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RESEARCH

Social and biological factors related to gender expression in 3-year-old children

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Abstract

Background: Gender identity (GI) is the unified and persistent self-perception on the male-female spectrum, and its acquisition is a multifactorial process. GI is generally consolidated around ages 3–4 years. The gender identity questionnaire for children (GIQC) aims to assess GI in both clinical and non-clinical populations. The aim of the research is to evaluate GI in relation to social and biological factors.

Methods: Single-center, prospective birth-cohort study enrolling those born at term, appropriate for gestational age. The GIQC was administered to the parents at age 3. The scoring was performed through the original coding scheme and the new coding scheme for the non-clinical group based on three scales: female typical behavior (FTB), male typical behavior (MTB), and cross-gender (CG). Anthropometrics, anogenital distances, and urinary hormone assessment were performed at birth, 3, 6 and 36 months.

Results: 86 children (males 53) participated. FTB, MTB, and CG scores differed significantly according to sex: boys (3.28 ± 0.59) scored higher than girls (2.45 ± 0.44) on MTB, while girls (3.41 ± 0.75) scored higher than boys (1.92 ± 0.61) on FTB. Girls (4.14 ± 0.64) scored higher than boys (3.66 ± 0.88) on the CG scale. Within the whole sample, the FTB scale showed a moderate negative correlation with MTB (r: −0.464, $P < 0.01$) and a positive one with CG (r: 0.377, $P < 0.001$) in the female population. Correlations exist between MTB and ano-scrotal distance (AGD-AS) in males, and between MTB and ano-clitoral distance (AGD-AC) in females.

Conclusion: Our findings confirm that by age 3, most children express differentiated sex-typed behavior according to the sex assigned at birth. In addition, androgenization appears to play a role in GI development in males.

Keywords: gender identity; hormones; minipuberty; anogenital distances; gender expression; gender identity questionnaire for children

Introduction

Gender identity (GI) refers to the internal sense of a person of being male, female, or something else (1), and its acquisition is a multifactorial process influenced by genetic, hormonal, neurobiological, social, and psychological factors. GI develops in early childhood and typically consolidates around ages 3–4 years (2). Indeed, children begin to develop a sense of GI at a very young age, becoming conscious of physical

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differences between boys and girls by around age 2 (3). Before their third birthday, most children can label themselves as either a boy or a girl (4), and by age 4, they generally have a stable sense of their GI.

Genetic makeup plays a role in GI, although the specific genes involved are not fully understood (5). Prenatal exposure to hormones, such as testosterone, influences GI, with higher levels of androgens leading to more masculine traits (6). Differences in brain structure and function have also been observed in individuals with different GIs (7, 8).

This process involves recognizing and internalizing societal gender norms and roles, often reinforced through interactions with parents, peers, and media.

In particular, it is known that the development of GI in children is significantly influenced by various social factors. Parental attitudes and behaviors, along with the reinforcement of gender norms, play a crucial role in shaping a child's understanding and expression of gender (9). The attitudes and behaviors of parents are pivotal in the early stages of GI development. Parents' reinforcement of traditional or non-traditional gender norms can either constrain or expand a child's exploration of gender roles (10). In addition, interactions of children with peers provide a social context in which gender norms are learned and reinforced. Exposure to media and prevailing cultural norms shapes children's perceptions of gender roles and identities (11). Media representations often reinforce stereotypical gender roles, which can influence children's understanding of gender. A child's self-perception and internal sense of GI are critical components of their overall gender development.

An important related concept is gender expression, which refers to the external presentation of one's gender through behaviors, clothing, hairstyle, voice, and interests. Gender expression may or may not align with societal expectations or with the individual's GI. In children, gender expression often reflects both personal preferences and environmental influences, and it can vary widely over time. Understanding gender expression as a separate but interconnected aspect of gender development helps provide a more comprehensive view of how children explore and communicate their gender.

However, gender-related behavior in humans is influenced by prenatal sex steroids, namely androgens. This evidence comes mainly from studies of girls and women with congenital adrenal hyperplasia who are exposed to high levels of androgens *in utero* (12, 13, 14, 15, 16, 17). Some studies of individuals with conditions that cause abnormalities in prenatal androgen exposure, and that relate prenatal androgen exposure to postnatal behavior in normally developing individuals, also support the conclusion that prenatal androgen exposure affects human neurobehavioral development (6, 15, 16, 17, 18, 19, 20, 21). In contrast, there is little evidence for neurobehavioral effects of early postnatal androgen exposure in humans. There are only a few studies relating androgen variation during minipuberty to variation in later sex-related behavior (15, 22).

Although it may be easier to assess exposure to androgens such as urinary testosterone when assessing minipuberty (23, 24, 25, 26), different aspects of physical development can be used to assess prenatal exposure to androgens. Anogenital distances (AGDs) (27) can be a marker of androgen exposure specific to early fetal development. In humans, AGDs are sexually dimorphic (22, 27) and appear to be sensitive to changes in fetal androgens (28, 29).

To study gender expression development in young children, it is essential to consider their natural inclination toward play and choice of playmates. Observing their daily lives offers deeper insight into how they interact with their social environment. Parental observations provide valuable information, and the gender identity questionnaire for children (GIQC) (30, 31) is a useful tool for this purpose.

The GIQC, developed by Johnson *et al.* (30), is a parent-report questionnaire designed to aid in the assessment of children with potential problems in their GI development. The Italian version of the same questionnaire was published in 2019 (31); it was also validated in a non-clinical population and has been recognized as a valuable tool for investigating GI in children between ages 3 and 12.

The aim of the study is to describe GI, as assessed by the GICQ, in a non-clinical population of 3-year-old children. Moreover, we aim to detect whether there are any correlations among GIQC scores in relation to the sociodemographic characteristics of the families, and to evaluate the relationship between hormone levels and anogenital distances (AGDs) during minipuberty.

Materials and methods

Study population

This study focuses on a subsample of the Modena cohort study. The Modena cohort study (26, 32) is an ongoing prospective birth-cohort study, which aims to assess perinatal and postnatal exposure to phthalates, and the association between this exposure and hormonal, anthropometric, and neurocognitive development in children aged 0–3 years, as well as phthalate exposure in mothers closely after delivery. The study enrollment phase started in 2019, and enrollment ended in October 2020. Newborns whose mothers were of legal age (>18 years old) at delivery, who understood the Italian language, and who had a singleton pregnancy were eligible to participate in the main cohort study. Other inclusion criteria based on infants' characteristics were

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term delivery (37–41 weeks postmenstrual age), infants appropriate for gestational age, and Apgar score >7 five minutes after birth. Follow-up visits are carried out at 3 (T3), 6 (T6), 15 (T15), and 36 (T36) months of age.

This study focuses mainly on the follow-up at 36 months. At this time, the parents of the children were asked to complete the Italian version of the GIQC (30). Parents of 89 children completed the questionnaire at T36, of which only 86 completed the questionnaire in full, thus determining the sample size for this sub-study. One child with neurodevelopmental disorders in the general cohort was not included in this cohort. The entire sample is of Caucasian ethnicity.

Sociodemographic data

At enrollment, a sheet of sociodemographic information about the family was filled in. The information collected focused on nationality, age, educational level, employment, and marital status of both parents.

More precisely, parental education was coded into three categories: 'none/primary/middle school', 'high school degree', and 'university or more'. Information on parental employment was grouped according to the European Socio-economic Groups (ESeG) (33) classification composed of nine categories (managers, professionals, technicians and associated professional employees, small entrepreneurs, clerks and skilled service employees, skilled industrial employees, lower-status employees, retired persons, and other not-employed persons). To simplify and group the job classes, we grouped them into four categories: 'not working', 'working class', 'middle class', and 'high class'. Marital status was coded as a categorical variable according to the different family constellations ('single parent', 'married', 'cohabiting', 'widowed', 'separated', 'divorced') and then recoded as a binary variable with the categories 'both parents cohabiting' and 'other'.

Children's anthropometric measurement and anogenital distances

Auxologic measurements were collected at all examinations: at birth (T0), at 3 months of age (T3), at 6 months of age (T6), and at 36 months of age (T36).

Body length was measured from the top of the head to the sole of the foot with the patient lying supine with hips and knees extended, using a calibrated stadiometer (Harpenden, Crymych; UK) to an approximation of 0.1 cm at T0, T3, and T6, while at T36 the height was measured using the same criteria but in a standing position. Infants were weighed naked or wearing as little clothing as possible and the diaper was removed; the measurement was rounded to the nearest 0.1 kg using a calibrated scale. Head circumference was measured with a tape measure from just above the glabella area to the area near the top of the occipital bone; the approximation was to the nearest 0.1 cm. WHO percentiles of anthropometric data at T0, T3, T6, and T36 were calculated.

The genital assessment included a description of the appearance of the external genitalia, measurement of anogenital distances (AGDs), and, in males, measurement of penile length (PL), and size and volume of testes. AGDs and PL were measured using a specific caliper, with the newborn/baby in the dorsal decubitus position with hips flexed. Two AGDs were collected in both males and females. In males, the ano-penile distance (AGD-AP) was measured from the anterior base of the penis to the center of the anus, and the ano-scrotal distance (AGD-AS) was measured from the posterior base of the scrotum to the center of the anus. Similarly, in females, the ano-clitorial distance (AGD-AC) was recorded as the distance between the clitoris and the center of the anus and the ano-fourchette distance (AGD-AF) was recorded as the distance from the posterior convergence of the fourchette to the center of the anus. Clinical assessment of testicular size and volume was carried out using a standard orchidometer. The PL was measured as the length of the flaccid penis, avoiding erection, with the penis slightly straightened and the distance between the lower edge of the pubic bone and the tip of the glans penis (excluding foreskin) measured from the dorsal side.

Urinary hormone assays

Urine samples were collected at birth (T0), 3 months (T3), and 6 months of age (T6). Spot urine was collected in pediatric urine bags attached to the skin during the examinations. Once the sample was obtained, it was collected by the researchers. Hormonal urine analysis was performed on fresh urine with regard to urinary FSH (uFSH), urinary LH (uLH), urinary testosterone (uT), and urinary estradiol (uE).

Each urine sample was analyzed by the Department of Laboratory Medicine and Pathological Anatomy of the University Hospital of Modena. All hormones analyzed were quantified using an electrochemiluminescence immunoassay 'ECLIA' (Cobas E601, Roche Diagnostics, Switzerland). The lower limit of detection for urinary gonadotropins (uGs) was 0.1 mIU/mL for both uFSH and uLH. The lower limits of detection for uTs and uE were 0.025 ng/mL and 18.4 pmol/L, respectively. Intermediate precision was <4.5% and <3.7% at concentrations ranging from 0.65 to 152 mIU/mL for uFSH, and <2.2% and <1.6% at concentrations ranging from 1.3 to 123 mIU/mL for uLH. For uT, intermediate precision was <14.8% and <2.1% at concentrations ranging from 0.063 to 14 ng/mL, and its conversion factor is ng/mL × 3.47 = nmol/L. For uE, Intermediate precision was <10.6% and <1.9% at concentrations

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ranging from 10.2 to 2,934 pg/mL, with a conversion factor of pg/mL × 0.00367 nmol/L.

Gender identity questionnaire for children (GIQC)

The GIQC (30, 34) is a parent-reported questionnaire covering a range of children's gender characteristics. The Italian version was validated for ages between 3 and 12 years by Caldarera and colleagues (31).

The questionnaire contained 16 items focusing on different gender-related behaviors.

Each item is scored on a 5-point scale for frequency of occurrence (three items also included a 'not applicable' option). Two different scoring methods can be applied to questionnaire results. Classically, for clinical populations, as reported by Johnson and colleagues (30), lower scores reflect more cross-gender (or less same-gender) behaviors, consistent with the fact that the assessment is aimed at establishing a diagnosis of GI disorder in accordance with the DSM-IV-TR (34). More recently, for non-clinical population, as in our population, Caldarera *et al.* (31) proposed a different scoring method, which assesses male and female behavior by assigning a score of 'female typical behavior (FTB)' to item '5', indicating a high frequency of female-typical behavior, and '1', indicating the absence of such behavior; the same was done for the male-typical behavior (MTB) items. For the remaining items, the scoring method presented by Johnson *et al.* (29) was used. On this basis, we determined three scales: i) a female-typical behavior (FTB) scale, ii) a male-typical behavior (MTB) scale, and iii) a cross-gender (CG) scale, intended to indicate cisgender (score of 5) versus cross-gender behavior (score of 1).

Statistical analysis

Data were expressed as mean and SD. A parametric statistical analysis was performed.

Comparison within two or more continuous variables was performed using the Pearson *t*-test and one-way ANOVA, respectively.

The chi-square test was used to relate categorical variables. Pearson correlation was used to relate continuous variables.

Significance was set at $P < 0.05$. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 20.0 (IBM SPSS Inc., USA).

Results

Description of the population

The study included 86 children: 53 males (61.6%) and 33 females, whose parents completed the GIQC in full at the T36 follow-up evaluations. The average age of the children was 3.08 ± 0.09 years. All the children were of Italian origin, and during the follow-up period (36 months), all grew adequately according to WHO growth charts, and none showed neurodevelopmental disorders.

Table 1 Parents' sociodemographic characteristics of enrolled infants stratified by child sex at T0.

Parents characteristics	Mothers (*n* = 86)	Fathers (*n* = 82)
Age at child's birth (median (IQR))		
	34.5 (31–38)	36.5 (34–40.75)
Education (*n* (%))		
None to middle school	0	2 (2.33%)
High school degree	78 (90.70%)	41 (47.67%)
University or more	8 (9.3%)	32 (37.21%)
Missing	0	11 (12.79%)
Work category (*n* (%))		
Unemployed	9 (10.47%)	0
Working class	29 (33.72%)	35 (40.70%)
Middle class	25 (29.07%)	9 (10.47%)
High class	23 (26.74%)	20 (23.26%)
Missing	0	22 (25.58%)

Table 1 shows the sociodemographic characteristics of the parents of the enrolled children. It should also be noted that all parents were living together at the time of enrollment.

Children anogenital distances

Anogenital distances (AGDs) of children over time are presented in Table 2.

Measurements were available at T0 for 84 newborns (53 males; 63%), at T3 for 79 infants (48 males; 60.8%), at T6 for 65 children (40 males; 61.5%), and at T36 for 67 children (42 males; 62.7%).

Table 2 Anogenital distances (AGD) by sex and PL of males at T0, T3, T6, and T36.

		T0	T3	T6	T36
Males (*n* = 53)		**(*n* = 53)**	**(*n* = 48)**	**(*n* = 40)**	**(*n* = 42)**
AGD-AS (cm)	Mean	1.93	3.62	3.86	4.61
	SD	0.64	0.88	1.03	0.88
AGD-AP (cm)	Mean	4.89	6.87	7.21	9.00
	SD	0.59	0.90	1.01	0.85
PL (cm)	Mean	2.42	2.80	2.82	3.82
	SD	0.63	0.59	0.65	0.62
Females (*n* = 33)		**(*n* = 31)**	**(*n* = 31)**	**(*n* = 24)**	**(*n* = 25)**
AGD-AF (cm)	Mean	1.17	1.75	2.12	2.16
	SD	0.29	0.52	0.70	0.74
AGD-AC (cm)	Mean	3.95	4.60	5.33	6.91
	SD	0.54	0.83	1.00	1.31

Abbreviations: AGD-AS, ano-scrotal distance; AGD-AP, ano-penile distance; AGD-AC, ano-clitorial distance; AGD-AF, ano-fourchette distance; PL, penile length.

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Table 3 Urinary sex hormone levels at T0, T3, and T6 in male and female infants.

		T0	T3	T6
Males (*n* = 53)		**(*n* = 48)**	**(*n* = 47)**	**(*n* = 43)**
uLH (mIU/mL)	Mean	0.49	0.23	0.17
	SD	0.78	0.25	0.14
uFSH (mIU/mL)	Mean	1.99	0.92	0.88
	SD	4.64	0.35	0.59
uT (ng/mL)	Mean	9.76	0.46	0.24
	SD	5.34	0.48	0.19
Females (*n* = 33)		**(*n* = 20)**	**(*n* = 32)**	**(*n* = 25)**
uLH (mIU/mL)	Mean	0.13	0.12	0.12
	SD	0.13	0.10	0.09
uFSH (mIU/mL)	Mean	1.69	2.45	3.14
	SD	2.46	2.14	2.49
uE (pg/mL)	Mean	449.85	20.99	22.63
	SD	655.55	8.07	12.27

Urinary hormones concentration

We evaluated at T0 68 samples (48 males; 70.6%), at T3 79 samples (47 males; 59.5%), and at T6 68 samples (43 males; 63.2%).

Table 3 summarizes sex urinary hormone values in males and females, respectively, at T0, T3, and T6. The measurements obtained are consistent with, and do not differ from, those of the general study population (26).

Gender identity questionnaire for children

Table 4 shows the frequencies of ratings for each GIQC item by sex assigned at birth. The data show similar trends in the frequency of answers in males and females for most items. Some differences can be seen in items 5 and 6, concerning imitation of female or male TV/movie characters. Descriptive statistics and comparisons between the two groups of boys and girls assigned at birth are shown in Table 5: most items showed significant differences between the groups. Item 15 was answered unanimously: no child at this age reported disliking their sexual anatomy.

However, according to the new scoring method proposed for the nonclinical population, we evaluated the three scales and compared the results by dividing the population by sex assigned at birth (Table 6). As expected, the results confirmed that boys scored higher than girls on the MTB scale ($P < 0.001$), while girls scored higher than boys on the FTB scale ($P < 0.001$). In addition, girls scored higher than boys on the CG scale ($P = 0.004$), indicating that they tended to be more gender-conforming than boys at 3 years of age. It should be noted that the CG scale had the highest mean scores compared to the FTB and MTB scales, although the variability was greater for boys in particular.

When evaluating the correlations between the scores on the different scales, it was shown that, in the whole population, FTB scale correlated positively with CG scale ($\rho = 0.377$, $P < 0.001$), and MTB scale correlated negatively with FTB scale ($\rho = -0.464$, $P < 0.001$). In addition, FTB scale correlated positively with CG scale in girls ($\rho = 0.381$, $P = 0.029$).

GIQC and social-demographic factors

We found that, in girls, the CG scale was negatively correlated with maternal age ($\rho = -0.430$, $P = 0.013$), whereas in boys this association was not found. On the other hand, in the male population, maternal education appeared to correlate with all scales. The highest mean scores were achieved by children of mothers with a university degree (or higher), reaching differences for the MTB scale (3.42 ± 0.59 vs 2.98 ± 0.50; $P = 0.09$).

GIQC and biological factors

We evaluated possible correlations among the three scales of the GIQC and the biological factors, in particular, prenatal androgenization by indirect measurement of AGDs and the urinary hormone levels assessed during minipuberty.

With regard to the correlations between the three scales of the GIQC and the AGDs in males (Table 7A), some positive correlations were observed, with the moderate correlations between MTB scale and the AGD-AS at T3 and T6 ($\rho = 0.414$, $P = 0.003$; and $\rho = 0.432$, $P = 0.005$; respectively) being particularly noteworthy.

In girls (Table 7B), there were weak positive correlations between MTB scale and AGD-AC at T0 ($\rho = 0.357$, $P = 0.049$), and CG scale correlated positively with AGD-AF at T3 ($\rho = 0.362$, $P = 0.045$).

On the other hand, no relevant correlations were found for hormone levels measured during minipuberty (Table 8A for males and Table 8B for females).

Discussion

This prospective longitudinal study of a nonclinical population of 3-year-old children of both sexes allowed us to describe the expression of GI during the period of its consolidation, using the GIQC and the possible role of different social and biological factors on the three scales evaluated by the questionnaire.

Analyzing the questionnaire according to the scoring scheme of Johnson and colleagues (30), we found that some of the items examined are unlikely to be applicable to our nonclinical 3-year-old population.

In particular, item 1 ('His/her favorite playmates are…') does not exhibit the stronger gender differences often observed in other contexts involving older children. Studies have shown that same-sex peer preference peaks in middle childhood (35, 36, 37, 38). Notably,

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Table 4 Frequencies for each item of the GIQC.

	Groups	
	Males	**Females**
Item 1: Favorite playmates	*n* = 52	*n* = 33
Same-sex	9.6%	6.1%
Equal	84.6%	90.9%
Cross-sex	5.8%	3.0%
Item 2: Play with girl-type dolls	*n* = 53	*n* = 33
Frequently/favorite toy	11.3%	78.8%
Once-in-a-while	41.5%	21.2%
Very rarely/never	47.2%	0%
Item 3: Play with boy-type dolls	*n* = 53	*n* = 33
Frequently/favorite toy	100%	30.3%
Once-in-a-while	0%	60.6%
Very rarely/never	0%	9.1%
Item 4: Experiments with cosmetics and jewelry	*n* = 53	*n* = 33
Frequently/favorite activity	3.8%	51.5%
Once-in-a-while	17.0%	33.3%
Very rarely/never	79.3%	15.2%
Item 5: Imitates female characters on TV/movies	*n* = 53	*n* = 33
Frequently/favorite activity	0%	36.4%
Once-in-a-while	3.8%	12.1.%
Very rarely/never	96.2%	51.5%
Item 6: Imitates male characters on TV/movies	*n* = 53	*n* = 33
Frequently/favorite activity	32.1%	9.1%
Once-in-a-while	11.3%	24.2%
Very rarely/never	56.6%	66.7%
Item 7: Plays sport with boys (but not girls)	*n* = 53	*n* = 33
Frequently/favorite activity	13.2%	9.1%
Once-in-a-while	17.0%	12.1%
Very rarely/never	69.8%	78.8%
Item 8: Plays sport with girls (but not boys)	*n* = 53	*n* = 33
Frequently/favorite activity	5.7%	15.2%
Once-in-a-while	13.2%	15.2%
Very rarely/never	81.1%	69.7%
Item 9: Roles in fantasy play	*n* = 32	*n* = 30
Same-sex	68.8%	83.3%
Equal	31.3%	16.7%
Cross-sex	0%	0%
Item 10: Plays 'girl-type' games	*n* = 53	*n* = 33
Frequently/favorite activity	5.7%	75.8%
Once-in-a-while	26.4%	18.2%
Very rarely/never	67.9%	6.1%
Item 11: Plays 'boy-type' games	*n* = 53	*n* = 33
Frequently/favorite activity	94.3%	18.2%
Once-in-a-while	3.8%	57.6%
Very rarely/never	1.9%	24.2%
Item 12: Dress-up play	*n* = 30	*n* = 24
Same-sex	80.0%	95.8%
Equal	20.0%	4.2%
Cross-sex	0%	0%
Item 13: States wish to be opposite sex	*n* = 53	*n* = 33
Frequently/every day	1.9%	0%
Once-in-a-while	1.9%	3.0%
Very rarely/never	1.9%	0%
Never	94.3%	97.0%
Item 14: States he is the opposite sex	*n* = 53	*n* = 33
Frequently/every day	0%	3.0%
Once-in-a-while	3.8%	3.0%
Very rarely/never	1.9%	6.1%
Never	94.3%	87.9%
Item 15: Talks about disliking sexual anatomy	*n* = 53	*n* = 33
Frequently/every day	0%	0%
Once-in-a-while	0%	0%
Very rarely/never	0%	0%
Never	100%	100%
Item 16: Talks about liking sexual anatomy	*n* = 53	*n* = 33
Frequently/every day	1.9%	0%
Once-in-a-while	7.6%	6.1%
Very rarely/never	18.9%	6.1%
Never	71.7%	87.9%

As in the original article (30), for items 1, 9, and 12, the response options 'always' and 'usually' were combined for the same-sex and cross-sex categories. For items 2–8 and 10–11, the response options 'as a favorite activity' or 'frequently' and 'rarely' or 'never' were combined. For items 13–14, the response options 'every day' and 'frequently' were combined. For items 1, 9, and 12, there was a non-applicable option (e.g., 'does not play with other children', 'does not play these games'). The variation in *n* between the items reflects the agreement with the not-applicable option.

Lam and colleagues (39) demonstrated that affiliation with same-sex peers increases throughout middle childhood and reaches its zenith in early adolescence. Moreover, gender differences in the developmental trajectory of this preference are well documented. Girls tend to exhibit a preference for same-sex peers at an earlier age compared to boys (40). By approximately 5 or 6 years of age, boys show a stronger preference for same-sex peers than girls, a trend observed across multiple cultures (40, 41, 42). This pattern is subtly reflected in our data, with girls scoring higher than boys on this item as early as 3 years old.

Items 7 and 8 ('He/she plays sports with boys/girls (but not girls/boys)' and 'He/she plays sports with girls/boys (but not boys/girls)') did not allow an effective response, as structured sport is rarely practiced at this age in Italy, in favor of other types of activity. In fact, these items received very similar responses for both sexes, with the responses mainly distributed between the options 'very rarely' and 'never'. For these reasons, we suggest that such items be reconsidered or omitted when using this questionnaire in population cohorts of preschool-aged children, particularly in cultural contexts where structured sports activities are not typical at this age. This adjustment would improve the measure's relevance and appropriateness for assessing gender development in early childhood.

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Table 5 GIQC mean scores for birth-assigned boys compared with birth-assigned girls (traditional item-based scoring for the clinical population). *P*-values in bold indicate statistical significance ($P < 0.05$).

Item	Males Mean (SD)	Female Mean (SD)	*P*
1 Favorite playmates (5 = boys; 1 = girls)	2.98 (0.57)	2.96 (0.30)	0.904
2 Play with girl-type dolls (5 = favorite toy; 1 = never)	2.49 (0.89)	3.88 (0.55)	**<0.001**
3 Play with boy-type dolls (5 = favorite toy; 1 = never)	4.47 (0.50)	3.24 (0.66)	**<0.001**
4 Experiments with cosmetics and jewelry (5 = favorite activity; 1 = never)	1.83 (0.85)	3.36 (1.37)	**<0.001**
5 Imitates female characters on TV/movies (5 = favorite activity; 1 = never)	1.32 (0.55)	2.61 (1.37)	**<0.001**
6 Imitates male characters on TV/movies (5 = favorite activity; 1 = never)	2.53 (1.40)	2.00 (1.03)	**0.047**
7 Plays sport with boys (but not girls) (5 = favorite activity; 1 = never)	1.79 (1.15)	1.63 (1.03)	0.514
8 Plays sport with girls (but not boys) (5 = favorite activity; 1 = never)	1.55 (0.93)	1.85 (1.18)	0.217
9 Roles in fantasy play (5 = same-sex; 1 = cross-sex)	2.45 (2.11)	3.73 (1.35)	**0.001**
10 Plays 'girl-type' games (5 = favorite activity; 1 = never)	2.02 (0.93)	3.79 (0.70)	**<0.001**
11 Plays 'boy-type' games (5 = favorite activity; 1 = never)	4.32 (0.73)	2.94 (0.66)	**<0.001**
12 Dress-up play (same-sex dress-up) (5 = same-sex; 1 = cross-sex)	2.38 (2.18)	3.06 (1.95)	0.135
13 States wish to be opposite sex (5 = every day; 1 = never)	1.11 (0.51)	1.06 (0.65)	0.570
14 States he is the opposite sex (5 = every day; 1 = never)	1.09 (0.40)	1.21 (0.65)	0.355
15 Talks about disliking sexual anatomy (5 = every day; 1 = never)	1.00 (0)	1.00 (0)	-
16 Talks about liking sexual anatomy (5 = every day; 1 = never)	1.40 (0.72)	1.18 (0.53)	0.115

Item 15, which addresses verbalized anatomical dysphoria, was initially defined in the first version (30) as a rudimentary attempt to measure this component of the DSM criteria. Subsequent validation of the questionnaire in Italian (31) indicates that the majority of participants did not express this concept. This is likely attributable to the fact that, in prepubertal children, particularly within our cohort of 3-year-olds, the perception and verbalization of anatomical dysphoria are exceedingly rare at such an early age (all participants selected the response 'never'). Given that our study focuses on a nonclinical, general population sample where gender dysphoria is not the primary concern, we believe that this item is not appropriate or relevant. Therefore, we recommend omitting this question when using the questionnaire in similar nonclinical populations, as it does not contribute meaningful information and falls outside the scope of typical gender development assessment.

Focusing on the three scales produced by the new scoring system (31) – MTB, FTB and CG – boys and girls scored significantly differently. As this is a nonclinical population, the scales expressing typically male (MTB) and typically female behavior (FTB) had higher scores for males and females, respectively. On the other hand, regarding the CG scale scores, these indicate lower gender conformity in boys compared to girls at age 3. Thus, in our cohort, contrary to what has been found in the literature, girls showed less variable behavior than boys; this difference may be related to the age of the test population. In fact, as already shown in the literature (30, 31), the younger the subject, the more gender-specific the scores tend to be, and this effect, which does not seem to have a linear trend, seems to be more pronounced in boys.

Table 6 GIQC mean scale score (new scoring method for the non-clinical population) as a function of sex. A *t*-test was used to assess the difference between the groups. All variables with $P < 0.05$ were considered statistically significant and shown in bold.

	Males ($n = 53$)		Females ($n = 33$)		
	Mean	SD	Mean	SD	*P*
FTB	1.92	0.61	3.41	0.75	**<0.001**
MTB	3.28	0.59	2.45	0.44	**<0.001**
CG	3.66	0.88	4.14	0.64	**0.004**

Considering the correlations found with social factors, we must emphasize that our study does not thoroughly explore all aspects of this domain. Specifically, we have information regarding parents and the family environment but not peers. Due to this limitation and the sample size of the cohort, we cannot definitively describe the correlations between these factors (particularly maternal age and education levels) and the scores of the three scales of the GIQC. To provide clear evaluations on this aspect, dedicated studies are necessary.

The scientific literature established an association between prenatal exposure to androgens and gender-related behavior in both animals and humans (6), but the effects of postnatal exposure (minipuberty) have not been fully explored. While Lamminmaki *et al.* (22) showed that androgen production in early childhood, as measured by urinary testosterone levels, is also associated with increased masculine play behavior in boys at 14 months, Pasterski and colleagues (43), studying healthy 3–4-year-old children, found that both AGD-AS and AGD-AP at birth and PL growth in the first 3 months independently predicted masculine behavior, suggesting that AGD reflects prenatal exposure to androgens, whereas PL indicates postnatal exposure to androgens.

Our results are conceptually consistent with those of Pasterski *et al.* (43), after separating them from the

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Table 7 Representation of the correlation matrix for AGDs and GIQC scales at different times. Correlation coefficients (*r*) and *P*-values (*P*) are presented in two rows for each gender: (A) indicates males and (B) indicates females. Pearson correlation coefficients range from −1 to 1, where 0 (white) represents no correlation, −1 (dark blue) represents a strong negative correlation, and 1 (dark red) represents a strong positive correlation. No correction for multiple comparisons was applied. *P*-values in bold indicate statistical significance ($P < 0.05$).

A		FTB	MTB	CG
T0	**AGD-AS**	0.277	−0.058	0.102
		0.045	0.682	0.465
	AGD-AP	0.226	0.076	0.206
		0.103	0.588	0.139
	PL	0.056	−0.046	0.087
		0.693	0.742	0.536
T3	**AGD-AS**	−0.095	0.414	−0.002
		0.521	**0.003**	0.992
	AGD-AP	−0.170	0.248	−0.167
		0.249	0.090	0.257
	PL	0.182	0.130	0.170
		0.222	0.383	0.252
T6	**AGD-AS**	−0.186	0.432	−0.197
		0.249	**0.005**	0.223
	AGD-AP	−0.002	0.306	−0.150
		0.991	0.055	0.355
	PL	0.067	0.197	0.069
		0.689	0.235	0.683
T36	**AGD-AS**	−0.115	0.011	−0.257
		0.469	0.945	0.100
	AGD-AP	−0.197	0.135	−0.025
		0.218	0.399	0.878
	PL	−0.141	0.097	0.150
		0.380	0.546	0.348
B		**FTB**	**MTB**	**CG**
T0	**AGD-AF**	0.074	0.224	0.247
		0.692	0.225	0.180
	AGD-AC	−0.085	0.357	−0.113
		0.649	**0.049**	0.546
T3	**AGD-AF**	0.084	0.217	0.362
		0.652	0.242	**0.045**
	AGD-AC	0.337	0.181	0.170
		0.063	0.330	0.361
T6	**AGD-AF**	0.269	−0.204	−0.029
		0.204	0.339	0.893
	AGD-AC	0.284	−0.098	−0.106
		0.179	0.650	0.621
T36	**AGD-AF**	−0.065	−0.025	0.006
		0.757	0.900	0.977
	AGD-AC	0.199	0.072	0.117
		0.351	0.738	0.586

Table 8 Representation of the correlation matrix for urinary hormone levels and GIQC scales at different sampling times. Correlation coefficients (*r*) and *P*-values (*P*) are presented in two rows for each gender: (A) indicates males and (B) indicates females. Pearson correlation coefficients range from −1 to 1, where 0 (white) represents no correlation, −1 (dark blue) represents a strong negative correlation, and 1 (dark red) represents a strong positive correlation. No correction for multiple comparisons was applied. *P*-values in bold indicate statistical significance ($P < 0.05$).

A		FTB	MTB	CG
T0	**uLH**	0.110	−0.104	−0.253
		0.457	0.482	0.082
	uFSH	0.276	0.098	0.112
		0.058	0.509	0.450
	uT	0.224	−0.046	−0.003
		0.126	0.758	0.986
T3	**uLH**	0.257	−0.092	0.016
		0.081	0.540	0.918
	uFSH	0.177	−0.088	0.028
		0.235	0.558	0.853
	uT	0.182	−0.095	0.110
		0.225	0.530	0.467
T6	**uLH**	0.150	−0.310	0.191
		0.336	**0.043**	0.219
	uFSH	−0.082	−0.203	0.003
		0.599	0.193	0.985
	uT	−0.128	−0.173	−0.151
		0.414	0.268	0.335
B		**FTB**	**MTB**	**CG**
T0	**uLH**	−0.279	0.175	−0.029
		0.233	0.459	0.905
	uFSH	0.026	0.351	−0.060
		0.913	0.129	0.801
	uE	0.056	0.079	−0.093
		0.815	0.741	0.696
T3	**uLH**	0.039	−0.026	0.034
		0.832	0.887	0.853
	uFSH	−0.019	−0.048	0.148
		0.918	0.795	0.420
	uE	0.224	0.122	0.262
		0.218	0.506	0.148
T6	**uLH**	0.076	0.193	−0.122
		0.718	0.356	0.562
	uFSH	0.072	0.134	0.378
		0.733	0.523	0.062
	uE	−0.276	0.121	0.275
		0.181	0.563	0.184

temporal effects of androgen exposure. In our cohort of 3-year-olds, there are positive correlations between the MTB scale and AGDs, especially AGD-AS, which has been described in the literature as the distance most affected by androgenization in the prenatal period (28, 29), and the larger, the greater the risk of infertility or hypogenitalism, at T3 and T6, while correlations with FTB scale are negative. Similarly, there are no significant correlations in the female population. This underlines the existence of both cerebral and genital imprinting determined by prenatal androgenizing hormones.

Before concluding, we highlight the main strengths and limitations of our study to provide essential context for

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interpreting the findings. A key strength is the study's integration within a well-characterized, prospective public birth cohort, enabling a systematic and relatively unbiased assessment of early gender-typical behavior in a nonclinical population.

Nonetheless, some limitations must be acknowledged. First, given the exploratory nature of some analyses and the relatively small sample size, we did not apply corrections for multiple comparisons/correlations, which increases the risk of Type I errors and the likelihood of false-positive findings. These results should, therefore, be interpreted with caution, and future studies with larger samples should incorporate appropriate statistical adjustments to confirm these preliminary associations. Second, the assessment of social and environmental factors was limited. As this was a secondary analysis of an existing dataset, we were constrained by the variables originally collected. In particular, the absence of theory-driven measures related to social context limits the interpretation of our findings within a broader psychosocial framework. Future research explicitly designed to examine gender development should include more comprehensive and theoretically informed constructs.

Despite these limitations, our findings offer novel insights into early expressions of gender-typical behavior and provide a valuable foundation for future investigations in this area.

In conclusion, the GIQC can be a valuable tool for assessing early gender-role behavior and gender expression in nonclinical populations already at the age of three. Our findings suggest that, by this age, most children can show that they express differentiated sex-typed behavior, with females exhibiting higher CG scores, that is, a lower tendency to experiment with cross-gender activities, than males. This difference is likely due to variations in the development of cognitive, affective, emotional, and social skills. In addition, androgenization appears to impact gender expression development in males, as indicated by the correlation between AGD-AS and MTB scores. These findings highlight the importance of a comprehensive and multidimensional understanding of GI but underscore the need for further research to better understand the underlying mechanisms and to validate our findings across broader populations.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. Lorenzo Iughetti is a Senior Editor of *Endocrine Connections*. Lorenzo Iughetti was not involved in the review or editorial process for this paper on which he is listed as an author.

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Ethical considerations

The study was approved by the Area Vasta Emilia Nord Ethics Committee (2018/num715). Informed consent to participation and publication of the data was obtained from the caregivers of all individual participants included in the study.

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