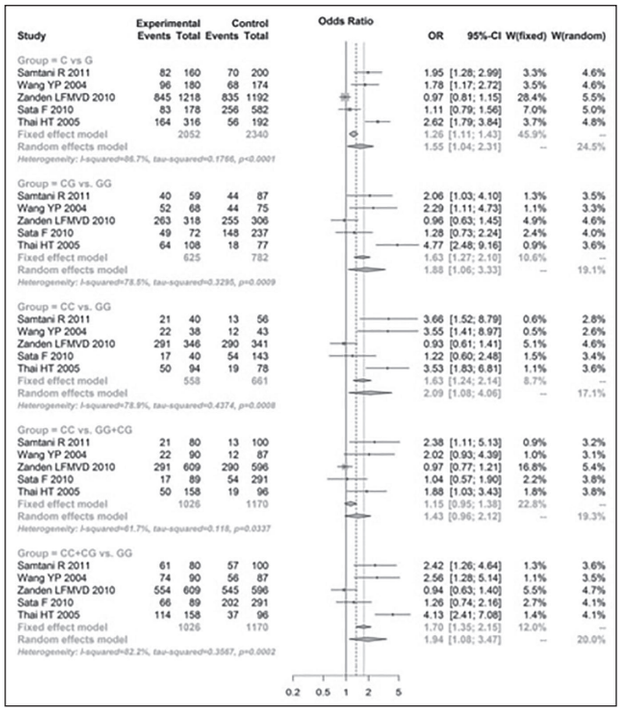

Fig. 3: Forest plots of different gene models of rs523349 OR, odds ratio; CI, confidence interval

ated with hypospadias in the different studies, but only rs523349 was examined multiple times Different gene models of this SNP were analyzed, including wild type vs. mutational, heterozygote vs. wild type homozygote, mutational homozygote vs. wild type homozygote, wild type homozygote vs. heterozygote + wild type homozygote, wild type homozygote + heterozygote vs. wild type homozygote. A random-effects model was used throughout our meta-analysis. In addition, the results of *SRD5A2* polymorphism in this meta-analysis indicated statistical significance, implying that *SRD5A2* is associated with the etiology of hypospadias. Additionally, the results of Egger's test demonstrated that a publication bias was found in mutational homozygote vs. wild type homozygote and wild type homozygote vs. heterozygote + wild type homozygote, but no statistical evidence of publication bias was found in other models. The stability of the results was confirmed by sensitivity analysis. We analyzed *SRD5A2* polymorphism rs523349. *SRD5A2* polymorphism rs523349 (V89L) is caused by a G-to-C transversion, leading to the substitution of valine for leucine at codon 89 (Jiang et al. 2010). Variant rs523349 has been reported to be associated with a higher prevalence of metabolic syndrome (Boer et al. 2016) and hypospadias (Samtani et al. 2010; Sata et al. 2010; Thai et al. 2005; Wang et al. 2013). The C allele results in significant decline in enzyme activity. In our study, the data showed that variant rs523349 was a potential risk factor of hypospadias.

However, because of some limitations, our study should be cautiously interpreted. The first one might influence the authenticity of the results. Second, publication bias analysis of rs523349 was not analyzed because there were not more than ten eligible studies with respect to the rs523349 gene. Third, the results of HWE showed that not all papers were consistent with HWE, indicating that the representative sample is not optimal in terms of accuracy In addition, no HWE results could also result from genotyping errors. Fourth, the inconsistency of the included study probably influences our results. For example, the study of Carmichael et al. also examined rs523349, which was the largest study performed. That study found no significant results about the SNP. However, we were unable to include the study because we could not access the individual results..

In conclusion, our meta-analysis confirms the significant association between *SRD5A2* gene polymorphisms and hypospadias. *SRD5A2* polymorphisms may represent an important risk factor for hypospadias. However, more quality and individual studies with large sample sizes studies should be performed to verify the results.

4. Experimental

4.1. Data sources and searches

We systematically searched electronic databases such as PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and Embase (http://www.embase.com). In addition, a manual search of the literature was carried out to identify more relevant studies. Medical subject headings (MeSH) or free-text words were used to select search terms. The keywords used for search were: ("*SRD5A2*" OR "steroid 5α-reductase type-2" OR "steroid 5α-reductase type II" OR "steroid 5 alpha-reductase 2" OR "3-oxo-5-alpha-steroid 4-dehydrogenase 2" OR "type II 5-alpha reductase") AND ("hypospadias" OR "hypospadia"). The deadline for the search was March 1, 2016.

4.2. Inclusion and exclusion criteria

The inclusion criteria were as follows: 1) the study was aimed to explore the correlation between *SRD5A2* and hypospadias; and 2) the study included information about the distribution of *SRD5A2* in both the hypospadias group and non- hypospadias group or the information including odds ratio (OR) and 95% confidence interval (CI), or data to calculate them. Studies were excluded if they were reviews, reports, comments, or letters.

4.3. Data extraction and quality assessment

Data were extracted independently by two investigators based on the inclusion criteria and any discrepancies were resolved by consensus with a third investigator. For each study, the following important information was extracted: the first author's name, publication year, study year, study area, patient's numbers in case group (hypospadias group) and control group (non-hypospadias group), analysis of single-nucleotide polymorphisms (SNPs) in *SRD5A2*, and number of participants with *SRD5A2* in both case and control group, etc. Newcastle-Ottawa Scale (NOS) was performed to assess the quality of the literature in the meta-analysis (Wells et al. 2000). NOS is a 9-point scale composed of the following ratings: ≥ 7 (high-quality), 4-6 (medium quality), and ≤ 3 (low quality).

4.4. Statistical analyses

Hardy-Weinberg equilibrium (HWE) tests were performed using χ^2 tests (Schaid and Jacobsen 1999). Statistical analysis was conducted using the software R 3.12, and the effect sizes were OR and 95% CI (Liu et al. 2013). A P-value of less than 0.05 was considered as statistically significant. Sensitivity analysis was conducted by removing studies one by one to confirm the robustness of the results (Ma et al. 2015). Egger's test was performed to test a potential publication bias (Egger et al. 1997a).

Conflicts of interest: None declared.

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