Low perinatal androgens predict recalled childhood gender nonconformity in men

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Abstract

The contributions of gender socialization and direct hormonal action on the brain in the development of human behavioral sex differences are subjects of intense scientific and social interest. Prior research indicates masculinized behavioral patterns in individuals with high prenatal androgen exposure raised as girls, but complementary evidence regarding individuals with low prenatal androgens raised as boys is critically lacking. We investigated recalled childhood gender nonconformity (CGN) in men (n = 65) and women (n = 32) with isolated GnRH deficiency (IGD) and typically developing men (n = 463) and women (n = 1207). IGD is characterized by low or absent gonadal hormone production after the first trimester of gestation until hormone replacement therapy initiation around the time of puberty, but external appearance is concordant with chromosomal and gonadal sex. Compared to typically developing men, men with IGD reported higher CGN, particularly if they also reported cryptorchidism at birth, a marker of low perinatal androgens. Women with IGD did not differ from typically developing women. These results suggest that early androgen exposure after the first trimester contributes to male-typical gender role behaviors in childhood.

Keywords: childhood gender nonconformity; isolated GnRH deficiency; sex differences; sex hormones; androgens

1. Introduction

Sex differences in gender role behaviors emerge early in development [1–3], persist across adolescence [2], and are among the largest observed human sex differences in psychology and behavior [4]. The developmental factors that shape these sexually differentiated phenotypes are the topic of vigorous debate [5–7]. Androgen action during critical periods may organize the central nervous system to later influence sexually differentiated behavioral phenotypes, including gender role behaviors [8,9]. Testicular androgen production in typical human males begins roughly eight weeks into gestation and remains elevated until just prior to birth, rising again during mini-puberty, the period a few months after birth when gonadal hormones are produced at near-adult levels [10–13] (Fig. 1).

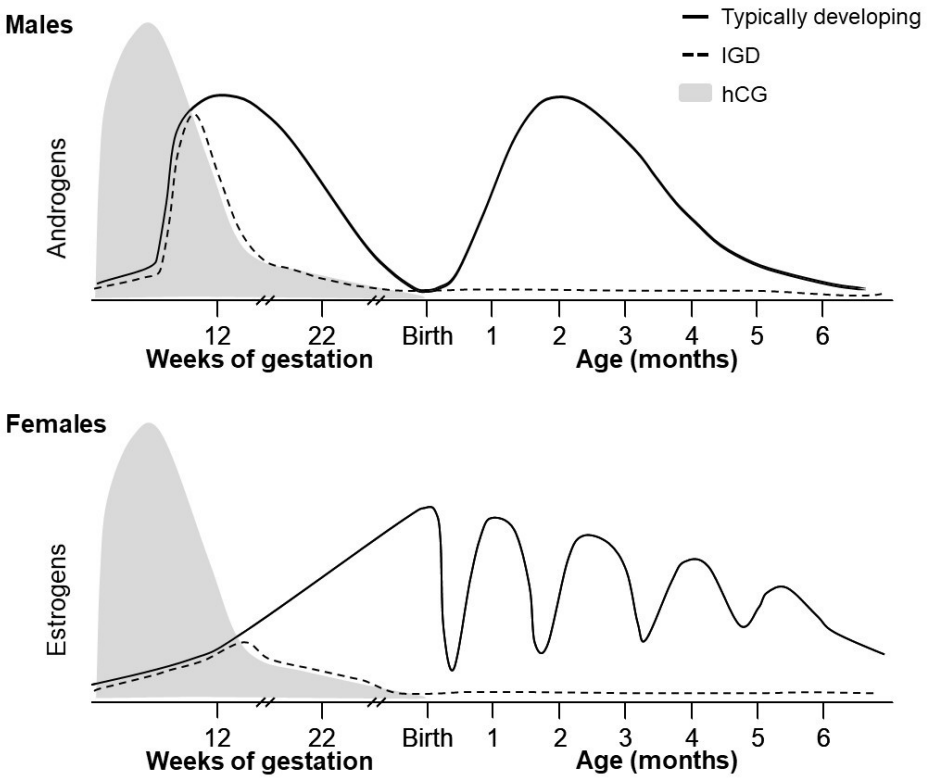

Figure 1. Approximate human chorionic gonadotropin (hCG, gray shading) and gonadal sex steroid hormone production in males (top) and females (bottom). In typical males, androgen production begins at roughly the 8th week of gestation with the differentiation of the bipotential gonads into testes [12–14], persisting until the 24th week [15]. In normal females, estrogen levels progressively increase across gestation, peaking perinatally, though ovarian activity transiently ceases in the immediate postpartum period between birth and the onset of mini-puberty [11]. In individuals with isolated GnRH deficiency (IGD), gonadal hormone production declines as circulating hCG levels wane. Figure adapted from Lanciotti et al. (2018).

In nonhuman mammals, androgen administration early in development masculinizes behavior and underlying neural structures [9,16,17]. Parallel experiments in humans would be unethical, but it is possible to investigate naturally occurring variation in androgen production. Here, it is necessary to disentangle the direct influence of gonadal hormones on the brain and behavior from their indirect effects via external anatomy and gender socialization (Fig. 2). If androgens influence behavior by shaping brain development directly, then among individuals raised as girls, those exposed to elevated androgens during their early development should exhibit more masculine behavior. In fact, girls who experienced elevated prenatal androgen levels due to congenital adrenal hyperplasia have been found to exhibit more male-typical play preferences and behaviors compared to unaffected girls [18–22]. Similarly, natal males whose gender was reassigned to female in infancy due to cloacal exstrophy or penile ablation during circumcision have also been found to exhibit behavioral masculinization in comparison to typically developing girls [23,24]. If androgens influence behavior by shaping brain development directly, then it should also be the case that, among individuals raised as boys, those exposed to reduced androgens during their early development should exhibit less masculine behavior. However, this complementary evidence is critically missing.

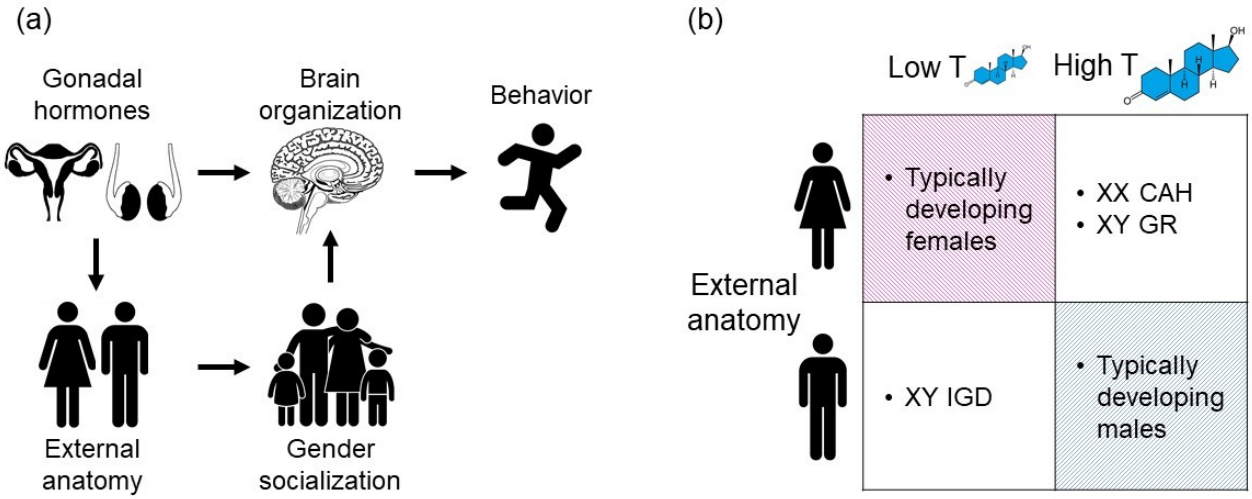

Figure 2. Some (a) putative causes of sex differences in behavior and (b) possible combinations of external anatomy and perinatal testosterone (T) exposure. Behavioral sex differences may result from direct effects of gonadal hormones on the brain (panel a, top row) and/or gender socialization (panel a, bottom row). These factors can be disentangled by examining individuals whose perinatal T exposure differs from that of typically developing individuals with similar external anatomy (unshaded cells in panel b). XX CAH = females with congenital adrenal hyperplasia, XY GR = males whose gender was reassigned to female in infancy, XY IGD = males with isolated GnRH deficiency.

Isolated GnRH deficiency (IGD) is a rare endocrine disorder characterized by the congenital lack of function or absence of a network of neurons in the hypothalamus that secrete gonadotropin-releasing hormone (GnRH) [25,26]. During the first trimester of gestation, abundant placental human chorionic gonadotropin (hCG) [27] binds to luteinizing hormone receptors in the fetal gonads to stimulate steroid hormone production [28,29]. In normal development, hypothalamic GnRH stimulates gonadal hormone production after the first trimester as placental hCG levels drop, but in individuals with IGD, gonadal hormone production ceases due to GnRH deficiency. Individuals with IGD also do not experience mini-puberty [11]l, and hormone replacement therapy is required to initiate puberty, though adrenarche occurs because adrenal androgen action is unaffected [26].

Because gonadal hormones decline in IGD after the 1st trimester when external genitalia have already sexually differentiated, external appearance is concordant with chromosomal and gonadal sex. IGD thus represents a rare human model that provides a unique opportunity to disentangle the direct influence of early gonadal hormones on the central nervous system and behavior from their indirect effects via external anatomy and gender socialization (Fig. 2b). Here, we utilize IGD to understand the effects of chronically low perinatal gonadal hormone action on sexually differentiated childhood play and gender role behavior.

2. Method

Participants

Typically developing participants (henceforth “control” participants) were recruited in two separate studies on hormones and behavior. A first set of men (*n* = 233) and women (*n* = 395)

were recruited at Michigan State University (see also [30–32]). A second set of men (*n* = 230) and women (*n* = 812) were recruited at Pennsylvania State University (see also [33,34]).

Participants with IGD were recruited in two ways. A first group were referred to the study by the Reproductive Endocrine Unit at Massachusetts General Hospital or the NICHD's Reproductive Physiology and Pathophysiology unit (henceforth "IGD-clinical"; *n* = 44). A confirmation of diagnosis was available for all IGD-clinical participants. A second group was recruited through posts on web-based IGD support groups and forums (henceforth "IGD-web"; *n* = 53). Though IGD-web participants were recruited on IGD-specific forums, we could not confirm their diagnoses with a physician.

All participants were 18 years or older, permanent residents of the United States, and fluent in English. All procedures were IRB-approved, and participants provided informed consent.

General procedure
Control participants completed the study at private computer workstations in the laboratory. Participants with IGD participated remotely and were instructed to complete all questionnaires in one sitting. Control participants were compensated with either course credit or monetary compensation, and participants with IGD received monetary compensation.

Questionnaires
The childhood gender nonconformity questionnaire (CGNQ [35]) measures recalled childhood behaviors, attitudes, and desires that have been found to differ between boys and girls. The CGNQ includes 24 questions representing several behavioral and psychological categories, including peer preferences, toy preferences, dress-up play, fantasy play, and career aspirations [36]. Based on categorizations of each response as either male- or female-typical, we coded and scored responses to each question as either gender-conforming (score of -1), gender-non-conforming (score of +1), or gender-neutral (score of 0). Responses were then scaled within sexes and averaged to create childhood gender nonconformity scores, with higher scores indicating higher recalled childhood gender nonconformity. Cronbach's α was 0.78 in both men and women. For further validation, we tested for sex differences on the subset of items (13 of 24) that were scored symmetrically and worded identically for men and women, and we also created a composite "CGNQ masculinity" score using this subset of items, multiplying women's scores by -1 to yield a measure of childhood gender masculinity that could be compared between sexes. All individual items exhibited significant sex differences in expected directions (**ESM Table 1**), as did CGNQ masculinity scores (Cohen's *d* = 3.00; **ESM Figure 1**). Comparisons of CGNQ masculinity across all groups are reported in **ESM Text 1** and **ESM Table 2**.

Control participants recruited from Pennsylvania State University and participants with IGD were administered a shortened version of the Klein Sexual Orientation Questionnaire (KSOQ [37]). Responses to questions about sexual feelings and activity over the last year were averaged to create a sexual orientation score ranging from 0 (exclusively heterosexual) to 6 (exclusively homosexual). Control participants recruited at Michigan State University were administered a sexual orientation questionnaire based on the Kinsey scale [38]. Responses to items on sexual attraction and sexual fantasies were averaged to create a sexual orientation score, again ranging from 0 to 6.

Data on cryptorchidism and microphallus at birth were available from patient files for a subset of IGD-clinical men. Previous reports estimate that 20-40% of men diagnosed with IGD present

with cryptorchidism or microphallus at birth [25], with the elevated rates seen in this patient population consistent with low or absent androgen exposure after the first trimester of pregnancy [39,40]. Seventeen IGD-clinical participants had data available for cryptorchidism with eight indicating it at birth; data for microphallus were available for 14 men, and 4 reported presenting with microphallus at birth (**Table 1**).

All questionnaires and scoring methods relevant to the present analyses have been uploaded as ESM.

Data analysis
Analyses for men and women were conducted separately. Control participants across studies were combined. As sibling pairs were recruited in the Michigan State University sample, we ran linear regressions as multilevel models with individuals nested within sibling units. Current age and sexual orientation were entered as covariates. Regressions simultaneously evaluated the effect of diagnosis (IGD vs. control) and IGD group (IGD-clinical vs. IGD-web) using orthogonal contrast coding, with one contrast term per comparison. All regression estimates are reported as standardized beta estimates.

Data and script files have been uploaded as ESM.

3. Results

Sample characteristics
Sample demographics are displayed in **Table 1.**

Table 1: Sample demographics for men (left columns) and women (right columns).

	Men			**Women**		
	Control (*n* = 463)	**IGD (clinical)** (*n* = 30)	**IGD (web)** (*n* = 35)	**Control** (*n* = 1,207)	**IGD (clinical)** (*n* = 14)	**IGD (web)** (*n* = 18)
Age (M, SD)	20.71 (4.45)	41.20 (15.85)	37.46 (13.90)	20.18 (3.98)	34.29 (9.41)	31.39 (6.22)
Race/ethnicity (%)						
White, non-Hispanic	80%	83%	69%	77%	71%	78%
Asian, non-Hispanic	9.7%	10%	8.6%	10%	7.1%	0%
Black, non-Hispanic	4.1%	0%	0%	5%	0%	0%
Hispanic	4.5%	3.3%	17%	5.9%	7.1%	22%
Other	1.3%	3.3%	5.7%	2.1%	14%	0%
CGN (M, SD)	-0.04 (0.33)	0.24 (0.44)	0.34 (0.59)	-0.00 (0.37)	0.02 (0.55)	0.35 (0.67)
Sexual orientation (M, SD)	0.25 (0.94)	0.31 (0.71)	0.94 (1.48)	0.37 (0.79)	0.36 (0.37)	1.37 (1.68)
Microphallus at birth (%)		29%				
Cryptorchidism at birth (%)		47%				

Men
Men with IGD had higher CGNQ scores than control men (estimate = 0.24, $p < 0.001$), and scores did not differ between IGD-clinical and IGD-web participants (estimate = -0.10, $p = 0.312$**; Figure 3a**). A post-hoc test comparing CGNQ scores in only IGD-clinical participants (for whom we had confirmation of diagnosis) and control men revealed higher CGNQ scores in IGD-clinical men (estimate = 0.17, $p = 0.003$).

To probe the robustness of our findings, we conducted two additional sets of analyses. First, we conducted analyses where we first tested for differences between IGD groups and if differences

were not significant, combined IGD groups and tested for IGD-control differences (**ESM Text 1**). Second, we restricted our main analyses to participants older than 23 to eliminate probable university students and thus minimize demographic differences observed between control and IGD participants (**ESM Text 2**). Results of these robustness checks were consistent with results of our main analyses.

We next compared men in the IGD-clinical group with and without cryptorchidism at birth to controls to further test a connection with low gonadal hormones. We created a variable with three levels: control men, men with IGD without cryptorchidism at birth, and men with IGD with cryptorchism at birth. Control men were set as the reference category, and thus regression coefficients provide estimates for the difference between each condition at birth (i.e., IGD-clinical with condition, IGD-clinical without condition) and control men. IGD-clinical men with cryptorchidism at birth (estimate = 0.12, p = 0.019), but not without (estimate = 0.03, p = 0.489), exhibited higher CGNQ scores than control men (**Figure 3b**). We repeated this analysis for microphallus present at birth, finding no differences (**Figure 3c**).

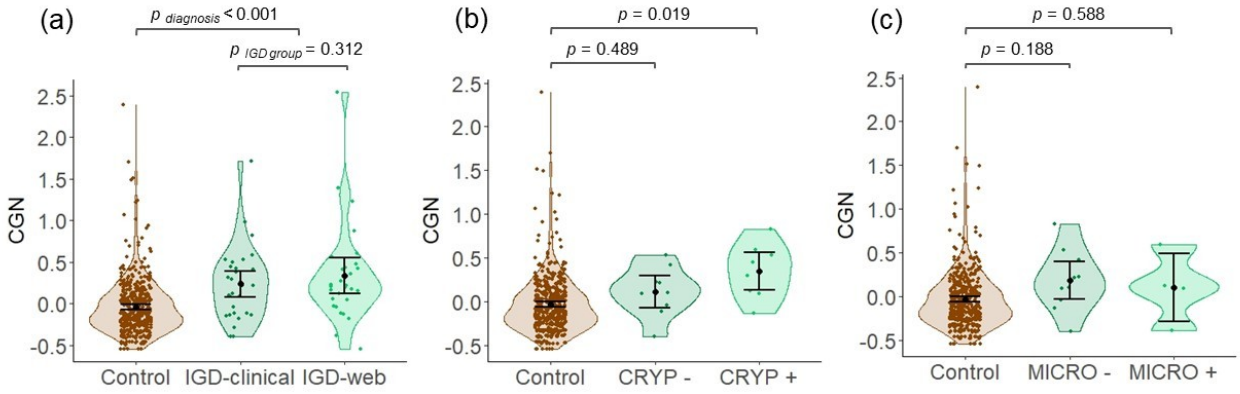

Figure 3. Childhood gender nonconformity (CGN) scores in men. Compared to control men, men with IGD reported significantly higher CGN (**a**), as did men with IGD born with cryptorchidism (CRYP +), a marker of low prenatal androgen exposure (**b**), but not microphallus (**c**). Vertical and horizontal jitter were added to points to aid in data visualization.

Women

Women with IGD had higher CGNQ scores than control women (estimate = 0.14, p = 0.003), but CGNQ scores were lower in IGD-clinical than in IGD-web women (estimate = -0.11, p = 0.014; **Figure 4**). A post-hoc test comparing CGNQ scores in only IGD-clinical and control women revealed no difference (estimate = -0.01, p = 0.673).

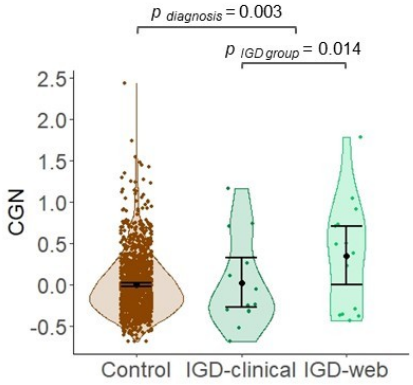

Figure 4. Childhood gender nonconformity (CGN) scores in women. CGN scores were higher in women with IGD than in control women, though this difference was driven by elevated CGN scores in the IGD-web, but not IGD-clinical, group. Vertical and horizontal jitter were added to points to aid in data visualization.

4. Discussion

We examined the effects of congenitally low gonadal steroids from the second trimester of gestation through mini-puberty on recalled childhood gender nonconformity by comparing typically developing men and women to those with IGD. We found less masculine recalled sex-typed childhood behaviors in men with IGD compared to unaffected men. This effect was robust to any differences in ascertainment bias between clinically- and web-recruited IGD groups, consistent across all robustness analyses, and most pronounced in IGD males with cryptorchidism at birth. These findings suggest that androgen action between the second trimester and neonatal period is important in the development of male-typical gender role behaviors in childhood.

Our data do not argue against a role of social learning in shaping child gender role behaviors [41–43], but they enable a unique convergence of evidence regarding a role of early androgen exposure. Experimental hormonal manipulations demonstrate that early androgen action masculinizes the brain and behavior in nonhuman mammals [16]. Prior studies in humans show greater behavioral masculinity in individuals raised as girls who experienced elevated perinatal androgens due to congenital adrenal hyperplasia [18–22] or having male-typical prenatal endocrine profiles but male-to-female sex reassignment in infancy [23,24]. The present findings of reduced behavioral masculinity in males with low perinatal androgens due to IGD adds complementary evidence that early androgen action promotes male-typical behavioral patterns in humans. This interpretation is strengthened because these patterns run counter to the general direction of gender socialization, which may be even more pronounced if parents are aware of their child's endocrine condition [22]. However, it is also important to note that men with IGD were more similar in recalled childhood gender role behaviors to control men than they were to control women (**ESM Table 2**; **ESM Figure 1**). This finding may reflect influences of testosterone in the first trimester, sex chromosome complement [44], and/or gender socialization.

Women for whom we had physician-confirmed IGD diagnosis did not differ from control women in recalled childhood gender nonconformity, suggesting that sex-typical childhood gender role behavior can develop in human females largely independently of ovarian hormone production after the first trimester. These results also provide further evidence that androgen action at or below female-typical levels (e.g., due to complete androgen insensitivity syndrome in XY

individuals [45] or IGD in those with XX karyotype) leads to female-typical childhood gender role behaviors. Some studies have found less female-typical behavior in women with Turner syndrome (TS), in whom gonadal hormone production is also chronically low [46]. However, women with TS experience a later decline in ovarian hormones and possess a single functioning X chromosome rather than two X chromosomes as women with IGD do. Behavioral differences between TS and IGD women may thus reflect these other endocrine and chromosomal differences [44,47].

Limitations

IGD is present in fewer than 1 in 10,000 live births [48,49], and hence recruiting samples with sufficient power presents a significant challenge. Although our sample sizes provide 80% power to detect small-to-medium effects (Cohen's *d* of 0.4 and 0.5 in men and women, respectively), it is possible that we were unable to detect some small differences between IGD and control individuals.

Our observational data also cannot demonstrate causal links between early sex hormone exposure and adult phenotypes. For example, we cannot rule out a role of differential treatment by parents or physicians. However, there is no sexual ambiguity in the clinical presentation of individuals with IGD [50], and IGD is not usually diagnosed until incomplete or absent pubertal development triggers referral to a physician [25,50]. IGD can be suspected in cases of cryptorchidism or micropenis, but our finding of no differences in CGN relative to micropenis presence, along with the relative conspicuousness of this trait, also argues against a major role of differential parental treatment (though this finding may also reflect the limited sample for which these data were available). It is also possible that demographic factors contributed to differences between individuals with IGD and control individuals. However, age and sexual orientation were statistically controlled, findings were robust when college-aged participants were excluded from analyses, and there is no compelling evidence suggesting a causal relationship between childhood socioeconomic status and childhood gender nonconformity [51].

Conclusion

We utilized IGD as a model to elucidate the effects of chronically low perinatal sex hormone exposure on the sexual differentiation of human behavior. Our results indicate that low gonadal sex hormone exposure in mid-to-late gestation and early infancy predicts higher recalled childhood gender nonconformity in men but not women. These results suggest that androgen action is critical to the organization of male-typical play and gender role behaviors, and that lower androgen levels associated with IGD in males, or with androgen production at or below sex-typical levels in females, is associated with more female-typical behaviors.

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7. Author contributions

KD, LLMW, RC, RB, AD, SMB, and DAP made substantial contributions to the conception and design of the study. HS, KD, RC, RB, AD, and DAP made substantial contributions to the acquisition of the data. TNS, KAR, JMB, SMB, and DAP made substantial contributions to the analysis and interpretation of the data. TNS and DAP wrote the initial draft of this manuscript. All authors have made substantial contributions in the revision of this manuscript, and approve the submitted version.

8. Competing interests

No authors have any competing interests to report.