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Environmental and Genetic Contributors to Hypospadias: A Review of the Epidemiologic Evidence

Suzan L. Carmichael[1], **Gary M. Shaw**[1], and **Edward J. Lammer**[2]

[1]Department of Pediatrics, Division of Neonatology, Stanford University School of Medicine, Stanford, CA

[2]Children's Hospital Oakland Research Institute, Oakland, CA

Abstract

This review evaluates current knowledge related to trends in the prevalence of hypospadias, the association of hypospadias with endocrine-disrupting exposures, and the potential contribution of genetic susceptibility to its etiology. The review focuses on epidemiologic evidence. Increasing prevalence of hypospadias has been observed, but such increases tend to be localized to specific regions or time periods. Thus, generalized statements that hypospadias is increasing are unsupported. Due to limitations of study designs and inconsistent results, firm conclusions cannot be made regarding the association of endocrine-disrupting exposures with hypospadias. Studies with more rigorous study designs (e.g., larger and more detailed phenotypes) and exposure assessment that encompasses more breadth as well as depth (e.g., specific endocrine-related chemicals) will be critical to make better inferences about these important environmental exposures. Many candidate genes for hypospadias have been identified, but few of them have been examined to an extent that enables solid conclusions. Further studies are needed that include larger sample sizes, comparison groups that are more representative of the populations from which the cases were derived, phenotype-specific analyses, and more extensive exploration of variants. In conclusion, examining the associations of environmental and genetic factors with hypospadias remain important areas of inquiry, although our actual understanding of their contribution to hypospadias risk in humans is currently limited.

Keywords

hypospadias; urogenital; genes; environment

I. Introduction

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). Hypospadias is a congenital malformation in which the urethral opening is on the ventral side of the penis, rather than at the tip. It is one of the most common congenital malformations, affecting about four to six males per 1,000 male births (Dolk and others, 2004; Paulozzi, 1999; Paulozzi and others, 1997). It ranges in severity from a urethral meatus that is slightly off center to a meatus in the perineal area. About 70% of cases are considered mild (the meatus is at or distal to the coronal sulcus; also referred to as 'first degree'), while the remaining

Corresponding author: Suzan L. Carmichael, Ph.D., Associate Professor, Department of Pediatrics, Division of Neonatal and Developmental Medicine, Stanford University, 1265 Welch Road, Rm X111, Stanford, CA 94305-5415, Telephone 650.736.0735, fax 650.721.5751, scarmichael@stanford.edu.

30% are considered moderate (the meatus is on the penile shaft; 'second degree') or severe (the meatus is in the penoscrotal or perineal area; 'third degree'). Some hypospadias cases can be attributed to known underlying genetic causes or syndromes (<10%), but most are idiopathic (Manson and Carr, 2003). The public health impact of hypospadias is significant, given that it usually involves surgical correction, and even after correction, individuals born with hypospadias may experience impaired sexual function and psychosocial difficulties related to sexuality and sexual activity later in life (Jugenburg and Kipikasa, 1988; Mieusset and Soulie, 2005). Hypospadias tends to be more common among babies born to women who are non-Hispanic white, have higher education, and are older and nulliparous, and babies with hypospadias are more likely to be small-for-gestational age (Manson and Carr, 2003).

Three questions that currently arise with regard to hypospadias are whether its prevalence is increasing, whether its etiology is related to exogenous exposures that influence endocrine function (endocrine disruptors), and what genes contribute to its etiology. This paper evaluates current knowledge related to these questions – clarifying dividing lines between what is known versus what is suspected. The focus is on the epidemiologic evidence; experimental evidence has been reviewed in more detail by others (Gray and others, 2004; Kalfa and others, 2011c; Kalfa and others, 2010; Yamada and others, 2006).

II. Trends in Prevalence

Several reports suggested hypospadias was increasing in Europe in the 1970s (Paulozzi, 1999). Concerns were renewed upon publication of a report that hypospadias prevalence had doubled in the Atlanta area and other parts of the United States from 1970–1993 (from about 1.5–2.0 per 1,000 total births, to about 3.5) (Paulozzi and others, 1997). This was the first report of an increase outside of Europe (Paulozzi and others, 1997). It was also the first study to report prevalence by severity of the phenotype. This was potentially an important addition given that trends in moderate to severe phenotypes may be less subject to reporting bias than mild cases, e.g., mild cases may be more completely reported over time if there is heightened awareness and therefore increased reporting of uncorrected mild cases. However, the percent of cases in the Atlanta study that had any information on severity went from about 20% to 50% during the study period, which undermines the interpretability of trends by severity (the trend among more severe cases could be attributable to improved reporting of severity).

The U.S. report was followed by data indicating that the previously reported increasing prevalences in Europe had stabilized by the 1980s and 1990s, with the exception of Denmark (Aho and others, 2000; Paulozzi, 1999). Prevalences were stable or declining in many other countries and regions during this time period (Canada, Australia, New Zealand, California, Israel, China, Mexico, South America) (Paulozzi, 1999). Prevalences were increasing only in France, Czechoslovakia and Japan.

The next wave of prevalence studies captured more recent observations, from the 1990s onward. Most reported relatively stable or declining prevalences, including Atlanta and Japan (Abdullah and others, 2007; Ahmed and others, 2004; Correa and others, 2007; Dolk and others, 2004; Elliott and others, 2011; Fisch and others, 2010; Ghirri and others, 2009; Kurahashi and others, 2004; Loane and others, 2011; Martinez-Frias and others, 2004; Porter and others, 2005). Increases were reported in Western Australia for all levels of severity (Nassar and others, 2007), and a continued increase has been confirmed in Denmark (Lund and others, 2009). The majority of registries contributing to the European Surveillance of Congenital Anomalies (EUROCAT) reported stable or declining prevalences during this time period. The report noted that any observed increasing prevalences could be

attributable to changes in reporting of mild cases over time (Dolk and others, 2004; Loane and others, 2011). Increases have also been reported in China, but initial prevalences were quite low (less than one per 1,000 male births), which suggests under-ascertainment of the condition (Jin and others, 2010; Li and others, 2012).

Potential increasing prevalences in hypospadias have been observed but such increases are localized to a few specific regions or time periods. Thus, generalized statements that hypospadias is increasing are clearly unsupported. Nevertheless, the areas that reveal changes in prevalence over time would be prime candidates for study to determine whether particular exposures are responsible. Incorporation of severity of phenotype into future studies of prevalence trends would be a useful adjunct whenever feasible, given concerns that changes in reporting of mild cases over time could bias observed trends.

III. Endocrine Disruption

Urethral closure depends on fetal conversion of testosterone to dihydrotestosterone (DHT) by steroid 5α-reductase type II, binding of the ligand DHT to the nuclear androgen receptor, and proper subsequent androgen receptor (AR) action. Hypospadias can be induced experimentally by exposures that interfere with androgen and estrogen synthesis and signaling pathways during sexual differentiation, including AR antagonists, inhibitors of 5α-reductase, and inhibitors of enzymes involved in steroid hormone synthesis (Gray and others, 2004; Noriega and others, 2005; Ostby and others, 1999)[105]. Thus, biologic plausibility and experimental evidence exists that endocrine disruptors, defined broadly as exogenous substances that interfere with hormones, may cause hypospadias in humans.

The purported increasing prevalence of hypospadias does not necessarily provide evidence supporting a connection between hypospadias and endocrine disruptors. Given the ubiquity, variety, and dynamic mix of endocrine-disrupting exposures in our environment over time, and even assuming there existed a generalized increased prevalence, drawing an inference of such a connection is tenuous at best and ecological fallacy at worst. Determining a possible connection between endocrine-disrupting exposures and hypospadias is best carried out in carefully designed epidemiologic studies that capture more individualized exposure assessments.

Environmental chemicals

The group of chemicals that has received the most attention with respect to hypospadias is pesticides, some of which are endocrine disruptors. A recent meta-analysis of seven studies indicated that maternal agricultural work or occupational exposure to pesticides was associated with a modest increase in risk of hypospadias – reporting an odds ratio of 1.4 (95% confidence interval (CI) 1.0, 1.8) for mother's occupation (Rocheleau and others, 2009). Three large occupation-based studies published since then, however, have not provided evidence for increased risk (Morales-Suarez-Varela and others, 2011; Nassar and others, 2010; Rocheleau and others, 2011).

Other groups of chemicals that include endocrine disruptors have also been evaluated. In particular, several studies have used job exposure matrices to assess probable maternal occupational exposure to specific groups of chemicals (Table 1). Use of such matrices tends to be more rigorous than simply asking mothers to recall exposure to specific types of compounds. A small questionnaire-based study in Italy found increased risks with probable exposure to several groups of chemicals (Giordano and others, 2010), but odds ratios were relatively imprecise and whether controls and cases were derived from the same underlying population is uncertain. A questionnaire-based study in England reported increased risk with phthalate exposure, but control participation was low (33%), which could undermine the

study's validity (Ormond and others, 2009). A large study in the United Kingdom based on birth defects registry data reported that hypospadias was not more likely than expected among infants born to women with any particular occupational exposures (Vrijheid and others, 2003). A large study in Australia based on occupational data from birth registration forms reported increased risk with heavy metals, but not other groups of chemicals (Nassar and others, 2010). A prospective cohort study conducted in Denmark reported increased risks with phthalates and alkylphenols (Morales-Suarez-Varela and others, 2011). Thus, some studies have suggested increased risk with certain groups of chemicals, but results are inconsistent.

Paternal exposures have also been examined

Evidence for increased hypospadias risk associated with paternal occupational exposures is modest. The recent meta-analysis of occupational exposure to pesticides included eight studies of paternal exposures; the odds ratio was 1.2 (95% CI 1.0, 1.4) (Rocheleau and others, 2009). Results from occupation-based studies of exposures to other groups of chemicals suggest increased risk with occupational exposure to biphenolic compounds, although the confidence intervals include one (Table 2). The premise underlying these studies of paternal exposures is often not stated but may include maternal exposure via seminal fluid (Rocheleau and others, 2009).

Although these studies are useful for generating clues, they must be interpreted with caution. They are limited in that they do not consider non-occupational sources of the chemicals, not all chemicals within each group have endocrine-disrupting properties, and the endocrine disruptors within each group may contribute to different (or even opposing) mechanisms of action, e.g., some endocrine disruptors within a group may have estrogenic properties while others are anti-estrogenic.

Some studies have attempted to examine exposures more directly. A study in Arkansas analyzed residential proximity to agriculture-related pesticide applications, which was estimated based on data related to phenology and average annual pesticide applications (Meyer and others, 2006). They examined many individual pesticides and also groupings of pesticides that were based on toxicologic evidence, including their specific endocrine-disrupting effects (e.g., anti-androgenic). Higher exposures were not associated with increased risk; in fact, almost all odds ratios were less than one, albeit imprecise. Several studies have measured maternal serum levels of DDE, a metabolite of DDT, a persistent pesticide used until the 1970s that has known estrogenic and anti-androgenic properties. These studies do not provide evidence for increased hypospadias risk with increased exposure (Bhatia and others, 2005; Carmichael and others, 2010; Flores-Luevano and others, 2003; Giordano and others, 2010; Longnecker and others, 2002), even during the 1960s when exposure was much higher than today (Longnecker and others, 2002). A study in India reported higher levels of DDE in cases than controls, but levels were measured up to ten years after birth (Shekharyadav and others, 2011). Cases also had significantly higher levels of HCH (hexachlorocyclohexane), but not any other organochlorine pesticide. Maternal levels of chlordane (Trabert and others, 2011) and PCBs (polychlorinated biphenyls) (McGlynn and others, 2009) were examined in a cohort of births from the 1960s, when levels were much higher than today. Chlordane was not associated with hypospadias (Trabert and others, 2011). Results suggested PCB exposure was associated with modestly increased risk (50–70% for the lowest versus higher quartiles), but the authors noted that the modest magnitude of the association, combined with lower current exposure levels, argue against PCBs being a substantial contributor to hypospadias risk. Other small studies measuring PCBs and other halogenated organic pollutants in maternal serum have not reported higher levels in mothers of hypospadias cases (Carmichael and others, 2010; Giordano and others, 2010). A study of maternal exposure to PBB (polybrominated

biphenyl), a brominated flame retardant, was negative (Small and others, 2009). However, it was based on maternal PBB levels that may have been measured many years before delivery, and hypospadias was based on self-report of the sons.

Other potential chemical exposure sources have also been investigated. A study in Great Britain reported that hypospadias was not increased among infants living within 2 km of landfill sites (Elliott and others, 2001). A study in South Africa observed no differences in birth prevalence of hypospadias in villages that were, versus were not, sprayed with DDT for malaria control (Bornman and others, 2010). Two studies have reported no association of hypospadias with residential water source levels of water disinfection by-products (Iszatt and others, 2011; Luben and others, 2008).

A study in Spain observed higher placental levels of organochlorine pesticides and total effective xenoestrogen burden from organohalogenated compounds in hypospadias cases relative to controls (Fernandez and others, 2007). However, the case group of 50 infants also included isolated cryptorchidism cases, so results should be considered inconclusive. Hypospadias and cryptorchidism may have some overlapping etiologic factors, but they are mechanistically quite distinct.

One study reported that maternal self-reported use of biocides was associated with increased hypospadias risk (e.g., insect repellants, ant powder, flea and lice treatments) (Dugas and others, 2010). The exposures were not associated with increased risk when examined one at a time, with the exception of insect repellants. A higher total number of exposures was associated with increased risk. The control participation was only 33% for this study (Ormond and others, 2009), which may undermine the validity of its findings.

Vegetarian diet and phytoestrogens

Phytoestrogens are naturally occurring plant substances that have structural and functional similarities to estrogen. One small epidemiologic study suggested that a maternal vegetarian diet and high intake of legumes (which are high in lignans, a type of phytoestrogen) were associated with increased risk of hypospadias (North and Golding, 2000). Phytoestrogen intake was proposed as an explanation for the finding, and the finding has frequently been used to support the hypothesized connection between endocrine disrupting exposures and hypospadias. The study was followed by an experimental study indicating that the phytoestrogen genistein, which is particularly high in soy products, could induce hypospadias (Vilela and others, 2007). However, several studies have not confirmed an increased risk of hypospadias with a vegetarian diet or low meat or fish consumption (Brouwers and others, 2006b; Giordano and others, 2010; Giordano and others, 2008; Ormond and others, 2009; Pierik and others, 2004; Shekharyadav and others, 2011), with two exceptions (Akre and others, 2008; Samtani and others, 2011). Given limitations regarding study designs, sample sizes, and incomplete dietary assessment, however, these studies are somewhat problematic for drawing meaningful inferences.

Exogenous sex hormones and fertility

Two studies have observed at least a two-fold increased risk of hypospadias among women who took progestins (i.e., natural progesterone and synthetic progesterone and testosterone derivatives that produce biologic effects similar to those of progesterone) for the purpose of preventing pregnancy loss or other complications (Carmichael and others, 2005; Kallen and others, 1992). Progestin intake for the purpose of contraception does not appear to be associated with increased risk of hypospadias (Carmichael and others, 2005; Wogelius and others, 2006). This inconsistency is not surprising, given that the dose and types of progestins used in oral contraceptives and for pregnancy-related issues are very different, as

are their mechanisms of action (Williams and Stancel, 1996). Maternal or paternal fertility problems or fertility-related procedures may also be associated with hypospadias (Bergh and others, 1999; Ericson and Kallen, 2001; Meijer and others, 2006; Silver and others, 1999; Sorensen and others, 2005b; Wennerholm and others, 1996), but it is uncertain whether the findings are due to underlying conditions, the procedures themselves or accompanying medications.

A few studies have examined whether hypospadias is associated with maternal intake of diethylstilbestrol (DES) a synthetic estrogen that was widely prescribed several decades ago. Early studies tended to be small and inconclusive (Henderson and others, 1976). However, more recent studies suggest that hypospadias risk may be increased among boys born to mothers who themselves were exposed to DES *in utero* (Brouwers and others, 2006a; Kalfa and others, 2011b; Klip and others, 2002; Pons and others, 2005), with one exception (Palmer and others, 2005). Each study has limitations, which is not surprising given the difficulty of studying trans-generational effects of a rare exposure. However, the findings are provocative and appear biologically plausible based on mechanistic and experimental data (Kalfa and others, 2011b).

Other medications

Certain medications may also confer endocrine-disrupting effects. Multiple studies have reported that valproic acid is associated with increased risk of hypospadias (Arpino and others, 2000; Diav-Citrin and others, 2008; Rodriguez-Pinilla and others, 2008). This association may be attributed to the gonadotropin-releasing hormone agonism of valproic acid, which has anti-androgenic effects long-term (Rodriguez-Pinilla and others, 2008). Corticosteroids, which have anti-androgenic properties, and loratadine, which has estrogenic properties, can both induce hypospadias experimentally (Ajayi and Fadiran, 1999; Lipworth, 1999; Negulescu and others, 1977; Reinisch and others, 1978; Willingham and others, 2006; Yucel and others, 2004a). They do not, however, appear to be associated with hypospadias risk in humans (Carmichael and others, 2009; CDC, 2004; Kallen and Olausson, 2006; 2007; Pedersen and others, 2008; Pedersen and others, 2006; Sorensen and others, 2005a).

Other factors

Other potential risk factors for hypospadias include placental abnormalities and maternal hypertension and pre-eclampsia (Akre and others, 2008; Akre and others, 1999; Boisen and others, 2005; Fujimoto and others, 2008). It is unknown – albeit plausible, and certainly worth further exploration – whether the co-occurrence of these conditions stems from common endocrine-related pathologies.

Summary

The available observational studies that can inform the question of whether endocrine disruptors are risk factors for hypospadias have thus far not provided solid evidence in support of the question. The studies have not been sufficiently rigorous or the results sufficiently consistent to justify a firm conclusion. Studies with more rigorous study designs (e.g., larger and detailed phenotypes) and exposure assessment that encompasses more breadth as well as depth (e.g., specific endocrine-related chemicals) will be critical to make better inferences about these important environmental exposures. For example, analyses that differentiate exposures more carefully based on their actual endocrine-disrupting properties (versus lumping large, broad categories of exposures together) will be particularly informative.

IV. Genetics

Heritability of hypospadias is high, at around 65–75%, and risk is estimated to be elevated 12- to 20-fold among first-degree relatives (Harris, 1990; Schnack and others, 2008). Most evidence from family-based studies points towards multifactorial (polygenic) inheritance of hypospadias, indicating that small effects of many genes and/or environmental factors act in concert to affect risk (Harris, 1990; Opitz, 1985). The current literature regarding genes and hypospadias has largely focused on candidate genes related to genital tubercle development and sex steroid metabolism. Experimental studies and case reports have provided many informative clues regarding potential relevant pathways and candidate genes, as reviewed by others (Kalfa and others, 2010; Kojima and others, 2010; Manson and Carr, 2003). The focus here is to summarize findings from epidemiologic studies in humans that included unaffected comparison groups.

Genital tubercle outgrowth and differentiation

Homeobox genes, bone morphogenetic proteins, and fibroblast growth factors all contribute to the development of the genital tubercle, the anlage for the penis (Baskin and others, 2004; Morgan and others, 2003; Yucel and others, 2004b). *WT1* also contributes to early development of the urogenital system (Kohler and others, 2007; Kohler and others, 2004; Kohler and others, 1999), and *WTAP* (Wilms tumor 1-associated protein) may promote *WT1* function. Studies have reported a greater frequency of mutations in cases than controls for *HOXA4, HOXA6, BMP4, BMP7, WT1* and *WTAP* among Chinese subjects (Chen and others, 2007), and *FGF8* and *FGFR2* but not *FGF10* or *BMP7* among Swedish subjects (Beleza-Meireles and others, 2007c), and one study did not observe difference in German subjects for *HOXA13* or *WTAP* (Utsch and others, 2003). The etiologic significance of these findings remains to be elucidated, given that the functional significance of many of the mutations are uncertain and attempts to replicate the findings in other study populations have not been published.

Fetal and placental biosynthesis and biotransformation of sex steroid hormones

SRD5A2 encodes steroid 5a-reductase type II, which converts testosterone (T) to DHT in the urethral seam(Kim and others, 2002). The V89L polymorphism (rs523349, +336G>C) has been investigated by several studies (Table 3). The C allele is associated with substantial reduction in enzyme activity (Samtani and others, 2010). Three studies have reported two- to three-fold increased risk associated with heterozygosity or homozygosity for the C allele, among Chinese, Caucasian and Indian subjects (Makridakis and others, 2000; Samtani and others, 2011; Thai and others, 2005). Two of these studies conducted phenotype-specific analyses and reported that results were similar regardless of severity (Samtani and others, 2011; Thai and others, 2005). A fourth study, of Japanese subjects, reported elevated risk only for severe cases (Sata and others, 2010). A fifth study, of Caucasians, reported no association (van der Zanden and others, 2010b). It is concerning (but not easily explained) that the study reporting no association was by far the largest (van der Zanden and others, 2010b). True population differences are possible but difficult to discern given the highly variable approach to control selection across the studies.

SRD5A1 is an isoform of *SRD5A2*. One small study did not observe differences in mutations *SRD5A1* among 10 cases and 49 controls (Tria and others, 2004).

CYP17 (p450c17) is critical to the biosynthesis and metabolism of androgens and estrogens (Sharp and others, 2004). A polymorphism in the 5′-untranslated promoter region, rs743572 (c.-34T>C), has been studied extensively (it is also referred to as the MspA1 polymorphism, with A2 signifying the variant allele). A study in India observed that relative to the T/T

genotype, the OR for the T/C genotype was 1.2 (95% CI 0.6, 2.5), and for C/C it was 2.1 (95% CI 0.8, 5.1) (Samtani and others, 2010). Results were more pronounced among moderate than severe cases. Another study in India included 91 cases and 132 controls selected from pediatric outpatient clinics; the ORs were 1.2 (0.6, 2.1) for T/C and 0.9 (0.4, 2.2) for C/C (Yadav and others, 2011). Results from these two studies were imprecise, likely due to the small sample sizes. An explanation for the differences in results is uncertain, although it could be influenced by the different approach to control selection.

HSD3B2 (3beta-hydroxysteroid dehydrogenase type II) is also essential to the biosynthesis of androgens and estrogens. A sequencing study of 90 moderate/severe cases and 100 controls suggested more mutations were present in cases than controls (Codner and others, 2004).

HSD17B3 encodes 17beta-hydroxysteroid dehydrogenase type 3, which is responsible for the conversion of androstenedione to testosterone. One study examined five SNPs in *HSD17B3* (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).

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). As a Phase I biotransformation enzyme, it also contributes to the detoxification of environmental toxicants. In one study, the variant allele of the *CYP1A1 m1* polymorphism (rs4646903, T3801C), which is associated with elevated enzyme activity, was protective against hypospadias, regardless of severity (Kurahashi and others, 2005). The odds ratio was 0.2 (95% CI 0.04, 0.7) for the CT genotype and 0.7 (95% CI 0.1–3.9) for the CC genotype. However, the *m1* polymorphism, as well as the *m2* polymorphism (rs1048943, A2455G), were not significantly associated with hypospadias in another study, although the associations with the variant alleles did tend to be in the protective direction (Shekharyadav and others, 2011). These two studies also examined *GSTM1* and *GSTT1,* which encode glutathione S-transferases, Phase II biotransformation enzymes and which each include a well-studied null deletion. In one study, the null deletions were not associated with hypospadias (Kurahashi and others, 2005). In the other study, the presence of both deletions (versus neither) was associated with increased risk (OR 2.3, 95% CI 1.1, 4.6) (Shekharyadav and others, 2011).

Regulation of sex steroid hormone biosynthesis and action

AR (androgen receptor), which is on the X chromosome, mediates the biologic effects of gonadal androgens (Hiort and Holterhus, 2000). Longer CAG repeats, which encode a polyglutamine tract in exon 1, are associated with reduced AR expression (Tut and others, 1997). One study reported longer CAG repeats among cases than controls (Lim and others, 2000), but others have not (Aschim and others, 2004; Muroya and others, 2001). One study reported longer GGN (glycine) repeats (also in exon 1) among cases (Aschim and others, 2004). Limited investigation of variants other than the CAG repeat as well as highly variable approaches to control selection make it difficult to draw firm conclusions from these results.

Two isoforms of the human estrogen receptor, *ESR1* and *ESR2* (alpha and beta) function analogously to the *AR*. They contribute to sex steroid levels and activity, and they interact with the AR to regulate gene expression (Beleza-Meireles and others, 2007b). Several studies have suggested that polymorphisms in *ESR1* and *ESR2* may be associated with hypospadias (Table 4). However, there are inconsistencies in results across studies, relatively few variants have been studied, and most studies have examined different sets of variants.

FKBP4 is essential to AR activity (Yong and others, 2006). One study examined two SNPs (rs1062478 and rs3021522); differences between cases and controls were not significant (Beleza-Meireles and others, 2007a). *SRY* (sex determining region Y) is critical to sexual differentiation; *SOX9* (SRY-box 9) is part of its downstream signaling cascade (Wang and others, 2004). One study did not observe any mutations in the exons or exon-intron boundaries of *SRY* or *SOX9* (Wang and others, 2004).

Other candidate genes

ATF3 (activating transcription factor 3) was identified through microarray expression studies demonstrating up-regulation of *ATF3* in penile tissue from boys with hypospadias (Liu and others, 2005; Wang and others, 2007) and in the genital tubercle of mice exposed to estrogen (Liu and others, 2006). Two studies have suggested a greater frequency of mutations in cases than controls (Kalfa and others, 2008b; van der Zanden and others, 2010b). A large study of Swedish subjects reported that variant alleles of three of eight studied *ATF3* SNPs were independently and significantly associated with hypospadias (rs3125289, rs11119982, rs1877474, all in intron 1) (Beleza-Meireles and others, 2008). A large study of Dutch subjects examined rs11119982 (van der Zanden and others, 2010b). The study reported that the odds ratio for the C allele was 0.8 (0.7, 1.0), and results were similar regardless of phenotype. In contrast, the odds ratio for the C allele was 1.5 (95% IC 1.3, 1.9) in the Swedish study (Beleza-Meireles and others, 2008).

The contribution of *MAMLD1* (mastermind-like domain containing 1, or *CXorf6*, on the X chromosome) to hypospadias was initially investigated because subjects who had microdeletions that involved *MAMLD1* as well as *MTM1*, the gene responsible for myotubular myopathy, had abnormal genital development (Fukami and others, 2006). The original sequencing study, along with three others, have suggested a greater frequency of mutations among cases than controls (Chen and others, 2010; Fukami and others, 2006; Kalfa and others, 2011a; Kalfa and others, 2008a). One study examined two SNPs (rs41313406, or c.856C>T; and rs2073043, or c.1766A>G) among Swedish subjects (Chen and others, 2010). Possession of both rare alleles (the T-G haplotype) was more frequent among cases than controls. This finding was confirmed by a subsequent study (Kalfa and others, 2011a). Combining the data from the two studies resulted in an odds ratio of 1.9 (95% CI 1.2, 3.0) for the T-G haplotype.

Recently, the first genome-wide association study (GWAS) of hypospadias was published (van der Zanden and others, 2010a). The study involved a pooled GWAS of 436 Dutch cases and 494 controls, followed by individual-level genotyping of 11 SNPs in the discovery sample and 220 additional Dutch cases and 328 Swedish cases. Dutch controls were derived from a separate case-control study; Swedish controls were derived from voluntary blood donors and from placental samples. The strongest finding was for the two SNPs that were individually genotyped for *DGKK* (rs1934179, which is intronic, and rs7063116, which is 21kb upstream). *DGKK* encodes diacylglycerol kinase K and is on the X chromosome. The variant alleles were associated with more than a two-fold increased risk of mild and moderate hypospadias in the Dutch and Swedish subjects; they were associated with severe hypospadias only among the Swedish subjects. The two SNPs were in high linkage disequilibrium, and their potential functional effect is unknown.

Summary

The list of biologically plausible candidate genes for hypospadias extends well beyond those that have been studied thus far. For many of the genes that have been examined in humans, studies have not gone beyond mutational screens of small sets of subjects, nor has the potential functional significance, if any, of most of the observed variants been investigated.

Few genes have been examined to an extent that enables comparisons across different study populations, but even then studies have often examined different sets of variants and had study design differences that complicate such comparisons, such as differences in racial-ethnic composition and highly variable approaches to control selection. Future studies will be better able to contribute to the current knowledge base by including larger sample sizes, comparison groups that are more representative of the populations from which the cases were derived, phenotype-specific analyses, and more extensive exploration of variants. It is also important that future endeavors include gene discovery approaches; certainly the initial findings from the recent GWAS (van der Zanden and others, 2010a), as well as a small array comparative genomic hybridization study (Tannour-Louet and others, 2010), have provided provocative findings that merit follow-up.

V. Conclusions & Recommendations

Extensive experimental evidence suggests that endocrine-disrupting exposures contribute to hypospadias etiology. The observational epidemiologic literature, however, falls short regarding whether (or which) environmental exposures contribute. There is highly publicized concern about these and other chemicals in our environment. Nevertheless, generalizations that focus on 'positive' findings rather than a more comprehensive view that incorporates null findings and study limitations should be avoided. It is important that future studies are designed to produce strong internal as well as external validity, as well as measurements that cover a broader range of chemicals, in a more direct way.

Extensive evidence suggests that genetic variation contributes to hypospadias, but again the observational literature does not provide firm evidence regarding which genetic variants contribute. In the area of genetic susceptibility, important challenges for future studies will be to incorporate a more in-depth inquiry of existing candidate genes and more attention to gene discovery tools. Another critical goal is to be able to interrogate interactions among the various endogenous, exogenous and genetic factors of interest. Thus far, studies have tended to be too small and/or lacking the data to make such inquiries.

Another lingering concern is whether phenotypic heterogeneity exists. Some studies suggest that heritability is somewhat higher for more severe hypospadias cases (Bauer and others, 1981; Opitz, 1985; Schnack and others, 2008; Sweet and others, 1974), while others do not (Brouwers and others, 2010; Fredell and others, 2002). Studies examining candidate genes have tended to report similar findings across the range of severity (Sata and others, 2010; Silver and Russell, 1999; Thai and others, 2005; van der Zanden and others, 2010a; Watanabe and others, 2007), as have studies examining descriptive risk factors such as age, parity and race-ethnicity(Brouwers and others, 2010; Carmichael and others, 2003). The association of hypospadias with low birthweight tends to be stronger, the more severe the phenotype (Brouwers and others, 2010; Carlson and others, 2009; Carmichael and others, 2003), but this may simply reflect the underlying severity of the condition, rather than different etiologies. Regarding embryology, more recent studies indicate that urethral closure 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). Thus, existing evidence tends to point toward similarity of etiology regardless of severity, but many studies have had sample sizes that limited the ability to detect phenotypic differences. The most prudent approach at this point is to consider phenotype-specific analyses whenever feasible.

Examining the associations of environmental and genetic factors with hypospadias remain important areas of inquiry, although our actual understanding of their contribution to hypospadias risk in humans is currently limited. A more solid understanding of their

contribution to hypospadias etiology will help guide the development of appropriate messages related to public health as well as individual-level risk, and it will facilitate the elucidation of underlying mechanisms, which are all important to its eventual prevention.

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Table 1

Odds ratios (95 percent confidence intervals) for associations of hypospadias with probable maternal occupational exposure to selected classes of chemicals that include endocrine disruptors, from studies using job exposure matrices.*

Study	Birth years and location of study population	Subject selection (case:control)	Pesticides	Phthalates	Organo-chlorines	Biphenols	Alkyl-phenols	Heavy metals	Any Exposure
Vrijheid et al. ’03	1980-96, England and Wales	Passive registry; malformed comparison group; (3,471:35,962)	0.8 (0.5–1.4)	0.9 (0.7–1.1)	1.0 (0.2–4.1)	0.7 (0.3, 1.9)	0.8 (0.7–1.1)	0.7 (0.4–1.2)	0.9 (0.7–1.0)
Ormond et al. ’08	1997-98, Southeast England	Case-control study with interview (471:490)	n.a.	3.1 (1.0–11)	n.a.	n.a.	n.a.	n.a.	n.a.
Nassar et al. ’09	1980–2000, Western Australia	Registry-based case-control study (1,202:2,583)	0.9 (0.5–1.7)	1.2 (0.8–1.7)	1.0 (0.2–4.9)	1.0 (0.4–2.5)	1.1 (0.8–1.5)	2.6 (1.3–5.2)	1.3 (1.0–1.7)
Giordano et al. ’10	Recruited 2005-07 (age 0–24 months), Italy	Case-control study with interview (80:80)	n.a.	3.6 (0.9–14.0)	n.a.	3.3 (0.3–33.6)	1.9 (0.9–4.0)	2.0 (0.4–9.0)	2.4 (1.1–5.6)#
Varela et al. ’11	1997–2009, Denmark	Cohort study with interview (262:45,341)	1.1 (0.9–1.3)	2.3 (0.9–3.7)	n.a.	0.8 (0.2–1.4)	2.3 (1.0–3.6)	1.2 (0.4–2.0)	1.8 (1.0–2.6)

*Odds ratios were adjusted for various covariates.

#Odds ratio for one versus zero exposure; odds ratio for more than one versus zero exposure was 4.1 (95% CI 1.3, 12.6).

Table 2

Odds ratios (95 percent confidence intervals) for associations of hypospadias with probable paternal occupational exposure to selected classes of chemicals that include endocrine disruptors, from studies using job exposure matrices.*

Study	Birth years and location of study population	Subject selection (case:control)	Pesticides	Phthalates	Organo-chlorines	Biphenols	Alkylphenols	Heavy metals	Any Exposure
Giordano et al. ’10	Recruited 2005-07 (age 0–24 months), Italy	Case-control study with interview (80:80)	n.a.	1.0 (0.5–2.3)	0.9 (0.3–2.7)	1.7 (0.3–10.7)	1.6 (0.7–3.7)	1.0 (0.3–3.6)	1.4 (0.6–3.2)#
Varela et al. ’11	1997–2009, Denmark	Cohort study with interview (262:45,341)	1.6 (0.8–2.4)	1.7 (0.9–2.5)	1.1 (0.4–1.8)	1.8 (0.8–2.8)	0.7 (0.3–1.1)	0.7 (0.2–1.2)	1.3 (0.7–1.9)

*Odds ratios were adjusted for various covariates.

#Odds ratio for one versus zero exposure; odds ratio for more than one versus zero exposure was 1.5 (95% CI 0.6–3.2)

Table 3

Association of hypospadias with the *SRD5A2* V89L (rs 523349, +336G>C) polymorphism.

Study	Subjects	Results overall	Results by phenotype
Samtani et al. 2011	80 cases, 100 controls from house-to-house surveys; Indian	CC: 3.6 (1.5, 8.8) CG: 2.1 (1.0, 4.1) GG: Reference	Similar for moderate and severe (too few mild to analyze separately)
Sata et al. 2010	89 cases, 291 controls born in Hokkaido; Japanese	CC: 1.2 (0.6, 2.5) CG: 1.3 (0.7, 2.3) GG: Reference	Mild/moderate: CC: 0.8 (0.3, 1.9) CG: 0.9 (0.5, 1.7) Severe: CC: 3.3 (1.0, 11.5) CG: 3.2 (01.1, 9.5)
Thai et al. 2005	158 cases, 96 ‘controls’; Caucasian (Sweden)	CC: 3.5 (1.8, 6.8) CG: 4.8 (2.5, 9.2) GG: Reference	Similar for mild, moderate and severe
Van der Zanden et al. 2010	609 cases, 596 controls from an unrelated case-control study; Caucasian (the Netherlands)	CC: 0.9 (0.6, 1.4) CG: 1.0 (0.6, 1.5) GG: Reference	Similar by severity (data not shown)
Wang et al. 2004	90 cases, 276 controls (“unrelated normal males”); Chinese	CC: 3.6 (1.4, 9.0) CG: 2.3 (1.1, 4.7) GG: Reference	n.a.

n.a. = not available

Table 4

Association of hypospadias with polymorphisms in *ESR1* and *ESR2*.

Study	Subjects	*ESR1*	*ESR2*
Aschim et al. 2005	51 cases, 186 controls who were military conscripts; Caucasian (Sweden)	n.a.	rs1256049 (1082G>A, a.k.a. *Rsa*I) AA: n.a. AG: 1.4 (0.4, 5.4) GG: Reference rs4986938 (1730G>A, a.k.a. *Alu*I) AA: 1.4 (0.6, 3.5) AG: 1.0 (0.5, 2.0) GG: Reference
Ban et al. 2008	59 cases, 286 controls born in Hokkaido; Japanese	rs2234693 (c.454– 397T>C, a.k.a. PvuII) & rs9340799 (c. 454- 351A>G, a.k.a. XbaI) – CC/AA genotype vs. others: 3.1 (1.2, 8.2)	rs944050 (2681-4A>G) GG: 0.8 (0.3, 1.8) GA: 0.4 (0.2, 0.8) AA: Reference
Beleza-Meireles et al. 2006	90 cases, 94 controls who were “voluntary blood donors”; primarily Caucasian (Sweden)	TA repeat n.s. ($p>0.05$)	Longer CA repeat ($p<0.05$) rs944050 (2681-4A>G) AG: 6.6 (0.8, 56.3) AA: Reference
Beleza-Meireles et al. 2007	354 cases, 380 controls who were “voluntary blood donors”; primarily Caucasian (Sweden)	n.a.	Studied CA repeat and 6 SNPs (excluding 944050, including rs2987983); only CA repeat was independently associated with increased risk
Van der Zanden et al. 2010	620 cases, 596 controls from an unrelated case- control study; Caucasian (the Netherlands)	rs6932902 (c.1236- 5602G>A) AA: 1.8 (0.8, 4.2) AG: 1.2 (0.9, 1.6) GG: Reference	rs2987983 (-13950T>C) GG: 0.8 (0.6, 1.3) GA: 0.8 (0.6, 1.0) AA: Reference
Watanabe et al. 2007	43 cases, 135 controls, including 82 boys seen for short stature and 53 fertile adult males; Japanese	Studied 8 SNPs; focused on 5 SNPs composing “AGATA” haplotype (includes rs6932902) – associated with increased risk ($p<0.05$)	n.a.s

n.s. = not significant; n.a. = not available