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Original Contribution

Congenital Abnormalities of the Male Reproductive System and Risk of Autism Spectrum Disorders

Ran S. Rotem*, Gabriel Chodick, Michael Davidovitch, Russ Hauser, Brent A. Coull, and Marc G. Weisskopf

* Correspondence to Ran S. Rotem, Department of Environmental Health, Harvard T.H. Chan School of Public Health, 401 Park Drive, Landmark Center, P.O. Box 15697, Boston, MA 02215 (e-mail: ran.rotem@mail.harvard.edu).

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Androgens have an extensive influence on brain development in regions of the brain that are relevant for autism spectrum disorder (ASD), yet their etiological involvement remains unclear. Hypospadias (abnormal positioning of the urethral opening) and cryptorchidism (undescended testes) are 2 relatively common male birth defects that are strongly associated with prenatal androgen deficiencies. Having either disorder is a proxy indicator of atypical gestational androgen exposure, yet the association between these disorders and autism has not been extensively studied. We analyzed male singleton live births ($n = 224{,}598$) occurring from January 1, 1999, through December 31, 2013, in a large Israeli health-care organization. Boys with autism, cryptorchidism, and hypospadias were identified via *International Classification of Diseases, Ninth Revision*, codes, with further verification of autism case status by review of medical records. In multivariable-adjusted analyses, the odds ratio for ASD among boys with either condition was 1.62 (95% confidence interval (CI): 1.44, 1.82). The odds ratio for boys with cryptorchidism only was 1.55 (95% CI: 1.34, 1.78), and that for boys with hypospadias only was 1.65 (95% CI: 1.38, 1.98). ASD risk was not elevated among unaffected brothers of hypospadias or cryptorchidism cases, despite familial aggregation of all 3 conditions, providing some indication for the possibility of pregnancy-specific risk factors driving the observed associations. Results suggest that in-utero hypoandrogenicity could play a role in ASD etiology.

androgens; autism; autism spectrum disorder; autistic disorder; cryptorchidism; hypospadias

Abbreviations: ASD, autism spectrum disorder; EMR, electronic medical record; ICD-9, *International Classification of Diseases, Ninth Revision*; MHS, Maccabi Health Services; SES, socioeconomic status.

Editor's note: *An invited commentary on this article appears on page 664.*

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with increasing prevalence worldwide (1). While genetics play a strong role in its etiology, evidence is increasing that environmental exposures also affect ASD risk (2–7). Androgens are known to affect fetal brain development, and a role for androgens in ASD etiology has been specifically proposed based on the participation of sex hormones in the regulation of neural domains involved in communication and social interaction that are relevant for ASD, as well as more indirectly based on the involvement of several genes that regulate sex hormone pathways in ASD pathogenesis (8–11). Thus, a better understanding of the contribution of in-utero androgen exposure in ASD etiology could have tremendous implications, both for understanding ASD etiology and for potentially generating interventions to reduce its burden.

Androgens also play pivotal roles in the development of the urethra and external genitalia in males. Androgen deficiency leads to the development of cryptorchidism (undescended testis) and hypospadias (abnormal positioning of the urethral opening on the ventral side of the penis) (12–14), two of the most common birth defects in newborn boys. An increasing prevalence of these anomalies has been reported over the past several decades (15, 16). Intriguingly, a correlation between county-level rates of male reproductive tract disorders and ASD has been observed ecologically (17), but to date this was only explored on an individual level in 1 study, and those investigators only considered

the less common hypospadias, not cryptorchidism (18). Thus, to further examine the association between androgen-dependent male reproductive system abnormalities and ASD, we explored the association between hypospadias and cryptorchidism and a clinical diagnosis of ASD in a large birth cohort in Israel.

METHODS

Study population

Maccabi Health Services (MHS) is Israel's second-largest integrated health-care organization, serving as both insurer and health-care provider to 2.1 million members (25% of the Israeli population). MHS is one of 4 insurers providing the equivalent universal coverage mandated by Israel's National Health Insurance Law. While transferring between health-care providers is possible, it is rare, and attrition is extremely low (1% per year), thus enabling long-term follow-up. MHS members are generally representative of the larger Israeli population, although the average monthly income in MHS is the highest among the 4 insurers. MHS physicians use an electronic medical record (EMR) system, which feeds into a central database with administrative and clinically oriented data. The database is linked to records from Israel's Central Bureau of Statistics, permitting linkage to additional information. The conduct of this study was approved by the MHS institutional review board (Helsinki Committee of Assuta Medical Center) and by the Office of Human Research Administration at the Harvard School of Public Health.

There were 248,082 male singleton pregnancies ending in a live birth that occurred in MHS from January 1, 1999, through December 31, 2013, among mothers who were MHS members throughout the year preceding the birthdate of their child. Our main analyses included 224,598 children, after we excluded children who left MHS before the end of follow-up (December 31, 2016) at an age younger than 8 years to avoid missing ASD diagnoses that may have been given to children after leaving MHS. However, all male singleton births were considered with censoring in sensitivity analyses. We searched MHS data through December 31, 2016, to identify male reproductive tract disorders and cases of ASD. We used specific *International Classification of Diseases, Ninth Revision* (ICD-9) codes to identify cases of cryptorchidism (752.51) and hypospadias (752.61). To have more specific exposure definitions, we excluded possible cases identified solely by the nonspecific codes 752.6 (hypospadias, epispadias, and other penile anomalies) and 752.5 (undescended and retractile testicle), since these broader codes include additional anomalies with unrelated pathogeneses. Use of these broader codes was phased out in the mid-1990s but continued intermittently until the early 2000s. We considered all diagnoses in a sensitivity analysis.

ASD case ascertainment

In the primary analyses, we considered as cases any children with an ICD-9 record of 299.x, indicating an ASD diagnosis (n = 3,992). A review of a random sample of 450 such cases by our coauthor (M.D.), who is head of the Child Development Department at MHS, confirmed the diagnosis for 90.4% of these cases.

In sensitivity analyses, we also used a stricter case definition. The Israeli National Insurance Law entitles children diagnosed with ASD to receive enhanced medical, psychological, and social services. To be eligible, the child's diagnosis must meet a set of strict criteria. The diagnosis must be based on: 1) a physical, neurological, and developmental assessment conducted by a pediatric neurologist or a pediatric psychiatrist; 2) meeting all *Diagnostic and Statistical Manual of Mental Disorders*, Fourth or Fifth Edition, criteria, and the provision of a detailed description of the tests and tools used in the evaluation; and 3) in addition to the medical evaluation, an independent psychological assessment that includes a detailed, age-appropriate developmental and cognitive assessment confirming the diagnosis of ASD. Additional observations from speech pathologists, occupational therapists, and social workers are incorporated into the evaluation process. Review of such cases by our coauthor (M.D.) confirmed 100% of the diagnoses that met these criteria. The ability to access governmental benefits incentivizes most families to go through this extensive evaluation process in order to confirm their child's ASD case status, but this requires the active involvement of the family. Families who do not require further assistance or are otherwise not interested in receiving additional services may choose to opt out.

Covariate information

We considered adjustment for several demographic covariates that could be related to both conditions and so potentially produce an association between the two: socioeconomic status (SES), minority group representation in the mother's residential enumeration area (a homogenous geographical unit of approximately 3,000 people), parental age, residential district, and calendar year.

Socioeconomic level was based on mothers' residential enumeration areas, as defined by the 2008 national census. Israel's Central Bureau of Statistics assigns a poverty index for each residential enumeration area (on a scale of 1–10) based on several parameters, including household income, percentage of recipients of income and unemployment supplements, educational qualifications, crowding, material conditions, and car ownership (19). Information on the percentages of residents in 4 minority groups (Ultraorthodox Jews, Israeli Arabs, Ethiopians, and Russians), on a scale of 1 (none) to 5 (very high), was also obtained for each enumeration area. Information on maternal age at birth was calculated on the basis of the mother's and child's birth dates as recorded in the EMRs. Paternal age was only available for 60% of the children in our cohort, and it was highly correlated with maternal age ($r = 0.8$, $P < 0.001$). Thus, we only adjusted for maternal age in our analyses. We considered the possibility of a secular trend in diagnoses by including an adjustment for child's birth year.

Our hypothesis was that the association between the male reproductive disorders and ASD is indirect—that is, that having either of the conditions is not, on its own, a direct risk factor for ASD but rather these conditions are caused by factors that also increase the risk of ASD (e.g., in-utero hypoandrogenicity). We considered additional models that adjusted for pregnancy-related factors—despite some of them possibly arising after onset of the male reproductive disorders—to evaluate whether any association between these disorders and ASD is independent of these conditions, under the assumption that if they

weren't, the adjusted association would likely be reduced. Fertility treatments, preeclampsia, diabetes, and in-utero growth restriction have been suggested to relate to risk of ASD and male reproductive tract disorders, possibly through shared risk factors acting prior to conception or in early gestation (20–33).

We used the EMRs for each mother in our cohort to obtain information on in vitro fertilization or treatment with other ovarian stimulation drugs (clomifene, gonadotropins). Births related to in vitro fertilization were defined as those that occurred 5–10 months after embryo transfer. Births associated with ovarian stimulation treatments were defined as those that occurred 5–12 months after the last date of dispensation of ovary-stimulating drugs. Importantly, while these treatments are recorded in the mother's EMRs, the underlying reason for receiving these treatments is often not related to a maternal infertility condition but rather to paternal factors.

We used ICD-9 codes 648.0 and 648.8 to obtain information on mothers diagnosed with gestational diabetes mellitus during pregnancy. For each mother, we additionally collected information on dispensing of insulin or metformin during pregnancy and on results of the 100-g oral glucose tolerance test, commonly used as a test for gestational diabetes. Mothers without a gestational diabetes diagnosis in their EMRs who purchased insulin or metformin during pregnancy or had at least 2 pathological values on the same oral glucose tolerance test were defined as having gestational diabetes. We identified mothers with prepregnancy diabetes as those with ICD-9 code 250.x or 790.2 given prior to conception, as well as those with blood tests showing abnormal blood glucose (2 random blood sugar tests within a 30-day period with values exceeding 200 mg/dL) or hemoglobin A_{1c} (>7.3%) values. Women who purchased insulin and/or other antidiabetic drugs regularly were additionally defined as having prepregnancy diabetes. We additionally used ICD-9 code 642.x to obtain information on preeclampsia (and hypertension) during pregnancy. Detailed information on birth weight (grams) and gestational age (weeks) was obtained from hospital birth records.

Statistical analysis

We used generalized estimating equations logistic regression models to evaluate whether a child diagnosed with cryptorchidism or hypospadias was at increased odds for also being subsequently diagnosed with ASD, using a robust (sandwich) variance estimator to obtain consistent standard errors for the effect estimates. We further accounted for geographical clustering by including indicator variables for each district. For analyses that considered birth weight and gestational age, we used multiple imputation by chained equations (34) to impute missing gestational age (4.7% of births) and birth weight (0.7%) values. We generated 20 imputed data sets on which we ran our analysis, and then combined the results by taking the average of the regression coefficients across data sets using Rubin and Schenker's formula (35) to estimate the variance. We initially used generalized additive logistic regression models with penalized splines (R 3.2.2, mgcv package; R Foundation for Statistical Computing, Vienna, Austria) to more flexibly model the associations between ASD and the continuous covariates of interest (gestational age, birth weight, SES, birth year, and maternal age at birth) and then used polynomial terms to account for the curvature observed in birth weight and SES with regard to ASD risk. We also conducted a sensitivity analysis using a Cox model with time since birth as the metameter, considering the entire cohort of male singleton births, and censoring at the time of the first indication of ASD, the date of the last MHS contact, or the end of follow-up, whichever came first.

To evaluate whether the results were susceptible to possible detection bias (i.e., that children diagnosed with ASD are examined more thoroughly for other conditions compared with typically developing children), we repeated the analysis after excluding hypospadias and cryptorchidism cases who were diagnosed with these conditions after the first date of ASD evaluation, even if the child received the final ASD diagnosis at a later date. We also repeated the analysis after excluding boys diagnosed with chromosomal anomalies or congenital anomalies affecting the nervous system (defined by ICD-9 codes 740–742 and 758). We additionally repeated the analyses restricting the cases to cryptorchidism and hypospadias diagnoses given during the first year of life, to exclude possibly milder hypospadias cases that were not detected near birth and to exclude cryptorchidism cases with late ascension of testes, which is considered a later developmental disorder (16). Finally, we examined the odds of ASD among brothers of boys with hypospadias or cryptorchidism to assess whether any association was child-specific or clustered in families. The final analysis was conducted in SAS 9.4 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

Characteristics of the study population are provided in Table 1. A total of 157,667 mothers gave birth to 224,598 singleton male newborns during 1999–2013. By the end of follow-up (December 31, 2016), 7,524 (3.4%) boys had been diagnosed with cryptorchidism and 4,141 (1.8%) with hypospadias (280 boys were diagnosed with both conditions). For the main analyses, we excluded 488 possible hypospadias cases and 305 possible cryptorchidism cases identified solely on the basis of the nonspecific ICD-9 codes. A total of 3,992 boys (1.8%) were diagnosed with ASD. Mean age at first autism diagnosis was slightly lower for hypospadias cases (4.92 years) than for cryptorchidism cases (5.04 years) and in comparison with boys who had neither birth defect (4.97 years), but the differences were statistically insignificant in 1-way analysis of variance ($P = 0.95$). There were 1,152 ASD cases that did not go through all of the steps needed to confirm our stricter ASD definition. These 1,152 children were generally comparable to the group that did meet the stricter ASD case definition, except that they were more likely to come from minority groups (excluded cases: 20.4% in an enumeration area with high minority representation; stricter definition cases: 12.8%) and, relatedly, a lower SES class (excluded cases: median SES score, 6; stricter definition cases: median SES score, 7).

In crude analyses using a binary variable to indicate a child who has either cryptorchidism or hypospadias, an elevated odds ratio was observed for ASD (Table 2). The odds ratio remained elevated after adjustment for demographic covariates

Table 1. Characteristics of the Study Population (Male Singletons Born in 1999–2013; $n = 224,598$) by Reproductive Tract Disorder Status, Israel, 1999–2016

Characteristic	Condition					
	Cryptorchidism[a]		Hypospadias[a]		Neither Defect	
	No. of Boys	%	No. of Boys	%	No. of Boys	%
All male newborns	7,524	3.4	4,141	1.8	213,213	5.3
District						
North	1,365	18.1	608	14.7	34,486	16.2
Sharon	1,599	21.3	889	21.5	44,206	20.7
South	1,398	18.6	628	15.2	34,165	16.0
Central	1,525	20.3	832	20.1	49,118	23.0
Jerusalem and Shfela	1,637	21.8	1,184	28.6	51,238	24.0
Assisted reproductive therapy						
Ovarian stimulation only[b]	396	5.5	244	6.2	9,870	4.8
In vitro fertilization[c]	329	4.4	208	5.0	7,055	3.3
Gestational diabetes	540	7.2	321	7.8	14,218	6.7
Prepregnancy diabetes	43	0.6	27	0.7	939	0.4
Preeclampsia and hypertension	245	3.3	117	2.8	4,927	2.3
Congenital and chromosomal anomalies[d]	181	2.4	93	2.3	1,575	0.7
Child's birth year						
1999	339	4.5	137	3.3	12,944	6.1
2000	443	5.9	154	3.7	13,404	6.3
2001	447	5.9	169	4.1	13,701	6.4
2002	489	6.5	219	5.3	13,777	6.5
2003	579	7.7	316	7.6	14,358	6.7
2004	512	6.8	318	7.7	14,333	6.7
2005	555	7.4	307	7.4	13,876	6.5
2006	542	7.2	293	7.1	13,798	6.5
2007	522	6.9	289	7.0	13,810	6.5
2008	543	7.2	323	7.8	14,039	6.6
2009	516	6.9	337	8.1	14,453	6.8
2010	521	6.9	293	7.1	15,131	7.1
2011	513	6.8	305	7.4	14,950	7.0
2012	503	6.7	348	8.4	15,211	7.1
2013	500	6.7	333	8.0	15,428	7.2
Maternal age, years[e]	30.92 (5.3)		31.44 (5.1)		31.19 (5.2)	
Gestational age, weeks[e,f]	38.86 (2.0)		38.84 (2.2)		39.15 (1.7)	
Birth weight, g[e,f]	3.23 (0.6)		3.23 (0.6)		3.34 (0.5)	
Socioeconomic status[g]	6.03 (6.0)		6.29 (6.0)		6.12 (6.0)	

[a] A total of 280 boys were diagnosed with both conditions.
[b] Births occurring 5–12 months after the date of the last dispensation of clomifene and/or gonadotropins for which there were no additional records indicating embryo transfer.
[c] Births occurring 5–10 months after embryo transfer.
[d] Chromosomal anomalies and congenital anomalies affecting the nervous system were based on *International Classification of Diseases, Ninth Revision*, codes 740.x–742.x and 758.x.
[e] Values are expressed as mean (standard deviation).
[f] Data on gestational age were available for 213,862 boys, and data on birth weight were available for 223,076 boys.
[g] Socioeconomic status was calculated on a scale from 1 to 10 (see Methods section) and is reported as mean (median) score.

and child's birth year. When hypospadias and cryptorchidism were considered separately in models adjusting for the other condition, elevated odds ratios were observed for both disorders (Table 2). Consistent results were obtained when we repeated the analyses using the stricter ASD case definition (Table 2), when we additionally included possible hypospadias and cryptorchidism cases identified by nonspecific ICD-9 codes, and when we analyzed the data with a Cox model using the entire birth cohort (data not shown).

Results of the sensitivity analyses are presented in Table 3. Additional adjustments for assisted reproductive therapy, gestational diabetes, preeclampsia, and prepregnancy diabetes did not materially alter the results. With further adjustment for birth weight and gestational age, the odds ratios were slightly attenuated yet remained elevated. Consistent effect estimates were observed when we excluded cases diagnosed with congenital anomalies affecting the nervous system or with chromosomal anomalies, and when we excluded hypospadias and cryptorchidism cases diagnosed after the first date of ASD evaluation. When restricting the data to only hypospadias and cryptorchidism diagnoses given during the first year of life, we observed more robust effect estimates. Among boys without hypospadias or cryptorchidism, having a sibling diagnosed with either of these conditions was not associated with a higher risk of ASD (brother with cryptorchidism: odds ratio = 0.99, 95% confidence interval: 0.79, 1.24; brother with hypospadias: odds ratio = 0.78, 95% confidence interval: 0.57, 1.08). In contrast, having a brother with cryptorchidism or hypospadias significantly increased the odds of being diagnosed with either of those conditions, and having a brother with ASD significantly increased the odds of ASD.

DISCUSSION

We used individual-level data from a national medical registry to examine the association between hypospadias and cryptorchidism and subsequent risk of ASD. ASD risk was elevated among children with either of these conditions. Results were stronger when we considered only male reproductive disorders diagnosed during the first year of life, suggesting that the main findings may have been somewhat attenuated by inclusion of milder cases. Intriguingly, the effect estimates we observed remained robust even after adjustment for several pregnancy-related factors, suggesting that whatever the antecedent factor is that causes the association between male reproductive disorders and ASD, it is likely acting through pathways that are independent of these variables.

Our results are in agreement with those of a recent ecological study showing elevated ASD incidence rates in areas with higher rates of congenital malformations of the male reproductive system (17). Our result for hypospadias also agrees with the only previous study that examined this at the individual level using national registry data from Sweden (18). The Swedish study did not consider cryptorchidism.

While future studies should attempt to replicate our findings in other cohorts, results of this study may have several important implications. The "extreme male brain" theory is a prominent theory on the origins of ASD, though not without controversy (36–39), suggesting that high fetal testosterone

Table 2. Odds Ratios for Autism Spectrum Disorder According to Reproductive Tract Disorder Status (n = 224,598), Israel, 1999–2016

Condition	ICD-9-Based Case Definition							Stricter ASD Case Definition						
	No. of Boys	Total No.	%	Crude		Multivariable-Adjusted[a]		No. of Boys	Total No.	%	Crude		Multivariable-Adjusted[a]	
				OR	95% CI	OR	95% CI				OR	95% CI	OR	95% CI
Neither	3,674	213,213	1.7	1.00	Referent	1.00	Referent	2,613	213,213	1.2	1.00	Referent	1.00	Referent
Either	318	11,385	2.8	1.62	1.45, 1.82	1.62	1.44, 1.82	227	11,385	2.0	1.63	1.42, 1.86	1.61	1.41, 1.85
Hypospadias[b]	124	4,141	3.0	1.68	1.40, 2.02	1.65	1.38, 1.98	98	4,141	2.4	1.87	1.53, 2.30	1.79	1.46, 2.20
Cryptorchidism[b]	204	7,524	2.7	1.53	1.33, 1.76	1.55	1.34, 1.78	137	7,524	1.8	1.44	1.21, 1.71	1.46	1.23, 1.74

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; ICD-9, *International Classification of Diseases, Ninth Revision*; OR, odds ratio.

[a] Results were adjusted for maternal age at delivery, maternal residential district, socioeconomic status, residential enumeration area minority group representation (percentages of residents in 4 minority groups: Ultraorthodox Jews, Israeli Arabs, Ethiopians, and Russians), and child's birth year.

[b] All models of hypospadias and cryptorchidism as individual exposures additionally adjusted for the other condition.

Table 3. Odds Ratios for Autism Spectrum Disorder According to Reproductive Tract Disorder Status in Different Sensitivity Analyses, Israel, 1999–2016[a,b]

Condition	Model 1[c]		Model 2[d]		Model 3[e]		Model 4[f]		Model 5[g]	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Neither	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Either	1.60	1.42, 1.79	1.53	1.36, 1.72	1.47	1.31, 1.66	1.54	1.36, 1.74	1.86	1.61, 2.13
Hypospadias	1.63	1.36, 1.96	1.56	1.30, 1.88	1.56	1.29, 1.88	1.57	1.29, 1.90	1.89	1.55, 2.32
Cryptorchidism	1.52	1.32, 1.76	1.45	1.25, 1.67	1.38	1.18, 1.60	1.47	1.26, 1.71	1.75	1.45, 2.11

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; ICD-9, *International Classification of Diseases, Ninth Revision*; IVF, in vitro fertilization; OR, odds ratio.

[a] Models considered all ASD cases identified using ICD-9 codes. All models adjusted for maternal age at delivery, socioeconomic status, residential enumeration area minority group representation (percentages of residents in 4 minority groups: Ultraorthodox Jews, Israeli Arabs, Ethiopians, and Russians), maternal residential district, and child's birth year.

[b] In footnotes c–g, n = total number of children considered; w, x, y, and z = number (%) of ASD cases among boys with neither condition (w), either condition (x), hypospadias (y), and cryptorchidism (z), respectively.

[c] Model 1: additional adjustment for use of assisted reproductive therapy (IVF and non-IVF), gestational diabetes, preeclampsia, and prepregnancy diabetes (n = 224,598; w = 3,674 (1.7%), x = 318 (2.8%), y = 124 (3.0%), and z = 204 (2.7%)).

[d] Model 2: model 1 adjustments, with additional adjustment for birth weight and gestational age (pooled results from 20 imputed data sets) (n = 224,598; w = 3,674 (1.7%), x = 318 (2.8%), y = 124 (3.0%), and z = 204 (2.7%)).

[e] Model 3: analysis restricted to hypospadias and cryptorchidism diagnoses given before the earliest date of ASD evaluation (n = 224,569; w = 3,674 (1.7%), x = 289 (2.5%), y = 116 (2.8%), and z = 181 (2.4%)).

[f] Model 4: analysis restricted to children without any indication of chromosomal anomalies or congenital anomalies affecting the nervous system (n = 222,795; w = 3,543 (1.7%), x = 287 (2.6%), y = 111 (2.7%), and z = 183 (2.5%)).

[g] Model 5: analysis restricted to only hypospadias and cryptorchidism diagnoses given during the first year of life (n = 219,948; w = 3,674 (1.7%), x = 215 (3.2%), y = 102 (3.4%), and z = 116 (3.0%)).

exposure is a risk factor for ASD (40, 41). The extreme male brain theory generally posits a unidirectional relationship with in-utero androgen levels, with an implied implication that reduced in-utero androgen levels could theoretically be protective against adverse neurodevelopmental outcomes. Because deficiencies in prenatal androgen signaling are thought to play a central role in the development of both cryptorchidism and hypospadias (12–14), this could suggest that children with these disorders would be at reduced risk for ASD. The fact that we observed an increased risk of ASD among children diagnosed with cryptorchidism and hypospadias indicates a potentially more complicated relationship between fetal androgenic environment and neurodevelopment, and could suggest that moderate fetal hypoandrogenicity may also be a risk factor for adverse neurodevelopmental effects.

While cryptorchidism and hypospadias share similar risk factors and altered developmental pathways (12–14), they differ with regard to the time of onset. Hypospadias depends chiefly on androgen signaling during the first trimester, normally occurring in weeks 8–12 of gestation (42). In contrast, descent of fetal testes is thought to occur in 2 separate hormonal and anatomical steps (16, 42). Early transabdominal descent occurs in weeks 8–15 of gestation and depends critically on appropriate stimulation by insulin-like hormone 3 from developing Leydig cells, with androgens and antimüllerian hormone playing more minor roles (42, 43). This initial descent process occurs normally even in humans and animal models with complete androgen insensitivity (44). The second, inguinoscrotal descent phase occurs in weeks 25–35, during which the testes migrate to the scrotum, and this phase is chiefly controlled by androgen signaling (42). Defects in this second phase of the descent account for the vast majority of cryptorchidism cases (44). Thus, while a decrease in androgenic hormone levels or a change in androgen action plays an important role in the etiology of both disorders, the effect is exerted in different critical time windows during gestation. The robust effect estimates observed in this study for both disorders may suggest overall stable in-utero androgen levels throughout pregnancy, potentially indicating that levels in critical windows of brain development are also implicated during other periods that are relevant for the development of the male reproductive system. Alternatively, it is also possible that androgens have important neurodevelopmental effects across all trimesters, although the precise influence of hormonal perturbation during specific periods of gestation remains to be further evaluated.

Whereas child-specific genetic factors associated with androgen production or action are possible common causes of male reproductive disorders and ASD, accumulating evidence strongly suggests that in-utero exposures or other maternal factors affecting the intrauterine environment play important roles in the etiology of the disorders. Cryptorchidism and hypospadias are clustered in families, but genetic abnormalities have been suggested to account for a small fraction of cases (15, 45). Familial aggregation studies of cryptorchidism seem to suggest pregnancy-specific effects, as twin brothers have higher concordance rates than full brothers, and rates are of equal magnitude in monozygotic and dizygotic twins (46). Similarly, an increasing body of evidence from animal and human studies suggests strong environmental links for hypospadias (15, 47). Notably, in the prior report from Sweden, Butwicka et al. (18) found a higher risk of ASD among brothers of boys with hypospadias and argued that this suggests genetic causes of the hypospadias-ASD association. However, environmental exposures can be

correlated over time. Shared maternal environmental exposures between pregnancies could also explain an elevated risk of ASD among brothers of boys with hypospadias or cryptorchidism. We did not see an elevated risk of ASD among brothers of boys with hypospadias or cryptorchidism—despite strong evidence in our data of familial aggregation of both male reproductive disorders and ASD separately—which in fact would suggest that parental genetics are not causing the association.

While different factors could cause prenatal fetal androgen deficiency, animal models have shown that one cause could be exposure to environmental endocrine-disrupting chemicals (12–14), several classes of which are known to exert antiandrogenic effects (48–54). This hypothesis is supported by our finding of no excess ASD risk among unaffected brothers of cryptorchidism or hypospadias cases, suggesting that the shared etiologies could be driven by pregnancy-specific exposures.

Strengths of this study include the use of prospectively collected medical information from a large population-based cohort with universal access to health-care services; the ability to use a subgroup of validated ASD diagnoses; the ability to adjust for parental and perinatal factors; and the ability to construct family clusters to evaluate familial aggregation of the disorders. The study also had several limitations. We did not have direct serum androgen measurements, and it is possible that the observed associations with ASD risk operate through alternative pathways other than androgen hormones. However, if the true causative factor is indeed androgens, we would expect an even stronger association with direct measurements. Additionally, the ICD-9 coding system does not differentiate between subtypes of ASD and does not contain information concerning the severity of the condition. Thus, we could not explore subtypes of ASD. Finally, while our analysis of unaffected siblings of cryptorchidism and hypospadias cases was done to gain insights regarding the underlying factors driving the observed association, our study design was not suited to separating shared genetic and environmental factors. Nonetheless, our study suggests that a hypoandrogenic in-utero environment is a potential risk factor for ASD, with implications that 1) maternal exposure to hormonally active compounds could increase ASD risk and 2) children diagnosed with disorders related to androgen insufficiency should be carefully monitored for the development of ASD symptoms to allow for early intervention.

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Author affiliations: Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts (Ran S. Rotem, Russ Hauser, Brent A. Coull, Marc G. Weisskopf); Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts (Russ Hauser, Marc G. Weisskopf); Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts (Brent A. Coull); Maccabi Institute for Research and Innovation, Maccabi Healthcare Services, Tel Aviv, Israel (Gabriel Chodick); and Child Development Department, Maccabi Healthcare Services, Tel Aviv, Israel (Michael Davidovitch).

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