Family based genetic study in proximal penile hypospadias with undescended testis

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Abstract. Objective: Hypospadias is known to have familial inheritance because of multifactorial etiology. Little is known about the genetic polymorphism involved in hypospadias. A few genetic associations have been reported but mainly in studies of small sample size and were based on case control studies. The aim of this study was to evaluate whether the genetic polymorphism usually seen in the affected siblings, also seen in other 1st degree relative of the family (father, mother, brothers). Methods: Prospective analysis of 60 cases of penoscrotal/ scrotal hypospadias with unilateral or bilateral undescended testis and their 1st degree relatives (father, mother and brother) was done. Inclusion criteria included cases of penoscrotal/ scrotal hypospadias with 46 XY karyotype. Exclusion criteria included cases of disorders of sexual differentiation, lost to follow up or not giving consent for study. Investigations include hormonal assessment of cases, karyotype, genitogram, and genetic assessment by allele-specific polymerase chain reaction. Results: Mean age at presentation was 3.7 years (range 2.2-7.5 years). Penoscrotal hypospadias with unilateral undescended testis was seen in 27(45%), with bilateral UDT in 9(15%), scrotal hypospadias with unilateral undescended testis (UDT) in 18(30%), with bilateral UDT in 6(10%). Mean level of serum testosterone, leutinising hormone (LH), follicular stimulating hormone (FSH) was 0.003 (ng/ml), 0.168 (ng/ml), 0.214(ng/ml) respectively. Most common genetic polymorphism seen was V89L polymorphism in SRD5A2 gene. Conclusion: Hypospadias is a congenital disorder having multifactorial etiology including familial inheritance, however mothers are a carrier for genetic polymorphism is highly unlikely.

Key words: Penoscrotal hypospadias, undescended testis, genetic polymorphism

Introduction

Hypospadias and cryptorchidism are the most common congenital malformations in boys, with prevalence at birth of 1%–9% and 0.4%–0.8%, respectively. The reported incidence of hypospadias with undescended testis is about 9%. Hypospadias clusters within families; 7% of hypospadias cases have a first, second, or third degree relative with hypospadias, whereas the expected rate of familial cases among the general population is 3%. This familial aggregation is believed to be caused by genetic rather than intrauterine environmental factors. Various genetic polymorphism has been reported in cases of hypospadias, for example in the genes encoding the androgen receptor (AR), steroid-5α-reductase (SRD5A2), hydroxy-δ-5-steroid dehydrogenase (HSD3B2) as well as in the genes coding for homeobox A4 and B6 (HOXA4 and HOXB6). Despite having evidence of familial pattern of inheritance, most studies done so far are only case control study that too involving hypospadias only.

Therefore, the aim of this study was to investigate whether the previously reported associations of single-nucleotide polymorphisms (SNPs) in genes involved in hormonal pathways are having a familiar pattern of inheritance in cases of penoscrotal/ scrotal hypospadias with undescended testis.

Materials and Methods

Prospective analysis of 60 cases of penoscrotal/ scrotal hypospadias with unilateral or bilateral undescended testis and their family members (father, mother, brother) registered in our urology clinic between December 2011 and May 2013 was done. Ethical clearance was taken for the study from ethical committee of our institute. Inclusion criteria included children with penoscrotal or scrotal hypospadias with unilateral or bilateral undescended testis, having father,
mother and brothers available for assessment. Exclusion criteria included children with confirmed diagnosis of disorders of sexual differentiation, children with multiple associated malformations, not giving consent and lost to follow up. Investigations included clinical examination, karyotype, genitogram, ultrasonography, magnetic resonance urography if required, hormonal assessment testosterone, LH, FSH. After informed parental consent, 3ml Intravenous blood was collected from cases and from their family members (father, mother and brothers) in EDTA-anticoagulated vacutainer for the purpose of the study. Genomic DNA was extracted from peripheral blood lymphocytes by phenol chloroform extraction method. Primers SRD5A2 were designed. The V89L polymorphism was analyzed using allele-specific PCR as described by Thai et.al and custom-synthesized primers (Sigma Aldrich Chemicals Pvt. Ltd, Bangalore, India), the nonspecific forward primer (5'-ACACGAGAGCCTGAAGC-3'), the nonspecific reverse primer (5'-TCGGTGCGCGCTCCACG-3'), and either the valine-specific (5'-AACGCTACCTGTGGAA GTAATGTA-3') or the leucine-specific (5'-ACGCTACCTGTGGAA GTAATGTA-3') primer. Amplifications were performed in a 0.2-mL, thin-walled tubes using 20 ng of DNA, 4 pm of each primer, 200 mM dinucleotide triphosphates, 10 X PCR buffer, 1.5 mM MgCl₂, and 0.5 units of DyNAzyme II DNA Polymerase (Thermo Scientific). The PCR reaction was carried out in a T-100 DNA Engine (Bio-Rad, Hercules, CA, USA) Thermal as follows: initial denaturation-94°C for 5 min, denaturation-94°C for 1 min, annealing-63°C for 1 min, extension-72°C for 1 min, final extension-72°C for 7 min and 4°C forever repeated for 40 cycles. Amplicons size were resolved in a 2% Agarose gel with the 100bp maker and stained with ethidium bromide, visualized under UV light, and photographed (Fig 1).

Results

Median age at presentation was 48.1 months (range 18 – 108 months). Penoscrotal hypospadias with unilateral undescended testis was seen in 27(45%), penoscrotal hypospadias with bilateral undescended testis in 9(15%), scrotal hypospadias with unilateral undescended testis in 18(30.0%) while scrotal hypospadias with bilateral undescended testis was seen in 6(10%) cases. Demographic profile, hormonal profile, karyotype are shown in table 1. Results of genetic analysis are shown in table 2. Mean level of serum testosterone, leutinising hormone (LH), follicular stimulating hormone (FSH) was 0.003 (ng/ml), 0.168 (ng/ml), 0.214 (ng/ml). Most common genetic polymorphism seen was V89L polymorphism in SRD5A2 gene.

### Table 1. Clinical diagnosis, hormone levels, dietary habits and maternal age of patients

<table>
<thead>
<tr>
<th>Clinical diagnosis</th>
<th>No of cases (n= %)</th>
<th>Mean age (months)</th>
<th>S.T (ng/ml)</th>
<th>LH (ng/ml)</th>
<th>FSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penoscrotal hypospadias with unilateral descended testis</td>
<td>27 (45%)</td>
<td>52.4</td>
<td>0.052</td>
<td>0.028</td>
<td>0.399</td>
</tr>
<tr>
<td>Penoscrotal hypospadias with bilateral descended testis</td>
<td>9 (15%)</td>
<td>55.5</td>
<td>0.051</td>
<td>0.281</td>
<td>0.244</td>
</tr>
<tr>
<td>Scrotal hypospadias with unilateral descended testis</td>
<td>18 (30%)</td>
<td>47.6</td>
<td>0.046</td>
<td>0.330</td>
<td>0.315</td>
</tr>
<tr>
<td>Scrotal hypospadias with bilateral descended testis</td>
<td>6 (10%)</td>
<td>36.8</td>
<td>0.041</td>
<td>0.369</td>
<td>0.327</td>
</tr>
<tr>
<td>Maternal age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;30 years</td>
<td>33</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>&lt;30 years</td>
<td>27</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dietary</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vegetarian</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nonvegetarian</td>
<td>41</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

S.T=Serum testosterone, LH=Leutinising hormone, FSH=Follicle stimulating hormone
**Discussion**

Hypospadias is defined as a malformation of the penis due to an incomplete development of the ventral part of the penis. This may include a defect in the developing urethra leading to the localization of the urinary meatus on the ventral aspect of the penis in a variable position from the glans to the perineum, defect in the ventral part of the prepuce, and inconstant ventral penile curvature mainly related to a defect in the ventral skin or, more rarely, the development of the corpus cavernosum. Hypospadias is the second most common congenital malformation in males, occurring in approximately 1 in 125 live male births. Androgens play a central role in male external genital development. Testosterone and its derivative 5 alpha-dihydrotestosterone are the two major androgens that mediate male sexual differentiation, and an alteration in the androgen sensitivity pathway has been identified in undermasculinized boys.

SRD5A2 encodes an enzyme that converts circulating testosterone in the genital tubercle to the more potent androgen dihydrotestosterone, which stimulates normal differentiation and development of the genital tubercle into the external genitalia. The V89L polymorphism (rs523349, +336G>C) has been investigated by several studies. Three studies have reported two to three fold increased risk associated with heterozygosity or homozygosity for the C allele, among Chinese, Caucasian and Indian population. Interestingly all the studies are only on case-control cohort based, as per our knowledge till date no study was conducted on family based trios. For some authors, the V89L variant of the SRD5A2 gene is a risk factor for hypospadias, whereas for others it is not.

So, in the present study we have analyzed the association of V89L polymorphism of SRD5A2 gene with family trios of unknown family background (i.e occupation). We are not able to find out significant association result in the family based trios study which is in consonance with study done by Tria et al. Our study also tried to correlate the dietary habit and maternal age as compounding factors for hypospadias with undescended testis but we could not find any association, may be because of small sample size of our study.

**Conclusion**

Hypospadias is a congenital disorder having multifactorial etiology including familial inheritance, however, mothers are a carrier for genetic polymorphism is highly unlikely.

**References**