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Journal
Andrology, 2(1)

ISSN
2047-2919

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Publication Date
2014

DOI
10.1111/j.2047-2927.2013.00165.x

Peer reviewed

HHS Public Access

Author manuscript

Andrology. Author manuscript; available in PMC 2015 January 01.

Published in final edited form as:

Andrology. 2014 January ; 2(1): 130–137. doi:10.1111/j.2047-2927.2013.00165.x.

Hypospadias and variants in genes related to sex hormone biosynthesis and metabolism

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Abstract

We examined whether variants in genes related to sex hormone biosynthesis and metabolism were associated with hypospadias in humans. We examined 332 relatively common tagSNPs in 20 genes. Analyses included 633 cases (84 mild, 322 moderate, 212 severe, 15 undetermined severity) and 855 population-based non-malformed male controls born in California from 1990–2003. We used logistic regression models to estimate odds ratios (OR) and 95 percent confidence intervals (CI) for each SNP. Several of the 332 studied SNPs had p<0.01: one in *CYP3A4*, four in *HSD17B3*, one in *HSD3B1*, 2 in *STARD3* 10 in *SRD5A2* and seven in *STS*. In addition, haplotype analyses gave several associations with p<0.01. For *HSD17B3*, 14-SNP and 5-SNP blocks had ORs of 1.5 (95% CI 1.1, 2.0, p<0.001) and 2.8 (95% CI 1.6, 4.8, p<0.001), respectively. For *SRD5A2*, 9-SNP, 3-SNP and 8-SNP blocks had ORs of 1.7 (95% CI 1.3, 2.2, p<0.001), 1.4 (95% CI 1.1, 1.8, p=0.008) and 1.5 (95% CI 1.2, 1.9, p=0.002), respectively. Our study indicates that several genes that contribute to sex hormone biosynthesis and metabolism are associated with hypospadias risk.

INTRODUCTION

Hypospadias is a common congenital malformation in which the urethral meatus is on the ventral side of the penis. Familial patterns suggest that genetic factors substantially contribute to its etiology (Schnack and others, 2008). Normal urethral closure, which occurs during the 8^{th}–14^{th} weeks of gestation, involves a continuous process of ventral fusion in the proximal to distal direction (Kurzrock and others, 1999; Seifert and others, 2008; Van Der Werff and others, 2000). This process requires fetal synthesis of testosterone, conversion to dihydrotestosterone (DHT), DHT binding to the androgen receptor (AR), and appropriate AR signaling. Estrogens, as well as progesterone, may interfere with this process in the fetus (Kim and others, 2004; Manson and Carr, 2003; Wright and others, 1983). We therefore

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hypothesized that variability in genes that contribute to fetal sex hormone biosynthesis and metabolism is associated with hypospadias risk. In particular, we hypothesize that lower testosterone or DHT levels or higher estrogen or progesterone levels would be associated with increased risk.

Many genes contribute to these pathways, and several have been examined by previous studies of hypospadias. *SRD5A2* is critical to conversion of testosterone to DHT in the urethral seam, and its variants have been associated with hypospadias in several small studies (Makridakis and others, 2000; Samtani and others, 2011; Sata and others, 2010; Thai and others, 2005) but not in one larger study (van der Zanden and others, 2010). Small studies have also suggested that variants in *CYP17A1* (Qin and others, 2012; Samtani and others, 2010), *HSD3B2* (Codner and others, 2004), *HSD17B3* (Sata and others, 2010), and *CYP1A1* (Kurahashi and others, 2005) are associated with hypospadias.

We examined whether variants in 20 genes that contribute to sex hormone synthesis and metabolism were associated with hypospadias (Table 1). Specifically, we examined over 300 relatively common variants in a large population of California male infants. Several of the genes have been examined previously for an association with hypospadias (as noted above) but most have not.

METHODS

The study population included all male infants born from 1990–2003 to mothers who were residents of eight California Central Valley counties (Fresno, Kern, Kings, Madera, Merced, San Joaquin, Stanislaus, and Tulare counties) and from 1990–1997 (7/1/1990–12/31/1997) to mothers who were residents of Los Angeles, San Francisco, and Santa Clara counties, reflecting counties where case ascertainment was actively being conducted by the California Birth Defects Monitoring Program (CBDMP). CBDMP staff ascertained cases by reviewing medical records at hospitals and genetic centers in the relevant California counties (Croen and others, 1991).

Cases were classified by severity, which was based on the reported anatomical position of the urethral opening. Mild cases were those for which the meatus was limited to the coronal or glanular penis (British Pediatric Association [BPA] codes 752.605, 752.625), moderate cases were those for which the meatus was on the penile shaft, and severe cases were those for which the meatus was at the peno-scrotal junction or perineal area (BPA codes 752.606, 752.607, 752.626, 752.627). Assignment of severity was finalized based on review by a medical geneticist (EJL or Dr. Cynthia Curry) (Carmichael and others, 2003). Cases for which the anatomical position was described as “not otherwise specified” (BPA codes 752.600, 752.620) were excluded. Cases having a known single gene disorder or chromosomal abnormality were excluded.

The underlying study population included 1,246,172 non-malformed live born male infants eligible for control selection. We randomly selected 931 controls with available newborn bloodspots for study, in proportion to the underlying birth population for that year, to give an approximate 2:1 ratio of controls to cases from Central Valley counties and a 1:1 ratio

from non-Central Valley counties. The ratio differed due to the presence of a secondary on-going study in the Central Valley that allowed for a larger control group. No control infant had a structural birth defect.

For cases and controls, information on the following descriptive covariates was derived from birth certificates: maternal race-ethnicity, education, age, and parity; plurality; and infant birthweight and gestational age at delivery. Cases and controls were linked with archived newborn bloodspots, which served as the source of DNA for genotyping. In total, 667 (88% of eligible) cases and 931 (93% of eligible) controls were available for genotyping.

Genomic DNA was extracted from bloodspots using MasterPure™ Complete DNA and RNA Purification Kit (Epicentre Biotechnologies Madison, WI) and 10 ng genomic DNA was then used for whole genome amplification (Qiagen Repli-g® kit). TagSNPs that assay known common SNPs either directly or indirectly via linkage disequilibrium among measured and unmeasured SNPs were selected using the Genome Variation Server (http://gvs.gs.washington.edu/GVS/). The program provided tagSNPs that cover common variation at r^2>0.80 across each candidate gene for a “cosmopolitan” population, including Hispanics. TagSNPs with minor allele frequencies (MAF) >10% were selected. SNPs were genotyped using a custom multiplex Illumina GoldenGate assay.

We started with 380 SNPs from the candidate genes in the GoldenGate assay. We excluded 29 SNPs for which the data indicated poor clustering of results and 2 SNPs with a call rate <90%. We also excluded 106 subjects (32 cases, 74 controls) with sample call rates <90%, leaving 635 cases (84 mild, 323 moderate, 213 severe, 15 unknown) and 857 controls for analyses. We then undertook a Hardy-Weinberg equilibrium test for each SNP among controls, which resulted in excluding 17 additional SNPs (p-value <0.005 among non-Hispanic whites or Hispanics), leaving 332 SNPs for analysis. All 3 SNPs for *CYP21A2* were excluded. The genes and number of SNPs per gene are described in Table 1.

We genotyped 106 ancestry informative marker (AIM) SNPs that were selected to discriminate Native American, African, and European ancestry (Choudhry and others, 2010; Gamboa-Melendez and others, 2012; Risch and others, 2009; Via and others, 2010). Four of the 106 AIMs were excluded because they had a call rate lower than 90%. To estimate individual ancestry estimates among cases and controls, we used the program Structure 2.1 (Falush and others, 2003; Pritchard and others, 2000). Structure was run using the admixture model with unlinked markers, with 50,000 burn-in iterations and 50,000 further iterations. We assumed three ancestral populations. Structure provided variables reflecting the proportions of Native American, African and European ancestry for each case or control. Given that the three proportions sum to one, we only incorporated two (Native American and African) into our analyses to adjust for potential population stratification.

We used logistic regression to examine the association of each candidate gene SNP with risk of hypospadias, comparing the homozygous and heterozygous variant genotypes with the homozygous wildtype genotype (the more frequent allele among all controls was designated as wildtype). We considered results to be significant if they had p<0.01. We considered the possibility for heterogeneity of results across ethnic groups by comparing risks among self-

identified non-Hispanic white and Hispanic subjects by examining models restricted to these two groups that contained product terms to estimate interaction (interaction was not assessed for "other" race-ethnicity, because it was a smaller group and heterogeneous). For SNPs for which the overall p-value for the product term was less than 0.10 (n=12), we focused on stratified results. We conducted analyses of all cases grouped together as well as separate analyses by severity of phenotype (mild, moderate, severe).

For the fifteen genes for which there were more than 5 SNPs (*COMT, CYP11A1, CYP17A1, CYP19A1, CYP3A4, HSD17B2, HSD17B3, HSD3B1, HSD3B2, SRD5A1, SRD5A2, STARD3, STS, SULT1E1, SULT2A1*), we examined haplotypes. We used Haploview 4.2 to determine the LD structure in the region and to define the haplotype blocks and their frequencies based on all subjects' genotypes (Barrett and others, 2005). The most common haplotype was the reference. Maximum likelihood estimates of odds ratios and their corresponding 95% confidence intervals (CI) were calculated from logistic regression models to estimate relative risks.

We also evaluated genetic risk scores created by combining high-risk SNPs. For each individual we counted the number of genes in which they carried an associated variant (based on $p<0.01$ as our criterion). For variants with ORs<1, the reference genotype (homozygous wildtype) was scored as the risk genotype. We calculated scores overall and separately by severity (applying the p-value criterion within each group, such that a different set of variants was scored within each group).

All odds ratios were adjusted for the two ancestral proportion variables and for maternal residence in the Central Valley (yes/no) due to the differing case-control ratio based on this variable that was inherent to the study design. In addition, non-stratified results were adjusted for maternal race-ethnicity (Hispanic, non-Hispanic white, or other). Two cases and two controls had missing race-ethnicity, such that SNP-based analyses included 855 controls and a maximum 633 cases (84 mild, 322 moderate, 212 severe, 15 uncertain after clinician review, which were included only in the analyses of all cases together).

RESULTS

Case mothers were more likely than control mothers to be non-Hispanic white, more highly educated, older, and nulliparous (Table 2). Cases were more likely to be low birthweight and delivered before 37 weeks of gestation.

Several SNPs had $p<0.01$ for at least one comparison (Table 3). One *CYP3A4* SNP was associated with increased risk, specifically for moderate hypospadias. For four *HSD17B3* SNPs, the heterozygous phenotypes were associated with risk, for moderate and severe hypospadias. For three of these SNPs, the odds ratios were even higher for the homozygous genotype (2- to 3-fold increased risk), but confidence intervals included one, likely due to smaller sample sizes for these comparisons. One *HSD3B1* SNP and two *STARD3* SNPs were associated with risk; results varied between whites and Hispanics and by severity. Ten *SRD5A2* SNPs were associated. As with *HSD17B3*, odds ratios were particularly high

among moderate and severe cases, and for the homozygous genotypes. Seven *STS* SNPs were associated with risk; results tended to be elevated regardless of severity.

High linkage disequilibrium was present among some of the SNPs reported in Table 3. For *HSD17B3* the pair-wise R-squared values for three of the four SNPs ranged from 0.72 to 0.99; values for the fourth SNP (rs2026001) were all 0.06. For *SRD5A2*, R-squared values ranged from 0.54–0.95, with the exception of rs725631 and rs765138 (range 0.01–0.17). The R-squared for the two *STARD3* SNPs was low (0.03). For *STS* SNPs, the range was 0.54–0.98.

Haplotype analyses among the overall study population gave even stronger results. In particular, for *HSD17B3*, 14-SNP and 5-SNP blocks had odds ratios of 1.5 (95% CI 1.1, 2.0, p<0.001) and 2.8 (95% CI 1.6, 4.8, p<0.001), respectively (Table 4). For *SRD5A2*, 9-SNP, 3-SNP and 8-SNP blocks had odds ratios of 1.7 (95% CI 1.3, 2.2, p<0.001), 1.4 (95% CI 1.1, 1.8, p=0.008) and 1.5 (95% CI 1.2, 1.9, p=0.002), respectively.

Based on the risk scores, a higher number of genes with risk-associated SNPs corresponded with higher ORs (Table 5). For example, among moderate cases a score of one or two was associated with a 2-fold increased risk, whereas a score of three was associated with a 4.5-fold increased risk.

DISCUSSION

This study indicates that SNPs in several genes that contribute to sex hormone biosynthesis and metabolism are associated with risk of hypospadias – *CYP3A4*, *HSD17B3*, *HSD3B1*, *SRD5A2*, *STARD3* and *STS*. However, the study did not indicate that SNPs in several other genes contribute to hypospadias – *COMT*, *CYP11A1*, *CYP17A1*, *CYP19A1*, *CYP1A1*, *CYP21A2*, *CYP3A7*, *HSD17B1*, *HSD17B2*, *HSD3B2*, *SRD5A1*, *STAR*, *SULT1E1*, *and SULT2A1*. Our discussion focuses on our findings for the six genes with significant results and the three additional genes that have been studied previously but were not significant in our study (*CYP1A1*, *CYP17A1*, and *HSD3B2*).

CYP1A1 contributes to the 2-hydroxylation of estrogens, which yields less estrogenic metabolites than the 4- and 16-alpha hydroxylation catalyzed by ***CYP3A4*** (Kurahashi and others, 2005). Two previous studies have examined two known functional polymorphisms in *CYP1A1* (rs4646903 and rs1048943). A study of 31 Japanese cases reported a protective association with rs4646903 (Kurahashi and others, 2005), whereas a study of 80 Indian cases did not provide evidence for association with rs4646903 or rs1048943 (Shekharyadav and others, 2011). Our study included rs1048943, as well as three other CYP1A1 SNPs, but found no evidence of association. We did observe an increased risk with one *CYP3A4* SNP for moderate cases. A study of 98 Japanese cases did not find evidence for an association with variants in *CYP1A1* or *CYP3A4* (Qin and others, 2012).

HSD17B3 is responsible for conversion of androstenedione to testosterone. One study examined five SNPs in *HSD17B3* among 89 Japanese cases (Sata and others, 2010). The SNP rs2066479 (+913G>A) was associated with increased risk, regardless of severity; the OR for the GA genotype was 1.5 (95% CI 0.9, 2.4), and for the AA genotype it was 3.1

(95% CI 1.4, 6.8). Our study included 56 *HSD17B3* SNPs. Three were associated with increased hypospadias risk; associations were strongest for the homozygous variant genotype and among moderate to severe cases. We did not include rs2066479 in our study, but we were able to obtain data on its R-squared value with one of our associated SNPs, rs12552648, from dbSNP; the R-squared value was near one. In our study, rs12552648 variant genotypes were associated with increased risk of moderate and severe hypospadias. One of our studied SNPs was associated with reduced hypospadias risk, and two haplotype blocks were also associated with increased risk.

HSD3B1 and HSD3B2 are important for synthesis of androgens and progesterone. *HSD3B2* mutations lead to impaired gonadal steroidogenesis and undermasculinized genitalia (Codner and others, 2004). *HSD3B1* has a similar function as *HSD3B2* but is the major form expressed in the placenta (Pezzi and others, 2003; Simard and others, 2005). In the current study, one of six SNPs in *HSD3B1* was associated with hypospadias, particularly among moderate cases; none of the five *HSD3B2* SNPs was associated. A study in Chile observed missense mutations in *HSD3B2* in two of 90 isolated moderate/severe hypospadias cases, versus none among 100 “healthy fertile male controls” (Codner and others, 2004).

SRD5A2 is critical to the conversion of testosterone to DHT in the urethral seam. The V89L polymorphism (rs523349 or +336G>C) has been associated with hypospadias in four small studies (Samtani and others, 2011; Sata and others, 2010; Thai and others, 2005; Wang and others, 2004) but not one large study (van der Zanden and others, 2010). The C allele confers substantial reduction in enzyme activity (Samtani and others, 2010). In our study, several *SRD5A2* SNPs were associated with hypospadias risk, but rs523349 did not make the $p<0.01$ cut-off. Among all cases, the OR for rs523349 was 1.1 (95% CI 0.8, 1.3) for the CG genotype and 0.8 (95% CI 0.6, 1.1) for the CC genotype, relative to GG. For mild cases, the respective ORs were 1.3 and 1.3, and for moderate cases, 1.0 and 1.0. For severe cases, the respective ORs were 1.0 (95% CI 0.7, 1.4) and 0.5 (95% CI 0.3, 0.9, $p=0.012$). Explanation of the differences across studies for this SNP is unknown. ORs for the SRD5A2 SNPs that did make our $p<0.01$ cut-off tended to be strongest for the homozygous variant genotypes.

The first and rate-limiting step in sex steroid biosynthesis is the conversion of cholesterol to pregnenolone, which involves ***STARD3*** (Tuckey and others, 2004). We observed an association of two of six *STARD3* SNPs with hypospadias, one with a 3-fold increased risk of severe hypospadias, and one with reduced risk of moderate and severe hypospadias; both results were only observed among Hispanics. ***STS*** contributes to the synthesis of biologically active estrogens. Seven of 20 SNPs we studied were associated with modestly (1.4-fold) increased risk of hypospadias. We are unaware of previous studies of hypospadias and genetic variation of *STARD3* or *STS*. ***CYP19A1*** catalyzes the last steps of estrogen synthesis. Its SNPs were not associated with hypospadias in our study, nor in another small study (Qin and others, 2012).

CYP17A1 (p450c17) is key to synthesis of androgens, estrogens and progestins (Miller, 2002). Case reports suggest that mutations in *CYP17* or reduced *CYP17* activity may be associated with hypospadias and male pseudohermaphroditism (Ammini and others, 1997;

Sherbet and others, 2003). Two small studies among Indian subjects examined the functional *CYP17A1* polymorphism rs743572; one reported increased risk (Samtani and others, 2010), whereas the other did not (Yadav and others, 2011). A small study of Japanese subjects reported an association of rs17115149 with hypospadias (Qin and others, 2012; Samtani and others, 2010). Our study did not provide evidence of an association of hypospadias with nine *CYP17A1* SNPs, including rs743572; we did not include rs17115149 in our study.

In summary, of the six genes with results that we considered significant (see Table 3), two contribute to estrogen metabolism or synthesis (*CYP3A4, STS*), three contribute to androgen synthesis (*HSD17B3, HSD3B1, SRD5A2*), and one is more generally involved in steroid synthesis (*STARD3*). Thus, they represent multiple aspects of sex hormone synthesis and metabolism. However, we do not know the actual functional consequences of the studied SNPs, since most tagSNPs are intronic.

The strengths of this study include its size, population-based controls, ancestry informative markers, and examination of multiple genes that contribute to a specific pathway. Many of the genes we examined have not been studied previously for their contribution to hypospadias, or if they have, previous studies tend to be small in number of subjects and variants investigated. Our approach of highlighting results with $p<0.01$ rather than a more conservative statistical criterion minimizes Type II errors (false negatives), but the trade-off is an increased possibility of false positive results (Type I error). Given that we had an *a priori* hypothesis about the studied genes, and most have not been studied extensively if at all in the context of hypospadias, this seemed an appropriate trade-off for the presentation of results. However, some of our results may be false positives, since the p-value criterion of <0.01 was not very conservative relative to the large number of comparisons we made. Thus, we emphasize the need for replication of our findings in additional study populations. Under-ascertainment of mild cases is a potential limitation of this study but would not alter the associations we observed for moderate or severe hypospadias. We considered all cases in our study regardless of whether they had other accompanying congenital malformations; most cases did not have other malformations and thus those with other malformations are unlikely to have driven our results. Our approach of investigating tagSNPs was justified given that it captures the majority of genetic variation and is cost-efficient, and that minimal examination of the studied genes in humans preceded the current study. However, many tagSNPs are intronic and have no known functional consequences. The SNPs we genotyped include one non-synonymous exonic SNP (*STARD3* rs1877031) and two 3′-UTR (untranslated region) SNPs (*SRD5A2* rs1042578 and rs9332975). Thus, some of the observed associations could be driven by linkage disequilibrium with other less common, unmeasured variants that do have functional (but still uncertain) consequences. In addition, several of the associations we have reported were for SNPs that were in relatively high disequilibrium and are therefore unlikely to be independent. Our study investigated confounding by race-ethnicity as well as effect modification for Hispanics versus non-Hispanic whites. A limitation of our study is the small numbers of subjects with other race-ethnicities, such as Asian or Pacific Islander.

In conclusion, this study observed substantial evidence for an association of hypospadias with certain genes that contribute to sex hormone biosynthesis and metabolism, especially *HSD17B3*, *SRD5A2*, and *STS*. Further studies are needed to verify these results and identify potential underlying causal variants.

Acknowledgments

We thank the California Department of Public Health Maternal Child and Adolescent Health Division for providing data, Dr. Cynthia Curry for her contributions to case review for some of the study subjects, and Dr. Shweta Choudhry for creating the ancestral proportion variables. This project was partially supported by NIH R01 ES017060 and CDC 6U01DD000489. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the California Department of Public Health.

References

Ammini AC, Sharma DC, Gupta R, Mohapatra I, Kucheria K, Kriplani A, et al. Familial male pseudohermaphroditism. Indian J Pediatr. 1997; 64(3):419–423. [PubMed: 10771866]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–265. [PubMed: 15297300]

Carmichael SL, Shaw GM, Nelson V, Selvin S, Torfs CP, Curry CJ. Hypospadias: trends and descriptive epidemiology in a California population. Epidemiol. 2003; 14:701–706.

Choudhry S, Que LG, Yang Z, Liu L, Eng C, Kim SO, et al. GSNO reductase and beta2-adrenergic receptor gene-gene interaction: bronchodilator responsiveness to albuterol. Pharmacogenet Genomics. 2010; 20(6):351–358. [PubMed: 20335826]

Codner E, Okuma C, Iniguez G, Boric MA, Avila A, Johnson MC, et al. Molecular study of the 3 beta-hydroxysteroid dehydrogenase gene type II in patients with hypospadias. J Clin Endocrinol Metab. 2004; 89(2):957–964. [PubMed: 14764821]

Croen LA, Shaw GM, Jensvold NJ, Harris JA. Birth defects monitoring in California: a resource for epidemiologic research. Paediatr Perinat Epidemiol. 1991; 5:423–427. [PubMed: 1754501]

Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 2003; 164(4):1567–1587. [PubMed: 12930761]

Gamboa-Melendez MA, Huerta-Chagoya A, Moreno-Macias H, Vazquez-Cardenas P, Ordonez-Sanchez ML, Rodriguez-Guillen R, et al. Contribution of common genetic variation to the risk of type 2 diabetes in the Mexican Mestizo population. Diabetes. 2012; 61(12):3314–3321. [PubMed: 22923468]

Kim KS, Torres CR Jr, Yucel S, Raimondo K, Cunha GR, Baskin LS. Induction of hypospadias in a murine model by maternal exposure to synthetic estrogens. Environ Res. 2004; 94(3):267–275. [PubMed: 15016594]

Kurahashi N, Sata F, Kasai S, Shibata T, Moriya K, Yamada H, et al. Maternal genetic polymorphisms in CYP1A1, GSTM1 and GSTT1 and the risk of hypospadias. Mol Hum Reprod. 2005; 11(2):93–98. [PubMed: 15579657]

Kurzrock EA, Baskin LS, Cunha GR. Ontogeny of the male urethra: theory of endodermal differentiation. Differentiation. 1999; 64:115–122. [PubMed: 10234808]

Makridakis NM, di Salle E, Reichardt JK. Biochemical and pharmacogenetic dissection of human steroid 5 alpha-reductase type II. Pharmacogenetics. 2000; 10(5):407–413. [PubMed: 10898110]

Manson JM, Carr MC. Molecular epidemiology of hypospadias: review of genetic and environmental risk factors. Birth Defects Res A Clin Mol Teratol. 2003; 67(10):825–836. [PubMed: 14745936]

Miller WL. Androgen biosynthesis from cholesterol to DHEA. Mol Cell Endocrinol. 2002; 198(1–2):7–14. [PubMed: 12573809]

Pezzi V, Mathis JM, Rainey WE, Carr BR. Profiling transcript levels for steroidogenic enzymes in fetal tissues. J Steroid Biochem Mol Biol. 2003; 87(2–3):181–189. [PubMed: 14672738]

Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155(2):945–959. [PubMed: 10835412]

Qin XY, Kojima Y, Mizuno K, Ueoka K, Massart F, Spinelli C, et al. Association of variants in genes involved in environmental chemical metabolism and risk of cryptorchidism and hypospadias. J Hum Genet. 2012; 57(7):434–441. [PubMed: 22648180]

Risch N, Choudhry S, Via M, Basu A, Sebro R, Eng C, et al. Ancestry-related assortative mating in Latino populations. Genome Biol. 2009; 10(11):R132. [PubMed: 19930545]

Samtani R, Bajpai M, Ghosh PK, Saraswathy KN. SRD5A2 gene mutations--a population-based review. Pediatr Endocrinol Rev. 2010; 8(1):34–40. [PubMed: 21037542]

Samtani R, Bajpai M, Vashisht K, Ghosh PK, Saraswathy KN. Hypospadias risk and polymorphism in SRD5A2 and CYP17 genes: case-control study among Indian children. J Urol. 2011; 185(6):2334–2339. [PubMed: 21511288]

Sata F, Kurahashi N, Ban S, Moriya K, Tanaka KD, Ishizuka M, et al. Genetic polymorphisms of 17beta-hydroxysteroid dehydrogenase 3 and the risk of hypospadias. J Sex Med. 2010; 7(8):2729–2738. [PubMed: 20059664]

Schnack TH, Zdravkovic S, Myrup C, Westergaard T, Christensen K, Wohlfahrt J, et al. Familial aggregation of hypospadias: a cohort study. Am J Epidemiol. 2008; 167(3):251–256. [PubMed: 18042671]

Seifert AW, Harfe BD, Cohn MJ. Cell lineage analysis demonstrates an endodermal origin of the distal urethra and perineum. Dev Biol. 2008; 318(1):143–152. [PubMed: 18439576]

Shekharyadav C, Bajpai M, Kumar V, Ahmed RS, Gupta P, Banerjee BD. Polymorphism in CYP1A1, GSTM 1, GSTT1 genes and organochlorine pesticides in the etiology of hypospadias. Hum Exp Toxicol. 2011; 30(10):1464–1474. [PubMed: 21300689]

Sherbet DP, Tiosano D, Kwist KM, Hochberg Z, Auchus RJ. CYP17 mutation E305G causes isolated 17,20-lyase deficiency by selectively altering substrate binding. J Biol Chem. 2003; 278(49): 48563–48569. [PubMed: 14504283]

Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH. Molecular biology of the 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase gene family. Endocr Rev. 2005; 26(4): 525–582. [PubMed: 15632317]

Thai HT, Kalbasi M, Lagerstedt K, Frisen L, Kockum I, Nordenskjold A. The valine allele of the V89L polymorphism in the 5-alpha-reductase gene confers a reduced risk for hypospadias. J Clin Endocrinol Metab. 2005; 90(12):6695–6698. [PubMed: 16174723]

Tuckey RC, Bose HS, Czerwionka I, Miller WL. Molten globule structure and steroidogenic activity of N-218 MLN64 in human placental mitochondria. Endocrinology. 2004; 145(4):1700–1707. [PubMed: 14715710]

Van Der Werff JFA, Nievelstein RAJ, Brands E, Luijsterburg AJM, Vermeij-Keers C. Normal development of the male anterior urethra. Teratology. 2000; 61:172–183. [PubMed: 10661906]

van der Zanden LF, van Rooij IA, Feitz WF, Vermeulen SH, Kiemeney LA, Knoers NV, et al. Genetics of hypospadias: are single-nucleotide polymorphisms in SRD5A2, ESR1, ESR2, and ATF3 really associated with the malformation? J Clin Endocrinol Metab. 2010; 95(5):2384–2390. [PubMed: 20215396]

Via M, De Giacomo A, Corvol H, Eng C, Seibold MA, Gillett C, Galanter J, et al. The role of LTA4H and ALOX5AP genes in the risk for asthma in Latinos. Clin Exp Allergy. 2010; 40(4):582–589. [PubMed: 20067482]

Wang Y, Li Q, Xu J, Liu Q, Wang W, Lin Y, et al. Mutation analysis of five candidate genes in Chinese patients with hypospadias. Eur J Hum Genet. 2004; 12(9):706–712. [PubMed: 15266301]

Wright, F.; Giacomini, M.; Riahi, M.; Mowszowicz, I. Antihormone activity of progesterone and progestins. In: Bardin, CW., editor. Progesterone and progestins. Raven Press; New York: 1983. p. 109-134.

Yadav CS, Bajpai M, Kumar V, Kumar Datta S, Gupta P, Ahmed RS, et al. Polymorphisms in the P450 c17 (17-Hydroxylase/17, 20-Lyase) gene: Association with estradiol and testosterone concentration in hypospadias. Urology. 2011; 78(4):902–907. [PubMed: 21676446]

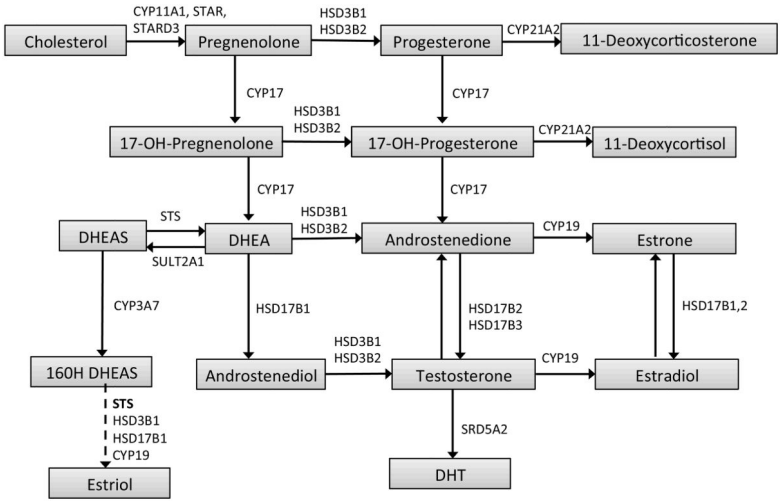

Figure 1.

Candidate genes from the sex steroid biosynthesis pathway.

DHEA = Dehydroepiandrosterone, DHEAS = DHEA-sulfate, see Table 1 for gene names

Table 1

Genes included in analyses.

Gene symbol	Gene name	Role	Number of SNPs analyzed (332 total)
COMT	catechol-O-methyltransferase	Inactivates estrogens	19
CYP11A1	cytochrome P450, family 11, subfamily A, polypeptide 1	Conversion of cholesterol to pregnenolone	5
CYP17A1	cytochrome P450, family 17, subfamily A, polypeptide 1	Conversion of pregnenolone and progesterone	9
CYP19A1	cytochrome P450, family 19, subfamily A, polypeptide 1	Conversion of androgens to estrogens	64
CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	Contributes to the 2-hydroxylation of estrogens	4
CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4	Contributes 4- and 16a-hydroxylation of estrogens	9
CYP3A7	cytochrome P450, family 3, subfamily A, polypeptide 7	Contributes to fetal supply of androgen precursors of estrogens by generating 16OH-DHEAS, the major precursor for placental estriol synthesis	3
HSD17B1	hydroxysteroid (17-beta) dehydrogenase 1	Inter-conversion of estrogens and androgens, e.g., conversion of estrone to estradiol	1
HSD17B2	hydroxysteroid (17-beta) dehydrogenase 2	Inter-conversion of estrogens and androgens; e.g., conversion of estradiol to estrone	51
HSD17B3	hydroxysteroid (17-beta) dehydrogenase 3	Inter-conversion of estrogens and androgens	56
HSD3B1	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1	Interconversion of androgens and progesterone-related hormones	6
HSD3B2	hydroxy-delta-5-ster0oid dehydrogenase, 3 beta- and steroid delta-isomerase 2	Interconversion of androgens and progesterone-related hormones	5
SRD5A1	steroid-5-alpha-reductase, alpha polypeptide 1	Isoform of SRD5A2	22
SRD5A2	steroid-5-alpha-reductase, alpha polypeptide 2	Conversion of testosterone to dihydrotestosterone	31
STAR	steroidogenic acute regulatory protein	Conversion of cholesterol to pregnenolone	1
STARD3	StAR-related lipid transfer (START) domain containing 3	Conversion of cholesterol to pregnenolone	6
STS	steroid sulfatase (microsomal), isozyme S	Contributes to placental generation of estriol by catalyzing conversion of sulfated steroid precursors to estrogens	20
SULT1E1	sulfotransferase family 1E, estrogen-preferring, member 1	Inactivates estrogens by sulfoconjugation	13
SULT2A1	sulfotransferase family, cytosolic, 2A, DHEA-preferring, member 1	Conversion of DHEA to DHEAS	7

Table 2

Descriptive characteristics of cases with hypospadias (n=633) and non-malformed controls (n=855).

	Percent of Controls (n)	Percent of Cases (n)
Maternal race-ethnicity		
White	31 (262)	43 (275)
Hispanic	52 (443)	35 (221)
Others	18 (150)	22 (137)
Maternal education		
< High school	39 (335)	26 (162)
High school	31 (264)	27 (173)
> High school	29 (249)	47 (296)
Unknown	1 (7)	<1 (2)
Maternal age		
< 25 years	46 (395)	30 (189)
25–34 years	43 (365)	52 (331)
35 or more years	11 (95)	18 (113)
Number of previous live births		
0	36 (309)	52 (331)
1	32 (276)	26 (163)
≥ 2	32 (270)	22 (137)
Unknown	0 (0)	<1 (2)
Infant birthweight		
≤ 2500 g	5 (42)	30 (192)
> 2500 g	95 (813)	70 (441)
Gestational age at delivery		
< 37 weeks	7 (60)	23 (143)
≥ 37 weeks	89 (758)	74 (468)
Unknown	4 (37)	3 (22)
Maternal residence in Central Valley		
No	45 (385)	63 (394)
Yes	55 (470)	37 (239)

Table 3

Association of hypospadias with selected SNPs.*

Gene, SNP (Alleles)	MAF (Controls)	Genotype	No. Controls	No. Cases	OR (95% CI) All Cases	P	No. Mild Cases	OR (95% CI) Mild Cases	P	No. Mode- rate	OR (95% CI) Moderate Cases	P	No. Severe Cases	OR (95% CI) Severe Cases	P
CYP3A4															
rs12333983 (T:A)	0.249	TT	489	354	Reference		50	Reference		178	Reference		118	Reference	
		TA	297	217	1.1 (0.9, 1.4)	0.318	30	1.4 (0.8, 2.3)	0.257	105	1.2 (0.8, 1.6)	0.366	75	1.0 (0.7, 1.4)	0.926
		AA	63	57	1.4 (0.9, 2.2)	0.123	2	NC		37	**2.1 (1.2, 3.5)**	**0.006**	18	1.0 (0.6, 1.9)	0.923
HSD17B3															
rs12552648 (C:T)	0.075	CC	730	495	Reference		71	Reference		253	Reference		158	Reference	
		TC	110	117	**1.6 (1.2, 2.2)**	**0.002**	13	1.1 (0.6, 2.2)	0.704	59	**1.9 (1.3, 2.8)**	**0.001**	43	1.6 (1.1, 2.5)	0.025
		TT	9	12	2.2 (0.9, 5.5)	0.101	0	NC		5	3.8 (1.1, 12.9)	0.035	7	2.6 (0.9, 7.8)	0.080
rs8190566 (A:G)	0.100	AA	693	473	Reference		68	Reference		249	Reference		144	Reference	
		AG	150	143	1.4 (1.1, 1.8)	0.017	16	1.2 (0.7, 2.3)	0.546	65	1.3 (0.9, 1.8)	0.200	59	**1.8 (1.2, 2.6)**	**0.003**
		GG	11	15	2.0 (0.9, 4.7)	0.093	0	NC		7	3.1 (1.1, 9.1)	0.038	8	2.7 (1.0, 7.3)	0.054
rs8190557 (C:T)	0.099	CC	694	471	Reference		68	Reference		249	Reference		142	Reference	
		TC	148	146	**1.5 (1.1, 1.9)**	**0.006**	16	1.2 (0.6, 2.2)	0.561	66	1.3 (0.9, 1.9)	0.110	61	**1.9 (1.3, 2.8)**	**0.001**
		TT	11	15	2.1 (0.9, 4.8)	0.088	0	NC		7	3.1 (1.1, 9.0)	0.039	8	2.8 (1.0, 7.5)	0.047
rs2026001 (C:A)	0.434	CC	279	256	Reference		39	Reference		125	Reference		86	Reference	
		AC	408	278	0.8 (0.6, 1.0)	0.026	28	**0.5 (0.3, 0.8)**	**0.008**	147	0.8 (0.6, 1.1)	0.251	97	0.8 (0.5, 1.1)	0.139
		AA	165	96	0.7 (0.5, 1.0)	0.029	17	0.9 (0.5, 1.8)	0.771	47	0.7 (0.5, 1.1)	0.105	29	0.6 (0.4, 1.0)	0.033
HSD3B1															
rs6203 (C:T)															
White	0.434	CC	89	94	Reference		10	Reference		66	Reference		16	Reference	
		TC	115	119	1.1 (0.7, 1.6)	0.739	22	1.8 (0.7, 4.1)	0.194	72	1.0 (0.6, 1.5)	0.883	21	1.0 (0.5, 2.1)	0.941
		TT	55	55	1.2 (0.7, 1.9)	0.531	13	2.1 (0.8, 5.4)	0.129	27	0.9 (0.5, 1.6)	0.693	15	1.7 (0.8, 3.9)	0.185
Hispanic	0.482	CC	126	41	Reference		5	Reference		14	Reference		22	Reference	
		TC	200	118	**2.0 (1.3, 3.0)**	**0.002**	14	1.5 (0.5, 4.4)	0.467	53	**3.0 (1.5, 5.8)**	**0.001**	47	1.4 (0.8, 2.5)	0.233
		TT	110	58	1.8 (1.1, 3.0)	0.016	6	1.3 (0.4, 4.7)	0.641	27	**2.9 (1.4, 6.1)**	**0.004**	23	1.2 (0.6, 2.4)	0.510
SRD5A2															
rs1042578 (G:A)	0.113	GG	675	443	Reference		61	Reference		215	Reference		156	Reference	
		AG	157	161	**1.4 (1.1, 1.8)**	**0.008**	22	1.5 (0.8, 2.6)	0.173	90	**1.6 (1.2, 2.2)**	**0.004**	47	1.2 (0.8, 1.8)	0.321

Gene, SNP (Alleles)	MAF (Controls)	Genotype	No. Controls	No. Cases	OR (95% CI) All Cases	P	No. Mild Cases	OR (95% CI) Mild Cases	P	No. Mode- rate	OR (95% CI) Moderate Cases	P	No. Severe Cases	OR (95% CI) Severe Cases	P
		AA	17	25	1.8 (1.0, 3.5)	0.069	1	NC		13	1.8 (0.8, 4.0)	0.145	9	2.4 (1.0, 5.8)	0.049
rs9332975 (A:G)	0.097	AA	700	463	Reference		62	Reference		226	Reference		163	Reference	
		AG	142	147	**1.4 (1.1, 1.9)**	**0.007**	21	1.6 (0.9, 2.9)	0.099	83	**1.7 (1.2, 2.3)**	**0.003**	41	1.2 (0.8, 1.7)	0.483
		GG	11	19	2.4 (1.1, 5.1)	0.031	1	NC		10	2.8 (1.1, 7.0)	0.032	7	3.3 (1.2, 9.3)	0.021
rs2281546 (T:G)	0.114	TT	675	439	Reference		61	Reference		213	Reference		155	Reference	
		TG	163	168	**1.4 (1.1, 1.9)**	**0.005**	22	1.4 (0.8, 2.5)	0.208	95	**1.6 (1.2, 2.2)**	**0.003**	48	1.2 (0.8, 1.8)	0.332
		GG	15	24	2.1 (1.0, 4.0)	0.038	1	NC		13	2.1 (0.9, 4.9)	0.066	8	2.5 (1.0, 6.3)	0.048
rs28383032 (C:T)	0.083	CC	720	482	Reference		63	Reference		238	Reference		169	Reference	
		TC	124	135	**1.5 (1.1, 2.0)**	**0.006**	20	1.5 (0.9, 2.8)	0.149	76	**1.7 (1.2, 2.4)**	**0.002**	37	1.2 (0.8, 1.8)	0.449
		TT	8	15	2.4 (1.0, 5.9)	0.051	1	NC		8	2.8 (1.0, 8.0)	0.057	5	3.1 (1.0, 10.2)	0.056
rs6543634 (T:G)	0.113	TT	675	455	Reference		61	Reference		220	Reference		162	Reference	
		TG	159	151	1.3 (1.0, 1.7)	0.041	21	1.5 (0.8, 2.6)	0.166	87	**1.6 (1.1, 2.2)**	**0.009**	41	1.0 (0.7, 1.5)	0.893
		GG	16	22	1.9 (0.9, 3.7)	0.073	1	NC		12	2.1 (0.9, 4.9)	0.074	8	2.5 (1.0, 6.3)	0.055
rs2268794 (T:A)	0.125	TT	648	422	Reference		60	Reference		201	Reference		152	Reference	
		AT	179	171	1.3 (1.0, 1.7)	0.021	20	1.2 (0.7, 2.1)	0.570	100	**1.6 (1.2, 2.2)**	**0.004**	48	1.1 (0.8, 1.7)	0.503
		AA	15	28	2.3 (1.2, 4.5)	0.013	1	NC		16	2.4 (1.1, 5.2)	0.028	9	2.8 (1.1, 7.0)	0.024
rs725631 (C:A)	0.349	CC	371	281	Reference		35	Reference		147	Reference		90	Reference	
		AC	350	270	1.1 (0.8, 1.3)	0.590	40	1.4 (0.8, 2.3)	0.250	126	0.9 (0.7, 1.3)	0.729	99	1.1 (0.8, 1.5)	0.720
		AA	117	67	0.8 (0.5, 1.1)	0.182	9	1.4 (0.6, 3.3)	0.390	40	1.0 (0.6, 1.6)	0.982	17	**0.4 (0.2, 0.8)**	**0.004**
rs7562326 (T:C)	0.099	TT	694	473	Reference		63	Reference		233	Reference		165	Reference	
		TC	147	141	1.3 (1.0, 1.7)	0.067	20	1.4 (0.8, 2.5)	0.223	78	1.4 (1.0, 2.0)	0.048	41	1.1 (0.7, 1.7)	0.643
		CC	10	18	2.5 (1.1, 5.7)	0.024	1	NC		11	**3.4 (1.3, 8.7)**	**0.010**	5	2.8 (0.9, 8.6)	0.079
rs765138 (C:A)	0.090	CC	692	533	Reference		70	Reference		264	Reference		187	Reference	
		AC	152	99	0.8 (0.6, 1.1)	0.228	14	1.4 (0.7, 2.7)	0.287	57	1.0 (0.7, 1.4)	0.969	25	**0.5 (0.3, 0.8)**	**0.005**
		AA	0	0	NC		0	NC		0	NC		0	NC	
rs519704 (G:A)	0.092	GG	705	474	Reference		64	Reference		233	Reference		165	Reference	
		AG	141	139	1.3 (1.0, 1.7)	0.041	19	1.3 (0.7, 2.4)	0.330	78	1.5 (1.1, 2.1)	0.019	40	1.1 (0.8, 1.7)	0.518
		AA	8	19	**3.2 (1.4, 7.7)**	**0.008**	1	NC		10	3.6 (1.3, 9.8)	0.013	7	**4.5 (1.5, 13.6)**	**0.007**
STARD3															
rs1874224 (A:C)															
White	0.021	AA	251	270	Reference		46	Reference		164	Reference		54	Reference	

Gene, SNP (Alleles)	MAF (Controls)	Genotype	No. Controls	No. Cases	OR (95% CI) All Cases	P	No. Mild Cases	OR (95% CI) Mild Cases	P	No. Mode- rate	OR (95% CI) Moderate Cases	P	No. Severe Cases	OR (95% CI) Severe Cases	P
		AC	11	4	0.3 (0.1, 1.1)	0.078	1	NC		3	0.4 (0.1, 1.7)	0.235	0	NC	
		CC	0	0	NC		0	NC		0	NC		0	NC	
Hispanic	0.019	AA	425	210	Reference		25	Reference		96	Reference		83	Reference	
		AC	17	11	1.3 (0.6, 2.9)	0.475	0	NC		1	NC		10	**3.4 (1.5, 8.0)**	**0.005**
		CC	0	0	NC		0	NC		0	NC		0	NC	
rs1877031 (T:C)															
White	0.312	TT	117	115	Reference		18	Reference		69	Reference		23	Reference	
		TC	117	125	1.2 (0.8, 1.7)	0.446	22	1.0 (0.5, 1.9)	0.903	75	1.3 (0.8, 2.0)	0.332	27	1.4 (0.7, 2.6)	0.359
		CC	21	27	1.5 (0.8, 2.8)	0.247	5	1.3 (0.4, 4.2)	0.613	19	1.9 (0.9, 3.9)	0.106	3	0.9 (0.2, 3.4)	0.869
Hispanic	0.448	TT	134	86	Reference		8	Reference		35	Reference		42	Reference	
		TC	210	87	**0.6 (0.4, 0.9)**	**0.009**	10	0.8 (0.3, 2.2)	0.710	47	0.8 (0.5, 1.3)	0.326	28	**0.4 (0.2, 0.7)**	**0.001**
		CC	88	38	0.6 (0.4, 1.0)	0.058	6	1.3 (0.4, 4.1)	0.642	11	0.4 (0.2, 0.8)	0.015	19	0.7 (0.4, 1.3)	0.214
STS															
rs5934740 (G:A)	0.389	GG	521	342	Reference		39	Reference		183	Reference		109	Reference	
		AA	329	288	**1.4 (1.1, 1.7)**	**0.004**	44	1.8 (1.1, 3.0)	0.015	138	1.2 (0.9, 1.6)	0.196	102	1.5 (1.1, 2.1)	0.010
rs5934842 (C:A)	0.367	CC	538	354	Reference		40	Reference		190	Reference		113	Reference	
		AA	309	269	**1.4 (1.1, 1.7)**	**0.004**	43	1.8 (1.1, 2.9)	0.018	127	1.2 (0.9, 1.6)	0.167	95	1.5 (1.1, 2.1)	0.013
rs5934913 (G:A)	0.393	GG	509	330	Reference		38	Reference		172	Reference		109	Reference	
		AA	327	290	**1.4 (1.1, 1.7)**	**0.002**	46	1.8 (1.1, 3.0)	0.013	142	1.3 (1.0, 1.7)	0.075	98	1.4 (1.0, 2.0)	0.026
rs6639811 (A:G)	0.368	AA	519	342	Reference		39	Reference		182	Reference		110	Reference	
		GG	299	268	**1.4 (1.1, 1.8)**	**0.003**	43	1.9 (1.2, 3.1)	0.011	126	1.2 (0.9, 1.6)	0.161	95	1.5 (1.1, 2.1)	0.012
rs3923341 (G:A)	0.374	GG	531	345	Reference		38	Reference		186	Reference		110	Reference	
		AA	315	285	**1.4 (1.2, 1.8)**	**0.001**	45	**2.0 (1.2, 3.2)**	**0.007**	136	1.3 (1.0, 1.7)	0.098	100	**1.6 (1.1, 2.1)**	**0.007**
rs17268974 (T:A)	0.254	TT	635	437	Reference		62	Reference		226	Reference		137	Reference	
		AA	215	193	**1.4 (1.1, 1.8)**	**0.003**	22	1.1 (0.7, 2.0)	0.664	95	**1.5 (1.1, 2.1)**	**0.009**	73	1.5 (1.1, 2.1)	0.021
rs5934937 (C:G)	0.353	CC	549	365	Reference		42	Reference		196	Reference		116	Reference	
		GG	297	268	**1.4 (1.1, 1.8)**	**0.002**	42	1.7 (1.0, 2.7)	0.040	126	1.3 (1.0, 1.7)	0.086	96	**1.6 (1.1, 2.2)**	**0.005**

*Results for SNPs with p-value <0.01 overall or within a specific phenotype are shown (ORs with p<0.01 are in **bold**). ORs are presented if all cells in the comparison had at least 3 observations; separate results for whites and Hispanics are shown if the p-value for interaction was <0.10. All odds ratios were adjusted for the two ancestral proportion variables, maternal residence in the Central Valley (yes/no), and maternal race-ethnicity (Hispanic, non-Hispanic white, or other) if the results were not already stratified.

MAF = minor allele frequency, NC = not calculated

Table 4

Association of haplotypes in *HSD17B3* and *SRD5A2* with hypospadias.

Gene, Block	Haplotype	Frequency of cases, controls	OR (95% CI)	p-value	SNPs
HSD17B3					
Block 1	CACATCCAGATGTC	0.440, 0.430	Reference		
	GTCACGCAAATTCT	0.181, 0.176	0.9 (0.8 – 1.2)	0.585	
	GTCACGCGAGCTCC	0.137, 0.165	0.8 (0.7 – 1.1)	0.132	rs1324196, rs6479179, rs12552648, rs8190566, rs1927883, rs1927882, rs8190557, rs2066485, rs913580, rs2243595, rs2253502, rs1810711, rs912461, rs407179
	CTCATCCAGATGTC	0.086, 0.119	0.9 (0.7 – 1.2)	0.361	
	GTTGTCTAAACTTC	0.107, 0.073	**1.5 (1.1 – 2.0)**	**0.006**	
	GTCGTCTAAACTTC	0.016, 0.014	1.1 (0.6 – 2.2)	0.734	
Block 2	CAGGG	0.744, 0.782	Reference		rs729390, rs1886260, rs8190541, rs8190540, rs2066475
	TGAAA	0.175, 0.166	1.0 (0.8 – 1.3)	0.704	
	CGGGG	0.036, 0.015	**2.8 (1.6 – 4.8)**	**0.0003**	
	CAAGG	0.016, 0.014	0.9 (0.5 – 1.8)	0.782	
Block 3	AGT	0.378, 0.381	Reference		
	ACC	0.236, 0.211	1.1 (0.9 – 1.4)	0.251	rs2479824, rs8190534, rs2257157
	GGT	0.201, 0.232	0.8 (0.7 – 1.0)	0.069	
	AGC	0.177, 0.171	1.0 (0.8 – 1.2)	0.864	
Block 4	GG	0.475, 0.435	Reference		rs7039978, rs2476923
	AA	0.435, 0.445	0.9 (0.8 – 1.1)	0.374	
	AG	0.089, 0.121	0.9 (0.7 – 1.1)	0.341	
Block 5	TAGGAGT	0.294, 0.265	Reference		rs8190531, rs2479822, rs8190530, rs11788785, rs16910694, rs999269, rs13302476
	CGGAAGA	0.250, 0.248	1.0 (0.8 – 1.3)	0.875	
	CGAGACT	0.234, 0.232	1.0 (0.8 – 1.2)	0.758	
	TGGGAGT	0.100, 0.107	1.0 (0.7 – 1.3)	0.772	
	TAGGCGT	0.076, 0.099	1.0 (0.8 – 1.4)	0.886	
	CGGGAGA	0.014, 0.012	1.5 (0.8 – 3.0)	0.239	
	CGGGAGT	0.010, 0.012	0.8 (0.4 – 1.7)	0.536	
Block 6	TAGAGGCAACGT	0.282, 0.252	Reference		rs2476927, rs4551481, rs8190522, rs1983828, rs280663, rs8190512, rs2479828, rs1119864, rs11788083, rs8190508, rs8190498, rs2183009
	CGGAATCAGTGT	0.238, 0.270	1.0 (0.8 – 1.2)	0.980	
	CGACATCGACTC	0.201, 0.207	0.9 (0.7 – 1.1)	0.448	

Gene, Block	Haplotype	Frequency of cases, controls	OR (95% CI)	p-value	SNPs
	CAGAAGTAACGT	0.096, 0.099	1.0 (0.8 – 1.3)	0.978	
	CGGAATCAACGT	0.056, 0.043	1.2 (0.8 – 1.8)	0.391	
	CGGAGGCAACGT	0.037, 0.039	1.1 (0.7 – 1.6)	0.791	
	CGACATCAACGC	0.019, 0.017	1.1 (0.6 – 2.0)	0.678	
SRD5A2					
Block 1	ATCCCTATA	0.322, 0.344	Reference		rs9332975, rs2281546, rs28383032, rs12470143, rs4952220, rs6543634, rs28383018, rs2268794, rs725631
	ATCTATGTC	0.258, 0.270	1.0 (0.8 – 1.2)	0.85	
	ATCTATATC	0.121, 0.158	0.9 (0.7 – 1.1)	0.337	
	GGTCCGAAC	0.130, 0.076	**1.7 (1.3 – 2.2)**	**0.0003**	
	ATCCATATC	0.082, 0.066	1.3 (0.9 – 1.7)	0.145	
	ATCCCTATC	0.011, 0.014	0.9 (0.5 – 1.6)	0.705	
	ATCCCGAAC	0.009, 0.014	0.6 (0.3 – 1.3)	0.219	
	AGCCATAAC	0.016, 0.008	1.0 (0.3 – 3.0)	0.979	
	GGCCCGAAC	0.010, 0.012	1.0 (0.5 – 2.2)	0.912	
Block 2	CG	0.449, 0.457	Reference		rs2300697, rs2300700
	TA	0.383, 0.415	0.9 (0.8 – 1.1)	0.413	
	TG	0.166, 0.127	1.3 (1.0 – 1.6)	0.044	
Block 3	GCT	0.461, 0.468	Reference		rs2208532, rs7594951, rs13395648
	ACT	0.401, 0.434	0.9 (0.8 – 1.1)	0.436	
	ATC	0.128, 0.083	**1.4 (1.1 – 1.8)**	**0.008**	
Block 4	TCCCCCGC	0.473, 0.503	Reference		rs7562326, rs2300703, rs2754530, rs2268799, rs28382999, rs765138, rs519704, rs523349
	TTTTCCGG	0.259, 0.261	1.1 (0.9 – 1.3)	0.519	
	CTCCACAC	0.131, 0.085	**1.5 (1.2 – 1.9)**	**0.002**	
	TTTTCAGG	0.073, 0.087	0.9 (0.6 – 1.2)	0.37	
	TTTTCCGC	0.038, 0.030	1.1 (0.7 – 1.7)	0.711	

Table 5

Association of risk scores overall and within specific phenotypes.*

	Score	No. Cases	No. Controls	OR (95% CI)
All Cases	0	121	218	Reference
	1	249	346	1.4 (1.1–1.9)
	2	203	221	2.3 (1.7–3.2)
	3	48	65	2.5 (1.6–4.1)
	4	12	5	8.3 (2.8–24.9)
Mild Cases	0	22	364	Reference
	1	40	388	1.7 (1.0–3.1)
	2	22	103	3.2 (1.6–6.4)
Moderate Cases	0	84	349	Reference
	1	154	340	2.3 (1.6–3.2)
	2	68	138	2.9 (1.9–4.4)
	3	16	24	4.5 (2.1–9.5)
	4	0	3	---
	5	0	1	---
Severe Cases	0	7	68	Reference
	1	57	354	1.9 (0.8–4.4)
	2	99	342	3.5 (1.5–8.2)
	3	47	82	8.7 (3.5–21.5)
	4	2	9	---

*Risk scores reflect the number of genes for which an individual had a variant genotype that had a p-value <0.01 (see Table 3 for variants that met this criterion). The maximum *possible* scores were 5, 2, 5, and 4 for all, mild, moderate and severe cases, respectively. All odds ratios were adjusted for the two ancestral proportion variables, maternal residence in the Central Valley (yes/no), and maternal race-ethnicity (Hispanic, non-Hispanic white, or other).