Paediatric and Perinatal Epidemiology
Affiliated to the Society for Pediatric and Perinatal Epidemiologic Research

doi: 10.1111/ppe.12022

Anogenital Distances in Newborns and Children from Spain and Greece: Predictors, Tracking and Reliability

Eleni Papadopoulou,[a,b,c,e] Marina Vafeiadi,[a,b,c] Silvia Agramunt,[a] Xavier Basagaña,[a,b] Kleopatra Mathianaki,[f] Polykseni Karakosta,[f] Arianna Spanaki,[f] Antonis Koutis,[f] Leda Chatzi,[f] Martine Vrijheid,[a,b,d] Manolis Kogevinas[a,b,d,e]

[a]*Centre for Research in Environmental Epidemiology (CREAL)*

[b]*IMIM (Hospital del Mar Research Institute)*

[c]*Department of Experimental and Health Sciences, Pompeu Fabra University, and*

[d]*CIBER Epidemiologia y Salud Pública (CIBERESP), Barcelona, Spain*

[e]*National School of Public Health, Athens*

[f]*Department of Social Medicine, Medical School, University of Crete, Heraklion, Greece*

Abstract

Background: Anogenital distance has been associated with prenatal exposure to chemicals with anti-androgenic effects. There are limited data in humans concerning descriptive patterns, predictors, and the reliability of measurement of anogenital distances. We examined anogenital distance measurements and their predictors in males and females and further estimated the reliability of these measurements.

Methods: Anogenital distances were measured in repeated time periods among 352 newborns and 732 young children in two cohorts, one in Crete, Greece and one in Barcelona, Spain. Mixed effect models were used to estimate the between-children, between- and within-examiners variance, as well as the reliability coefficients.

Results: Genitalia distances were longer in males than in females. Anogenital distances in both sexes increased rapidly from birth to 12 months, while the additional increase during the second year was small. Birthweight was associated with an increase of 1.9 mm/kg [95% CI 0.1, 3.8] (CI, confidence interval) in the anogenital distance measured from the anus to anterior base of the penis in newborn males, 2.9 mm/kg [95% CI 1.8, 3.9] in anoclitoral distance and 1.0 mm/kg [95% CI 0.0, 2.0] in anofourchettal distance in newborn females, after adjustment for gestational age. In children, body weight was the main predictor of all genitalia measurements. Moreover, anogenital distances at birth were associated with the corresponding distances at early childhood. High reliability coefficients (>90%) were found for all anogenital distances measurements in males and females.

Conclusions: Anogenital distances are strongly related to gestational age and birthweight and later, to growth. They track through early life and are highly reliable measures in both sexes.

Keywords: *anogenital distance, cohort study, dioxins, endocrine disruption, environmental contaminants.*

Anogenital distance has been established as a measure of fetal androgen action, and a sensitive marker of endocrine disruption in newborn animals.[1–3] Increased androgen concentrations lead to increased anogenital distance hence male animals have greater anogenital distance than females.[4] In animal studies, neonatal anogenital distance is predictive of adult anogenital distance and other androgen-responsive outcomes.[2,5] Prenatal or lactational exposure to anti-androgens, like 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), are linked to a reduction of anogenital distance in animals.[6–8] The use of anogenital distance in epidemiological studies is increasing and methods for its reliable measurement are still being developed.

In humans, birthweight is identified as a major determinant of anogenital distance.[9–12] Genitalia distances are sexually dimorphic and are increasing rapidly from birth to 12 months.[9,11–13] Studies examining the effect of prenatal exposures to anogenital distance have suggested that it may serve as a biological marker of fetal androgen disruption.[14–18]

Similar to other anthropometric measurements, the reliability of anogenital distances is influenced by measurement error. High reliability has been reported for male newborns;[10] however, poor reliability values were found in small training studies.[11–13] Anogenital

Correspondence: Manolis Kogevinas, Centre for Research in Environmental Epidemiology (CREAL), Doctor Aiguader, 88, 08003 Barcelona, Spain.
E-mail: kogevinas@creal.cat

distance is a morphologically small distance. Measurement error can therefore be a serious problem as small magnitude differences can be expected.[19]

We examined anogenital distance measurements and their predictors on a large sample of newborns and young children in two cohorts and assessed whether anogenital distances measured at birth correspond to measurements in early childhood. We further estimated the reliability of anogenital distance measurements in males and females.

Methods

Study population

This study includes newborns and children from two cohorts, the mother–child cohort in Crete, Greece (Rhea study) and the Hospital del Mar-New Generis study in Barcelona, Spain (Hmar study), that were part of the Newborns and Genotoxic exposure risks (NewGeneris) EU project.[20]

The Rhea study

The Rhea study examines prospectively a population-based cohort of pregnant women and their children at the prefecture of Heraklion, Crete, Greece.[21] Women who became pregnant within a year, starting from February 2007 were contacted at four maternity clinics. Mothers were recruited at around 12 weeks of gestation, when attending the first standard ultrasound examination. The inclusion criteria were: to be residents in the study area; pregnant women aged >16 years; to have the first visit at hospital or private clinics at the time of the first standard ultrasound examination at 10–13 week of gestation and with no communication handicap. During the study recruitment period, 1765 eligible women were approached, of whom 1610 (91%) agreed to participate and 1317 (82%) were followed until delivery. As the Rhea study had already started at the time of the incorporation of the anthropometric measurements, only a small number of children were measured at birth ($n = 165$) in the maternity clinics, and most of them were measured postnatally at home ($n = 732$).

The Hospital del Mar study – NewGeneris (Hmar study)

The Hospital del Mar study includes women with singleton pregnancies enrolled at delivery, from October 2008 until March 2010. Women less than 18 years old, with multiple pregnancies or with pregnancy complications (HIV/B hepatitis/C hepatitis infections, urgent C-sections, post-partum excessive haemorrhage) were excluded. Anthropometric measurements of the newborns were collected between birth and 2 days. A total of 281 women with their newborns participated and 187 of them with complete information were included in this analysis.

Study sample

One hundred sixty-five newborns of the Rhea study and 187 newborns of the Hmar study were included in this analysis. During their first-to-second year of life 732 children of the Rhea study were measured at home visits and of those, 112 had also been measured at birth. Twins ($n = 33$, 4%) and children with incomplete anthropometric measures were excluded from this analysis (five from Rhea study and three from Hmar study). All the included newborns and young children were phenotypically normal and none of them had major medical problems. Information on maternal and paternal ethnicity and country of origin was collected by interviews.

Both studies were conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethical committee of the University Hospital in Heraklion, Crete, Greece or by the Clinical Research Ethical Committee at Hospital del Mar (CEIC). Written informed consent was obtained from all women participating in the studies.

Anthropometric measurements and gestational age

The measurements protocol of anogenital distances was based on that used by Swan *et al*[18] and was modified to include measurements in both males and females.[11,22] In males, anogenital distance (AGD) was measured from the anterior base of the penis to the centre of the anus and anoscrotal distance (ASD) from the posterior base of the scrotum to the centre of the anus (Figure 1). Additionally, penis width (PW) was recorded in male participants. Likewise, in females, anoclitoral distance (ACD) was recorded as the distance between clitoris and the anus centre and anofourchettal distance (AFD) as the distance measured from the posterior convergence of the fourchette to the centre of the anus (Figure 1).

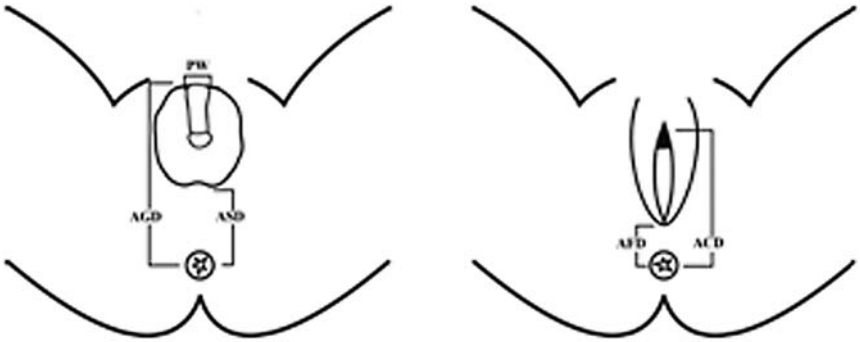

Figure 1. Schematic diagram of measurements done, by sex. Boys, AGD: anogenital distance, from the anterior base of the penis to the centre of the anus; ASD: anoscrotal distance, from the posterior base of the scrotum to the centre of the anus; PW: penis width. Girls, ACD: anoclitoral distance, from the clitoris to the anus centre; AFD: anofourchettal distance, from posterior convergence of the fourchette to the centre of the anus (original source: Salazar-Martinez *et al*.[11]).

Weight, length, abdominal and head circumferences were also measured. The mean value of three repeated measurements was used for genitalia measurements and the mean of two repeated measurements was used for other anthropometric measurements. The same protocol and joint training was followed in both studies, except abdominal circumference that was not recorded at the Hmar study. One examiner conducted all the anthropometric measurements in the Hmar study and six examiners in the Rhea study.

Gestational age was based on the interval between the last menstrual period and the date of delivery for 84% of the subjects. When the menstrual estimate of gestational age was inconsistent by 7 or more days with the first trimester ultrasound measurement, a quadratic regression formula describing the relationship between crown–rump length and gestational age was used instead.[23]

Anogenital distances and PW were measured with a Vernier digital calliper in increments of 0.01 mm (TESA Cal C/PROOF 150MM IP67, TESA Technology, Switzerland). An electronic scale readable to increments of 0.001 kg was used to measure child's weight (SECA model 354, Seca Corporation, Hamburg, Germany) and a measuring mat was used for the length (Seca model 210, Seca Corporation, Hamburg, Germany).

Reliability study

Within the Rhea study, we conducted a reliability study on the genitalia measurements. Thirteen males and 17 females (mean age: 23 months) participated and 1460 measurements were done in total, by two examiners (EP and MV). They were singleton births, randomly selected among the youngest children of the birth cohort.

Each child was measured by both examiners, at two scheduled home visits, one visit for each examiner. Each examiner did 10 repeated blind measurements per visit, resulting in two sets of 10 measurements for each distance. Thus we collected 40 measurements for each girl (for ACD and AFD) and 60 measurements for each boy (for AGD, ASD and PW). To ensure that the examiner was not biased, the instrument's screen was covered and the measurement was read and recorded by the assistant. Examiners were therefore blind concerning their own measurements.

Statistical analysis

We examined summary statistics for all anthropometric measurements, gestational age and age at examination. Normality was evaluated by the Shapiro–Wilk goodness of fit test. Differences between countries were tested using *t*-test for normally distributed data and the Mann–Whitney non-parametric test for non-normally distributed data. Box plots were used to illustrate the distribution of the genitalia measurements by age group and sex. A slope of increase from birth to 12 months and from 12 to 24 months was estimated for all genitalia measurements, by linear and restricted cubic splines.

Univariate linear regression models were formed to estimate the association between measurements of genitalia (AGD, ASD, PW, ACD and AFD) and other anthropometric measurements. Multivariate models of birthweight and gestational age for newborns, as well

as weight and age at examination for children were mutually adjusted. These variables were included a priori in the models of the other anthropometric factors (length, abdominal circumference and head circumference). Statistically significant anthropometric variables (P-value < 0.05) in the multivariable models were considered as major anthropometric predictors of AGD, ASD, PW, ACD and AFD. We did stratified analyses by country to identify potential country differences in predictors of anogenital distance.

On a subsample ($n = 112$) of our population we applied Generalized Additive Models to explore the shape of the relationship of repeated measurements of anogenital distances and PW measured at birth and at the first-to-second year of life, after adjustment for confounders. (P gain defined as the difference in normalised deviance between the GAM model and the linear model for the same predictor <0.10). Partial correlation coefficients were used to describe the relationship between repeated measurements. Data analysis was performed using STATA version 10.0 (StataCorp. 2007. *Stata Statistical Software: Release 10*. College Station, TX: StataCorp LP).

Reliability study

Mixed-effects models were fitted to estimate the variance components for AGD, ASD, PW, ACD, and AFD by using the 'xtmixed' command in STATA version 10.0. Anogenital distance measurements were included as dependent variables, while children ($n = 30$) and examiners were accounted as random-effect variables.

The model splits the total variance into three variances: variance because of child, variance because of examiner and residual variance. The variance components were calculated as ratios of the total variance and presented as percentages of the total between-children variance, represented the per cent of variability because of differences between children. Between-examiner variance represented the variability of the measurements because of differences between examiners measuring the same child. Within-examiner variance represented the per cent of variance because of differences in repeated measurements of the same child examined by the same examiner.

The reliability coefficient is equivalent to the between-children and between-examiner components divided by the total variance.[24] This coefficient measures the reproducibility of the measurement and a reliability coefficient of 1 indicates that the measurements are free of measurement error.

We also assessed the impact of the number of repeated measurements on the reliability coefficients. The relationship between the number of repetitions per individual and the reliability of the measure was determined from the Spearman–Brown equation.[25] Thus, the reliability of the mean of m replicate measurements (ρ_m) was obtained as

$$\rho_m = \frac{m\rho}{1+(m-1)\rho},$$

where m is the number of the repetitions and ρ is the reliability of a single measurement.

Results

Description of anogenital distances

The anthropometric characteristics of 1084 children participating in the study are presented in Table 1. The mean gestational age of newborns was 38.4 weeks (SD = 1.5) and 57 of them (16%) had a preterm delivery (<37 weeks). Newborns from the Rhea study had lower birthweights (mean = 3.11 kg, SD = 0.4 vs. mean = 3.37 kg, SD = 0.4) and higher prevalence of low birthweight ($n = 11$, 6.7% vs. $n = 2$, 1.1%) than in the Hmar study. Genitalia distances in males and females measured at birth were longer in Greece compared with Spain. In the Rhea study, all mothers were Caucasian (Greek 93.6%, Albanian 2.7%, other 3.7%). Therefore, we assessed ethnic differences of anogenital distances in the Hmar study where the ethnic diversity was larger (Caucasian 48.4%, Hispanic 19.7%, Asian 13.3%, Middle Eastern 12.2%, other 6.4%). Boys of non-Caucasian mothers ($n = 45$) had shorter ASD compared with boys of Caucasian mothers ($n = 48$) (mean = 22.7, SD = 4.3 mm vs. mean = 24.7, SD = 5.1 mm). No difference was found between paternal ethnicity groups. In the group of children measured during the first-to-second year of life, the mean values of AGD, ASD and PW for males and of ACD and AFD for females were higher than for the corresponding measurements of newborns.

The distribution of anogenital distances and PW is described by age group and sex in Figure 2. AGD from birth to 12 months was increasing rapidly (β spline = 2.8 mm/month [95% CI 2.6, 3.0 mm]; CI, confidence interval) while after the first year the increase was minor (β spline = –0.1 mm/month [95% CI –0.2,

Table 1. Anthropometric characteristics and anogenital distances of 1084 children measured at birth or at first-to-second year of age, by country

	Newborns						Children	
	Rhea study, Crete, Greece ($n = 165$)		Hospital del Mar study, Barcelona, Spain ($n = 187$)		All newborns ($n = 352$)		Rhea study, Crete, Greece ($n = 732$)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age at examination (months)	Birth		Birth		Birth		17.5	6.8
Gestational age (weeks)	38.5[a]	1.2	39.0	1.8	38.7	1.6	38.2	1.4
Preterm delivery, *n* (%) (gestational age <37 weeks)								
Yes	26	15.8	31	16.6	57	16.2	164	22.4
No	139	84.2	156	83.4	295	83.8	568	77.6
Weight (kg)	3.11[a]	0.4	3.37	0.4	3.25	0.4	11.2	2.1
Low birthweight, *n* (%) (birthweight<2500 kg)								
Yes	11[b]	6.7	2	1.1	13	3.7	42	5.7
No	154	93.3	185	98.9	339	96.3	690	94.3
Length (cm)	49.9	2.0	50.1	1.9	50.0	2.0	82.1	8.2
Head circumference (cm)[c]	34.8	1.8	34.0	2.0	34.8	1.9	47.5	3.0
Abdominal circumference (cm)	31.3	1.8	NA	NA	31.3	1.8	44.1	3.5
AGD males (mm)	50.9[a]	4.8	46.4	4.6	48.5	5.2	80.5	7.8
ASD males (mm)	27.1[a]	4.4	23.6	5.1	25.2	5.1	39.8	7.3
PW males (mm)	11.2[a]	0.99	10.1	0.8	10.6	1.0	14.0	1.7
ACD females (mm)	35.3	2.9	34.6	3.3	34.9	3.1	49.8	7.1
AFD females (mm)	14.4	3.0	13.8	2.5	14.1	2.8	21.7	3.9

[a]*P*-value < 0.05 of *t*-test for differences between studies.

[b]*P*-value < 0.05 of chi-square test for differences between studies.

[c]Values are median (interquartile range).

ACD, anoclitoral distance; AFD, anofourchettal distance; AGD, anogenital distance; ASD, anoscrotal distance; NA, not available; PW, penis width.

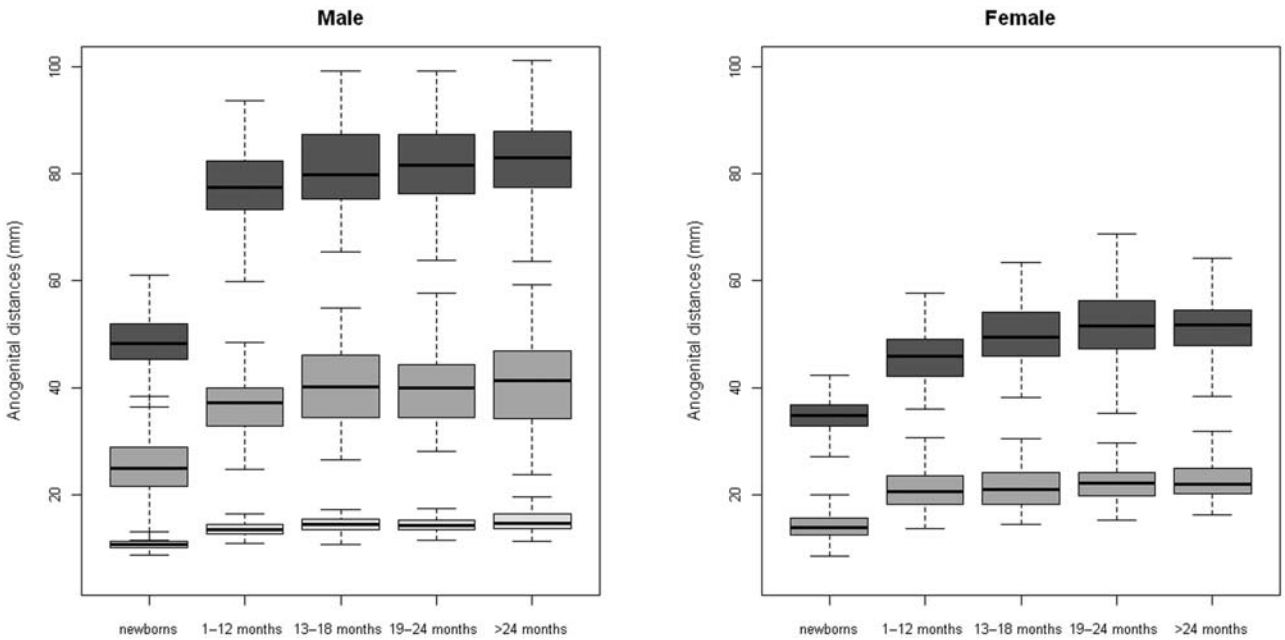

Figure 2. Distribution (median, IQR, minimum, maximum) of AGD, ASD, and PW for 374 males and ACD, AFD for 358 females, by age group: newborns, <12 months, 13–18 months, 19–24 months and over 24 months. ACD, anoclitoral distance; AFD, anofourchettal distance; AGD, anogenital distance; ASD, anoscrotal distance; IQR, interquartile range; PW, penis width.

0.1 mm]). Similarly, for ASD the change per month was 1.2 mm [95% CI 1.1, 1.3 mm] till 12 months and 0.1 mm [95% CI –0.1, 0.2 mm] after 12 months. For ACD and AFD in females, the increase during the first year of life was 1.2 mm/month [95% CI 1.1, 1.4 mm] and 0.7 mm/month [95% CI 0.6, 0.7 mm] respectively, while the additional increase until the second year was small (β spline = 0.2 mm/month [95% CI 0.0, 0.3 mm] and β spline = 0.0 mm/month [95% CI –0.1, 0.1 mm]).

Predictors of anogenital distances

The associations between anthropometric measures and anogenital distances in newborns and children are presented in Table 2 for males and Table 3 for females. Gestational age was associated with an increase of 0.7 mm/week [95% CI 0.2, 1.2] in AGD and 0.8 mm/week [95% CI 0.3, 1.3] in ASD of newborn males, after adjustment for birthweight. Birthweight was associated with an increase of 1.9 mm/kg [95% CI 0.1, 3.8] in AGD in newborn males. For ASD we observed a 1.5 mm/kg [95% CI –0.3, 3.3] increase while not statistically significant. PW was positively associated with head circumference after adjusting for gestational age and birthweight (β = 0.2 mm/cm [95% CI 0.0, 0.3]). Similarly, in male children weight was associated with increase in AGD, ASD and PW, after adjustment for age.

Body weight was associated with an increase in ACD and AFD of both newborn and young females, after adjustment for gestational age and age respectively (Table 3).

Further, we evaluated differences by ethnicity in the Hmar study. Birthweight was associated with an increase in AGD in Caucasian males (β = 3.4 mm/kg [95% CI 0.5, 6.3]) but not for non-Caucasian males (β = –0.4 mm/kg [95% CI –4.0, 3.2]). A similar trend was found for ASD (β = 2.7 mm/kg [95% CI –0.4, 5.7] and β = 0.5 mm/kg [95% CI –3.1, 4.1] respectively). The association of birthweight with ACD and AFD for Caucasian newborn females was 4.4 mm/kg [95% CI 2.5, 6.2] and 1.4 mm/kg [95% CI –0.4, 3.3] while the corresponding associations for non-Caucasian females were 2.0 mm/kg [95% CI –0.1, 4.1] and 0.4 mm/kg [95% CI –1.2, 2.0].

Association between measurements at birth and at first year of life

In a subsample of children who were repeatedly measured ($n = 112$), we evaluated the association between anogenital distances at birth and in early childhood. The adjusted GAM models indicated that

Table 2. Regression coefficients for univariate and adjusted associations between anthropometric characteristics and AGD and ASD among 179 newborn and 374 young males

	Males							
	AGD (mm)				ASD (mm)			
	Univariate		Adjusted		Univariate		Adjusted	
	β	[95% CI]	β	[95% CI]	β	[95% CI]	β	[95% CI]
Newborns ($n = 179$)								
Gestational age (weeks)	0.9	[0.4, 1.4]	0.7[a]	[0.2, 1.2]	0.9	[0.4, 1.4]	0.8[a]	[0.3, 1.3]
Birthweight (kg)	2.9	[1.2, 4.7]	1.9[b]	[0.1, 3.8]	2.6	[1.0, 4.3]	1.5[b]	[−0.3, 3.3]
Birth length (cm)	0.8	[0.4, 1.2]	0.5[c]	[−0.0, 1.1]	0.6	[0.2, 1.0]	0.2[c]	[−0.3, 0.8]
Head circumference (cm)	0.8	[0.2, 1.3]	0.4[c]	[−0.2, 1.0]	0.7	[0.2, 1.2]	0.3[c]	[−0.3, 0.9]
Abdominal circumference (cm)[d]	1.0	[0.5, 1.5]	−0.2[c]	[−1.0, 0.6]	0.8	[0.3, 1.3]	0.1[c]	[−0.7, 0.9]
Children ($n = 374$)								
Gestational age (weeks)	−0.1	[−0.6, 0.5]	0.0[c]	[−0.5, 0.5]	−0.3	[−0.8, 0.3]	−0.2[c]	[−0.7, 0.3]
Birthweight (kg)	1.3	[−0.4, 3.0]	−0.0[c]	[−0.0, 0.0]	0.5	[−1.1, 2.1]	−0.6[c]	[−2.2, 1.0]
Age at examination (months)	0.3	[0.2, 0.4]	−0.3[a]	[−0.5,−0.2]	0.2	[0.1, 0.3]	−0.1[a]	[−0.3, 0.1]
Weight (kg)	1.6	[1.2, 1.9]	2.4[b]	[1.9, 3.0]	1.1	[0.7, 1.4]	1.4[b]	[0.8, 1.9]
Length (cm)	0.3	[0.2, 0.4]	−0.1[c]	[−0.4, 0.2]	0.2	[0.1, 0.3]	−0.2[c]	[−0.5, 0.1]
Head circumference (cm)	1.2	[0.8, 1.6]	−0.0[c]	[−0.6, 0.5]	0.8	[0.5, 1.2]	0.0[c]	[−0.5, 0.6]
Abdominal circumference (cm)	0.7	[0.5, 1.0]	−0.1[c]	[−0.4, 0.2]	0.5	[0.3, 0.7]	−0.0[c]	[−0.3, 0.3]

[a]Models of newborns are adjusted for birthweight and models of children are adjusted for weight.
[b]Models of newborns are adjusted for gestational age and models of children are adjusted for age at examination.
[c]Models of newborns are adjusted for gestational age and birthweight and models of children are adjusted for age and weight at examination.
[d]$n = 84$ males.
95% CI, 95% Confidence Intervals; ACD, anoclitoral distance; ASD, anoscrotal distance; β, regression coefficient.

the relationship was linear (P gain > 0.10). The scatter plot and the fitted line of the association between repeated measurements of ASD are presented in Figure 3. Similar patterns were observed for all anogenital distances. For males ($n = 61$), AGD and ASD measured at birth were positively associated with the corresponding measurement at the first year of life, after adjustment [correlation coefficient = 0.19 ($P = 0.170$) and correlation coefficient = 0.63 ($P < 0.001$)]. Similarly, the adjusted correlation coefficient between repeated measurements of PW was 0.53 ($P < 0.001$). For females ($n = 51$), ACD and AFD measured at birth were positively associated with ACD and AFD measured at 1 year of life, after adjustment [correlation coefficient = 0.32 ($P = 0.035$) and correlation coefficient = 0.53 ($P < 0.001$)].

Reliability of anogenital distances

The contribution of the variance components to the total variance is shown in Table 4. In males, AGD and ASD were mainly affected by the between-children variance. For PW, only 75% of the variance was explained by differences between children, while the remaining 25% was because of the within-examiner variance. In females, 70–80% of the variance was because of between-children variation, while another 10% was explained by the within-examiner variance. For AFD in females, the between-examiner variance also contributed to the total variability of the measurements (22%). Reliability coefficients of anogenital distances in males and females were over 0.90, and for PW was 0.75. The reliability of the measurements increases when the average of 2 or 3 repeated measurements.

Comment

In this study we report anogenital distance measurements in male and female newborns and young children from Greece and Spain. Child growth was

Table 3. Regression coefficients for univariate and adjusted associations between anthropometric characteristics and ACD and AFD among 173 newborn and 358 young females

	Females							
	ACD (mm)				AFD (mm)			
	Univariate		Adjusted		Univariate		Adjusted	
	β	[95% CI]	β	[95% CI]	β	[95% CI]	β	[95% CI]
Newborns ($n = 173$)								
Gestational age (weeks)	0.4	[0.1, 0.7]	0.1[a]	[–0.2, 0.4]	0.1	[–0.2, 0.3]	–0.1[a]	[–0.3, 0.2]
Birthweight (kg)	3.0	[2.0, 3.9]	2.9[b]	[1.8, 3.9]	0.9	[–0.0, 1.9]	1.0[b]	[–0.0, 2.0]
Birth length (cm)	0.7	[0.5, 1.0]	0.5[c]	[0.2, 0.8]	0.3	[0.1, 0.6]	0.4[c]	[0.1, 0.7]
Head circumference (cm)	0.7	[0.4, 1.0]	0.4[c]	[0.1, 0.7]	0.2	[–0.0, 0.5]	0.2[c]	[–0.1, 0.5]
Abdominal circumference (cm)[d]	0.5	[0.1, 0.8]	–0.3[c]	[–0.8, 0.3]	0.1	[–0.3, 0.5]	–0.4[c]	[–1.0, 0.2]
Children ($n = 358$)								
Gestational age (weeks)	–0.3	[–0.8, 0.3]	–0.4[c]	[–0.9, 0.1]	0.1	[–0.3, 0.3]	–0.0[c]	[–0.3, 0.3]
Birthweight (kg)	0.4	[–1.4, 2.3]	–1.2[c]	[–2.9, 0.5]	0.4	[–0.6, 1.5]	–0.3[c]	[–1.3, 0.7]
Age at examination (months)	0.3	[0.2, 0.4]	–0.1[a]	[–0.2, 0.1]	0.1	[0.1, 0.2]	–0.1[a]	[–0.2, 0.0]
Weight (kg)	1.6	[1.3, 1.9]	1.8[b]	[1.3, 2.3]	0.6	[0.4, 0.8]	0.8[b]	[0.5, 1.1]
Length (cm)	0.4	[0.3, 0.4]	0.2[c]	[–0.1, 0.5]	0.1	[0.1, 0.2]	–0.1[c]	[–0.3, 0.1]
Head circumference (cm)	1.2	[0.8, 1.5]	–0.0[c]	[–0.5, 0.5]	0.6	[0.4, 0.7]	0.3[c]	[0.0, 0.6]
Abdominal circumference (cm)	0.7	[0.5, 0.9]	0.1 [c]	[–0.2, 0.4]	0.3	[0.2, 0.4]	0.0[c]	[–0.1, 0.2]

[a]Models of newborns are adjusted for birthweight and models of children are adjusted for weight.
[b]Models of newborns are adjusted for gestational age and models of children are adjusted for age at examination.
[c]Models of newborns are adjusted for gestational age and birthweight and models of children are adjusted for age and weight at examination.
[d]$n = 81$ females.
95% CI, 95% Confidence Intervals; ACD, anoclitoral distance; ASD, anoscrotal distance; β, regression coefficient.

positively associated with all genitalia measurements. Genitalia distances at birth were associated with the corresponding distance in early childhood. Estimates of anogenital distances in males and females are highly reliable when conducted by experienced examiners.

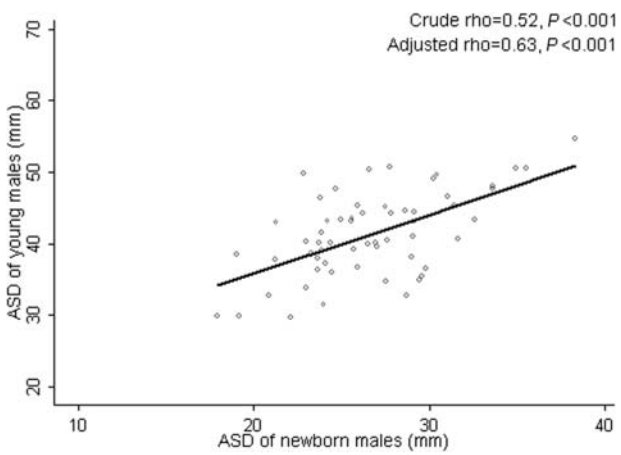

Figure 3. Scatter plot and fitted line of the association between repeated measurements of ASD (anoscrotal distance) (in mm) of males at birth and at first year of life ($n = 61$).

Our measurements in newborns were similar to previously published studies,[12,15,26] although differences were observed.[8,10,14,18] In the Hmar study non-Caucasian newborn males had shorter genitalia distances compared with Caucasians, which has been reported also by Sathyanarayana *et al*.[12] Along with racial differences, variation of anogenital distance could also be explained by different environmental backgrounds and different methodologies, as a common measurement protocol is not established yet for epidemiological studies. However, in our study the latter explanation is unlikely as a common protocol and training was applied in the two studies.

We found that anogenital distances of males and females measured at birth are associated with the corresponding measure at early childhood. Recently anogenital distance measured in adult men was linked to semen parameters and fertility, providing evidence for the clinical significance of anogenital distance as a biomarker of reproductive disorders;[27,28] however,

Table 4. Sources of variance and reliability coefficients of anogenital distance measurements for males and females, and penis width (PW) in males participating in the validation study ($n = 1460$ measurements)

	Percentage of total variance			Reliability coefficient		
	Between-children	Between-examiners	Within-examiner	For single measurement	For 2 repetitions	For 3 repetitions
Anogenital distances						
Males ($n = 13$)						
AGD	88.3	0.8	10.9	0.89	0.94	0.96
ASD	90.9	5.3	3.8	0.96	0.98	0.99
PW	74.9	0.0	25.1	0.75	0.86	0.90
Females ($n = 17$)						
ACD	77.4	13.6	9.0	0.91	0.95	0.97
AFD	69.1	21.5	9.4	0.91	0.95	0.97

ACD, anoclitoral distance; AFD, anofourchettal distance; AGD, anogenital distance; ASD, anoscrotal distance.

knowledge is still limited. Our longitudinal data suggest that anogenital distance tracks from birth to early childhood, hence measurements at birth may be linked to later reproductive disorders. In animals anogenital distance is an established lifelong indicator of prenatal androgen exposure,[29–31] although in humans more human studies are needed to strengthen this hypothesis. This is the first study to show an association between genitalia distances measured at birth and at first year of life in females. Animal studies suggest that changes in neonatal anogenital distance may indicate permanently altered phenotype in adult female rats, but no study in humans exists.[2]

We found that body weight is a strong determinant of AGD and ASD in males and ACD in females at birth and at early childhood. Moreover, early postnatal growth was linked to a rapid increase of genitalia distances from birth to the first year of life where it reaches a plateau. Human descriptive studies have reported that the genital size is in part determined by body dimensions.[9–11] Prenatal exposure to known endocrine disrupting chemicals may affect fetal growth and might also be linked to impaired postnatal growth.[32–34] On the other hand, such *in utero* exposures may have an effect on the reproductive system, expressed as reduced anogenital distance.[14,16–18,26] Thus, endocrine disruptors may affect both growth and anogenital distance, as suggested by some animal studies depending on the studied exposure.[35,36] As fetal, child growth and anogenital distance are associated, it could be hard to differentiate the effects, mostly depending on the studied exposure. In an effort to eliminate the variability because of body size, percentiles of anogenital distance for weight have been also proposed for children.[31]

The reliability coefficients of anogenital distances in both males and females were 0.90, meaning that 90% of the total variability is true variation, while the remaining proportion (<10%) is attributable to measurement error.[37] Thus a single measurement is likely to represent the 'true' anogenital distance, as it carries small measurement error. As anogenital measures depend on distinct landmarks on soft tissues, AGD and ASD of males are reasonably easy to measure, because of easy identifiable borders. On the other hand, ACD and AFD distances of females have less clear borders and it could be harder for the examiners to track the same landmarks. Thus, measurement error could increase. For PW, the 25% of the total variability was attributable to within-examiner variance, and the most likely explanation is penis irritation (including penile erection) because of repeated measurements. However, taking into account that genitalia dimensions are small compared with other anthropometric measurements, anogenital distance measurements have comparable reliability to well-proven reliable anthropometric measures.[38,39] If it is determined that the reliability is poor because of errors introduced by the measurement procedure, one way to improve the reliability is to replicate the measurement and then report the average.

In conclusion, we found that anogenital distancesare associated with child's growth regardless of age. We reported for both males and females that neonatal anogenital distance is associated with the corresponding distances measured in early childhood. When con-

ducted by experienced examiners, measurements of these distances are highly reliable. Future research in humans should focus on the possible effect of *in utero* exposure to endocrine disruptors on genitalia distance measurements at early life and reproductive dysfunction in later life.

Acknowledgements

The study was supported by the Research Pilot Project Grants of CREAL Internal Projects (Call 2010) on Environmental Health, Centre for Research in Environmental Epidemiology (CREAL), Barcelona, Spain.

Figure 1 is a modified version of the original published by Salazar-Martinez *et al*.[11] and BioMed Central is duly identified as the original publisher.

References

1 Gray LE, Ostby J, Furr J, Wolf CJ, Lambright C, Parks L, *et al*. Effects of environmental antiandrogens on reproductive development in experimental animals. *Human Reproduction Update* 2001; 7:248–264.
2 Hotchkiss AK, Lambright CS, Ostby JS, Parks-Saldutti L, Vandenbergh JG, Gray LE, Jr. Prenatal testosterone exposure permanently masculinizes anogenital distance, nipple development, and reproductive tract morphology in female Sprague-Dawley rats. *Toxicological Science* 2007; 96:335–345.
3 Kratochwil K. Development and loss of androgen responsiveness in the embryonic rudiment of the mouse mammary gland. *Developmental Biology* 1977; 61:358–365.
4 Baum MJ, Woutersen PJ, Slob AK. Sex difference in whole-body androgen content in rats on fetal days 18 and 19 without evidence that androgen passes from males to females. *Biology of Reproduction* 1991; 44:747–751.
5 Bowman CJ, Barlow NJ, Turner KJ, Wallace DG, Foster PM. Effects of in utero exposure to finasteride on androgen-dependent reproductive development in the male rat. *Toxicological Science* 2003; 74:393–406.
6 Jin MH, Ko HK, Hong CH, Han SW. In utero exposure to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin affects the development of reproductive system in mouse. *Yonsei Medical Journal* 2008; 49:843–850.
7 Ohsako S, Miyabara Y, Nishimura N, Kurosawa S, Sakaue M, Ishimura R, *et al*. Maternal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) suppressed the development of reproductive organs of male rats: dose-dependent increase of mRNA levels of 5alpha-reductase type 2 in contrast to decrease of androgen receptor in the pubertal ventral prostate. *Toxicological Science* 2001; 60:132–143.
8 Ohsako S, Miyabara Y, Sakaue M, Ishimura R, Kakeyama M, Izumi H, *et al*. Developmental stage-specific effects of perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure on reproductive organs of male rat offspring. *Toxicological Science* 2002; 66:283–292.
9 Ozkan B, Konak B, Cayir A, Konak M. Anogenital distance in Turkish newborns. *Journal of Clinical Research in Pediatric Endocrinology* 2011; 3:122–125.
10 Romano-Riquer SP, Hernandez-Avila M, Gladen BC, Cupul-Uicab LA, Longnecker MP. Reliability and determinants of anogenital distance and penis dimensions in male newborns from Chiapas, Mexico. *Paediatric and Perinatal Epidemiology* 2007; 21:219–228.
11 Salazar-Martinez E, Romano-Riquer P, Yanez-Marquez E, Longnecker MP, Hernandez-Avila M. Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. *Environmental Health* 2004; 3:8.
12 Sathyanarayana S, Beard L, Zhou C, Grady R. Measurement and correlates of ano-genital distance in healthy, newborn infants. *International Journal of Andrology* 2010; 33:317–323.
13 Thankamony A, Ong KK, Dunger DB, Acerini CL, Hughes IA. Anogenital distance from birth to 2 years: a population study. *Environmental Health Perspectives* 2009; 117:1786–1790.
14 Huang PC, Kuo PL, Chou YY, Lin SJ, Lee CC. Association between prenatal exposure to phthalates and the health of newborns. *Environment International* 2009; 35:14–20.
15 Longnecker MP, Gladen BC, Cupul-Uicab LA, Romano-Riquer SP, Weber JP, Chapin RE, *et al*. In utero exposure to the antiandrogen 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) in relation to anogenital distance in male newborns from Chiapas, Mexico. *American Journal of Epidemiology* 2007; 165:1015–1022.
16 Suzuki Y, Yoshinaga J, Mizumoto Y, Serizawa S, Shiraishi H. Foetal exposure to phthalate esters and anogenital distance in male newborns. *International Journal of Andrology* 2012; 35:236–244.
17 Miao M, Yuan W, He Y, Zhou Z, Wang J, Gao E. In utero exposure to bisphenol-A and anogenital distance of male offspring. *Birth Defects Research. Part A, Clinical and Molecular Teratology* 2011; 91:867–872.
18 Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, *et al*. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environmental Health Perspectives* 2005; 113:1056–1061.
19 Arnqvist G, Martensson T. Measurement error in geometric morphometrics: empirical strategies to assess and reduse its impact on measures of shape. *Acta Zoologica Academiae Scientiarum Hungaricae* 1998; 44:73–96.
20 Merlo DF, Wild CP, Kogevinas M, Kyrtopoulos S, Kleinjans J. NewGeneris: a European study on maternal diet during pregnancy and child health. *Cancer Epidemiology, Biomarkers& Prevention* 2009; 18:5–10.
21 Chatzi L, Plana E, Daraki V, Karakosta P, Alegkakis D, Tsatsanis C, *et al*. Metabolic syndrome in early pregnancy and risk of preterm birth. *American Journal of Epidemiology* 2009; 170:829–836.
22 Callegari C, Everett S, Ross M, Brasel JA. Anogenital ratio: measure of fetal virilization in premature and full-term newborn infants. *The Journal of Pediatrics* 1987; 111:240–243.
23 Westerway SC, Davison A, Cowell S. Ultrasonic fetal measurements: new Australian standards for the new millennium. *The Australian and New Zealand Journal of Obstetrics & Gynaecology* 2000; 40:297–302.

24 Laenen A, Vangeneugden T, Geys H, Molenberghs G. Generalized reliability estimation using repeated measurements. *The British Journal of Mathematical and Statistical Psychology* 2006; 59:113–131.
25 Lord FM, Novick ML. *Statistical Theories of Mental Health Scores*. Reading, MA: Addison-Wesley Publishing, 1968.
26 Torres-Sanchez L, Zepeda M, Cebrian ME, Belkind-Gerson J, Garcia-Hernandez RM, Belkind-Valdovinos U, *et al*. Dichlorodiphenyldichloroethylene exposure during the first trimester of pregnancy alters the anal position in male infants. *Annals of the New York Academy of Sciences* 2008; 1140:155–162.
27 Eisenberg ML, Hsieh MH, Walters RC, Krasnow R, Lipshultz LI. The relationship between anogenital distance, fatherhood, and fertility in adult men. *Plos ONE* 2011; 6:e18973.
28 Mendiola J, Stahlhut RW, Jorgensen N, Liu F, Swan SH. Shorter anogenital distance predicts poorer semen quality in young men in Rochester, New York. *Environmental Health Perspectives* 2011; 119:958–963.
29 van den Driesche S, Scott HM, MacLeod DJ, Fisken M, Walker M, Sharpe RM. Relative importance of prenatal and postnatal androgen action in determining growth of the penis and anogenital distance in the rat before, during and after puberty. *International Journal of Andrology* 2011; 34:e578–e586.
30 MacLeod DJ, Sharpe RM, Welsh M, Fisken M, Scott HM, Hutchison GR, *et al*. Androgen action in the masculinization programming window and development of male reproductive organs. *International Journal of Andrology* 2011; 33:279–287.
31 Welsh M, Saunders PT, Fisken M, Scott HM, Hutchison GR, Smith LB, *et al*. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *The Journal of Clinical Investigation* 2008; 118:1479–1490.
32 Chen SQ, Chen JN, Cai XH, Chen GR, Gao Y, Ge RS, *et al*. Perinatal exposure to di-(2-ethylhexyl) phthalate leads to restricted growth and delayed lung maturation in newborn rats. *Journal of Perinatal Medicine* 2010; 38:515–521.
33 Hertz-Picciotto I, Charles MJ, James RA, Keller JA, Willman E, Teplin S. In utero polychlorinated biphenyl exposures in relation to fetal and early childhood growth. *Epidemiology* 2005; 16:648–656.
34 Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, *et al*. Exposure to phthalates and phenols during pregnancy and offspring size at birth. *Environmental Health Perspectives* 2011; 120:464–470.
35 Ema M, Miyawaki E, Hirose A, Kamata E. Decreased anogenital distance and increased incidence of undescended testes in fetuses of rats given monobenzyl phthalate, a major metabolite of butyl benzyl phthalate. *Reproductive Toxicology* 2003; 17:407–412.
36 Gyekis J, Anthony K, Foreman JE, Klein LC, Vandenbergh DJ. Perinatal nicotine exposure delays genital development in mice. *Reproductive Toxicology* 2010; 29:378–380.
37 Marks GC, Habicht JP, Mueller WH. Reliability, dependability, and precision of anthropometric measurements. The Second National Health and Nutrition Examination Survey 1976–1980. *American Journal of Epidemiology* 1989; 130:578–587.
38 Johnson W, Cameron N, Dickson P, Emsley S, Raynor P, Seymour C, *et al*. The reliability of routine anthropometric data collected by health workers: a cross-sectional study. *International Journal of Nursing Studies* 2009; 46:310–316.
39 Ulijaszek SJ, Kerr DA. Anthropometric measurement error and the assessment of nutritional status. *British Journal of Nutrition* 1999; 82:165–177.