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Genetic and environmental influences on pubertal hormones in human hair across development

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ABSTRACT

Puberty is a complex biopsychosocial process that can affect an array of psychiatric and medical disorders emerging in adolescence. Although the pubertal process is driven by neuroendocrine changes, few quantitative genetic studies have directly measured puberty-relevant hormones. Hair samples can now be assayed for accumulation of hormones over several months. In contrast to more conventional salivary measures, hair measures are not confounded by diurnal variation or hormonal reactivity. In an ethnically and socioeconomically diverse sample of 1286 child and adolescent twins and multiples from 672 unique families, we estimated genetic and environmental influences on hair concentrations of testosterone, DHEA, and progesterone across the period of 8–18 years of age. On average, male DHEA and testosterone were highly heritable, whereas female DHEA, progesterone, and puberty were largely influenced by environmental components. We identified sex-specific developmental windows of maximal heritability in each hormone. Peak heritability for DHEA occurred at approximately 10 years of age for males and females. Peak heritability for testosterone occurred at age 12.5 and 15.2 years for males and females, respectively. Peak heritability for male progesterone occurred at 11.2 years, while the heritability of female progesterone remained uniformly low. The identification of specific developmental windows when genetic signals for hormones are maximized has critical implications for well-informed models of hormone-behavior associations in childhood and adolescence.

1. Introduction

1.1. Overview

Puberty involves two distinct periods of hormonal changes marked by rising hormone levels: adrenarche and gonadarche (Patton and Viner, 2007). Adrenarche and gonadarche are biologically and developmentally dissociable processes characterized by maturation of the hypothalamic-pituitary-adrenal (HPA) and −gonadal (HPG) axes, respectively (Saenger and Dimartino-Nardi, 2001). Specifically, adrenarche begins with the expansion of the inner layer of the adrenal cortex, the zona reticularis, which causes a subsequent increase in dehydroepiandrosterone (DHEA) and its sulphate ester (DHEA-S; Hui et al., 2009). The typical onset of adrenarche is between six and nine years of age in females, and begins approximately one year later in males (Blakemore et al., 2010). Gonadarche begins approximately two years after adrenarche onset with the increase of sex hormones, including testosterone and progesterone. In both boys and girls, rising concentrations of DHEA cause the onset of body odor and pubic hair growth, while rising sex hormones drive the emergence of secondary sex characteristics (Havelock and Auchus, 2004; Hiort, 2002). However, hormone levels are not synonymous with somatic metrics of pubertal development, such as Tanner stages, as hormones are present pre- and post-puberty, and there is overlap in hormone concentrations across pubertal stages.

The extent to which genetic variation accounts for individual differences in the hormonal outputs of adrenarche and gonadarche is unclear. Quantitative genetic designs use genetic similarities between relatives (e.g., twins) to disentangle genetic and environmental effects on variation in an outcome. Although the intuition might be that a biological variable such as hormone levels is strictly heritable, it is possible that a highly constrained set of genes causes the typical rise in hormone levels, while variation about this average is entirely due to environmental input. Indeed, some level of environmental influence is

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to be expected, as pubertal hormones respond to physiological (Hoffman et al., 1996) and psychological stress (Lennartsson et al., 2012). The current paper reports results from quantitative genetic models of pubertal development, testosterone, progesterone, DHEA, and their covariation, in a population representative sample of child and adolescent twins.

1.2. Prior quantitative genetic studies

Previous twin studies have identified significant additive genetic influences on adolescent testosterone for both sexes, with three out of five studies indicating higher heritability in males (Harden et al., 2014b; Harris et al., 1998; Hoekstra et al., 2006; Koenis et al., 2013; Van Hulle et al., 2015). A handful of previous studies indicate that variation in DHEA levels in adolescence contain a large heritable component in males and females (Li et al., 2016; Van Hulle et al., 2015). Similarly, pubertal development, as measured using secondary sex characteristics, has been estimated to be at least 60% heritable in both sexes (Harden et al., 2014b; Hoekstra et al., 2006; Mustanski et al., 2004).

Many of the previous studies included participants spanning the adolescent age range, but age-specific sex differences in biosynthetic pathways suggest that the heritability of hormonal levels may change with development. For example, DHEA is the primary precursor to testosterone biosynthesis for pre-pubertal children and for females across the lifespan, but not for post-pubertal males (Granger et al., 1999). This likely reflects the fact that testosterone switches from primarily adrenal to gonadal in origin during puberty for males only. In support of developmental specificity of genetic effects, the heritability of testosterone was found to increase for males (64%–78%) and decrease for females (70%–51%) from ages 9–12 (Koenis et al., 2013). The key research question, then, is whether different estimates of heritability are obtained across adolescence. This research goal can be contrasted with previous studies that described an aggregate estimate of adolescent heritability. Identifying ages at which disparate heritability estimates are obtained may mark biologically relevant components of the pubertal transition that uniquely predict adolescent behavior.

1.3. Measurement of hormones in hair

Quantitative genetic research on hormone levels is complicated by measurements that reflect state- and trait-specific processes. DHEA, testosterone, and progesterone all display diurnal patterns characterized by peak levels in the morning, followed by a steady decline across the day (Granger et al., 2003; Hucklebridge et al., 2005; Liening et al., 2010; Matchock et al., 2009). There is also variation in hormones across days; one previous study estimated the correlation between testosterone samples taken at the same time of day two days apart at 0.62 (Harden et al., 2016). Although multiple salivary or blood samples can be collected to estimate individual differences in basal hormone levels, this can be costly and may decrease participant compliance.

Hair sampling is a recently developed method that provides a measure of long-term (i.e., several months) free hormone output using a single, non-invasive sample (Gao et al., 2015). Each centimeter of hair is thought to reflect 1 month of hormone accumulation, given an average growth rate of 1 cm per month (Wennig, 2000). Indeed, the association in humans (age range: 21–53) between a 1-cm hair sample and salivary cortisol collected three times daily over a 1-month period was highest when the salivary average included all 4 weeks, and gradually decreased as a function of the number of weeks excluded from the average (Short et al., 2016). This supports the model of hair as a marker of accumulated hormone exposure. Hair cortisol has high test-retest reliability across wide intervals (e.g., $r = 0.73$ across 1 year in a sample with mean age = 30.6; Stalder and Kirschbaum, 2012) but is not associated with salivary measures of reactive cortisol, diurnal slope, or the cortisol awakening response (Grass et al., 2015, [mean participant age = 25]; Short et al., 2016). Other hair hormones have yet to be comprehensively evaluated with regard to intensive salivary measurements.

1.4. Current study

The current study sought to identify developmentally-specific and sex-specific genetic and environmental etiologies of hair biomarkers of testosterone, progesterone, and DHEA in a population representative sample of child and adolescent twins. Based on results from previous twin studies, we hypothesized that testosterone and DHEA would be more heritable in males overall, and generally expected genetic influences on hormones to differ across adolescence.

2. Material and methods

2.1. Participants

Participants were drawn from the Texas Twin Project, a population-based study of school-aged twins in central Texas (Harden et al., 2013). Parents of twin families were contacted by mail and invited to participate in an in-lab study. The current study draws from a final sample of $n = 1286$ individual twins (from 672 unique families) who provided hair samples. Repeat observations were available for 111 participants, for a total of $i = 1397$ (813 female) non-missing observation points. The twins were in grades 3 through 12 and ranged in age from 7.80 to 19.47 years ($M = 12.34$, $SD = 2.77$). Two families had repeat triplets with two observations missing for the repeat visit (contributing 5 pairs each),[1] one family had quadruplets (6 pairs), 24 families had triplets (3 pairs), 3 families had triplets with two missing observations (2 pairs), one family had two sets of twins (2 pairs), and 61 pairs were repeat observations (2 pairs), for a total of 798 twin pairs (266 monozygotic [MZ] pairs [108 male, 158 female] and 532 dizygotic [DZ] pairs [112 male, 148 female, and 272 opposite-sex]). Both twins provided hair specimens for 681 pairs out of the 798 twin pairs. Thirty-one potential participants were excluded on the basis of reported oral contraception use (see Supplement S1.2. for effects on hormone levels) and eight potential participants were excluded for reported endocrine problems (numbers not included in totals above). Sixty-three percent (63%) of the twins identified as non-Hispanic White, 18.6% identified as Hispanic/Latino, 4.2% identified as African American, and 14.2% identified as another race/ethnicity. Of the participating families, 33.4% reported receiving some form of means-tested public assistance (e.g., food stamps) since the twins' birth.

2.2. Measures

2.2.1. Zygosity

Opposite-sex twin pairs were classified as DZ. Same-sex twin pair zygosity was assessed using responses to ratings about the twins' physical similarities (e.g., facial appearance). High school aged twins, parents, and two research assistants completed the ratings. Parents and high school aged twins additionally rated how often the twins are mistaken for one another. These ratings were entered into a latent class analysis (LCA) that was used to obtain zygosity classifications. LCA has been reported to accurately determine zygosity > 99% of the time (Heath et al., 2003). In the present study, for a subset of 153 twin pairs that were genotyped, LCA accurately determined zygosity > 95% of the time.

[1] As twin models in M*plus* are specified to examine pairwise combinations (e.g., Twin 1 with Twin 2) families with repeat observations, triplets, quadruplets or multiple twin sets were analyzed as multiple pairwise combinations. In the case of triplets, this was achieved by examining three pairwise associations: Twin 1 with Twin 2; Twin 2 with Twin 3; Twin 1 with Twin 3.

2.2.2. Pubertal development

Participants rated their pubertal development on the Pubertal Development Scale (Petersen et al., 1988). Boys and girls rated their growth in height, growth in body hair, and skin changes, such as pimples, on a 4-point scale (1 = *Not Yet Begun* to 4 = *Has Finished Changing*). Males additionally rated deepening of voice and growth of facial hair, while girls rated breast development and if they had started menstruating (1 = *No,* 4 = *Yes*). A total pubertal status score was calculated by averaging across the items. Pubertal status spanned the full range (1–4) in both sexes (Fig. S1). Forty participants were missing pubertal development scores due to incomplete survey response. On average, girls ($M = 2.55$, $SD = 0.98$) reported more advanced pubertal status than boys ($M = 2.07$, $SD = 0.77$). Girls also reported their age at menarche (in years). Fifty-five percent (55%) of girls reported having begun menstruating, and among menstruating girls, the average age at menarche was 12.01 years ($SD = 1.38$ years).

2.2.3. Hair hormones

Hair samples 3 mm in diameter were taken from the posterior vertex of the scalp. The first 3 cm of hair (weight = 7.5 mg) was analyzed as a marker of hormone accumulation over a 3-month period. Hair was not collected or analyzed from 28% of participants (70% of whom were male), because their hair was shorter than the 3-cm requirement, the hair sample collected was less than the required weight of 7.5 mg, or the participant declined to provide a hair sample. As a result, these participants were included as having missing hormone data if their co-twin provided a useable hair sample. Samples were stored in aluminum foil packets at room temperature before being shipped to Dr. Clemens Kirschbaum's laboratory at Technical University Dresden. Participants were instructed not to use any hair products that are not rinsed out of the hair on the day of the appointment.

Hair samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Whole, nonpulverized hair was washed in isopropanol following wash and steroid extraction procedures describe elsewhere (Gao et al., 2013). The test-retest stability was examined in the 111 repeat participants and indicated high consistency for female progesterone and male DHEA (see Supplement S1.4). The inter- and intra-assay coefficients of variation (CVs) have been reported as < 10% for all hair hormones (Gao et al., 2013). The inter- and intra-assay coefficients of variation (CVs) have been found to be < 15% by Dr. Kirschbaum (personal communication) but were not computed for the current sample.

The lower limit of detection was 0.1 pg/ml for testosterone, DHEA, progesterone, and estradiol. Samples were assayed for estradiol only in the fourth year of data collection, and estradiol was not examined further, as only 36 of the 384 samples produced values above the detection limit. There were 398 (28.5%) samples below the sensitivity threshold for testosterone, 331 (23.7%) samples below threshold for progesterone, and 138 (9.9%) samples below threshold for DHEA. Binomial regressions with age as the predictor indicated that younger participants were significantly more likely to have concentrations below the sensitivity threshold (0 = above threshold, 1 = below threshold) for testosterone (*log odds* = −0.07, 95% Confidence Interval (CI) = [−0.11, −0.02], $p = .003$), progesterone (*log odds* = −0.11, 95% CI = [−0.16, −0.06], $p < .001$), and DHEA (*log odds* = −0.17, 95% CI = [−0.24, −0.09], $p < .001$).

The same binomial regressions were then run with pubertal development as a predictor. These analyses indicated that participants at earlier pubertal stages were more likely to have below-threshold hormone levels for progesterone (*log odds* = −0.34, 95% CI = [−0.48, −0.20], $p < .001$), and DHEA (*log odds* = −0.21, 95% CI = [−0.41, −0.01], $p = .040$). This trend was also in the expected direction for testosterone (*log odds* = −0.09, 95% CI = [−0.21, 0.04], $p = .173$). A subset of 460 participants (age range = 14–19) had both hair and salivary testosterone. In line with prior cortisol findings (e.g., Short et al., 2016), the phenotypic association between a single saliva and hair testosterone sample was small ($r = 0.05$; Grotzinger et al., *in press*).

2.3. Analyses

Monte Carlo simulations based on the characteristics of the current sample were conducted to determine the best approach to modeling hormone concentrations below the sensitivity threshold (see Supplement S1.3). Tobit models—an analytic approach that estimates parameters based on the assumption that values at the lower limit of detection indicate true values occupying an unobserved lower tail of a normal distribution—were most likely to produce 95% confidence intervals that captured the genetic and environmental variance components specified in the generating models (Table S1). Participants with below-threshold values were, therefore, included in all models, and data were analyzed using Tobit models estimated with robust weighted least squares (WLSMV). For twin models, this involved specifying a left-censored distribution for both twins. Participants with missing pubertal development scores, or with co-twins that did not provide hair samples, were included in analyses using full information maximum likelihood (FIML). A winsorizing procedure was used to replace extreme hair hormone values by the highest observed score within 3 standard deviations of the sample mean. This involved replacing 11 outliers for hair testosterone, 7 outliers for hair progesterone, and 24 outliers for hair DHEA. The pattern of twin correlations was robust to these analytic decisions, as it was highly consistent regardless of whether outlying values were winsorized, excluded, or included at the observed value. Distributions of all three hair hormones were positively skewed, and hormonal measurements were log-transformed to approximate normal distributions in the range of values above-detection limits and then standardized.

As hair assays were performed annually, all models controlled for the year hair samples were analyzed (i.e., batch effects) using a set of nominal variables. Sex was effects coded (−0.5 = female, 0.5 = male), and age was mean centered prior to calculating its quadratic effect. Scores on the pubertal development scale were analyzed using either all covariates or all covariates except linear and quadratic effects of age. Notably, removing variance in pubertal status due to age changes the meaning of the variable; it represents pubertal *timing*, i.e., the extent to which a child/adolescent is more or less developed than his or her same-aged peers. Standard errors and model fit statistics were corrected for non-independence among twins and repeat observations using the complex sampling option. Biometric models of the twin correlations were examined using structural equation modeling (SEM) in M*plus* version 7.4. Model fit was evaluated using the root mean square error of approximation (RMSEA) and the comparative fit index (CFI).

2.3.1. Twin model specification

Hair-based assays are particularly appealing for twin models, as they may produce greater stability in twin estimates relative to salivary or blood-based measures. As hormonal responses to the environment are likely heritable (i.e., via gene × environment interaction), genetic estimates will be unstable to the extent that they are contingent on environmental exposures within and across twin pairs. In the former case, if twins in a pair are differentially exposed to hormone-affecting contexts, this will inflate heritability estimates. Conversely, if both twins within a pair are exposed to the same environment, this will increase estimates of shared environmental influence. Across twins, if only a subset of pairs have been exposed recently to relevant environments, this will aggregate across theoretically distinct genetic effects on hormone variation (e.g., stress reactive versus baseline hormone variation). Using hair-based assays as an aggregate measure of hormone concentrations across several months may then protect against *ACE* estimates that are overly contingent on unmeasured, fluctuating environments.

Quantitative genetic models were fit to the data to determine variance attributable to additive genetic (*A*); shared environmental (*C*);

Table 1
Cross-twin correlations for hair hormones, pubertal status and pubertal timing.

	Monozygotic		Dizygotic		
	Male	Female	Male	Female	Opposite-sex
Pubertal Status	.69 (.58, 0.79)	.78 (.69, 0.88)	.65 (.55, 0.76)	.74 (.64, 0.83)	.64 (.57, 0.72)
Pubertal Timing	.28 (.05, 0.51)	.40 (.22, 0.57)	.30 (.16, 0.44)	.23 (.04, 0.42)	.13 (−0.04, 0.30)
DHEA	.78 (.64, 0.92)	.58 (.45, 0.71)	.28 (.14, 0.42)	.66 (.50, 0.82)	.37 (.27, 0.47)
Progesterone	.67 (.55, 0.78)	.62 (.54, 0.70)	.91 (.77, 1.00)	.91 (.69, 1.00)	.46 (.36, 0.56)
Testosterone	.94 (.74, 1.00)	.62 (.50, 0.73)	.65 (.48, 0.82)	.42 (.29, 0.55)	.49 (.38, 0.60)

Note. All models included race as a covariate and allowed for sex-specific means and residual variances in outcomes. Hormonal outcomes were regressed on analytic batch and all outcomes except pubertal status were regressed on sex-specific effects of age and age-squared. Additive genetic influences are approximately estimated as twice the difference between monozygotic and dizygotic correlations (i.e., $2 \times [r_{MZ} - r_{DZ}]$). That dizygotic twin correlations for progesterone are larger than the monozygotic correlations is likely due to a combination of imprecision in point estimates and measurement error. Due to missing survey data, pubertal outcomes are based on a total sample size of $i = 1357$ observation points. All hair hormones were based on a total sample size of $i = 1397$ observation points. Below assay sensitivity threshold observations (398 below threshold for testosterone, 331 for progesterone, and 138 for DHEA) were including in analyses using Tobit models. 95% confidence intervals are given in parentheses.

and non-shared environmental factors, including error variance, unique to each twin (*E*). The *ACE* factors were standardized. As MZ and DZ pairs share approximately 100% and 50% of their genes, respectively, *A* factors are fixed to correlate at 1.0 for MZ pairs and 0.5 for both opposite-sex and same-sex DZ pairs. Correlations between *C* factors are fixed to 1.0 in all twin pairs. In order to estimate sex-specific *ACE* estimates, opposite-sex DZ twins were ordered so that Twin 1 was always male. Hormones, pubertal status, and pubertal timing were examined separately in a series of five univariate models. Although the primary focus of the paper was to examine developmental shifts in *ACE* estimates across childhood and adolescence, we first report univariate models that provided the average of the biometric estimates across the observed age range, in order to compare estimates to the existing literature. All five models provided good fit to the data (CFIs ≥ 0.987, RMSEAs ≤ 0.028).

Nonparametric local structural equation models (LOSEM) were also used to examine differences in sex-specific *ACE* estimates across age (Briley et al., 2015). LOSEM uses a weighting kernel and bandwidth that assigns a higher weight to observations in closer proximity to the focal value of a continuous moderator (in this case, age). Multiple structural equation models were estimated that differed only with respect to the assigned focal value of age, which ranged from 8 to 18 years at intervals of 0.10 (i.e., 180 individual models). All participants were included for each of the 180 models, though twins that were closer to the focal age value for a particular model were given greater weight. Age was excluded as a covariate, because the LOSEM models restricted the variance in age, resulting in age effects estimated near 0. Pubertal timing was, therefore, calculated by residualizing pubertal status for sex-specific linear and quadratic effects of age prior to fitting LOSEMs to the data. Race was also excluded as a covariate for LOSEM models, because there were limited numbers of African American twins for certain age groups.

3. Results

3.1. Effects of covariates on hair hormones

Table S2 reports descriptive statistics by sex for each hair hormone, and Table S3 reports the number of individuals across age and pubertal status. In order to examine the effect of covariates, models were run that included age, sex, race, age^2, and an age × sex interaction on each hair hormone individually. We first estimated the separate effects of age and sex to yield easily interpretable main effects (Table S4). DHEA and testosterone were both significantly higher in males. In addition, an age × sex interaction was identified for DHEA and progesterone (Fig. S2). Relative to males, female DHEA started lower at age 8 but rose more rapidly across age, such that mean levels were approximately equivalent across sexes by age 18. Progesterone levels were largely stable across age for both sexes, with modest decreases across age observed for males and a slight increase in progesterone levels observed for females. Testosterone levels evinced modest increases across age for both sexes, though these trends were not significant. Race was included as a covariate for hormones and puberty in the remaining parametric models, and the effect of sex was taken into account by allowing for separately estimated means across males and females. Sex-specific effects of age and age^2 were also estimated unless otherwise noted. Phenotypic models estimating the association between pubertal status and hormones are presented in the Supplement (S1.5; Fig. S3). For females, the correlation between pubertal development and hair hormones was largest for DHEA ($r = 0.32$, $SE = 0.04$, $p < .001$), followed by progesterone ($r = 0.15$, $SE = 0.04$, $p < .001$), and testosterone ($r = 0.02$, $SE = 0.04$, $p = .564$). For males, DHEA was also the most strongly associated with pubertal development ($r = 0.16$, $SE = .04$, $p < .001$), followed by testosterone ($r = 0.10$, $SE = 0.05$, $p = .034$), and a small, negative association with progesterone ($r = -0.06$, $SE = 0.05$, $p = .279$).

3.2. Univariate twin results

3.2.1. Twin correlations

Cross-twin correlations are summarized in Table 1. The correlations between MZ twins' hormone levels were generally high, suggesting low levels of non-shared environmental input. The difference between MZ and DZ correlations in DHEA and testosterone was higher for males than females, indicating larger genetic influences in males. Opposite-sex DZ correlations were approximately equivalent to same-sex DZ correlations, indicating that the same genes and environmental factors affect these outcomes in both sexes.

3.2.2. Univariate twin models

An initial test of scalar sex differences—that is, differences in the age-independent variance that could be accounted for by *ACE* factors—indicated that, relative to corresponding male estimates, there was significantly more variance in female DHEA and significantly less variance in female progesterone and pubertal status. For this reason, we compare proportions of variance, as opposed to unstandardized variance, accounted for by *ACE* factors across the sexes (summarized in Table 2; displayed in Fig. 1). DHEA levels were significantly more heritable in males (63%) than females (10%). Female testosterone was influenced by a significantly larger non-shared environmental effect (38% vs. 7% in males). Testosterone was primarily heritable in males (55%) and females (44%). Variation in pubertal status and progesterone was largely due to shared environment in both sexes, and variance in pubertal timing was predominantly attributable to non-shared environmental influences.

Table 2
Proportion of variance explained by ACE factors and sex differences from univariate twin models.

	Males			Females			Males - Females			
	h^2	c^2	e^2	h^2	c^2	e^2	Δh^2	Δc^2	Δe^2	Δphenotypic variance
Pubertal Status	.16 (.17)	.53 (.13)***	.31 (.06)***	.09 (.21)	.69 (.17)***	.22 (.06)***	.07 (.33)	−0.16 (.26)	.09 (.09)	−0.41 (.06)***
Pubertal Timing	.05 (.34)	.40 (.21)**	.55 (.22)*	.36 (.21)	.06 (.19)	.58 (.06)***	−0.31 (.55)	.34 (.38)	−0.03 (.24)	−0.03 (.03)
DHEA	.63 (.14)**	.09 (.10)	.28 (.06)***	.10 (.21)	.54 (.17)**	.36 (.06)***	.53 (.14)***	−0.45 (.11)***	−0.08 (.07)	−0.34 (.06)***
Progesterone	.04 (.18)	.74 (.14)***	.22 (.05)***	.26 (.24)	.40 (.22)	.34 (.04)***	−0.22 (.41)	.34 (.36)	−0.12 (.07)	.13 (.05)**
Testosterone	.55 (.30)	.38 (.22)	.07 (.10)	.44 (.17)*	.18 (.12)	.38 (.06)***	.11 (.35)	.20 (.27)	−0.31 (.11)**	.10 (.08)

Note. All models included race and sex-specific effects of age and age-squared as covariates and allowed for sex-specific means and residual variances in outcomes, with the exception of excluding age effects from the pubertal status model. Hormonal outcomes were additionally regressed on analytic batch. The last column on the right reports the difference in the residual variance across sexes, and the adjacent three columns report differences in proportions of variance attributable to *ACE* factors. Standard errors are given in parentheses.*** significantly different than zero at $p < .001$; ** $p < .01$; * $p < .05$

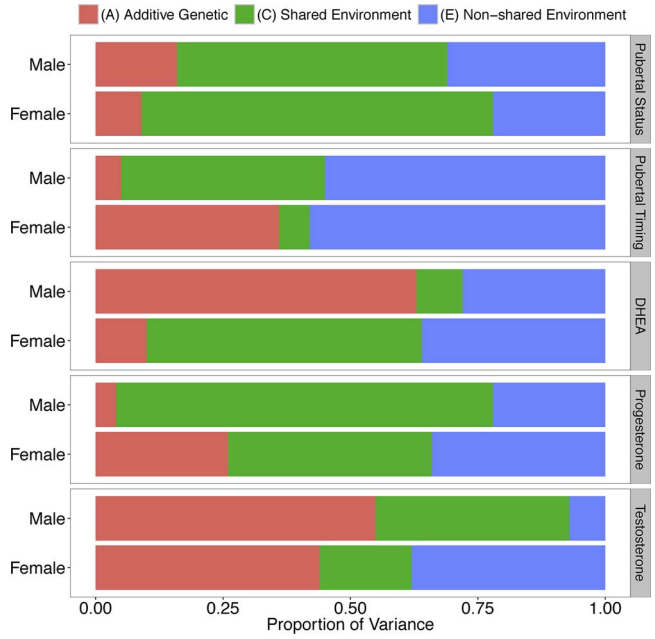

Fig. 1. Proportion of variance in each outcome explained by genetic (*A*) and environmental (*C* or *E*) factors from univariate models that allowed for sex differences in all *ACE* paths.

3.2.3. Nonparametric age moderation models

Univariate LOSEMs were then used to examine differences in sex-specific *ACE* estimates across age. Unstandardized variance by sex is depicted in Fig. 2, and proportions of variance are shown in Fig. S4. Age ranges of heightened heritability were calculated for each focal value of age for which the heritability estimate + 1 *SE* included the maximum observed heritability. Estimates for pubertal timing indicated that variability was due to a combination of genes and environment, but that the highest levels of heritability were observed at age 11.6 for females (50% heritable; age range: 10.9–13.2) and age 13.2 for males (34% heritable; age range: 11.2–18.0). Male progesterone was moderately heritable from ages 8–14, with a peak of 65% heritability at age 11.2 years (age range: 8.6–12.7). Conversely, variation in female progesterone was almost entirely attributable to environmental influences across the age range, with a maximum heritability of 38% (age range = 8.0–13.6). The heritability of male DHEA steadily declined across age, reaching levels of ~20% by age 16. The heritability of female DHEA peaked at age 10, followed by a drop to near 0 by age 14. Notably, DHEA was highly heritable at its peak in both sexes (99% at age 9.8 in males [age range: 8.0–10.4], 89% at age 10 in females [age range: 8.0–11.9]). Male testosterone evinced a gradual rise in heritability starting at age 9, followed by a peak of 100% heritability at age 12.5 (age range: 10.9–13.9), and subsequent decline to 0% heritability by age 15. Female testosterone showed a similar, but later, pattern starting at age 10, and reaching 100% heritability at age 15.2 (age range: 13.8–16.4).

3.3. Bivariate twin models

A series of six bivariate correlated factors models were used to examine genetic and environmental sources of covariation between DHEA, progesterone, testosterone, and pubertal status. Bivariate associations with pubertal timing are not reported, as the phenotypic associations were estimated near 0 in both sexes (Fig. S3), and twin models indicated this was not due to countervailing genetic and environmental inputs. Table 3 reports correlations between additive genetic (r_A), shared environmental (r_C), and non-shared environmental factors (r_E), as well as the contributions to phenotypic correlations using the within-sex standardized *ACE* path estimates.

Additive genetic predictors of DHEA and pubertal status were highly correlated in females ($r_A = 0.99$); however, this large correlation partly reflected the limited variance in both female DHEA and pubertal status explained by additive genetic factors. There was a significant correlation, via shared environmental pathways, between progesterone and a number of outcomes: male testosterone (phenotypic $r = 0.14$), female pubertal status (.18), and DHEA in males (.18) and females (.08). In addition, there were significant non-shared environmental correlations between progesterone and male DHEA (.09), and testosterone in males (.13) and females (.10).

Bivariate results revealed five significant sex differences. The shared environmental correlation between testosterone and progesterone was significantly higher in males ($\Delta = 0.49$, $SE = 0.19$, $p = .008$), as was the corresponding contribution to the phenotypic correlation ($\Delta = 0.21$, $SE = 0.07$, $p = .001$). Conversely, the shared environmental correlation between progesterone and pubertal status was significantly higher in females ($\Delta = -0.44$, $SE = 0.17$, $p = .010$). The correlation between non-shared environmental predictors of DHEA and progesterone ($\Delta = 0.46$, $SE = 0.20$, $p = .018$), and the contribution to the observed correlation ($\Delta = 0.13$, $SE = 0.06$, $p = .036$), was higher in males. Overall, results from bivariate twin models indicate that observed correlations among hormones characteristics of adrenarche and gonadarche reflect shared genetic *and* environmental influences.

4. Discussion

4.1. Summary and interpretation of windows of maximal heritability

The current paper presents cross-sectional findings from the largest twin study of pubertal hormones to date, and the first genetically informative study of a novel measure: concentrations of puberty-relevant hormones in hair. On average, genetic influences on DHEA were higher in males, and testosterone was highly heritable in both sexes. Variation in progesterone and both pubertal status and timing were largely

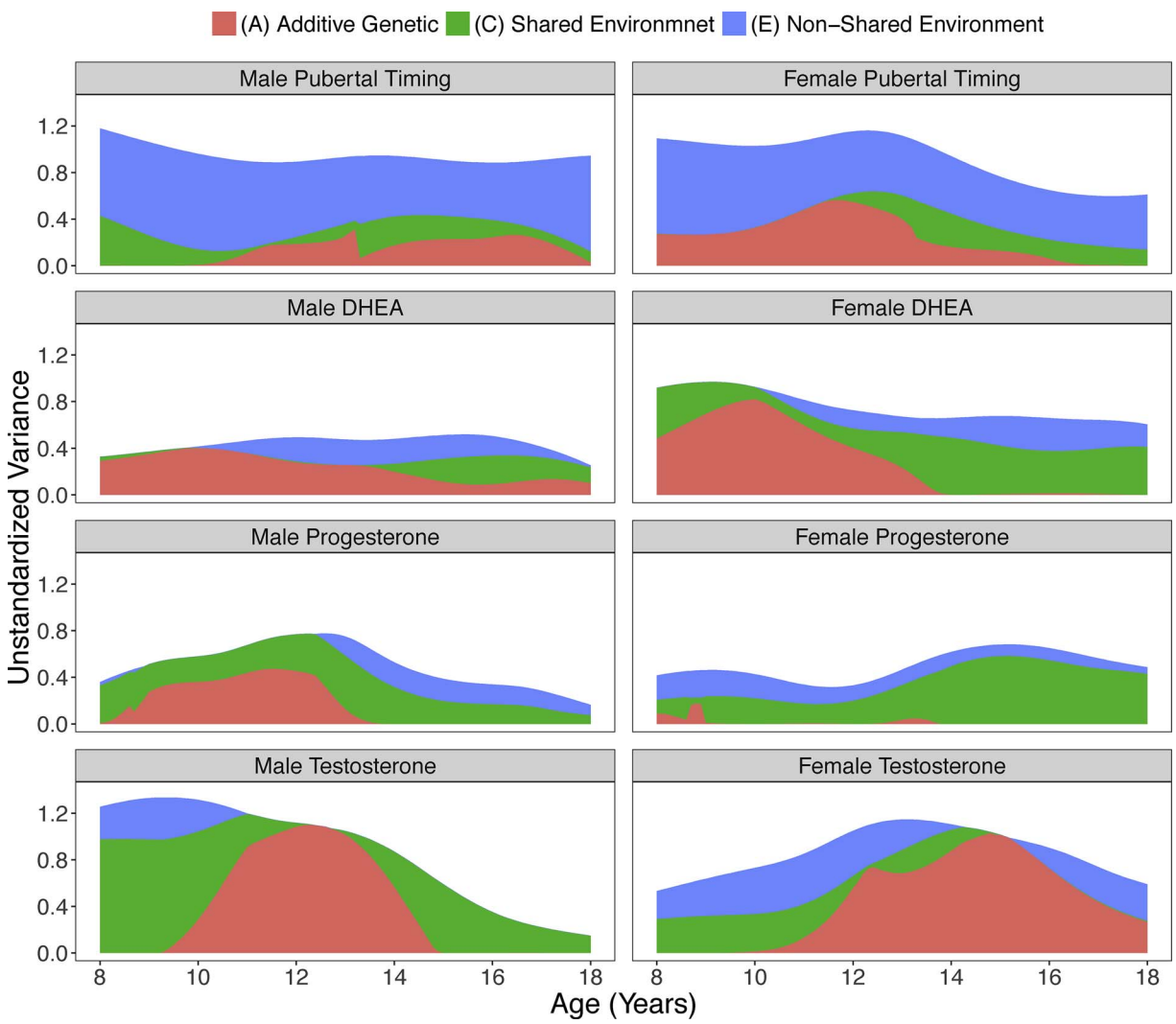

Fig. 2. Unstandardized variance explained by *ACE* components from univariate models estimated using LOSEM across age.

attributable to environmental influences. Age-specific analyses, however, indicated that these broad estimates aggregated over dramatic developmental differences. In both sexes, the heritability of DHEA peaked at age 10 and subsequently declined. For testosterone, heritability was near 0 at age 8, followed by an increase to near 100% heritability at age 12.5 years in males and 15.2 years in females. This suggests that genetic effects on pubertal hormones vary across adolescence and are characterized by specific periods of heightened heritability. Consequently, point estimates of heritability for pubertal hormones in age-heterogeneous child and adolescent samples are likely to be of limited value, beyond providing an indication of the "typical" heritability of the phenotype across a wide range of ages.

Periods of heightened hormonal heritability might reflect genetic influences on variation in the degree to which a hormone is synthesized, variation in the onset of a hormonal event (i.e., intercept), and variation in the speed at which this hormonal event progresses (i.e., tempo or slope). Additionally, gene × environment interactions, when not modeled in a classical twin model, will inflate estimates of *A* variance when the environmental moderator is unique to each twin. Thus, heightened hormonal heritability could also reflect heightened genetically-associated responsiveness to environmental inputs. These interpretive ambiguities are not unique to hair samples but are also present for cross-sectional designs using salivary samples.

It is plausible, but not definitive, that the current analyses were largely picking up on heritability in the onset and tempo of gonadarche and adrenarche. In the most compelling example of this account, genetic influences on male testosterone were identified between 9 and 15 years of age, which coincides exactly with the range of gonadarche onset in males (Blakemore et al., 2010). Conversely, given earlier onset of gonadarche in females, it may be surprising that the peak heritability of testosterone occurred earlier in males, relative to females. For females, it may be that variation in testosterone reflects the rate of hormonal change, rather than the onset of this change. Future research should examine whether hormones more closely linked to female pubertal development (e.g., estradiol, androstenedione) produce earlier windows of maximal heritability. For both sexes, the peak heritability of DHEA at 10 years of age closely followed adrenarche onset, which is typically 6–9 years of age in females and 7–10 years of age in males (Blakemore et al., 2010). The delay between onset of adrenarche and peak heritability observed in the current sample might be partly due to the age range of our participants, which began near the end of when adrenarche typically begins. It is also possible that adrenarche is a more gradual process involving multiple events beyond the initial onset of adrenal maturation. Genetic signals for DHEA, as well as for female testosterone, might then reflect a combination of onset and progression through adrenarche and gonadarche, respectively.

4.2. Potential applications for research and clinical practice

Knowledge of when hormonal biomarkers are maximally heritable

Table 3
ACE correlations and contributions to phenotypic correlations.

	r_A				r_C				r_E			
	DHEA	T	P	Puberty	DHEA	T	P	Puberty	DHEA	T	P	Puberty
DHEA	–	.21 (−0.57, 0.99)	−0.27 (−1.32, 0.77)	.99*** (.54, 1.44)	–	−0.05 (−0.60, 0.50)	.17* (.02, 0.31)	.22 (−0.17, 0.60)	–	.03 (−0.16, 0.22)	−0.11 (−0.37, 0.14)	−0.69 (−1.98, 0.60)
T	.29 (−0.05, 0.63)	–	.02 (−0.47, 0.51)	.04 (−0.90, 0.99)	.04 (−0.30, 0.39)	–	−0.22 (−0.57, 0.13)	.01 (−0.48, 0.48)	−0.13 (−1.10, 0.83)	–	.28* (.03, 0.54)	.02 (−0.31, 0.36)
P	−0.26 (-0.70, 0.18)	.01 (−0.65, 0.68)	–	−0.10 (−0.92, 0.71)	.62* (.09, 1.16)	.27** (.07, 0.46)	–	.41* (.06, 0.76)	.35* (.06, 0.64)	1.00 (−0.53, 2.53)	–	.16 (−0.14, 0.46)
Puberty	.65 (−1.04, 2.34)	.69 (−0.38, 1.78)	−0.53 (−4.42, 3.37)	–	.27 (−1.18, 1.72)	−0.06 (−0.53, 0.42)	−0.03 (−0.33, 0.26)	–	.15 (−0.28, 0.58)	.06 (−0.62, 0.74)	−0.08 (−0.37, 0.20)	–
	A contribution				*C* contribution				*E* contribution			
	DHEA	T	P	Puberty	DHEA	T	P	Puberty	DHEA	T	P	Puberty
DHEA	–	.04 (−0.14, 0.22)	−0.04 (−0.16, 0.07)	.33 (−0.02, 0.67)	–	−0.02 (−0.19, 0.16)	.08* (.01, 0.15)	.11 (−0.13, 0.34)	–	.01 (−0.06, 0.08)	−0.04 (−0.13, 0.05)	−0.12 (−0.28, 0.03)
T	.17 (−0.01, 0.36)	–	.01 (−0.15, 0.16)	.01 (−0.25, 0.27)	.01 (−0.06, 0.08)	–	−0.07 (−0.17, 0.04)	< 0.01 (−0.25, 0.27)	−0.02 (−0.15, 0.11)	–	.10* (.01, 0.19)	.01 (−0.09, 0.10)
P	.04 (−0.15, 0.23)	−0.01 (−0.09, 0.07)	–	−0.03 (−0.25, 0.19)	.18* (.04, 0.35)	.14** (.05, 0.24)	–	.18* (.02, 0.33)	.09* (.01, 0.17)	.13** (.03, 0.22)	–	.04 (−0.04, 0.11)
Puberty	.07 (−0.39, 0.52)	.11 (−0.20, 0.20)	−0.01 (−0.21, 0.19)	–	.07 (−0.31, 0.44)	−0.03 (−0.25, 0.20)	−0.02 (−0.24, 0.19)	–	.05 (−0.09, 0.18)	.01 (−0.11, 0.13)	−0.02 (−0.10, 0.05)	–

Note. Values for males are reported on the lower diagonals, and for females on the upper diagonals. The top rows report correlations between *ACE* components and the bottom rows the raw *ACE* contribution to observed phenotypic correlations. *ACE* estimates were standardized within sex. 95% confidence intervals are presented in parentheses. *** significantly different than zero at $p < .001$; ** $p < .01$; * $p < .05$

can inform research designs and has potential clinical applications. Most straightforwardly, power to identify specific genetic loci increases with increasing heritability. Power considerations are particularly critical for genome-wide association studies (GWAS), as the individual effect sizes of genetic loci are very small. Even for large adult cohorts, GWAS has resulted in the ability to detect only three independent genetic loci for testosterone ($N = 3225$, Jin et al., 2012; $N = 8938$, Ohlsson et al., 2011) and no genetic loci for estradiol ($N = 1703$, Prescott et al., 2012). Future studies should, therefore, carefully consider the age range of selected cohorts, as power will likely be maximized when cohorts are taken from relatively narrow age bands that vary by the hormone under consideration. Power may remain static across ages only if the number of individual genetic loci affecting the trait increases during periods of increased heritability, while the average effect size across trait-affecting loci is constant. Even in this scenario, it would still be advantageous for genome-wide research to focus on age ranges of maximum heritability, as genetic loci that are offline at different ages would otherwise remain undiscovered (regardless of sample size). Investigations of environmental determinants of hormone concentrations might also seek to recruit participants during ages when environmental influences are high. It is currently unclear what these environments might be, though prior research points to the importance of the intrauterine environment. For example, prenatal maternal smoking was found to predict adult daughters' testosterone levels (Kandel and Udry, 1999). Aspects of the prenatal environment, such as nicotine exposure, might then interact with factors unique to adolescence (e.g., pubertal onset) to shape adolescent testosterone levels and correspondingly increase latent estimates of environmental influence.

Periods when hormone heritability increases dramatically has three possible implications for hormone-behavior associations. First, it may be that the increase in heritability results in a corresponding increase in the phenotypic correlation. Second, the phenotypic association may remain constant if there is a corresponding decrease in environmental contributions to the hormone-behavior association. Finally, the phenotypic association may also remain stable if the increase in hormone heritability is unrelated to the genetic components of the behavioral outcome. Whether hormonal biomarkers are especially predictive of individual differences in behavior during developmental periods when hormones are maximally heritable remains an intriguing hypothesis for future research. The present findings may also be leveraged to improve clinical decision-making if clinicians know that, in a population-based sample of adolescents, elevations or depressions in hormonal levels are more likely to reflect environmental inputs at some ages than at others.

The current findings regarding the developmental genetics of pubertal hormones might also offer insights into the mechanisms underlying behavioral development and psychiatric morbidity. Animal models suggest that pubertal hormones structurally reorganize the brain during developmentally sensitive periods, particularly in subcortical areas and reward-related systems (reviewed by Blakemore et al., 2010). For example, rats castrated before puberty have been shown to have higher levels of androgen receptors in the amygdala than rats castrated post-puberty (Romeo et al., 2000), indicating that hormone levels fine-tune their receptor systems within specific developmental windows during puberty. An emerging literature suggests hormone-driven reorganization of the brain also applies to puberty in humans (Schulz et al., 2009). For example, testosterone levels in adolescence are associated with decreased gray matter volumes in the hippocampus and increased amygdala volume in both sexes (Neufang et al., 2009). DHEA levels have also been associated with increased cortical thickness specifically from ages 4–13 (Nguyen et al., 2013). As variation in pubertal hormones appears to be highly heritable during periods when the brain is particularly sensitive to hormonal input, it is critical for future research to identify what causes these genetic signals to come online.

4.3. Limitations and future research

Future research will need to examine the reliability and validity of hair measures of pubertal hormones in general, with a particular focus on more female-relevant hormones (i.e., progesterone, estradiol). In our sample, self-reported pubertal development was only modestly associated with hormone variation, consistent with previous literature on salivary measures of hormones (for review see, Harden et al., 2014a). This finding likely reflects measurement error, to some extent, but also underscores that observable metrics of the development of secondary sex characteristics at puberty (e.g., deepening of voice) fail to capture the entirety of the biological shifts occurring in adolescence. The Pubertal Development Scale may also have failed to capture relevant, hormonally influenced changes in physical characteristics (e.g., testicular growth), and future research should incorporate both physician and self-report ratings of development. These results should generally be treated with caution as hair based assays are novel and yet to be extensively researched. Limited information on the reliability of hair assays makes it particularly difficult to interpret estimates of non-shared environment, as these estimates may represent influences on hormonal variation unique to the twin, measurement error, or a combination of the two. That is, when reliability of the assay is high, non-shared environmental influences are less likely to reflect measurement error.

Future research should examine the replicability of our cross-sectional findings using a longitudinal design that examines the heritability of intra-individual hormone change across adolescence. It will also be informative to examine DHEA in a younger sample of twins to examine trends in heritability prior to, and at the onset of, adrenarche. Additional studies on adolescent estradiol in hair are also needed to determine whether values below the detection limit were unique to the current sample. It may be that hair samples obtained from adolescents are useful only for select hormones (e.g., testosterone but not estradiol). That dizygotic twin estimates for progesterone were lower than monozygotic estimates also indicates imprecision in these estimates.

5. Conclusions

Adolescence is a unique developmental period that involves sweeping biological, psychological, and social changes that reverberate through the rest of the lifespan. The current study adds to a presently sparse literature on genetic variation in hormones and is the first to identify developmental periods of heightened genetic influences on pubertal hormones. Future work may benefit from testing whether these periods of heightened heritability mark periods of reorganizational effects on neural systems that canalize behavioral and cognitive trajectories.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Contributors

K. P. Harden and E. M. Tucker-Drob developed the study concept and design. A. Grotzinger, M. Patterson, and F. Mann performed data collection; data collection was supervised by K. P. Harden, E. M. Tucker-Drob, and J. Tackett. A. Grotzinger performed the data analysis

and interpretation under the supervision of K. P. Harden and E. M. Tucker-Drob. A. Grotzinger drafted the manuscript, and all authors provided critical revisions. All authors approved the final version of the manuscript for submission.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.psyneuen.2018.02.005.

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