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Gender role behavior in children with XY karyotype and disorders of sex development

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Abstract

Children exhibit gender-typical preferences in play, toys, activities and interests, and playmates. Several studies suggest that high concentrations of pre- and postnatal androgens contribute to male-typical behavior development, whereas female-typical behavior develops in the absence of high androgens levels. This study aims to explore the consequences of hypoandrogenization on gender-typical behavior in children who have an XY karyotype and disorder of sex development (DSD). Participants included 33 children (ages 2–12 years) with an XY karyotype and DSD; 21 reared as girls and 12 reared as boys. Children's preferred activities and interests and playmate preferences were assessed with parent report questionnaires, a structured free-play task, and choice of a toy to keep as a gift. Participant's responses were compared to those of children recruited in a pre-school and elementary school survey ($N=166$). In this study, the degree of hypoandrogenization as indicated by genital stage and diagnosis showed a significant relationship to nearly all of the gender-related behaviors assessed, supporting the hypothesis that masculinization of gender role behavior is a function of prenatal androgen exposure. Despite the fact that children with partial androgen effects reared as girls showed increased "boyish" behaviors, they did not show increased signs of gender identity confusion or instability on a group level. We conclude that androgen exposure plays a decisive role in the development of gender-typical behavior in children with XY karyotype and DSD conditions.

Keywords: Intersexuality; Disorder of sex development; Gender role; Psychosexual development; Sex differences; Play; Androgens

Introduction

Gender-typical behavior in humans is influenced by biological, psychological, social, and cultural factors. These factors are associated with differences between boys and girls in certain behaviors (e.g., aggressive and pro-social behaviors), as well as gender-typical preferences in toys and activities, playing habits, and friends (Bosinski, 2000; Hines, 2004; Ruble et al., 2006). Given the complexity of influences on psychosexual development, it is difficult to estimate the effect size of each factor (e.g., biological, social, cultural, individual) on gender-related behavior and preferences (Houk et al., 2004; Iervolino et al., 2005).

In humans, gonadal hormones are thought to play an important role in the development of gender-related behaviors. Androgen effects on the developing brain and consequent behaviors have been documented in a range of mammals (Arnold, 2002; Dohler et al., 1984; Hines and Collaer, 1993; Lephart et al., 2001; Sato et al., 2004). High concentrations of prenatal androgens contribute to male-typical behavior development, whereas female-typical behavior develops in the absence of high levels of androgens (Breedlove et al., 1999; Collaer and Hines, 1995; Hines, 2002; Hines et al., 2002; Hrabovszky and Hutson, 2002).

Disorders of sex development (DSD) provide a unique opportunity to study the effects of prenatal androgen exposure and gender-specific socialization on the development of gender-related behavior. Several studies of girls with congenital adrenal hyperplasia (CAH), an enzymatic defect in adrenal steroid synthesis resulting in high levels of prenatal androgens that lead to genital masculinization in affected female children, show that they differ markedly in gender-related behavior from unaffected girls (Berenbaum, 1999; Cohen-Bendahan et al., 2005). Girls

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with CAH, as a group, show an increased preference for typical "boys' toys" (Berenbaum and Hines, 1992; Berenbaum and Snyder, 1995; Dittmann et al., 1990; Slijper, 1984) and male playmates (Berenbaum and Snyder, 1995), show more aggressive behavior (Berenbaum and Resnick, 1997), and are less interested in maternal rehearsal play, feminine make-up and accessories (Dittmann et al., 1990; Ehrhardt and Meyer-Bahlburg, 1981; Leveroni and Berenbaum, 1998).

Children with an XY karyotype may also be affected by DSD leading to physical hypoandrogenization. In androgen insensitivity syndrome (AIS), the testes produce normal to high levels of androgens, however, functioning of the androgen receptor is completely (cAIS) or partially (pAIS) impaired, affecting physiological masculinization of the urogenital tract and the external genitalia. Defects in androgen biosynthesis [e.g., 17β-hydroxysteroid-dehydrogenase-3 deficiency (17βHSD3)], 5α-reductase-2 deficiency (5αRD), and gonadal dysgenesis cause insufficient androgen production to induce normal male-typical anatomical development (Bahceci et al., 2005; Hiort et al., 2002; Hiort and Holterhus, 2003). These conditions lead to a lack of androgen action, and the phenotype of affected individuals may range from predominantly male to typically female (Ahmed et al., 2000; Boehmer et al., 1999; Galli-Tsinopoulou et al., 2003; Hiort et al., 1996; Holterhus et al., 2000; Melo et al., 2003; Simpson and Rajkovic, 1999; Sinnecker et al., 1996, 1997; Thiele et al., 2005).

Compared with the number of studies of girls with excessive androgen exposure in prenatal life, studies assessing the effects of androgen insensitivity or lack of androgens on gender-typical behavior in children with 46,XY karyotype are scarce (al-Attia, 1996; Cohen-Kettenis, 2005; Cohen-Kettenis and Pfäfflin, 2003; Hines et al., 1998, 2003; Mendonca et al., 2000; Wilson, 2001; Zucker, 1999).

The aim of this study was to evaluate gender-related activities, play preferences, and interests of prepubertal children with 46,XY karyotype and DSD with varying prenatal androgen exposure and to compare them with same-age children without such conditions. Following a dose–response hypothesis we assume that masculinization of gender-related behavior is a function of prenatal androgen effects. If prenatal androgen exposure contributes to the development of gender role behavior, then individuals with an XY karyotype with complete hypoandrogenization (i.e., complete androgen insensitivity syndrome; cAIS) would exhibit the least masculine behavior followed by those with partial hypoandrogenization. In addition to the biological influences of androgens on gender role development, sex of rearing may support gender-typical behavior; i.e., more female-typical and/or less male-typical behavior in those with partial androgen exposure reared as girls compared to those reared as boys.

Methods

Recruitment of the DSD group

75 children ages 3 to 12 with a 46,XY karyotype and a condition causing either insufficient androgen production (e.g., 17β-hydroxysteroid-dehydrogenase-3 deficiency, 5α-reductase 2 deficiency, XY gonadal dysgenesis), incomplete responsiveness to androgens (cAIS, pAIS), or those diagnosed with an XY-intersex-condition with ambiguous genitalia of unknown etiology were identified from the molecular genetic laboratory database in Lübeck (O. H.). Nine patients were contacted directly by the authors as they received services in the center in Lübeck. Because of data protection law, the other 66 participants had to be contacted via their local pediatric endocrinologist to whom we sent information about the study. The local pediatric endocrinologist, in turn, sent information about the study to eligible families. Mailings included an information sheet that described the study, an invitation to participate, and a reply card so that families could contact the study center for either further information or to decline participation.

Written informed consent was obtained from all participating parents and verbal assent from the children. The study was approved by the Ethics Committee on Human Studies of the Medical Faculty of the University of Lübeck. The study took place either in the study center in Lübeck or at the families' homes. Diagnostic information came from our molecular genetic laboratory and clinical information from the child's pediatric endocrinologist.

Eight of nine patients (88.9%) who received clinical care in Lübeck agreed to participate. For 7 of the other 66 families, the referring physician could not be located, and an additional seven families were lost to follow up by their endocrinologist. One child had died and parents were not contacted. Of the remaining 51 families, 23 participated (45.1%), 6 families (11.8%) rejected participation via the reply card, and 22 families (43.1%) did not contact the study center and it is uncertain whether or not information about the study was mailed. In addition to recruitment from the laboratory database, two participants were recruited via patient support groups. Medical data for these patients came from medical records parents obtained from their physicians in charge.

Recruitment of the control group

For comparative purposes, a school and pre-school based survey was conducted, including five pre-schools and five elementary schools in northern Germany. The schools were selected using socio-demographic small-area characteristics to include families from all social strata. Flyers with information about the study and a reply card were distributed in the classrooms and children asked to take them home. Consent from the school board was attained prior to data collection.

Parents were informed that the purpose of the study was to collect general information on children's psychosexual development. The study was explained at parent–teacher meetings in schools and parents were given the opportunity to ask a staff member for further information. Altogether, 1800 flyers were distributed in the schools. 266 families contacted the study center and received the questionnaires and a pre-paid envelope. 166 (62.4%) families returned completed questionnaires to the study center.

Measures

Demographics

Demographic characteristics of both groups included age and sex of rearing of the child, number, age and sex of siblings, parental education, marital status, and nationality of mothers and fathers. We used these variables to assess the comparability of the study and control group.

Sex-typed activities and interests

To assess sex-typed activities and interests we used multiple instruments: (1) the parent report questionnaire of children's preferred activities and interests ("Fragebogen zu Aktivitäten and Interessen", FAI), (2) observation of sex-typed toy play (structured free-play task), and (3) child's selection of a toy to keep. The control group children received only the FAI.

(1) Activities and Interests Questionnaire ("Fragebogen zu Aktivitäten & Interessen"; FAI). To assess children's preferred activities and interests, preferences in gender-typical games, and dressing-up in role play, we constructed a parent report questionnaire. We adopted the methodology from existing questionnaires, i.e., the CGPQ—Child Game Participation Questionnaire

(Meyer-Bahlburg et al., 1994a) but developed culturally adapted items suitable for German children. Parents were asked to indicate the frequency (0 never; 1 seldom; 2 often; 3 very often) with which the child generally participates in 52 activities and interests. Activities and interests that had shown significant differences between boys and girls in the control group were considered "sex-typed" and included in the questionnaire (Table 1). We calculated girl-typical and boy-typical activity scores based on a sum of the items falling into each category divided by the sum of all activities. Gender scores are expressed as a percentage of activities/interests categorized as girl-typical (FAI-female) and boy-typical (FAI-male) (range 0–100).

(2) Structured free-play task. Play behavior was observed in a structured free-play task (modified from Berenbaum and Snyder, 1995; Zucker et al., 1982). The selection of toys was based on interviews with parents on their children's toy preferences and on lists of bestsellers from toy stores. For typical "boys' toys" we chose four play figures (science fiction figurines, warriors), six cars, a toy pistol, and a tool box. The typical "girls' toys" included play figures (woman and a horse), a Barbie doll with clothing, a baby doll with several care products, and cooking accessories.

Toys were arranged in a standard order on the floor or a suitable table with the child absent. The child was then brought into the room and asked to play freely for 10 min with the toys. The observer stayed in the background with a stopwatch, measuring the time the child played with toys typical for boys or for girls. The parents were not present during the structured free-play task. For each child we calculated a score reflecting the percent of total time of playing with male-typical toys (range 0–100).

(3) Toy to keep. After study participation the child chose a "toy to keep" among five toys, which were coded for gender typicality, based on expert opinion (child development professionals, parents). This procedure was modified from Berenbaum and Snyder (1995). Higher scores indicate higher attraction to male-typical toys. We used two different sets of age-appropriate toys. Children aged 2 to 6 years chose from a play figure (princess with horse and coach), a children's book "Flowers," a kaleidoscope, a children's book "Airplanes", and a truck with trailer. The set for children aged 7 to 12 years included a set of plastic beads, a board game, a kaleidoscope, playing cards featuring "Car Monsters", and a Lego "Star Wars" building set.

Table 1
Gender-typical items "Fragebogen zu Aktivitäten & Interessen (FAI)" [a]

Typical boys' activities and interests	Typical girls' activities and interests
Basketball	Arts and crafts
Boxing/fighting	Ballet
Building models	Dancing
Climbing trees	Dressing up as fairy
Competition games	Dressing up as princess
Dressing up as alien	Dressing up as witch
Dressing up as cowboy	Dressing up as woman
Dressing up as man	Gymnastics
Dressing up as pirate	Horse back riding
Hiking	Loose elastic [b]
Hunting	Make up as fairy
Martial arts	Make up as princess
Playing with construction crane	Make up as woman
Playing cowboy and Indian	Needle work
Playing soldiers	Painting and drawing
Playing spaceman	Playing doctor
Playing with electric train set	Playing hairdresser
Playing with Lego	Playing nurse
Playing with Playmobil	Playing princess
Playing with telescope	Playing school
Playing with toy cars	Playing store
Playing with toy trucks	Playing with cuddly animals
Playing with toy weapons	Playing with dolls
Soccer	Playing with doll's house
	Playing with toy kitchen
	Singing
	Sprucing up
	Working with clay

[a] Translated from German.
[b] A gymnastic game using a long elastic band; in Germany, it is very popular among girls.

Gender-typical behavior and attitudes

We developed a German version of the Child Behavior and Attitudes Questionnaire (CBAQ; Meyer-Bahlburg et al., 1994b) according to international translation standards (Medical Outcomes Trust, 1997) and carried out two separate backward and forward professional translations with e-mail conferencing to reconcile discrepancies. The German translation is a short version of the CBAQ and consists of 30 items for boys and 29 items for girls. The Femininity Scale measures the extent of typical feminine behavior (bipolar; 17 items; high scores = feminine). Examples for items concerning typical feminine behavior are "He (she) plays with girl-type dolls such as baby or Barbie dolls", and "He (she) plays house."

The Cross-Gender Scale measures the extent of cross-gender behavior which may indicate confusion or instability of gender identity (unipolar; 10 items; high scores = cross-gender).

An example for items concerning cross-gender behavior is "He (she) is called a sissy (tomboy) or similar names by other people." In contrast to the original questionnaire which uses 5- and 8-point scales, the German modification uses two 5-point answering scales, measuring parents' agreement with an item (from "I strongly agree" to "I strongly disagree") and the frequency of the activity (from "daily" to "once every three months or less"). Both the control and the DSD group answered the questionnaire.

Playmate preference

In addition to the FAI, we asked parents of both groups two questions about their children's playmate preferences: (1) who does your child presently make friends with most often? (with boys/with girls/with boys and girls); (2) does your child have a male or a female best friend? (no/yes, a male best friend/yes, a female best friend/don't know).

Data analysis procedures

We analyzed the association of potentially confounding socio-demographic variables (age, parents' educational attainment, presence of brothers and sisters) with all outcome variables using bivariate statistics. In case of significant associations, the relevant variable would be included in multivariate procedures, in the absence of significant associations we used one-way ANOVA to test for group differences. If there were overall group effects we used post-hoc testing with Scheffé's multiple comparison procedure for post-hoc testing and Duncan's test for subgroup homogeneity. We used the Kruskall–Wallis or *U*-test in case of nominal data.

Results

Description of DSD sample

The clinical diagnosis of a DSD-syndrome was supported by genetic testing and/or specific histological findings for 24 children. Nine children were diagnosed as "XY DSD of unknown etiology" based on a clinical evaluation by an experienced pediatric endocrinologist, laboratory findings, and imaging results (see Table 2).

The 33 participating children with DSDs were classified into one of two major groups:

DSD-C-F Children with complete hypoandrogenization, including complete androgen insensitivity syndrome (cAIS) and complete steroid biosynthesis defect (for

Table 2
Description of study sample

Child #	Age (year)	Karyotype	Clinical diagnosis	Genetic findings/Histology	Sinnecker score [a] (phenotype at birth)	Mullerian remnants (+/−)	Comorbidity
DSD-C-F: children with complete hypoandrogenization; reared as girls							
(1)	8.8	46,XY	46,XY DSD (cAIS)	AR-mutation R774H	5	−	
(2)	3.5	46,XY	46,XY DSD (cAIS)	AR-mutation R774H	5	−	
(3)	3.11	46,XY	46,XY DSD (cAIS)	AR-mutation R855G	5	−	
(4)	3.10	46,XY	46,XY DSD (cAIS)	AR-mutation R615H	5	−	
(5)	12.1	46,XY	46,XY DSD (cAIS)	AR-mutation A870G	5	−	
(6)	8.7	46,XY	46,XY DSD (complete steroid biosynthesis defect)	CYP11A1-mutation L288X	5	?	
DSD-P-M: children with partial hypoandrogenization; reared as boys							
(7)	3.3	46,XY	46,XY DSD (pAIS)	AR-mutation Δ409–411	2	−	Hearing deficit, developmental retardation
(8)	4.5	46,XY	DSD of unknown etiology, related to a syndrome	Irrelevant SRD5A-mutation	2	−	Hexadactyly, hearing deficit, congenital heart defect
(9)	8.10	46,XY	46,XY DSD (pAIS)	AR-mutation L712P	3	−	
(10)	7.7	46,XY	46,XY DSD (pAIS)	AR-mutation L 712P	1	−	
(11)	5.9	46,XY	46,XY DSD (pAIS)	AR-mutation L 712P	1	−	
(12)	7.2	46,XY	46,XY DSD (17β-HSD-3 deficiency)	HSD17B3-mutation R80E	2	−	
(13)	10.6	46,XY	46,XY DSD (pAIS)	AR-mutation S597 R	2	−	
(14)	11.5	46,XY	DSD of unknown etiology	Mutational analysis negative for AIS and 17β-HSD-3-deficiency	2	−	
(15)	4.0	45,X/ 46,XY	Sex chromosome DSD (mixed gonadal dysgenesis)	Gonadal histology: left rudimentary testicular tissue, remnants of epididymidis, remnants of fallopian tube, streak gonad	2	(+)	
(16)	4.1	46,XY	46,XY DSD (pAIS)	AR-mutation H885Y	2	−	
(17)	3.12	46,XY	DSD of unknown etiology	Micropenis	1	?	
(18)	8.8	46,XY	DSD of unknown etiology, related to a syndrome	Mutational analysis negative for AIS and 17β-HSD-3-deficiency; micropenis	1	No information	Hearing loss, developmental delay, ADHD
DSD-P-F: children with partial hypoandrogenization; reared as girls							
(19)	10.4	46,XY	DSD of unknown etiology	Mutational analysis negative for AIS and 17β-HSD-3-deficiency	2	−	
(20)	8.9	46,XY	46,XY DSD (pAIS)	AR-mutation Ile841Ser	4	−	
(21)	5.2	46,XY	46,XY DSD (17β-HSD-3 deficiency)	HSD17B3-mutation R 80 N1305	4	−	
(22)	7.11	46,XY	DSD of unknown etiology, related to a syndrome	Mutational analysis negative for AIS and 5αRD	5	−	Growth hormone deficiency, hypothalamic hypercortisolism, hyperprolactemia, glucose-6-phosphat-dyhydrogenase deficiency
(23)	6.1	46,XY	DSD of unknown etiology	Mutational analysis negative for AIS and 5αRD	5	−	
(24)	5.4	46,XY	Suspected 46,XY DSD (defect in testicular development)	Mutational analysis negative for AIS and 5αRD Both gonads in scrotum Gonadal histology: predominately immature Sertoli cells, isolated atypical germ cells, otherwise regular testicular structures	Phenotype not documented	+	
(25)	9.5	46,XY	46,XY DSD (pAIS)	AR-mutation V866 M	2	−	
(26)	7.11	46,XY	46,XY DSD (ovotesticular DSD)	Gonadal histology: ovotestis left; immature tissue from epididymidis, remnants of ductus deferens, isolated primitive tubuli	5	−	

Table 2 (*continued*)

Child #	Age (year)	Karyotype	Clinical diagnosis	Genetic findings/Histology	Sinnecker score [a] (phenotype at birth)	Mullerian remnants (+/−)	Comorbidity
(27)	2.2	46,XY	46,XY DSD (defect in testicular development)	Gonadal histology: immature gonadal tissue with ovarian stroma cells, remnants of fallopian tubes, immature testicular	3	No information	Difficult postnatal adaptation with amnion infection syndrome and transient adrenal insufficiency; congenital heart defect (VSD)
(28)	3.8	46,XY	46,XY DSD (defect in testicular development)	Gonadal histology: partial gonadal dysgenesis (no details available)	4	No information	
(29)	3.6	46,XY	46,XY DSD (defect in testicular development)	Mutational analysis negative for AIS and 5αRD Gonadal histology: right immature ovary, fallopian tubes, tissue of epididymidis, left immature testicular tissue and epididymidis	3	+	
(30)	10.10	45,X/46, XY	Sex Chromosome DSD	Gonadal histology: immature testicular tissue with normal tubuli seminiferi, normal epididymidis, remnants of fallopian tubes	4		
(31)	6.2	46,XY	46,XY DSD (defect in testicular development)	Gonadal histology: ovary left, ovary tissue right and testicular tissue in inguinal canal	3	+	
(32)	4.5	45X/46X idic (Yq)	Sex Chromosome DSD	Gonadal histology: streak gonads	2	−	
(33)	10.9	46,XY	46,XY DSD (pAIS)	AR-mutation V745 M	3	−	ADHD

DSD-C-F: children with complete hypoandrogenization, reared as girls.
DSD-P-M: children with partial hypoandrogenization, reared as boys.
DSD-P-F: children with partial hypoandrogenization, reared as girls.
cAIS: complete androgen insensitivity syndrome.
compl. andr.-biosynthesis defect: complete androgen biosynthesis defect.
pAIS: partial androgen insensitivity syndrome.
17β-HSD-3: 17β-hydroxysteroid dehydrogenase-3 deficiency.
[a] Phenotype at birth was classified according to Sinnecker as (1) male, (2) predominately male, (3) ambiguous, (4) predominately female, and (5) female (Sinnecker et al., 1996).

further description see Hiort et al., 2005). All six children in this group had a Sinnecker score[1] of 5 (Sinnecker et al., 1996, 1997), as rated by the physician at birth, and all were reared as girls.

DSD-P Children with partial hypoandrogenization, including 9 children with partial AIS (pAIS; 6 reared male, 3 female), 2 children with 17β-HSD3 (1 reared male, 1 female), 7 children with partial gonadal dysgenesis (1 reared male, 6 female), and 9 children with XY DSD of unknown etiology based on a clinical diagnosis but without established molecular diagnosis (4 reared male, 5 female). Within DSD-P, we further distinguished between children reared as boys (DSD-P-M, 12 children) and those reared as girls (DSD-P-F, 15 children). In subgroup DSD-P-M, four children had a Sinnecker score of 1 (male phenotype), seven children a Sinnecker score of 2 (predominantly male), and one child a Sinnecker score of 3 (ambiguous). In this group, the mean Sinnecker score at birth was 1.8 (SD 0.6).

In subgroup DSD-P-F, three children had a Sinnecker score of 5 (female), four children a Sinnecker score of 4 (predominantly female), four children a Sinnecker score of 3 (ambiguous), and three children a Sinnecker score of 2 (predominantly male). The mean Sinnecker score of this group was 3.5 (SD 1.1), differing significantly (p 0.000) from the DSD-P-M group. For a detailed summary on all patients see Table 2.

Mean children's age was 6.2 years (SD 2.82; range 2;2[2] to 12;10). Twenty-eight of the children lived with both biological parents, four with their single mother, and one with the mother and her new partner; twenty-two had at least one sibling. Families' educational attainment was relatively high: in 17 families (51.5%) at least one parent held a university or graduate degree; in only 4 families (12.1%) neither or only one parent had at least reached high school level (10 years of education).

The majority of parents were of German nationality, except 4 mothers and 4 fathers who were citizens from other European countries and 2 mothers of African origin.

[1] Sinnecker developed the score to assess the external genitals and described the severity of the masculinization defect in 46,XY individuals, ranging from 1 (male) to 5 (female) (Sinnecker et al., 1996, 1997).

[2] One family with a child younger than 3 years was included because this family was very enthusiastic to participate in the study and we did not want to miss parents' information about their child.

Statistical analysis of demographic characteristics did not reveal differences between subgroups.

Description of control group

166 families participated in the control group (89 children reared as boys, 77 reared as girls). Mean age of the children was 7.7 years (SD 2.24), which was significantly higher than the mean age in the study group (Table 3).

No significant associations were found for age, parental education level, or whether the child had a male or female sibling with any of the gender variables measured. We therefore did not control for these demographic variables in the analyses of variance.

Gender role behavior

Sex-typed activities and interests

(1) FAI. DSD subgroups differed significantly in preferences for activities and interests (Table 4). Compared with boys from the control group (CO-M), the study group children with partial hypoandrogenization reared as boys (DSD-P-M) showed similar scores in female-typical activities (FAI-female) and male-typical activities (FAI-male). Study group children with partial hypoandrogenization reared as girls (DSD-P-F) showed a significantly lower score in female-typical activities and a significantly higher score in male-typical activities, compared to girls from the control group (CO-F). In DSD-P-F, scores were higher on the FAI-female scale but lower on the FAI-male scale compared to DSD-P-M, however, differences failed to reach significance.

Children with complete hypoandrogenization (DSD-C-F) showed a significantly lower score in male-typical activities and a significantly higher score in female-typical activities than those with partial hypoandrogenization reared as girls (DSD-P-F).

(2) Structured free-play task. The structured play situation was not part of the pre-school and school survey; comparison data are therefore unavailable.

The subgroups DSD-P-M and DSD-P-F did not differ significantly in play behavior: children with partial hypoandrogenization reared as boys did not differ from those reared as girls regarding play behavior (Table 4). Both subgroups spent the majority of time playing with toys typical for boys. Children with complete hypoandrogenization (DSD-C-F) played only 21.3% of the time with toys typical for boys.

(3) Toy to keep. The "toy to keep" was not part of the pre-school and school survey; comparison data are therefore unavailable.

The only group of children which preferred typical girls' toys was DSD-C-F, the children with complete hypoandrogenization (Table 4). In addition, this group showed the most homogeneous behavior in respect of toy selection of all groups indicated by a small variance (SD 0.5). Children with partial hypoandrogenization tended to choose a male-typical toy to keep, regardless of whether they were reared as boys or as girls.

Gender-typical behavior and attitudes (CBAQ-G)

Results regarding the Femininity Scale showed significant differences between subgroups (Table 4). Study group children reared as boys (DSD-P-M) had scores similar to boys of the control group (CO-M). Children with complete hypoandrogenization (DSD-C-F) had scores comparable to the girls from the control group (CO-F), whereas children with partial hypoandrogenization reared as girls (DSD-P-F) had significantly lower scores. Children with partial hypoandrogenization reared as girls (DSD-P-F) exhibited substantial variation in gender-related behavior and had significantly higher scores on the Femininity Scale than children with partial hypoandrogenization reared as boys (DSD-P-M).

Regarding cross-gender behavior, we found significant differences among the subgroups, which were explained by higher scores on the Cross-Gender Scale in study group children with partial hypoandrogenization reared as girls (DSD-P-F). Two girls had very high scores on that scale (2.33 and 2.85 SD above the mean), possibly indicating insecurity in gender identity. Comparison between groups with the two outliers excluded did affect the results; without them we did not find a significant difference between DSD-P-F and all other subgroups.

Table 3
Demographics

	Study group (N=33)	Control group (N=166)	Significance level/test
Sex/Gender (of rearing)			
Male	12 (36.4%)	89 (53.6%)	ns (U-test)
Female	21 (63.6%)	77 (46.4%)	
Age	Mean: 6.2 years (SD 2.82)	Mean: 7.7 years (SD 2.24)	** (t-test)
Number of siblings	Mean: 1.06 (SD 1.17)	Mean: 1.41 (SD 1.04)	ns (t-test)
Only child	11 (33.3%)	22 (13.3%)	
Number of brothers	Mean: 0.55 (SD 0.79)	Mean: 0.72 (SD 0.80)	ns (t-test)
Number of sisters	Mean: 0.48 (SD 0.67)	Mean: 0.68 (SD 0.75)	ns (t-test)
Educational attainment of family (highest level of both parents [a])	Median: 4.00 (SD 1.08)	Median: 3.00 (SD 1.01)	ns (U-test)
Marital status			ns (U-test)
Married/parents living together	29 (87.9%)	132 (79.5%)	
Single mother	4 (12.1%)	33 (19.9%)	
No information	0	1 (0.6%)	
Nationality of mother			** (U-test)
German	28 (84.8%)	160 (97.0%)	
Other European countries	3 (9.1%)	4 (2.4%)	
Others	2 (6.1%)	1 (0.6%)	
No information		1 (0.6%)	
Nationality of father			ns (U-test)
German	28 (84.8%)	151 (91.0%)	
Other European countries	3 (9.1%)	4 (2.4%)	
Others	0	3 (1.8%)	
No information	2 (6.1%)	8 (4.8%)	

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

[a] From 1=low educational level (no secondary school qualifications) to 4=high educational level (degree from a university or graduate school).

Table 4
Gender-typical activities, free-play task, toy to keep, and Femininity and Cross-Gender Scale in children with DSD and children of the control group

	CO-M	DSD-P-M	DSD-P-F	DSD-C-F	CO-F	Differences among groups (ANOVA)	Significant differences between subgroups (Scheffé's test)	Homogeneity of subgroups (Duncan's test)
	M (SD)	*M* (SD)	*M* (SD)	*M* (SD)	*M* (SD)			
N	89	12	15	6	77			
FAI-female	26.8 (8.7)	34.0 (12.4)	40.8 (15.5)	58.4 (7.0)	55.8 (10.2)	*F* 90.472 *p* 0.000	CO-M < CO-F*** CO-M < DSD-C-F*** CO-M < DSD-P-F*** CO-F > DSD-P-F*** CO-F > DSD-P-M*** DSD-C-F > DSD-P-F* DSD-C-F > DSD-P-M***	1. CO-M; DSD-P-M 2. DSD-P-M; DSD-P-F 3. CO-F; DSD-C-F
FAI-male	46.9 (8.1)	44.0 (13.5)	34.7 (13.7)	15.7 (6.6)	17.2 (8.1)	*F* 123.743 *p* 0.000	CO-M > CO-F*** CO-M > DSD-C-F*** CO-M > DSD-P-F*** CO-F < DSD-P-F*** CO-F < DSD-P-M*** DSD-C-F < DSD-P-F*** DSD-C-F < DSD-P-M***	1. DSD-C-F; CO-F 2. DSD-P-F 3. DSD-P-M; CO-M
Free-play task	No data from comparison group available	66.7 (27.7)	65.3 (34.9)	21.3 (25.3)	No data from comparison group available	*F* 3.512 *p* 0.044		1. DSD-C-F 2. DSD-P-F; DSD-P-M
Toy to keep	No data from comparison group available	3.5 (1.4)	3.5 (1.7)	1.5 (0.5)	No data from comparison group available	*F* 4.503 *p* 0.019	DSD-C-F < DSD-P-F* DSD-C-F < DSD-P-M*	1. DSD-C-F 2. DSD-P-F; DSD-P-M
CBAQ-G Femininity Scale	58.1 (6.7)	58.5 (4.2)	68.0 (10.4)	79.0 (3.0)	79.0 (7.6)	*F* 92.525 *p* 0.000	CO-M < CO-F*** CO-M < DSD-C-F*** CO-M < DSD-P-F*** CO-F > DSD-P-F*** CO-F > DSD-P-M*** DSD-C-F > DSD-P-M*** DSD-P-F > DSD-P-M*	1. CO-M; DSD-P-M 2. DSD-P-F 3. DSD-C-F; CO-F
CBAQ-G Cross-Gender Scale	22.3 (6.0)	21.2 (5.7)	28.9 (12.5)	20.4 (5.3)	22.7 (5.4)	*F* 3.718 *p* 0.006	CO-M < DSD-P-F* CO-F < DSD-P-F*	1. DSD-C-F; DSD-P-M; CO-M; CO-F 2. DSD-P-F

CO-M: control group boys; DSD-P-M: partial hypoandrogenization, reared as males; DSD-P-F: partial hypoandrogenization, reared as females; DSD-C-F: complete hypoandrogenization, all reared as girls; CO-F: control group girls; inter-group comparison: Scheffé's test, only significant results are stated: $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; homogenous subgroups: Duncan's test; FAI-female: Fragebogen zu Aktivitäten & Interessen, score: percentage of female-typical activities and interests of total activities and interests; FAI-male: Fragebogen zu Aktivitäten & Interessen, score: percentage of male-typical activities and interests of total activities and interests; toy to keep: score of toy the child chose as a present (range 1 = typical girl's toy–5 = typical boys' toy); free-play task: percent of total time of playing with male-typical toys in a structured free-play task; CBAQ-G Femininity Scale: score the child reached on the Femininity Scale of the German version of the Child Behavior and Attitudes Questionnaire; CBAQ-G Cross-Gender Scale: score the child reached for cross-gender behavior on the Cross-Gender Scale of the German version of the Child Behavior and Attitudes Questionnaire.

Playmate preferences

For all study group children and 164 (of 166) control group children, parents were able to report their children's preferred friendship. Boys and girls of the control group showed a strong tendency to play with children of their own sex (Table 5). In contrast, many of the children from our study group preferred to play with children of both sexes. While boys of the control group nearly never chose girls as "preferred playmate", boys with partial hypoandrogenization (DSD-P-M) showed a higher percentage in choosing girls, followed by girls with partial hypoandrogenization, girls with complete hypoandrogenization, and girls of the control group.

In four study group children (12.1%) and six (3.6%) control group children, parents were unable to report the sex of their child's "best friend". The parents of eight study group children (24.2%) and 33 control group children (19.9%) answered that their child had no "best friend". Boys and girls of the control group obviously preferred children of their own sex as "best friend". In general, in study group children, the tendency to chose a child of ones own sex as "best friend" was less strong (Table 5). Only study group children with complete hypoandrogenization (DSD-C-F) exclusively chose girls as best friends (but this statement is based on only three children with valid data).

Discussion

Discussion of main findings

In this study, androgen effects showed a significant relationship to nearly all of the gender-related behaviors we

Table 5
Playmate preferences

Preferred friendship with					
	Girls % [a] (*N*)	Boys % [a] (*N*)	Both girls and boys % [a] (*N*)	Missing % [b] (*N*)	Significant group differences (overall ANOVA p 0.000)
CO-M	2.3% (2)	72.4% (63)	25.3% (22)	2.2% (2)	CO-M/CO-F***
DSD-P-M	8.3% (1)	33.3 (4)	58.3% (7)	(0)	CO-M/DSD-P-M*
DSD-P-F	46.7% (7)	(0)	53.3% (8)	(0)	DSD-P-M/DSD-P-F*
DSD-C-F	50.0% (3)	(0)	50.0% (3)	(0)	CO-F/DSD-P-F*
CO-F	79.2% (61)	(0)	20.8% (16)	(0)	

Sex of best friend						
	Female % [a] (*N*)	Male % [a] (*N*)	Both female and male [c] % [a] (*N*)	No best friend % [b] (*N*)	Missing/unknown % [b] (*N*)	Significant group differences (overall ANOVA p 0.000)
CO-M	6.3% (4)	85.7% (54)	7.9% (5)	22.5% (20)	6.7% (6)	CO-M/CO-F***
DSD-P-M	42.9% (3)	57.1% (4)	(0)	25.0% (3)	16.7% (2)	CO-F/DSD-P-F*
DSD-P-F	63.6% (7)	27.3% (3)	9.1% (1)	20.0% (3)	6.7% (1)	
DSD-C-F	100.0% (3)	(0)	(0)	33.3% (2)	16.7% (1)	
CO-F	84.4% (54)	6.3% (4)	9.4% (6)	16.9% (13)	(0)	

CO-M: control group boys; DSD-P-M: partial hypoandrogenization, reared as males; DSD-P-F: partial hypoandrogenization, reared as females; DSD-C-F: complete hypoandrogenization, all reared as girls; CO-F: control group girls; only significant results are stated: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$ (chi-square test).

[a] Percentages calculated from valid answers only (not "missing/unknown/no best friend").

[b] Percentages calculated from overall-*N* of each subgroup.

[c] The questionnaire did not include the answering category "female and male", but several parents chose to mark both the "female" and "male" category.

assessed, supporting our hypothesis that masculinization of gender role behavior is a function of prenatal androgen exposure. Overall, the results show an orderly correlation of behaviors with the degree of presumed prenatal androgen exposure. This finding is compatible with the hypothesis of organizational effects of prenatal androgens on brain structures and functions of the developing brain in individuals with XY karyotype and DSD.

XY DSD children with complete hypoandrogenization (DSD-C-F) showed girl-typical behaviors and preferences and did not differ significantly from girls of the control group. Children with partial prenatal androgen effects reared as girls (DSD-P-F) exhibited far more boy-typical behaviors and interests than girls of the DSD-C-F subgroup. Boys with partial androgen effects (DSD-P-M) showed the most boy-typical behavior compared to the other two DSD subgroups; they had somewhat but not significantly lower scores for male-typical behaviors compared to control boys.

The two groups of children with partial hypoandrogenization reared as boys or as girls differed much less from each other than boys and girls did in the control group. Slight differences in the female and male activity scores (FAI) remained non-significant, and there were no significant differences in free-play task and the toy to keep test; both subgroups showed a preference to play with boy-typical toys. Only the Femininity Scale of the CBAQ-G revealed statistically significant differences with children with partial hypoandrogenization reared as girls showing higher scores for feminine behaviors compared to those reared as boys. We speculate that the CBAQ-G assesses more complex behaviors, which are likely to be influenced by socialization effects and role modeling. An alternative interpretation may be that responses to this parent-administered questionnaire may reflect parental expectations, emphasizing gender-typical behaviors congruent with the assigned sex. In contrast, objective measurements such as observations of behaviors are independent of parental subjective appraisal.

Regarding playmate preferences children with partial androgen effects played significantly more often with children of both sexes in comparison to control group children who largely preferred to play with children of their own sex.

The effect of the sex of rearing within the subgroups of children with partial androgen effects appears to support the hypothesis that hormone effects prenatally may be modified by socialization effects (Bosinski, 2000, 2005; Lytton and Romney, 1991; Meyer-Bahlburg, 1993, 2002; Snow et al., 1983; Zucker, 1999, 2002a).

Given the small sample size we were unable to stratify the group with partial hypoandrogenization further and the degree of prenatal androgen exposure in individual children in this group remains speculative. The lack of expected differences between boys and girls in the study group in most measures of gender-related behaviors may be explained by similar levels of androgen exposure but also to a larger variance in behaviors in study group children with partial androgen effects compared to control group children, blurring the usual differences between boys and girls.

Despite the fact that gender-related behavior of children with partial androgen effects reared as girls showed highly significant differences from control girls, they did not show increased scores on Cross-Gender Scale (CBAQ-G) on a group level; however, high scores in two girls may indicate gender identity confusion. This result may indicate that opposite sex behavior is not necessarily a sign of gender identity confusion or instability. This finding underlines results from studies on girls with CAH (Meyer-Bahlburg et al., 2004; Zucker et al., 1996) and provides further evidence that gender role behavior and gender identity are different constructs (Money, 1994).

Other studies found increased rates of gender identity confusion among individuals with DSD (Bosinski, 2005; Cohen-Kettenis and Pfäfflin, 2003; Meyer-Bahlburg, 1993, 2005; Reiner, 2005; Slijper et al., 1998; Wilson, 2001; Wilson et al., 1993; Wisniewski and Migeon, 2002). More recently, reviews on gender identity in this population in the adult age group showed a high risk for gender dysphoria in some groups with partial undervirilization but not in complete androgen insensitivity (Cohen-Kettenis, 2005; Hines et al., 2003; Mazur, 2005; Reiner, 2005, Wisniewski et al., 2000). Longitudinal studies on the developmental trajectory of atypical gender role behavior in children and later gender dysphoria are not available. We believe that these children should be closely followed and parental anxieties concerning the child's development and behavior addressed (Beh and Diamond, 2000; Brown and Warne, 2005; Cohen-Kettenis and Pfäfflin, 2003; Houk and Lee, 2005; Lee, 2001; Lee and Houk, 2005; Meyer-Bahlburg, 2002; Thyen et al., 2005; Warne et al., 2005; Zucker, 2002b).

Limitations of the study

One limitation of the study consists in the rather small sample size of participating children and families, causing low statistical power and restricting statistical analytic options. The study is, however, the largest on gender roles in children with XY DSD as yet. Secondly, our definitions for the subgroups were based on aspects related to hormone effects (DSD-C versus DSD-P) and on sex of rearing. While DSD-C resulted in a very homogenous group, subgroup DSD-P included boys and girls with a great variety of diagnoses and clinical cases without molecular genetic findings. Because of the complexity of unknown intervening factors it is difficult to generalize the findings to the entire population of children with XY karyotype and DSD with partial androgen effects. A third limitation of this study pertains to the clinical sample from which participants were selected: they predominately came from a well-educated, middle class background. This may limit generalizability of our results beyond this population.

Conclusions

Effects of early androgen action on the developing brain appear to have a substantial impact on gender-related behaviors and development in early childhood. In some conditions, such as cAIS or complete androgen biosynthesis defect, sex assignment to the female gender appears to be the best strategy (Lee et al., 2006). However, great efforts should be made to ascertain the diagnosis because other conditions such as 17β-hydroxysteroid-dehydrogenase-3 deficiency and 5α-reductase-2 deficiency may also present with an almost normal female genital phenotype in infancy and yet belong to the group of conditions with partial androgen exposure prenatally and significant masculinization during puberty. Both groups, those reared as girls and those reared as boys, show large variability in gender-related behavior. Possible indications of gender confusion or insecurity appear to be rare and to occur in this study only in children who had been exposed to prenatal androgens and assigned to the female sex.

Recommendations for future research studies include longitudinal designs that follow children from an early age and include data on parental expectations, views, and parenting style to evaluate the modifying effect of socialization on gender dimorphism. Registries may help to identify and follow such children on a long-term basis (Ahmed et al., 2004).

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