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Caucasian male infants and boys with hypospadias exhibit reduced anogenital distance

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BACKGROUND: Animal models of endocrine dysfunction have associated male genital defects with reduced anogenital distance (AGD). Human studies have correlated shorter AGD with exposure to putative endocrine disruptors in the environment but have not examined AGD in hypospadiac boys. We measured AGD in boys with hypospadias and those with normal genitals.

METHODS: Data were collected prospectively on boys undergoing urologic procedures at the University of California San Francisco and the Children's Hospital of Oakland, CA, USA. Data included age, race, height, weight, BMI, urologic diagnoses and AGD. To minimize any potential effects of race on observed AGD, we examined only Caucasian boys. Differences between boys with hypospadias and those with normal genitals were examined through two-tailed Student's *t*-tests.

RESULTS: One hundred and nineteen Caucasian boys ranging in age from 4 to 86 months underwent AGD measurement, of which 42 and 77 were boys with normal genitals and hypospadias, respectively. The mean ($\pm$SD) AGD of boys with hypospadias was 67 $\pm$ 1.2 versus 73 $\pm$ 1 mm for boys with normal genitals ($P = 0.002$). In these age-unmatched patient groups, there were also differences in age, height and weight ($P = 0.0001$, 0.0002 and 0.0004, respectively). After age matching (all <2 years of age), boys with hypospadias ($n = 26$) still featured a shorter AGD than boys with normal genitals ($n = 26$; 62 $\pm$ 2 versus 68 $\pm$ 2 mm respectively, $P = 0.033$) but the differences in age, height and weight were no longer significant.

CONCLUSIONS: In humans, hypospadias may indeed be associated with reduced AGD. Additional studies are needed to corroborate these preliminary findings and to determine their etiology.

Key words: hypospadias / perineum / endocrine disruptors / anogenital distance

Introduction

Hypospadias, an abnormality in which the urethra opens onto the ventral aspect of the penis rather than at the tip, has an incidence among live male births ranging from <1% in the USA (Baskin *et al.*, 2001) to over 4% in Denmark (Boisen *et al.*, 2005). Endocrine disruptors (ED) are postulated to cause hypospadias and cryptorchidism in multiple species, including humans (Skakkebaek *et al.*, 2001). Animal studies of putative ED have associated male genital defects with reduced anogenital distance (AGD; Parker *et al.*, 1984; Wise *et al.*, 1991; Mylchreest *et al.*, 1998; Gray *et al.*, 1999). In humans, AGD has been shown to be a sexually dimorphic measure of genital development (Salazar-Martinez *et al.*, 2004; Torres-Sanchez *et al.*, 2008; Thankamony *et al.*, 2009; Sathyanarayana *et al.*, 2010). Moreover, AGD also correlates with adult testicular function as measured by sperm production (Eisenberg *et al.*, 2011; Mendiola *et al.*, 2011). Human studies have attempted to correlate reduced male AGD with exposure to candidate ED (Swan, *et al.*, 2005; Longnecker *et al.*, 2007). To our knowledge, only one report to date has examined AGD in boys with hypospadias but this study featured a patient group that was heterogeneous with regard to age, race and urologic diagnoses (Hsieh *et al.*, 2008). We sought to re-examine the relationship between human AGD and hypospadias using a more homogeneous patient group.

Materials and Methods

Patient groups

Upon University of California San Francisco (UCSF) institutional review board approval of this study as 'exempt' from requiring informed consent, cross-sectional data were collected on boys undergoing operations for urologic conditions at the UCSF and the Children's Hospital of Oakland, CA, USA. Boys with Tanner Stage 2 development or higher, buried penis, chordee without hypospadias, cryptorchidism, imperforate anus, exposure to corticosteroids or growth hormone or known syndromes were excluded. Collected data included AGD, age, height, weight, race and urologic diagnoses. Percentiles for weight-for-age, height-for-age, weight-for-height and BMI-for-age were calculated based on 2000 CDC growth charts (Kuczmarski *et al.*, 2002). To account for prematurity, age at measurement was adjusted based on gestational age at birth. Normal genitals were defined as the absence of hypospadias, epispadias and chordee and complete descent of both testes. All boys meeting inclusion and exclusion criteria and undergoing urologic surgery at UCSF and the Children's Hospital of Oakland from 8 August 2006 through 18 October 2007 underwent measurement.

Measurement of AGD

Measurements were performed with patients intubated and supine with one hip flexed at a 90° angle. AGD was defined as the distance from the center of the anus to the most caudal point of the penoscrotal junction (Longnecker *et al.*, 2007). AGD measurements were performed by tying a suture to the anal temperature probe, laying it along the perineum to the penoscrotal junction, marking the length and then measuring the marked suture against a pair of calipers.

Statistical analysis

Comparisons between boys with hypospadias versus those with normal genitals were performed using two-tailed *t*-tests. A *P*-value of <0.05 was considered significant. The reproducibility of duplicate AGD measurements taken by a single, versus multiple, observer was calculated using coefficients of variation (CV).

Results

Patient characteristics

One hundred and nineteen Caucasian boys were measured. Forty-two boys had normal genitals, whereas 77 boys had hypospadias (Table I). Boys ranged in age from 4 to 86 months. Of the 42 boys with normal genitals, 14 underwent anesthesia for hydrocele repair, 5 for meatoplasty (repair of meatal stenosis), 3 for herniorraphy and the remaining 20 were anesthetized to undergo circumcisions or revisions of circumcisions. Boys with normal genitals were older (23 versus 12 months for boys with hypospadias) and consequently heavier and taller. The mean AGD of hypospadiac boys was shorter than that of boys with normal genitals (67 ± 1.2 versus 73 ± 1 mm respectively, $P = 0.002$, Table I).

We performed another analysis in which boys were matched by age (<2 years). Twenty-six boys who were Caucasian, under the age of 2 years and had normal genitals were compared with 26 age-matched Caucasian boys with hypospadias (Table I). Of the 26 boys with normal genitals, 8 underwent anesthesia for hydrocele repair, 2 for herniorraphy and the remaining 16 were anesthetized to undergo circumcisions or revisions of circumcisions. There were no significant differences between these age-matched boys with or without hypospadias in mean age, BMI, height, weight or percentiles for weight-for-age, height-for-age or weight-for-height ($P > 0.05$). The mean AGD of these age-matched boys with hypospadias versus those with normal genitals was 62 ± 2 and 68 ± 2 mm, respectively ($P = 0.033$).

Reproducibility of measurement of AGD

The reproducibility of duplicate AGD measurements taken by a single versus multiple observers was calculated using CVs, which were 3.3 and 8.7% for intra-observer and inter-observer comparisons, respectively.

Discussion

In many mammals, the AGD and related measures of perineal length are the result of the hormone-mediated development of the genital tubercle, genital swellings and cloacal membrane into the penis or clitoris, scrotum or labia majora and anus, respectively. AGD features sexual dimorphism, with males having a longer AGD (Salazar-Martinez *et al.*, 2004). In fact, female mice located *in utero* adjacent to two male fetuses feature a longer AGD than do females which are not next to male fetuses (McDermott *et al.*, 1978). Alterations in human AGD thought to be linked to endocrine abnormalities were first noted in girls with congenital adrenal hyperplasia, who had longer AGD than girls without the disorder (Bongiovanni, 1962).

The testicular dysgenesis hypothesis postulates that cryptorchidism, hypospadias, male infertility and testicular cancer are on the rise because of environmental factors affecting male reproductive health (Skakkebaek *et al.*, 2001). Suspected causes include compounds, such as ED, which may be disturbing normal endocrine development in multiple species, including humans.

Besides causing hypospadias in experimental models, known and putative ED have been reported to decrease male AGD in animal models (Parker *et al.*, 1984; Wise *et al.*, 1991; Mylchreest *et al.*, 1998; Gray *et al.*, 1999). Human studies to date have yielded mixed results. For example, *in utero* exposure to phthalate may be associated with reduced AGD in human male infants (Swan *et al.*, 2005). In contrast, another study of *in utero* exposure to degradation products of the insecticide 1,1,1,-trichloro-2,2,-bis(p-chlorophenyl)ethane (DDT) did not show an association with shortened human male AGD (Longnecker *et al.*, 2007). To our knowledge, only two reports have shown an association between cryptorchidism and shortened AGD (Swan *et al.*, 2005; Hsieh *et al.*, 2008), with the latter study also demonstrating reduced AGD in boys with hypospadias. However, Hsieh *et al.* (2008) relied on a clinical cohort which featured patients of various ages, urologic diagnoses (for controls) and races, all of which may plausibly influence AGD. We applied several linear regression models incorporating such variables with AGD (data not shown). However, owing to the strong collinearity between several variables (such as age) and AGD, when both variables are placed in the regression model it became unstable.

Hence, we sought to measure AGD in boys with hypospadias or normal genitals and eliminate possible confounding factors by matching race and age. It is possible that unidentified factors influencing AGD were not taken into account, which would result in residual

Table I Characteristics of Caucasian male infants and boys in a study of AGD.

Parameter	Not age matched			Age matched (<2 years of age)		
	Normal genitals	Hypospadias	*P*-value*	Normal genitals	Hypospadias	*P*-value*
n	42	77	n/a	26	26	n/a
Age (months)	23 ± 2.5	12 ± 1.5	0.0001	11 ± 1	10 ± 1	0.54
Height (cm)	82 ± 2	72 ± 1.5	0.0002	73 ± 2	72 ± 2	0.73
Weight (kg)	12 ± 0.5	9.6 ± 0.2	0.0004	9.5 ± 1.1	8.8 ± 0.4	0.37
BMI (kg/m^2)	17.3 ± 3.5	17.8 ± 0.25	0.3	17.5 ± 0.4	17.2 ± 0.5	0.57
Weight-for-age (%)	47 ± 4	44 ± 5	0.74	45 ± 6.5	44 ± 13	0.94
Height-for-age (%)	43 ± 4	42 ± 4	0.87	43 ± 6	39 ± 8	0.68
Weight-for-height (%)	57 ± 3.5	51 ± 4	0.31	60 ± 6	44 ± 7	0.1
AGD (mm)	73 ± 1	67 ± 1.2	0.002	68 ± 2	62 ± 2	0.033

Data are mean ± SD.
*Two-tailed Student's *t*-tests.

confounding. We restricted 'controls' to patients with hydroceles, phimosis, meatal stenosis or incomplete circumcisions to avoid other urologic diagnoses (i.e. cryptorchidism) which may also arise from, or cause, androgen imbalance. After matching patients by age, we found no significant differences in overall patient size as assessed by weight, height and BMI or percentiles for weight-for-age, height-for-age or weight-for-height between boys with hypospadias and normal genitals. We did, however, find that hypospadiac boys had shortened AGD relative to normal controls (62 and 68 mm, respectively, $P = 0.033$). Because of insufficient power, we were unable to correlate the precise severity of hypospadias with AGD. Ongoing work is addressing this issue.

It was not possible to blind observers to the boys' diagnoses because any hypospadias was evident during perineal measurement. This may have led to observer bias, although the magnitude of observed differences in the perineal length (~10%) suggests that any bias would not change overall findings.

The current study may feature selection bias. If the patients we examined are not representative of boys with hypospadias or normal genitals, our findings may be invalid. For example, not all boys with hypospadias present for surgical correction. We excluded patients with buried penis because it is difficult to locate the true penoscrotal junction in affected boys. Boys with chordee but no hypospadias were excluded because chordee may represent the mild end of the chordee-hypospadias spectrum, and hence it is unclear how to best classify these patients. It is possible that boys with very mild hypospadias are not as frequently referred to pediatric urologists for surgical repair. Collectively, exclusion of boys with 'mild' penile anomalies may have magnified the observed effect estimates. Future studies will examine the AGD of boys with chordee. In addition, we restricted our primary analysis to Caucasian boys and thus the current findings may not be representative of other racial groups.

Previous studies have measured AGD using conscious children (Bongiovanni, 1962; Callegari *et al.*, 1987; Salazar-Martinez *et al.*, 2004; Swan *et al.*, 2005; Longnecker *et al.*, 2007). Such assessments can be difficult because of intentional and unintentional resistance to examination. In the current study, all measurements were performed under anesthesia and we believe this permitted more accurate assessment of the perineal length. Although intra-observer reliability was good, with a CV of 3.3%, inter-observer reliability was lower, with a CV of 8.7%. These issues highlight the need to determine the optimal methods to measure AGD.

Mean AGD for boys (<2 years) with normal genitals in our study (68 mm) differed from that in another report (median AGD of 45.4 mm), likely because the mean age and weight of boys with normal genitals in the current study (11 months, 9.5 kg) were greater than that of boys in the study by Longnecker *et al.* (2007; <2 days, weight range: 2.5–5.1 kg). Defining the normative ranges of AGD will require additional studies.

Previous human studies have used a variety of measurements when referring to AGD: the distance from the anus to the most posterior, midline point of the scrotum (Salazar-Martinez *et al.*, 2004), the span from the anus to the most inferior, midline point of the penoscrotal junction and the length from the anus to the most superior, midline point of the penopubic junction (Swan *et al.*, 2005). The biological significance and reproducibility of these various perineal measurements remains to be determined. Moreover, given the technical challenges of measuring AGD in awake boys and the small absolute magnitude of differences between hypospadiac and normal boys, AGD measurements may not be practical outside the research/operating room setting.

Even if reduced AGD in boys with hypospadias is not related to ED, at a minimum it represents a previously unrecognized anatomic abnormality that is likely linked to abnormal androgen signaling. To our knowledge, this report is the first to describe a clinically relevant association between AGD and hypospadias in a demographically homogeneous group, namely Caucasian infants and young boys. Finally, shortened perineal length in the setting of hypospadias may be a subtle component of global phenotypic changes in under-virilized boys. Coupled with AGD data from the adult literature, AGD may be a useful marker for normal genital development and function (Eisenberg *et al.*, 2011; Mendiola *et al.*, 2011).

Conclusions

Our findings support an association between hypospadias and reduced AGD. The preliminary data provided by our small series should encourage ongoing efforts to confirm these findings and establish the etiology of this association.

Authors' roles

M.H.H. was involved in study conception. M.H.H. and L.S.B. were involved in study design. A.B.H., J.M.W., G.E.T. and L.S.B. were involved in study execution. M.H.H. and M.L.E. contributed to data analysis. M.H.H., M.L.E. and L.S.B. contributed to manuscript writing.

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Conflict of interest

None declared.

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