Longitudinal Study of Hormonal and Physical Development in Young Twins

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Context and Objective: Information on the correlation of normative reproductive hormone levels with physical development (Tanner stages) during puberty and on the influences of genes and environment on variation in these hormones and Tanner stages is limited.

Design, Setting, and Participants: One hundred twelve healthy 9-year-old twin pairs (n = 224) took part in a longitudinal study, of which 89 pairs participated again at age 12 years (n = 178).

Main Outcome Measures: Morning urinary LH, FSH, estradiol, and salivary testosterone levels, determined by competitive immunoassays, were measured. Tanner stages were determined through physical examination.

Results: Over the 3-year interval, all hormone levels showed a 2- to 9-fold increase. LH and FSH at age 9 years predicted sex-specific Tanner stages at age 12 years in both boys and girls. Most of the associations between hormone levels at age 9 years and physical development at 12 years were explained by genetic influences. FSH in 9-year-old boys correlated with all hormone levels and Tanner stages at age 12 years. Moderate to high heritability estimates were found for hormone levels at both ages and in both sexes. In girls a shift from environmental (age 9 years) to genetic influences (age 12 years) was found for estradiol and pubic hair development, and for breast development a shift in the opposite direction was seen.

Conclusions: During development LH and FSH (and testosterone in boys) levels predict secondary sexual characteristics in boys and girls 3 years later. These correlations are largely due to genes that are involved in both early pubertal hormone levels and subsequent physical development. (***J Clin Endocrinol Metab*** **98: E518–E527, 2013)**

Puberty is characterized by the development of secondary sexual characteristics and physical growth. The general hypothesis on the endocrine onset of puberty is thought to proceed in a series of steps that concern the reactivation of the hypothalamus-pituitary-gonadal (HPG) axis (see, for example, Refs 1–4): first, the pituitary is stimulated by the hypothalamus (via GnRH neurons) to secrete nightly LH and FSH surges 1–2 years before the physical onset of puberty. After some time, LH and FSH pulses also occur during the day. These hormones in turn

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2012-3361 Received September 14, 2012. Accepted January 2, 2013.
First Published Online February 21, 2013

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Abbreviations: A, additive genetic component; BMI, body mass index; C, common environmental component; D, nonadditive genetic component; d^2, estimate of nonadditive genetic influences; DZ, dizygotic; E, unique environmental component; h^2, estimate of additive genetic effects (ie, heritability); HPG, hypothalamus-pituitary-gonadal; MZ, monozygotic; Rc, common environmental correlation; Re, unique environmental correlation; Rg, genetic correlation; Rph, phenotypic (observed) correlation; Tanner-B, breast development; Tanner-P, penis development; Tanner-PH, pubic hair development; Tanner-T, testes development.

stimulate the gonads (the testis or ovaries) to produce estrogen and testosterone, which play an important role in the onset of physical (secondary sexual characteristics) and behavioral changes.

Reproductive hormones regulate important changes during puberty. Knowledge on relative influences of genes and environment on individual differences in these hormone levels will provide relevant information on the (endocrine) regulation of puberty. Twin studies are widely used in various fields (see, for example, Ref 5 for assessment of genetic influences on sex hormones in adult males) to estimate the extent to which variation in a certain trait is caused by genetic and/or environmental factors. Monozygotic (MZ) twins are genetically identical and share (nearly) 100% of their genetic material, whereas dizygotic (DZ) twins share, like siblings, on average 50% of their segregating genes. If for a certain variable MZ twins are more alike than DZ twins, it can be inferred that the variable is influenced by genetic factors (see Ref 6 for an overview of twin research, and see Refs 7–9 for a more detailed description). There are some studies that report on the heritability of reproductive hormones in prepubertal boys [5–11 years old (10)], and testosterone in teenagers [(14–21 years old) (11); 12 years old (12)]. These studies found moderate to strong contributions of genetic effects on the variation of reproductive hormone levels. However, to our knowledge, the longitudinal aspect of genetic influences on hormone levels during this critical period of development has not been investigated.

In the current study, the development of HPG axis hormones during puberty is described in a longitudinal twin study (n = 224 individuals). LH, FSH, estradiol, testosterone, and secondary sexual characteristics were measured at 9 (baseline) and 12 (follow-up) years of age. The twin design allowed an estimate of the extent to which variation in reproductive hormones, and the extent to which correlations among hormones and physical development are driven by genes during puberty.

Materials and Methods

Participants

Healthy twins (224 individuals) were recruited from The Netherlands Twin Register (13). They took part at age 9 and 12 years (n = 178 individuals) in an ongoing longitudinal study on the development of cognition and brain structure and function (14) (Table 1). The Central Committee on Research involving Human Subjects of The Netherlands approved the study. Written informed consent was obtained from parents and children.

Analysis of hormone levels

Participants were asked to collect first morning urine and saliva immediately after waking up on 2 consecutive weekdays. Analyses were carried out by the endocrinological laboratory of clinical chemistry of the VU University Medical Center (Amsterdam, The Netherlands).

Determination of urinary LH, FSH, and estradiol, and salivary testosterone have been described elsewhere (15). Urinary LH, FSH, and estradiol levels were divided by creatinine level to correct for variations in urine excretion rate. A creatinine correction has been demonstrated to enhance the detection of LH surges (16). Creatinine concentrations were measured by the Jaffé method (Modular; Roche Diagnostics, Mannheim, Germany). The interassay coefficient of variation was 2.2% at 5.9 mmol/L and 1.7% at 12.5 mmol/L.

Assessment of Tanner stages

Secondary sexual characteristics of puberty were measured by a trained researcher at the University Medical Center Utrecht (Utrecht, The Netherlands) on the basis of the characteristics of sexual development devised by Marshall and Tanner (17, 18). In boys, genital stage was divided in penis and testes development. Testes volume was reported on a 4-item scale; at age 12 years, testes volume was measured with an orchidometer. When children felt uncomfortable with the assessment, they were asked to point out their status on black and white photographs of the different puberty stages accompanied by an explanation by the researcher (only at age 12 years; 16 girls, 28 boys).

Statistical analyses

Differences in hormone levels over time and between boys and girls were tested using structural equation modeling with the software package OpenMx (19) to control for the dependency of the data.

Hormone levels assessed at 2 days, available for more than 90% of the participants, were averaged. A log transformation was applied to normalize the data. Many of the 9-year-old children had LH levels that were below the detection limit (58 girls, 50 boys), but these low levels still provide information. These values were included in the genetic analysis by setting all values that were below the detection limit to a half of the detection limit. To check whether this procedure influenced the results, all genetic analyses were repeated with undetectable levels left out. Dissimilar results are reported in footnotes. After the log transformation, all data were normally distributed, except LH in 9-year-old girls (Kolmogorov-Smirnov $Z = 1.369$, $P = .047$).

Genetic analysis

Relative influences of genetic factors and environmental factors can be examined by comparing within-pair correlations between MZ and DZ twins. When an MZ correlation is twice as high as a DZ correlation, this indicates that a variable is largely influenced by genetic factors. The proportion of variance in a trait that can be attributed to genetic factors is termed heritability. In addition to genetic factors, resemblance between twins can arise from common environment, which comprises those environmental factors that induce similarity in children growing up in the same family. The presence of common environmental factors is suggested when correlations in DZ twins are larger than half the MZ correlation [as implemented in a full model that allows estimation of genetic (A), shared environmental (C), and nonshared environmental effects (E) (ie, ACE model) (6)]. When the MZ correlations are more than twice the DZ correlations, there is a suggestion for nonadditive genetic influences [epistasis

Table 1. Normative Hormone Levels and Tanner Stages in 9- and 12-Year-Old Boys and Girls

n (MZ/DZ/DOS Individuals)	Age 9 y 46/44/20		Age 12 y 40/34/15		
	Mean (SD)	n	Mean (SD)[a]	n	Increase (x-Fold)
Boys[b]					
Age, y	9.10 (0.10)	110	12.13 (0.24)	89	3.04 (0.23)[c]
BMI,[d] kg/m^2	16.21 (1.36)	103	18.54 (1.99)	87	
LH, U/mmol creatinine	0.02 (0.04)	59[e]	0.17 (0.14)	89	6.9
FSH, U/mmol creatinine	0.25 (0.16)	110	0.44 (0.26)	89	1.8
E2, pmol/mmol creatinine	126.55 (121.58)	108	205.61 (138.54)	89	1.6
T, pmol/L	24.98 (25.32)	106	74.23 (86.54)	86	3.0
Tanner	n per stage	n	n per stage	n	
Tanner-P 1/2/3/4/5	100/5/1/1/0	107	20/37/21/5/0	83	
Tanner-T 1/2/3/4	98/8/1/0	107	16/37/12/3	68	
Tanner-PH 1/2/3/4/5	96/10/0/0/0	106	24/31/22/6/0	83	
n (MZ/DZ/DOS Individuals)	**Age 9 50/42/20**		**Age 12 40/34/15**		
	Mean (SD)	**n**	**Mean (SD)[a]**	**n**	**Increase (x-Fold)**
Girls[f]					
Age	9.10 (0.09)	112	12.17 (0.28)	89	3.07 (0.26)[c]
BMI, kg/m^2[d]	16.32 (1.99)	106	18.80 (2.99)	82	
LH, U/mmol creatinine	0.02 (0.03)	53[g]	0.21 (0.21)	85	9.3
FSH, U/mmol creatinine	0.47 (0.27)[h]	112	0.92 (0.47)[h]	87	1.9
E2, pmol/mmol creatinine	116.13 (77.96)	112	366.77 (285.73)[h]	87	3.2
T, pmol/L	31.04 (24.04)[h]	108	58.00 (36.07)	88	1.9
Tanner	n per stage	n	n per stage	n	
Tanner-B 1/2/3/4/5	89/20/0/0/0	109	10/16/36/17/7	86	
Tanner-PH 1/2/3/4/5	91/17/0/0/0	108	17/19/18/24/5	83	

Abbreviations: DOS, dizygotic opposite sex; E2, estradiol; T, testosterone. Values below the detection limit were excluded from the descriptives, therefore, the reported LH levels are an overestimation of actual levels in 9-year-old boys and girls.

[a] All hormone levels were increased at age 12 compared with age 9 ($P < .0001$).

[b] In total, 224 individuals participated in this study, with 46 DZ males at age 9 years. However, no hormone data were available for 1 DZ male twin pair, leading to a total n of 222.

[c] Mean (SD) duration between measurement 1 and measurement 2 (in years).

[d] Based on BMI tables for Dutch children described elsewhere (40), 14 girls (12.5%) and 4 boys (3.6%) at age 9 years were considered to be overweight, and 2 girls (1.8%) were considered to be underweight. At age 12 years, 15 girls (16.9%) and 9 boys (10.1%) were considered to be overweight, and 1 girl (1.2%) was underweight.

[e] n below detection limit = 50.

[f] At follow-up, 14 girls (16%) had attained menarche, of which 3 reported to have regular cycles. None of the participants used oral contraceptives.

[g] n below detection limit = 58.

[h] Significantly higher values in girls, see text.

or dominance, implemented in a model that allows estimation of additive genetic effects (A), nonadditive genetic effects (D), and nonshared environmental effects (E) (ie, ADE model) (7)]. Unique environmental influences are not shared with other family members and also contain the measurement error (7). The proportion of the total variance that can be attributed to genetic or environmental factors gives estimates of (univariate) heritability (h^2), unique environmental influence, and common environmental influence (in the case of an ACE model), or nonadditive genetic influences (d^2) (in the case of an ADE model). In the latter case, we present estimates of broad heritability ($h^2 + d^2$).

Ordinal data, such as Tanner stages, were analyzed under the assumption that there is an underlying continuous liability with mean 0 and variance 1 to the observations. Four thresholds divide the liability into the 5 Tanner stages (4 for testes development); hence, 4 thresholds (3 for testes development) were estimated. Estimates of thresholds are based on the prevalence of the Tanner stages in the sample (8).

To increase power, heritability was estimated from bivariate models (8) in which variables assessed at age 9 and 12 years were analyzed simultaneously. The phenotypic correlation (Rph) among continuous traits (hormone levels), between ordinal Tanner stages, and among Tanner stages and hormone levels, were estimated in OpenMx (19). Due to low variability in Tanner stages at age 9 years, we chose to compute the correlations of hormone levels at age 9 and 12 years with Tanner stages at age 12 years only. To estimate correlations among hormone levels and Tanner stages, hormone levels were normalized and categorized in five groups.

Significant phenotypic correlations were decomposed in a genetic (Rg) and (unique) environmental (Re and Rc) component (see Supplemental Fig. 1, published on The Endocrine Society's

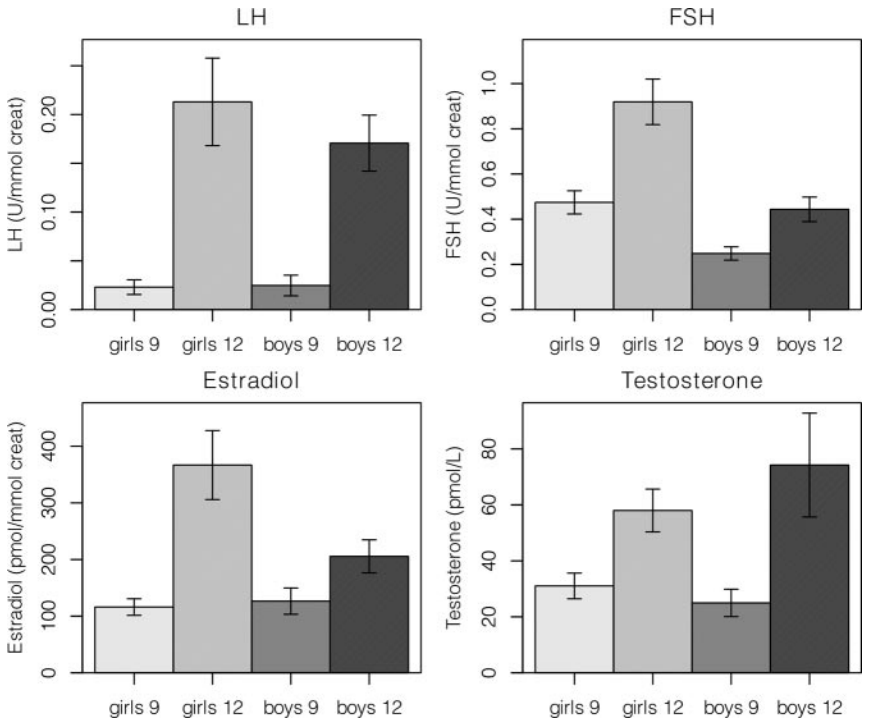

Figure 1. Normative hormone levels in 9- and 12-year-old boys and girls. LH, FSH, estradiol, and testosterone levels increased between age 9 and 12 years, in both boys and girls. At age 9 and 12 years, girls had higher FSH levels than boys; testosterone at age 9 years and estradiol at age 12 years were higher in girls compared with boys. Error bars represent 95% confidence interval.

Journals Online web site at http://jcem.endojournals.org). The magnitude of these underlying components is based on the comparison of cross-trait/cross-twin correlations for MZ and DZ twins. For example, if the cross-correlation between one hormone of twin A and another hormone of twin B is larger in MZ than in DZ twins, this indicates that a common genetic factor (partly) influences both hormones. The extent of the shared genetic influences is reflected by the magnitude of the genetic correlation. Rc and Re are interpreted likewise (8).

Because the mechanism underlying hormone levels (and subsequent physical changes) may differ in boys and girls, we chose to present the results for boys and girls separately. Data from boys and girls from DZ opposite-sex pairs were included with the data from the male and female DZ twins and thus contribute to the estimation of mean and variances, but we did not include their covariance. See Supplemental Table 1 for twin correlations.

All data analyses were carried out with structural equation modeling in the software package OpenMx (19). All data were included in the analyses, regardless of whether subjects participated once or twice in the study. Parameters were estimated by full-information maximum likelihood. Tests of significance of parameters were carried out by comparing the model fits of a model including that parameter to a model in which the parameter estimate was constrained at zero. The goodness of fit of different models was evaluated by comparing differences in log likelihood.

Results

Normative hormone levels at ages 9 and 12 years

For both boys and girls, all hormone levels were increased at age 12 years compared with age 9 years ($P <$ .0001) (Table 1 and Fig. 1; Supplemental Figures 2 and 3). The highest increase was seen for LH (~7-fold in boys, ~9-fold in girls). FSH levels increased about 2-fold in both boys and girls. Testosterone increased 3-fold in boys, approximately 2-fold in girls, whereas for estradiol this was the other way around (~2-fold increase in boys, ~3-fold in girls). At age 9 years, girls had higher levels of FSH ($P <$ .0001) and testosterone ($P = .04$) than boys. At age 12 years, girls had higher levels of FSH ($P <$ .0001) and estradiol ($P <$ .0001) than boys.

At age 9 years, most of the children were in Tanner stage 1 [boys: 91%, 92%, and 93% for penis (Tanner-P), testes (Tanner-T), and pubic hair (Tanner-PH) development, respectively; girls: 82% and 84% for breast (Tanner-B) and Tanner-PH development], ie, they had not yet started pubertal development. At age 12 years, most girls were in Tanner stage 3 (Tanner-B, 42%) or 4 (Tanner-PH, 29%) whereas most boys were in stage 2 (Tanner-P: 45%; Tanner-T: 54%; Tanner-PH: 37%). As can be seen in Table 1, at age 9 years, girls were only slightly further in their pubertal development than boys, whereas at age 12 years, girls were further developed than boys (based on Tanner stages).

Genetic analyses of hormone levels and Tanner stages

Significant genetic influences were found for variation in all hormones in boys and girls at both ages, except for estradiol at age 9 years in girls (Table 2; for unstandardized estimates see Supplemental Table 2). Overall, genetic influences were higher in boys compared with girls. Genetic influences did not change much over time, except for estradiol: in boys, the genetic influences seemed to become smaller over time. In girls, we found a significant influence of common environmental factors for estradiol at age 9 years. At age 12 years, these common environmental influences were no longer significant, and a significant proportion of the variance could be explained by genetic factors.

Over the 3-year interval, estimates of factors that influenced physical development changed. In boys, genetic influences for Tanner-T development were higher at age 12 years, whereas genetic influences for pubic hair development decreased over time. In girls, influences on Tanner-B develop-

Table 2. Influences of Genes and Environment on Hormone Levels and Physical Development of Boys and Girls During Puberty

	Model[a]	(Broad) Heritability (95% CI)	Common Environment (95% CI)	Unique Environment (95% CI)
Boys				
Age 9 y				
LH	ADE	**0.83** (0.66–0.90)	—	0.17 (0.10–0.34)
FSH	ADE	**0.89** (0.78–0.94)	—	0.11 (0.06–0.22)
E2	ACE	**0.72** (0.39–0.91)	0.13 (0.00–0.45)	0.14 (0.08–0.28)
T	ADE	**0.64** (0.30–0.81)	—	0.36 (0.19–0.70)
Tanner-P	ACE	0.09 (0.00–1.00)	0.87 (0.00–1.00)	0.04 (0.00–0.98)
Tanner-T	ACE	**0.40** (0.04–0.96)	0.51 (0.00–0.91)	0.09 (0.00–0.38)
Tanner-PH	ACE	**0.81** (0.07–0.99)	0.07 (0.00–0.84)	0.12 (0.00–0.97)
Age 12 y				
LH	ADE	**0.93** (0.85–0.96)	—	0.07 (0.04–0.15)
FSH	ADE	**0.95** (0.89–0.97)	—	0.05 (0.02–0.11)
E2	ACE	**0.45** (0.12–0.77)	0.12 (0.00–0.40)	0.43 (0.23–0.88)
T	ADE	**0.78** (0.59–0.88)	—	0.22 (0.12–0.41)
Tanner-P	ACE	0.09 (0.00–0.93)	0.30 (0.00–0.66)	0.62 (0.33–1.00)
Tanner-T	ACE	**0.72** (0.27–1.00)	0.18 (0.00–0.47)	0.10 (0.01–0.31)
Tanner-PH	ACE	0.33 (0.00–0.90)	0.49 (0.00–0.84)	0.19 (0.07–0.43)
Girls				
Age 9 y				
LH	ADE	**0.70** (0.46–0.83)[b]	—	0.30 (0.17–0.54)
FSH	ADE	**0.43** (0.06–0.68)	—	0.57 (0.32–0.94)
E2	ACE	0.02 (0.00–0.50)	**0.65** (0.14–0.79)	0.32 (0.20–0.52)
T	ADE	**0.70** (0.43–0.84)	—	0.30 (0.16–0.57)
Tanner-B	ACE	**0.72** (0.15–1.00)	0.23 (0.00–1.00)	0.05 (0.00–1.00)
Tanner-PH	ACE	0.29 (0.00–0.97)	**0.67** (0.07–0.97)	0.04 (0.00–0.38)
Age 12 y				
LH	ADE	**0.65** (0.37–0.81)	—	0.35 (0.19–0.63)
FSH	ADE	**0.56** (0.25–0.76)	—	0.44 (0.24–0.75)
E2	ACE	**0.54** (0.04–0.89)	0.28 (0.00–0.71)	0.19 (0.10–0.37)
T	ADE	**0.51** (0.05–0.76)	—	0.49 (0.24–0.95)
Tanner-B	ACE	0.21 (0.00–0.33)	**0.69** (0.08–1.00)	0.10 (0.00–0.20)
Tanner-PH	ACE	**0.80** (0.22–0.95)	0.06 (0.00–0.57)	0.14 (0.04–0.39)

Abbreviations: CI, confidence interval; E2, estradiol; T, testosterone; —, not modeled. Standardized estimates of genetic and environmental influences on hormone levels and Tanner stages at age 9 and 12 years in boys and girls. Genetic influences are reported as broad heritability (h^2 + d^2) when variables were modeled in an ADE model and as heritability (h^2) when variables were modeled in an ACE model; see column 2. Estimates are derived from a bivariate genetic model. Unstandardized estimates can be found in Supplemental Table 2. Bold-typed estimates of genetic and common environmental effects are significantly different from zero.

[a] Based on twin correlations (Supplemental Table 1), an ACE model was fitted for estradiol in boys and girls and all Tanner stages in both boys and girls. For the other variables, an ADE model was fitted. Column 3 contains an estimate of additive genetic influences (heritability) in the case of an ACE model and an estimate of additive + nonadditive genetic effects in the case of an ADE model.

[b] Estimate was not significantly different from zero in the data set in which values below the detection limit were excluded.

ment showed a shift over time: from genetic at age 9 years to common environmental influences at age 12 years. For Tanner-PH development in girls, environmental influences at age 9 years shifted to genetic influences at age 12 years.

Associations between hormone levels

Correlations between hormone levels differed between age 9 and 12 years and between boys and girls (Fig. 2). All significant correlations were positive (Table 3). In 9-year-old boys, LH correlated only with FSH (Rph = 0.62), whereas at age 12 years, LH correlated with FSH, estradiol, and testosterone (Rph = 0.32, 0.24, 0.49, respectively). In boys only, FSH at age 9 years correlated with all reproductive hormones at age 12 years (Rph = 0.30, 0.51, 0.25, 0.29 for FSH at age 9 years with LH, FSH, estradiol, and testosterone at age 12 years, respectively). In 9-year-old girls, LH correlated with FSH (Rph = 0.64) and estradiol (Rph = 0.31); at age 12 years, LH levels correlated with FSH, estradiol, and testosterone levels (Rph = 0.56, 0.50, 0.40). In girls, LH at age 9 years correlated with LH and estradiol at age 12 years (Rph = 0.40, 0.40).

Bivariate genetic analyses showed that in both boys and girls phenotypic correlations were driven by genetic factors influencing both hormones (Table 3). In addition, in boys a common environmental correlation influenced estradiol levels at age 9 and 12 years (Rc =

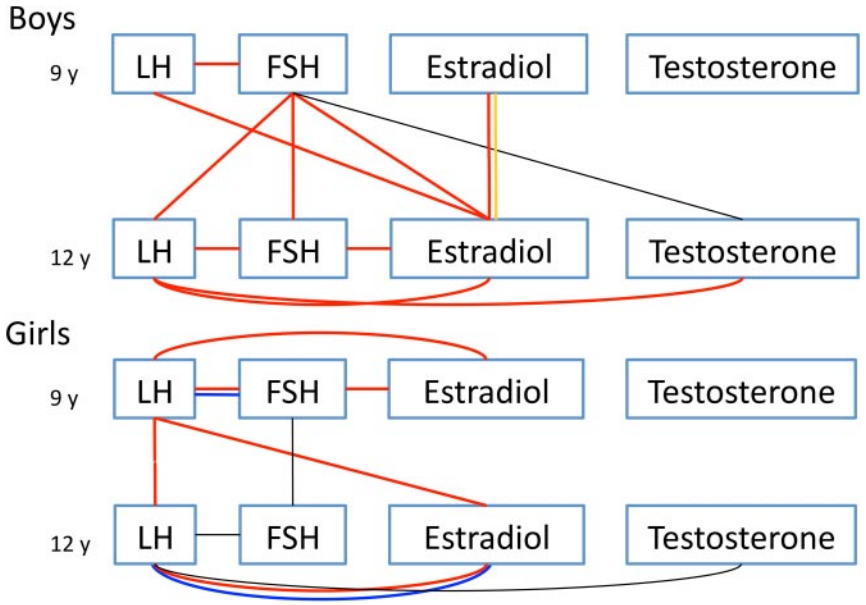

Figure 2. Significant phenotypic correlations between hormone levels during pubertal development in boys and girls at age 9 and 12 years (see also Table 3). Red: significant genetic correlation (Ra); orange: significant common environmental correlation (Rc); blue: significant unique environmental correlation (Re); black: significant phenotypic correlation that could not be specified to genetic or environmental correlations.

1.00). In girls a unique environmental correlation was observed between LH and FSH at age 9 years (Re = 0.59) and between LH and estradiol at age 12 years (Re = 0.68).

Associations between hormone levels and Tanner stages

Significant phenotypic correlations were found between hormone levels at age 9 years and Tanner stages at age 12 years (Table 3 and Fig. 3). In boys, LH at age 9 years correlated with Tanner-P and Tanner-PH development at age 12 years (Rph = 0.29, 0.29); FSH at age 9 years with Tanner-P, Tanner-T, and Tanner-PH development at age 12 years (Rph = 0.34, 0.33, 032); and testosterone at age 9 years with Tanner-PH development at age 12 years (Rph = 0.42). Correlations between hormone levels at age 12 years and Tanner stages at age 12 years were found for LH with Tanner-P (Rph = 0.39) and Tanner-PH development (Rph = 0.33), and for testosterone with Tanner-P, Tanner-T, and Tanner-PH development (Rph = 0.40, 0.44, 0.39) (Fig. 3).

In girls we found that LH levels at age 9 years correlated with Tanner-B and Tanner-PH development at age 12 years (Rph = 0.56, 0.44) and FSH at age 9 years with Tanner-B development at age 12 (Rph = 0.44). At age 12 years, LH, estradiol, and testosterone levels were correlated with both Tanner-B (Rph = 0.55, 0.70, 0.30) and Tanner-PH development (Rph = 0.46, 0.72, 0.33).

In boys and girls, at both ages, correlations between hormone levels and physical development were mainly driven by genetic factors. The correlation between estradiol at age 12 years and Tanner-B development at age 12 years was mainly driven by common environmental factors (Rc = 1.00).

Discussion

In this paper we described the results of a longitudinal twin study on the development of LH, FSH, and estradiol levels in morning urine, testosterone levels in morning saliva, and Tanner stages in healthy boys and girls at age 9 (at baseline) and 12 years (at follow-up). Because all participants were the same age, variation in hormone levels represents individual developmental differences only and cannot be explained by age differences between the participants. Moreover, the longitudinal aspect allowed us to study the correlations of hormone levels at age 9 years with physical development at age 12 years. We find that at age 9 years, LH and FSH levels in both boys and girls, and testosterone levels in boys, predict secondary sexual characteristics (as described by Tanner stages: breast and pubic hair development in girls; penis, testes, and pubic hair development in boys) at age 12 years. These correlations are largely due to genetic factors that are involved in both early hormone levels and subsequent physical development.

The genetic overlap between genes that regulate hormone levels at age 9 years and physical development at age 12 years implies that certain genes are involved in both the first processes of puberty (as represented by increases in LH and FSH secretion) and subsequent development of secondary sexual characteristics. These significant (genetic) correlations over time suggest that early hormone levels may have a predictive value at the individual level of physical development later during development. This was, however, not the aim of this study because we report correlations that illustrate predictive values on a group level only. Correlations between hormone levels and physical development, both measured at age 12 years, were as expected (20) and correspond with previous reported correlations (4).

To the best of our knowledge, we are the first to measure heritability of reproductive hormone levels in children at age 9 years and again at age 12 years. The only other study to date reported moderate (LH, FSH, estra-

Table 3. Significant Associations Between Hormone Levels and Tanner Stages in Boys and Girls During Puberty

	Rph (95% CI)	Model	Rg (95% CI)	Re (95% CI)
Boys				
LH 9–FSH 9	**0.62** (0.48–0.73)	ADE-ADE	**0.70** (0.53–0.83)	0.26 (−0.13–0.60)
LH 9–E2 12	**0.24** (0.03–0.43)	ADE-ACE	**0.54** (0.33–1.00)	0.22 (−0.23–0.59)
FSH 9–LH 12	**0.30** (0.06–0.50)	ADE-ADE	**0.31** (0.05–0.53)	0.34 (−0.12–0.65)
FSH 9–FSH 12	**0.51** (0.31–0.66)	ADE-ADE	**0.56** (0.35–0.71)	0.35 (−0.14–0.70)
FSH 9–E2 12	**0.25** (0.03–0.45)	ADE-ACE	**1.00** (0.07–1.00)	−0.27 (−0.64–0.25)
FSH 9–T 12	**0.29** (0.06–0.49)	ADE-ADE	0.24 (−0.05–0.50)	0.35 (−0.10–0.67)
E2 9–E2 12[a]	**0.32** (0.12–0.50)	ACE-ACE	**1.00** (0.36–1.00)	−0.40 (−0.68–0.03)
LH 12–FSH 12	**0.32** (0.10–0.52)	ADE-ADE	**0.32** (0.07–0.53)	0.03 (−0.39–0.45)
LH 12–E2 12	**0.24** (0.02–0.44)	ADE-ACE	**0.41** (0.08–1.00)	−0.30 (−0.65–0.17)
LH 12–T 12	**0.49** (0.28–0.65)	ADE-ADE	**0.55** (0.31–0.74)	0.09 (−0.35–0.50)
FSH 12–E2 12	**0.29** (0.08–0.48)	ADE-ACE	**1.00** (0.23–1.00)	−0.06 (−0.49–0.40)
LH 9–Tanner-P 12	**0.29** (0.03–0.50)	ADE-ACE	**1.00** (0.14–1.00)	−0.15 (−0.82–0.49)
LH 9–Tanner-PH 12	**0.29** (0.02–0.52)[b]	ADE-ACE	0.61 (−1.00–1.00)	0.28 (−0.45–0.82)
FSH 9–Tanner-P 12	**0.34** (0.09–0.55)	ADE-ACE	**1.00** (0.22–1.00)	−0.11 (−0.61–0.44)
FSH 9–Tanner-T 12	**0.33** (0.04–0.46)	ADE-ACE	**0.94** (0.59–1.00)[b]	0.79 (−0.24–0.86)
FSH 9–Tanner-PH 12	**0.32** (0.06–0.53)	ADE-ACE	**1.00** (0.48–1.00)	0.01 (−0.62–0.64)
T 9–Tanner-PH 12	**0.42** (0.18–0.62)	ADE-ACE	**1.00** (0.31–1.00)	−0.24 (−0.68–0.37)
LH 12–Tanner-P 12	**0.39** (0.10–0.61)	ADE-ACE	**1.00** (0.34–1.00)	−0.50 (−1.00–0.25)
LH 12–Tanner-PH 12	**0.33** (0.01–0.59)	ADE-ACE	0.43 (−1.00–1.00)	−0.04 (−0.71–0.66)
T 12–Tanner-P 12	**0.40** (0.16–0.60)	ADE-ACE	**1.00** (0.45–1.00)	−0.13 (−0.52–0.32)
T 12–Tanner-T 12	**0.44** (0.15–0.65)	ADE-ACE	**0.58** (0.20–1.00)	−0.05 (−0.70–0.65)
T 12–Tanner-PH 12	**0.39** (0.13–0.60)	ADE-ACE	**1.00** (0.23–1.00)	−0.15 (−0.69–0.48)
Tanner-P 12–Tanner-T 12[a]	**0.70** (0.51–0.83)	ACE-ACE	**1.00** (0.68–1.00)	0.03 (−0.63–0.59)
Tanner-P 12–Tanner-PH 12[a]	**0.30** (0.04–0.53)	ACE-ACE	1.00 (−1.00–1.00)	−0.45 (−0.84–0.14)
Girls				
LH 9–FSH 9	**0.64** (0.51–0.74)	ADE-ADE	**0.71** (0.38–1.00)	**0.59** (0.29–0.79)[b]
LH 9–E2 9	**0.31** (0.11–0.49)[b]	ADE-ACE	**1.00** (0.28–1.00)[b]	0.05 (−0.25–0.38)
LH 9–LH 12	**0.40** (0.19–0.57)	ADE-ADE	**0.50** (0.17–1.00)[b]	0.15 (−0.24–0.51)
LH 9–E2 12	**0.40** (0.20–0.57)	ADE-ACE	**1.00** (0.28–1.00)	−0.04 (−0.43–0.36)
FSH 9–E2 9	**0.23** (0.04–0.41)	ADE-ACE	**1.00** (0.23–1.00)	0.18 (−0.13–0.47)
FSH 9–FSH 12	**0.23** (0.03–0.41)	ADE-ADE	0.48 (−0.03–1.00)	−0.00 (−0.36–0.36)
LH 12–FSH 12	**0.56** (0.37–0.70)	ADE-ADE	0.32 (−0.10–0.65)[c]	0.48 (−0.04–1.00)[c]
LH 12–E2 12	**0.53** (0.33–0.68)	ADE-ACE	**0.59** (0.17–1.00)	**0.68** (0.38–0.84)
LH 12–T 12	**0.40** (0.19–0.57)	ADE-ADE	0.47 (−0.04–1.00)	0.32 (−0.10–0.65)
LH 9–Tanner-B 12	**0.56** (0.33–0.73)	ADE-ACE	**1.00** (0.56–1.00)	0.27 (−0.40–0.78)
LH 9–Tanner-PH 12	**0.44** (0.19–0.64)	ADE-ACE	**1.00** (0.36–1.00)	−0.42 (−0.81–0.20)
FSH 9–Tanner-B 12	**0.44** (0.51–0.51)	ADE-ACE	**0.91** (0.13–1.00)	**0.68** (0.17–0.95)
LH 12–Tanner-B 12	**0.55** (0.29–0.74)	ADE-ACE	**0.78** (0.31–1.00)	0.28 (−0.71–1.00)
LH 12–Tanner-PH 12	**0.46** (0.20–0.67)	ADE-ACE	**0.40** (0.22–0.85)[b]	0.82 (−0.19–1.00)[c]
E2 12–Tanner-B 12[a]	**0.70** (0.52–0.82)	ACE-ACE	1.00 (−1.00–1.00)	−0.13 (−0.70–0.51)
E2 12–Tanner-PH 12[a]	**0.72** (0.54–0.91)	ACE-ACE	**1.00** (0.63–1.00)	**0.73** (0.18–0.96)
T 12–Tanner-B 12	**0.30** (0.07–0.51)	ADE-ACE	**1.00** (0.19–1.00)	−0.28 (−0.83–0.47)
T 12–Tanner-PH 12	**0.33** (0.09–0.53)	ADE-ACE	0.35 (−1.00–1.00)	0.57 (−0.07–0.88)
Tanner-B 12–Tanner-PH 12[a]	**0.74** (0.59–0.85)	ACE-ACE	0.77 (−0.60–1.00)	0.06 (−0.63–0.75)

Abbreviations: CI, confidence interval; E2, estradiol; T, testosterone. Significant phenotypic correlations (Rph) between hormones at age 9 and 12 years and between hormones at age 9 and 12 years and Tanner stages at age 12 years are given. The extent of overlap in genetic (Rg) and unique environmental influences (Re) acting on both variables are given with their 95% confidence intervals. Bivariate ADE (ADE for both variables), bivariate ACE models (ACE for both variables), and mixed models were fitted, based on the twin correlations per variable. Rg is an estimate of the broad genetic correlation for bivariate ADE models and the additive genetic correlation in other cases. Bold-typed correlations are significant at $P < .05$.

[a] In the bivariate ACE models, a common environmental correlation was estimated. It was only significantly explaining the correlation between estradiol at ages 9 and 12 years in boys [Rc = 1.00 (0.30–1.00)] and the correlation between breast development and estradiol at age 12 years in girls [Rc = 1.00 (0.63–1.00)].

[b] Correlation was not significant in the data set in which values below the detection limit were excluded.

[c] Correlation was significant in the data set in which values below the detection limit were excluded.

diol) to high (testosterone) heritability estimates in a group of 35 boys aged between 5 and 11 years (10). We find moderate to high heritability estimates for hormone levels at both ages and in both sexes. Interestingly, based on our longitudinal setup, in girls we find a shift from significant common environmental influences at age 9 years to significant genetic influences at age 12 years on estradiol and Tanner-PH development and vice

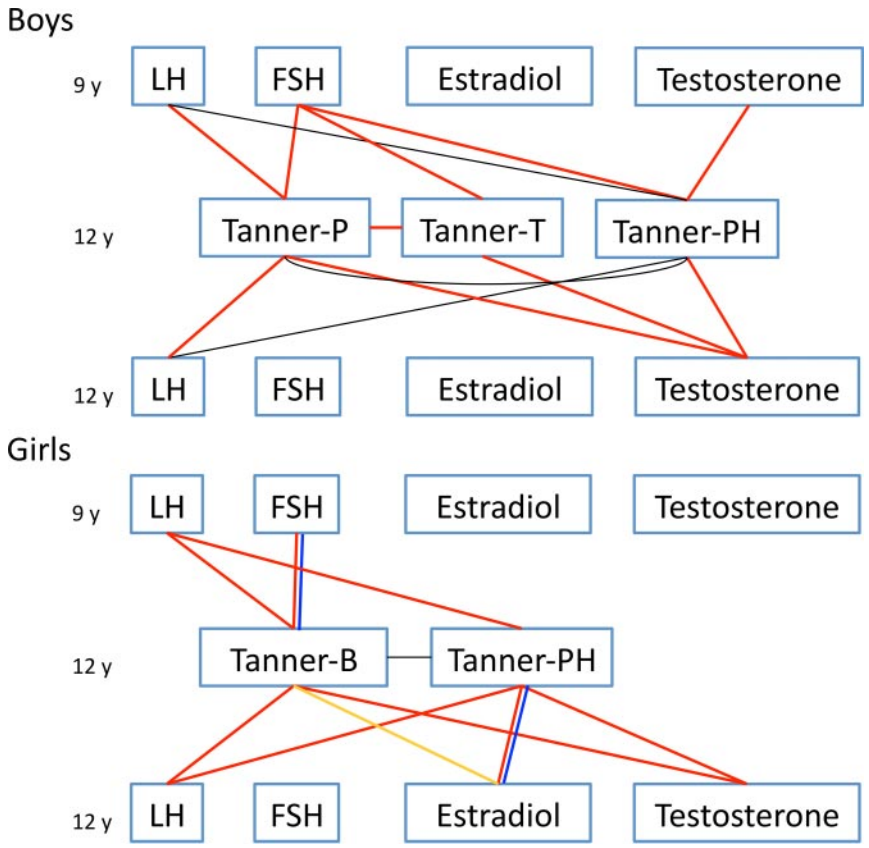

Figure 3. Significant phenotypic correlations between secondary sexual characteristics at age 12 years (middle) and hormone levels at age 9 years (top) and 12 years (bottom) in boys and girls (see also Table 3). Red: significant genetic correlation (Ra); orange: significant common environmental correlation (Rc); blue: significant unique environmental correlation (Re); black: significant phenotypic correlation that could not be specified to genetic or environmental correlations. For the sake of clarity, correlations between hormones are omitted from the figure; see Fig. 2 for these correlations.

versa for Tanner-B development. Importantly, in boys, heritability estimates and genetic variance (Supplemental Table 2) of hormone levels (except for estradiol) increased over time. At both ages, estimates of genetic variance for LH and FSH levels in boys were higher than in girls.

These changes in heritability estimates over time may be related to different stages of development at ages 9 and 12 years: boys develop from prepubertal to early pubertal stages and girls from early to middle pubertal stages. Therefore, processes involved in the maintenance and development of hormone levels and physical posture may differ at age 9 and 12 years, and between boys and girls of the same age [because boys mature at a later age (17, 18, 21)]. HPG axis-related hormones involved in puberty increase and reach peak levels at different developmental stages and are responsible for specific physical changes that come with puberty. For example, LH and FSH levels increase during sleep prior to physical changes but reach a plateau before full maturation (Tanner stage V) (see Supplemental Figs. 2 and 3) and may even decrease before full maturation is reached (22–27). Testosterone in boys and estrogen levels in both boys and girls start to increase after initiation of physical development (4, 22, 25, 26, 28–31) and may decrease before full maturation is reached [estrogen in girls (4, 31)]. Thus, the increase in reproductive hormone levels during puberty is not only reaching stable adult levels: hormone levels may decrease at the end of puberty, and physical development continues after hormone levels have reached their peak levels. Accordingly, pubertal stages may reflect specific developmental steps toward adulthood. For example, LH levels in Tanner stage II have a more initiation-related function, whereas in Tanner stage IV, LH has a preservative function. This suggests that different stages of puberty are differently influenced by genetic and/or environmental factors. In addition, earlier it has been suggested that testosterone levels are influenced by age-dependent factors as based on the finding that testosterone levels of fathers and sons did not correlate (11). We find that this may also be true for other reproductive hormones. In addition, it has been suggested that FSH is more related to general development, whereas LH is related to pubertal development (15). This is in agreement with our current finding that in boys at age 9 years FSH correlated with all other hormones and Tanner stages at age 12 years, whereas in 9-year-old girls LH was correlated with hormone levels and Tanner stages at age 12 years (see Figs. 2 and 3). Different developmental windows between the sexes may explain the central role of FSH in boys vs LH in girls.

Moreover, in addition to different developmental windows, differences in heritability estimates between boys and girls may be caused by the following 2 explanations. First, the relative influences of genes and environment on pubertal development probably differ between boys and girls because of differences in biological mechanisms. During puberty testosterone (mainly produced by the testes in boys) plays an important role in masculine development, whereas estrogen (mainly produced by the developing follicles in girls) plays an important role in feminine development. However, both hormones are present in both

sexes due to conversion of androgens to testosterone in girls and estrogens in boys and because other organs also produce sex hormones (eg, Refs 3, 32, and 33). In addition to differences in the production of sex hormones, LH and FSH are also differently regulated in boys and girls. Release of both LH and FSH is in part regulated by inhibin-A (in girls) and inhibin-B (in boys and girls). Inhibins A and B are present in different amounts in boys and girls (34). This suggests that there are different pathways not only for testosterone and estrogen synthesis but also for LH and FSH secretion. Second, a sex-specific interaction between a hormone and the environment could be involved. Moreover, one can also think of a time-specific hormone-environment interaction. For example, we found that common environmental factors have a high (age 9 years) to moderate (age 12 years, not significant) influence on estradiol levels in girls but are estimated to be low for estradiol levels in boys. Because estradiol plays a different role in girls vs boys, dietary intake (35) or other common environmental factors may have a higher influence on estradiol levels in girls.

The results described in this paper are based on a representative sample of the Dutch population. As such, we did not exclude children being overweight or obese, although excess adiposity may influence hormonal parameters and pubertal onset; extensive literature exists on the (possibly nonlinear) relationship between body mass index (BMI) and menarche (eg, Refs 21 and 36–38). Because in the current cohort, the percentage of children being overweight is representative of the Dutch population, the (genetic) results should be interpreted for the population as a whole, including overweight children.

The results of this study should be interpreted with some caution. The relatively small sample size may potentially lead to instable heritability and environmental estimates. Another source of instability may be differences in developmental stages between children of the same age because small changes in pubertal development may lead to large effects on variance components (39).

In summary, hormone levels at age 9 years are predictive of physical development at age 12 years, in part due to a shared genetic origin. Especially FSH in 9-year-old boys and LH in 9-year-old girls seem to predict hormone levels and secondary sexual characteristics at age 12 years. Variance in hormone levels and physical development was explained by different mechanisms (ie, genetic or environmental) at ages 9 and 12 years in girls, which stresses the importance of a specific developmental window when studying the heritability of pubertal markers. The third measurement of this longitudinal study is on its way and may provide more information on the development of hormone levels and genetic and environmental factors that influence these processes.

Acknowledgments

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This work was supported by the Netherlands Organization for Scientific Research Grants NWO 433-09-220 (to H.E.H.P.), NWO 51.02.060 (to H.E.H.P.), 668.772 (to D.I.B.); NWO-MagW 480-04-004 (to D.I.B.); and NWO/SPI 56-464-14192 (to D.I.B.); the European Research Council Grant ERC-230374 (to D.I.B.); and the High Potential Grant from Utrecht University (to H.E.H.P.).

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Disclosure Summary: The authors have nothing to disclose.

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