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A49T, R227Q and TA repeat polymorphism of steroid 5 alpha-reductase type II gene and Hypospadias risk in North Indian children

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ABSTRACT

Background/Aims: Hypospadias is a common congenital error of genital development, the frequency of which is increasing. As androgens have a significant role in the development of the male urethra, we sought to investigate the association between functional polymorphisms of SRD5A2 gene in relation to hypospadias.
Methods: We examined DNA samples of 96 cases and 105 controls for SRD5A2-A49T, R227Q and TA repeat gene polymorphisms.
Result: Absence of 49T locus and 227Q locus was observed in the present study. At the (TA) n repeat site, TA (0) allele was observed to be the most common allele in both cases (91.7%) and controls (90%). TA (9/9) genotype exhibited an odds ratio of 3.03 (95% C.I. = 0.18–50.14, $p = 0$) with respect to only middle phenotypes. Analysis of the demographic data depicted the agricultural background aspect of the parents of the cases. 72.27% of the cases (affected with Hypospadias) have parents having agriculture as a primary occupation.
Conclusion: As longer TA repeats are associated with lower enzymatic activity and lower DHT levels as reported among Caucasians, this polymorphism may have an effect (rather small) in predisposing the population of the present study to the risk of Hypospadias of lesser severity. Due to small sample size, the 3.03 O.R. is not significant and a larger sample is needed to validate the results.
Large scale screening of Hypospadias and other 46 X,Y disorders of sexual development is needed especially in India, where the majority of the population is from agricultural background. The results of the present

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study are likely to assist the health planners to initiate screening of Hypospadias among the farmer community to combat the risk of Hypospadias.

Introduction

Hypospadias is a common congenital anomaly of genital development in which the urethral orifice is found on the ventral side of the penis rather than at the tip. The incidence of hypospadias is 1 in 125 to 300 live male births in different ethnic populations (Paulozzi et al., 1997; Myrianthopoulos and Chung, 1974), and has reportedly increased during the last few decades. Hypospadias may be classified as simple (glanular or penile) or severe (scrotal or perineal) based on the anatomical location of the urethral meatus. It is regarded as a complex disorder caused by genetic and environmental influences. As formation of the male urethra/male external genitalia during the first trimester of gestation is fully androgen dependent, therefore, it appears a more reasonable theory to explain Hypospadias as an abnormality in the androgen metabolic pathway. Thus, the biosynthesis of testosterone (T) and its conversion to dihydrotestosterone (DHT), a more active androgen, play a crucial role in inducing the formation of internal/external genitalia. Genetic polymorphisms in genes controlling androgen action and biosynthesis of testosterone and dihydrotestosterone are likely to be important in the etiology of hypospadias.

The type II steroid 5 alpha-reductase enzyme encoded by SRD5A2 gene on chromosome 2 irreversibly metabolizes the conversion from testosterone to DHT (Di Salle et al., 1998). It is known that certain SRD5A2 polymorphisms may encode for 5 alpha reductase enzyme variants with different activities, probably because of altered mRNA stability (Reichardt et al., 1995).

A49T polymorphism (an alanine residue at codon 49 which is replaced by a threonine) increases by 5-fold the steroid 5 alpha-reductase activity in vitro (Makridakis et al., 1999). The T variant has been found to be more prevalent in Caucasians (3.5%) than African–American, Asians or Hispanics (Jaffe et al., 2000). Till date, only one study by Silver and Russell (1999) conducted among Caucasian men demonstrated the presence of A49T in homozygous form among severe cases of Hypospadias and in heterozygous form among mild cases.

The SRD5A2 gene harbors a polymorphic site at the 3′ untranslated region (3′ UTR) where a variable number of dinucleotide TA repeat length exists. The 3′ untranslated region of the SRD5A2 gene contains either no TA repeats [(TA) 0] or 9 [(TA)9] or 18 [(TA)18] repeats. Variations in the length of these dinucleotide repeats have been reported to influence the enzymatic activity of SRD5A2 (Bharaj et al., 2000). No data exists till date concerning its study on Hypospadias. According to Scorilas et al. (2001), increase in TA lengths may be associated with relative messenger instability and decreased levels of the 5 alpha reductase activities. In contrast, study by Allen et al. (2003) suggest longer TA repeats (TA (9)/TA (9)) to be associated with elevated enzymatic activity. However, longer repeats in Caucasians have been linked to down-regulation of the enzyme activity, thereby lowering the DHT production (Kantoff et al., 1997a).

Another mutation of SRD5A2 gene, R227Q variant is known to significantly reduce the Steroid 5 alpha reductase activity by 3.2% of the normal activity found mainly in the Chinese and Japanese populations (Forti et al., 1996).

The present study investigated the role of all these polymorphism in Hypospadias causation as these may alter (reduce) the dihydrotestosterone levels (DHT).

Our previous case–control study (Samtani et al., 2001) highlighted the role of leucine allele of V89L polymorphism in the manifestation of Hypospadias. In the present study, the association of A49T, R227Q and TA repeat sequences of SRD5A2 gene in Hypospadias causation is assessed using a case–control design.

Method

The protocols of the study are approved by the Departments Ethics Committee of the institution (University of Delhi) and All India Institute of Medical Sciences (AIIMS).

A total of 96 boys with isolated hypospadias 4–10 years old were prospectively entered in the study. All patients included were 46, XY. Patients with undescended testis, intersex conditions or known endocrine

abnormalities were excluded from the study. All cases were diagnosed at the Department of Pediatric Surgery. All India Institute of Medical Sciences. Pre-operative position of ureathral meatus was noted and phenotypes were graded as mild, medium or severe.

A total of 105 geography (Northern-India), ethnicity (Caucasians), language (Indo-European) matched controls from the general North Indian population without hypospadias or any history of genital abnormalities were collected via house to house surveys. Demographic and clinical data pertaining to the reproductive profile of the mother and family history of genital abnormalities were recorded for cases and controls.

3 ml intra-venous blood samples were collected in ethylenediaminetetraacetic acid coated tubes after receiving informed written consent from the parents for both cases and controls. Genomic DNA was extracted from blood using Qiagen DNA extraction kits (Qiagen). A49T and TA repeat sequences were genotyped using the protocol of Yang et al. (2002). For genotyping of R227Q polymorphism, protocol of Guang et al. (1999) was used.

Odds ratio (OR) as a measure of the relative risk of hypospadias and 95% confidence interval were calculated by standard method (Woolf, 1995). The prevalence of polymorphism was compared between patients and controls with the use of chi-square test. $p < 0.05$ was taken to be statistically significant.

Results

Demographic data for the 2 groups are outlined in Table 1. Significant differences were observed between cases and controls with respect to diet ($p < 0.05$). Analysis of the demographic data depicted the agricultural background aspect of the parents of the cases. 72.27% of the cases (affected with Hypospadias) have parents having agriculture as a primary occupation (Fig. 1).

On the basis of hypospadias severity, the cases of the present study ($N = 101$) were categorized into three groups, of which, 9.9% were of Mild phenotype, 34.6% constitute the Middle phenotype and 55.4% were Severe phenotypic cases (Table 2).

With respect to polymorphisms, the SRD5A2 gene was found to be polymorphic at the loci (TA repeats) in both Hypospadias cases and controls. Both the studied groups i.e. cases and controls were in Hardy–Weinberg equilibrium with respect to the genotypic distribution of TA repeats ($p > 0.05$). At the (TA)n repeat site,

Table 1
Demographic characteristics—potential risk factors and confounders for hypospadias, unadjusted ORs, and 95% CIs.

Characteristic	Cases $N = 101$	Controls $N = 110$	Odds ratio 95% C.I.	*p*-value
Maternal age at first birth				
Less than 20 years	22 (21.8%)	16 (14.5%)	1.00	
21–30 years	76 (75.2%)	85 (77.2%)	1.57 (0.73–3.37)	0.32
More than 30 years	03(2.9%)	09 (8.2%)	0.24 (0.05–1.04)	0.09
Age at menarche				
<14 years	33(32.7%)	38(34.5%)	1.00	
≥14 years	68(67.3%)	72(65.5%)	1.08 (0.61–1.92)	0.884
Birth weight of the affected child (g)				
Normal (≥2500 g)	76(75.2%)	93(84.5%)	1.00	
<2500	25(24.8%)	17(15.5%)	1.8 (0.9–3.57)	0.11
Folic acid supplements in pregnancy				
No	41 (51.25%)	46 (46%)	1.00	
Yes	39 (48.75%)	54 (54%)	0.8 (0.44–1.46)	0.58
Diet				
Non-vegetarian	29 (36.25%)	62 (62%)	1.00	
Vegetarian	51 (63.75%)	38 (38%)	2.86 (1.5–5.27)	0.001

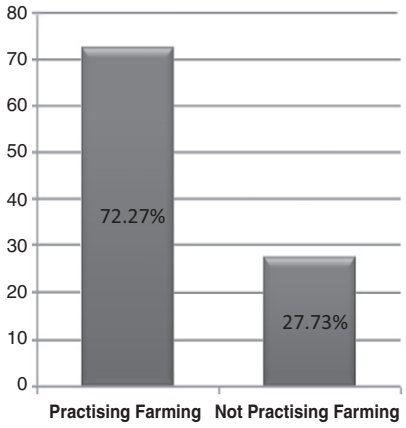

Fig. 1. Frequency of parental agriculture background (cases).

TA(0) allele was observed to be the most common allele in both cases (91.7%) and controls (90%) while TA(9) allele occurred at a very low frequency among cases (8.3%) and controls (10%). TA (0/0), TA (0/9), and TA (9/9) genotypes were observed at frequencies of 84.37%, 14.58% and 1.04% for cases and 80.95%, 18.1%, 0.95% for controls, respectively. Compared to TA (0/0) genotype, TA (0/9), TA (9/9) and TA (0/9) + TA (9/9) genotypes exhibited an odds ratio of 0.77 (95% C.I. = 0.36–1.64, $p = 0.57$), 1.04 (95% C.I. = 0.06–17.05) and 0.787 (95% C.I. = 0.377–1.642, $p = 0.579$) fold risk respectively (Table 3).

Further, in order to estimate the risk of a particular genotype (TA(0/0), TA (0/9), TA (9/9) in the development of severe and middle hypospadias, odds ratio was calculated between severe phenotype and controls, and also between middle phenotype and controls using a 2 × 2 contingency table. Compared to TA (0/0) genotype, TA (0/9), TA (9/9) and [TA (0/9) + TA (9/9)] genotypes exhibited an odds ratio of 0.95 (95% C.I. = 0.34–2.63, $p = 1$), 3.03 (95% C.I. = 0.18–50.14, $p = 0$) and 1.14 (95% C.I. = 0.43–3.00, $p = 0.803$) fold risk respectively with respect to middle phenotypes (Table 4).

Further, monomorphism at A49 locus and R227 locus was observed in the present study.

Discussion

Enzyme 5 alpha reductase type 2 encoded by SRD5A2 gene, converting testosterone to a more potent dihydrotetosterone, is required in the genito-urinary tract for normal development of the male external genitalia. The frequency distribution of 3 phenotypes of Hypospadias based on severity suggests that our study population was more prone to exhibit severe hypospadias. Of all the markers (i.e. A49T, R227Q, TA repeats) considered in the present study, the SRD5A2 gene was found to be polymorphic only for TA repeats in both

Table 2
Frequency distribution of Hypospadias cases on the basis of phenotypic severity.

	Phenotypic variability ($N = 101$)	Frequency distribution (%)
Mild phenotype ($n = 10$)		9.90
1.	Coronal ($n = 8$)	
2.	Glandular ($n = 2$)	
Middle phenotype ($n = 35$)		34.65
3	Mid-penile ($n = 13$)	
4.	Distal penile ($n = 22$)	
Severe phenotype ($n = 56$)		55.44
5.	Penoscrotal ($n = 38$)	
6.	Proximal penile ($n = 9$)	
7.	Scrotal ($n = 9$)	

Table 3
Genotypic and allelic distribution of SRD5A2 gene polymorphisms.

Genotype	Cases	Controls	Crude odds ratio	95% confidence interval	*p*-value
SRD5A2 (TA(n) repeats	$N = 96$	$N = 105$			
TA(0/0)	81 (84.38%)	85 (80.95%)	1.00	Reference	
TA(0/9)	14 (14.58%)	19 (18.10%)	0.77	0.36–1.64	0.57
TA(9/9)	01 (1.040%)	01 (0.950%)	1.04	0.06–17.05	1
TA(0/9) + TA(9/9)	15 (15.62%)	20 (19.05%)	0.787	0.377–1.64	0.579
SRD5A2 A49T	$N = 96$	$N = 105$	Crude odds ratio	95% C.I	*p*-value
AA	96	105	–	–	–
AT	0	0	–	–	–
TT	0	0	–	–	–
SRD5A2 R227Q	$N = 96$	$N = 105$	Crude odds ratio	95% C.I	*p*-value
RR	96	105	–	–	–
RQ	0	0	–	–	–
QQ	0	0	–	–	–

Hypospadias cases and controls. Variations in the length of these dinucleotide repeats (TA repeats) have been reported to influence the enzymatic activity of SRD5A2 gene (Bharaj et al., 2000). As longer repeats in Caucasians have been linked to down-regulation of the enzyme activity, thereby lowering the DHT production which lowers the cancer risk (Kantoff et al., 1997b), it can be hypothesized that in population of the present study, longer TA repeats may lead to lower DHT levels which may subsequently lead to abnormal genitalia formation causing Hypospadias. Though the result of the present study did not indicate a direct association of TA repeats with the risk of Hypospadias, it raises the possibility that genetic alterations in the 5-alpha reductase activity at the TA repeat locus may have a role in causing disorders related to external genitalia formation. Due to small sample size, the O.R. is not significant and a larger sample is needed to validate the results. Further studies will be required to examine the role of TA repeats in Hypospadias progression.

The A49T missense substitution has been reported to significantly increase the apparent maximal steroid 5α-reductase activity leading to an increase risk of prostate cancer (due to higher interprostatic DHT) in different populations (Zeigler-Johnson et al., 2002). The frequency of the T allele (rather small) has also been observed in most European groups; however, the findings of Zeigler-Johnson et al. (2002) suggest the tendency of A49T mutation to carry through certain ethnic groups. On comparing A49T frequencies across various ethnic groups, the occurrence of the T allele was found to be rare in all groups ranging from a frequency of 0 among Asians to 0.025 in Caucasians. Overall, T allele frequency of 0.025 for Caucasians, 0.014 for African–Americans, 0.005 for Ghanians and 0.009 for Senegalese is observed (Zeigler-Johnson et al., 2002). Monomorphism was observed in the present study with respect to A49T locus. Going by the frequency distribution of A49T polymorphism across various populations of the world, zero frequency of T allele is observed in most ethnic groups.

Table 4
Distribution of SRD5A2 (TA repeats) by Hypospadias severity.

Phenotype	SRD5A2 gene (TA n (repeat)			
	TA(0/0) Crude ORs	TA(0/9) Crude ORs 95% C.I.	TA(9/9) Crude ORs 95% C.I.	TA(0/9) + TA(9/9 Crude ORs 95% C.I.
Middle phenotype ($N = 35$)	1.00	0.95 (0.34–2.63) $P = 1$	3.03 (0.18–50.14) $P = 0.44$	1.14 (0.43–3.00) $P = 0.803$
Severe phenotype ($N = 56$)	1.00	0.76 (0.31–1.87) $P = 0.659$	NAN	0.72 (0.3–1.7) $P = 0.519$
Controls (%)	80.95%	18.1%	0.95%	19.05%

Due to small sample size, mild phenotype cases were eliminated from analysis.

Going by theory, as T allele is known to increase the enzymatic activity, higher DHT/T ratio, it is likely to be a protective phenomenon for Hypospadias causation. In that case, one would expect T allele frequency to rise in most populations of the world due to its protective effect. As this is not the case observed, two possible theories can be hypothesized to suggest its low frequency in most populations. One of them could be due to the deleterious effect of T allele rendering it to be so lethal that the individual fails to survive with its mere presence. Second, A49T mutation might have been recently introduced into the human genome and since it being a recent phenomenon; one would expect its frequency to rise in populations in times to come. Considering the latter hypothesis to be true, extensive epidemiological studies and functional analysis should be pursued for the rare 49 T missense substitution of the SRD5A2 gene.

Study by Guang et al. (1999), examined the role of R227Q mutation in isolated hypospadias cases among patients of Mongoloid ethnicity. Sasaki et al. (2003) observed homozygosity for Q allele among two brothers of Vietnamese origin (Mongoloid), one presented with scrotal hypospadias with a bifid scrotum and a small penis and the other presented with a small penis but otherwise normal male genitalia having abnormally high T/DHT ratio. Consistent with the results of the present study, absence of R227Q locus was also observed among Greenlanders and Scandinavians (Caucasians) (Giwercman et al., 2008). However, a study by Vilchis et al. (1997) suggested that the mutation at codon 227 impaired normal 5 alpha-SR2 function, thus leading to the phenotypical expression of 5 alpha reductase deficiency, an autosomal recessive disorder with variable expressivity including isolated hypospadias. In Indian context, population based screening of R227Q mutation for 46,XY DSD cases/ambiguous genitalia especially among the Mongoloid groups of North Eastern is needed.

The etiology of hypospadias is often assumed to be multifactorial, implicating a gene–environment interaction in the development of this anomaly. Vegetarianism was found to be associated with hypospadias in the present study. These results are in agreement with a previous report (North and Golding, 2000) which suggests that vegetarians have a greater exposure to phytoestrogens than omnivores thereby supporting the possibility that phytoestrogens have a deleterious effect on the developing male reproductive system. An alternative explanation for the association of hypospadias with vegetarianism might be related to the 'unnatural' chemicals (used as fertilizers and pesticides, and which act as endocrine disrupters) present in many fruits and vegetables.

On the basis of parental occupational status, a major group of children (72.27%) affected with hypospadias had their parents from rural background having agriculture as their primary occupation i.e. individuals adhering to agricultural background are likely to be exposed to pesticides which are endocrine disruptors and anti-androgenic in nature. Though our study lacks the estimate of pesticide levels among cases and also lack of agriculture based controls, its role in Hypospadias causation cannot be ruled out as fetal exposure to endocrine disruptors (EDs) with estrogen-like or anti androgen-like activity found among pesticides could be a risk factor for Hypospadias. Large scale screening of Hypospadias and other 46 X,Y disorders of sexual development is needed especially in India, where the majority of the population is from agricultural background. The results of the present study are likely to assist the health planners to initiate screening of Hypospadias among the farmer community to combat the risk of Hypospadias.

References

Allen, N.E., Reichardt, J.K., Nguyen, H., Key, T.J., 2003. Association between two polymorphisms in the SRD5A2 gene and serum androgen concentrations in British men. Cancer Epidemiol. Biomarkers Prev. 12 (6), 578–581.

Bharaj, B., Scorilas, A., Giai, M., Diamandis, E.P., 2000. TA repeat polymorphism of the 5alpha-reductase gene and breast cancer. Cancer Epidemiol. Biomarkers Prev. 9 (4), 387–393.

Di Salle, E., Giudici, D., Radice, A., et al., 1998. PNU 157706, a novel dual type I and II 5 alpha reductase inhibitor. J. Steroid Biochem. Mol. Biol. 64, 179.

Forti, G., Falchetti, A., Santoro, S., Davis, D.L., Wilson, J.D., Russell, D.W., 1996. Steroid 5 -reductase 2 deficiency: virilization in early infancy may be due to partial function of mutant enzyme. Clin. Endocrinol. (Oxf.) 44, 477–482.

Giwercman, Y.L., Giwercman, A., Pederson, H.S., Toft, G., Lundin, K., et al., 2008. Polymorphisms in genes regulating androgen activity among prostate cancer low-risk Inuit men and high-risk Scandinavians. Int. J. Androl. 31 (1), 25–30.

Guang, W., Zhou, L., Zeng, R., Du, C., 1999. Detection of R277Q mutation of SRD5 alpha 2 gene by amplification refractory mutation system. Chin. J. Med. Genet. 16 (6), 390–391.

Jaffe, J.M., Malkowicz, S.B., Walker, A.H., MacBride, S., Peschel, R., Tomaszewski, J., Van Arsdalen, K., Wein, A.J., Rebbeck, T.R., 2000. Association of SRD5A2 genotype and pathological characteristics of prostate tumors. Cancer Res. 60, 1626.

Kantoff, P.W., Febbo, P.G., Giovannucci, E., Krithivas, K., Dahl, D.M., Chang, G., Hennekens, C.H., Brown, M., Stamper, M.J., 1997a. A polymorphism of the 5 alpha reductase gene and its association with prostate cancer: a case–control analysis. Cancer Epidemiol. Biomarkers Prev. 6, 189–192.

Kantoff, P.W., Febbo, P.G., Giovannucci, E., Krithivas, K., Dahl, D.M., Chang, G., Hennekens, C.H., Brown, M., Stampfer, M.J., 1997b. A polymorphism of the 5 alpha-reductase gene and its association with prostate cancer: a case–control analysis. Cancer Epidemiol. Biomarkers Prev. 6 (3), 189–192.
Makridakis, N.M., Ross, R.K., Pike, M.C., Crocitto, L.E., Kolonel, L.N., Pearce, C.L., Henderson, B.E., Reichardt, J.K., 1999. Association of missense substitution in SRD5A2 gene with prostate cancer in African–American and Hispanic men in Los Angeles, USA. Lancet 354, 975–978.
Myrianthopoulos, N.C., Chung, C.S., 1974. Congenital malformations in singletons: epidemiologic survey. Report from the Collaborative Perinatal Project. Birth Defects Orig Artic Ser 10, 1.
North, K., Golding, J., 2000. A maternal vegetarian diet in pregnancy is associated with hypospadias. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. BJ. Int. 85 (1), 107–113.
Paulozzi, L.J., Erickson, J.D., Jackson, R.J., 1997. Hypospadias trends in two US surveillance systems. Pediatrics 100, 831.
Reichardt, J.K., Makridakis, N., Henderson, B.E., Yu, M.C., Pike, M.C., Ross, R.K., 1995. Genetic variability of the human SRD5A2 gene: implications for prostate cancer risk. Cancer Res. 55, 3973–3975.
Samtani, R., Bajpai, M., Vashisht, K., Ghosh, P.K., Saraswathy, K.N., 2001. SRD5A2 and CYP17 gene polymorphisms—role in isolated cases of hypospadias among Indian children. J. Urol. 185, 2334–2339.
Sasaki, G., Ogata, T., Ishii, T., et al., 2003. Micropenis and the 5 alpha reductase-2 (SRD5A2) Gene. Mutation and V89L polymorphism analysis in 81 Japanese patients. J. Clin. Endocrinol. Metab. 88 (7), 3431–3436.
Scorilas, A., Bharaj, B., Gial, M., Diamandis, E.P., 2001. Codon 89 polymorphisms in the human 5α-reductase gene in primary breast cancer. Br. J. Cancer 84, 760–767.
Silver, R.I., Russell, D.W., 1999. 5alpha-reductase type 2 mutations are present in some boys with isolated hypospadias. J. Urol. 162, 1142–1145.
Vilchis, F., Canto, P., Chávez, B., Ulloa-Aguirre, A., Méndez, J.P., 1997. Molecular analysis of the 5 alpha-steroid reductase type 2 gene in a family with deficiency of the enzyme. Am. J. Med. Genet. 69 (1), 69–72 (3).
Woolf, B., 1995. On estimating the relation between blood group and disease. Am. J. Hum. Genet. 19, 251.
Yang, C., Hamajima, N., Iwata, H., et al., 2002. A49T, V89L and TA repeat polymorphisms of steroid 5-alpha-reductase type II and breast cancer risk in Japanese women. Breast Cancer Res. 4 (4), R8.
Zeigler-Johnson, C.M., Walker, A.H., Mancke, B., et al., 2002. Ethnic differences in the frequency of prostate cancer susceptibility alleles at SRD5A2 and CYP3A4. Hum. Hered. 54 (1), 13–21.

Abbreviations list

DHT: Dihydrotestosterone
T: Testosterone
SRD5A2 gene: Steroid 5 alpha reductase type II gene