Familial Correlations in Postmenopausal Serum Concentrations of Sex Steroid Hormones and Other Mitogens: A Twins and Sisters Study

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Background: Serum concentrations of some hormones are risk factors for certain cancers, but little is known about their familial associations especially for females.

Methods: We measured serum concentrations of estradiol (E_2), testosterone (T), SHBG, prolactin, and IGF-I for 645 Australian female postmenopausal twins and their sisters [182 monozygotic (MZ) and 107 dizygotic (DZ) pairs and 67 nontwin sisters] using well-established immunoassays. After suitable transformation and adjusting for age, body mass index (BMI), and time since menopause, familial correlations and proportions of variance attributed to genetic (h^2) and nongenetic factors common to sisterships (c^2) were estimated under the classic twin multivariate normal model using FISHER.

Results: For all serum concentrations except prolactin, MZ, DZ, and sister pairs were correlated ($P < 0.001$). MZ correlations were in the range 0.5–0.7, and for all serum concentrations, there were no differences between DZ and sister correlations. MZ correlations were greater than DZ and sister correlations for log SHBG ($P = 0.0001$), IGF-I ($P = 0.0002$), and square-root T ($P = 0.007$) but not log E_2 ($P = 0.3$), and the respective h^2 estimates were 0.56 (SE = 0.14), 0.53 (0.17), 0.39 (0.14), and 0.14 (0.16). For log E_2 and square-root T, c^2 estimates were 0.39 (0.14) and 0.22 (0.12).

Conclusion: There are strong familial correlations in postmenopausal SHBG, IGF-I, and to a lesser extent T, which are consistent with a genetic etiology. For E_2, and to a lesser extent T, correlations are consistent with substantial nongenetic familial factors. The latter might include maternal effects. **(*J Clin Endocrinol Metab* 94: 4793–4800, 2009)**

Circulating sex hormones estradiol (E_2) and testosterone (T), other hormones such as IGF-I and prolactin, and SHBG have been shown to be important biomarkers for breast cancer. The risk of breast cancer for postmenopausal women has been found by overview analysis to be positively associated with serum E_2 and T concentrations and negatively associated with SHBG concentrations (1). For E_2 and SHBG, the relationships with breast cancer risk have been partly but not wholly explained by their associations with body mass index (BMI) (2). These findings

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Abbreviations: BMI, Body mass index; CI, confidence interval; DZ, dizygotic; E_2, estradiol; HRT, hormone replacement therapy; MZ, monozygotic; T, testosterone.

have led to serum estrogen concentrations being assessed as intermediate markers of breast cancer risk by studies of genetic polymorphisms (3) and also to being considered for inclusion in an integrative approach to risk evaluation for widespread use (4). IGF-I has been found to be associated with mammographic density and breast cancer risk with the association being more pronounced for premenopausal women (5, 6). Prolactin has recently been reported to be associated with the risk of estrogen-receptor-positive disease for postmenopausal women (7).

Although the mean concentrations of these biomarkers are associated with age and, for some measures, BMI and time since menopause, the reasons for their large residual variances are not understood. The few twin and family studies that have tried to estimate the relative importance of genetic and environmental influences on variation in concentrations of various sex hormones and/or other mitogens have either involved only males (8–10) or presented results for males and females combined (11).

The purpose of this study of the above serum concentrations of sex hormones and other mitogens in postmenopausal Australian twins and their sisters was to measure twin and sister correlations and, hence, estimate the contributions of genetic and shared familial environmental factors to explain residual variance under an extension of the equal environments assumption of the classic twin model. By including the sisters of twins, we enabled an extra contrast to assess the relative roles of genes and common sistership in explaining familial correlations.

Subjects and Methods

Participants

Participants were selected from sisterships identified through both monozygotic (MZ) and dizygotic (DZ) female twin pairs recruited to the Australian Twins and Sisters Mammographic Density Study (12). In brief, twin pairs were identified from and initially approached by the Australian Twin Registry and then asked to invite their sisters also to participate. Participation involved a blood sample collected at specified pathology laboratories and a structured questionnaire by telephone interview.

Women were eligible for this study of postmenopausal women if they neither had a menstrual period nor used hormone replacement therapy (HRT) within the 12 months before the interview that preceded blood collection. The questionnaire collected information including weight, height, smoking history, alcohol consumption, reproductive history, cessation of menstruation, use of oral contraceptives and HRT, and family history of cancer.

The zygosity of twin pairs was determined by a standard question that describes the differences between identical and nonidentical pairs. For pairs whose answers were contradictory, or were uncertain, zygosity was determined using additional questions and methods for classifying responses that have been shown to give 95% agreement with zygosity based on blood typing in middle-aged adults (13–15).

This study was approved by The University of Melbourne Research Ethics Committee, and informed consent was obtained for each participant.

Blood collection, serum preparation, and transportation

Subjects were asked to donate an extra 8.5 ml blood for this study, in addition to the standard request of 27 ml for the Australian Twins and Sisters Mammographic Density Study. The extra blood was collected in an SST gel tube and left to clot at room temperature for 45 min to 3 h before centrifugation at $1500 \times g$ for 10 min. Four 1-ml fractions of serum were prepared and immediately stored at −20 C. Serum fractions were transported on a monthly basis (or as required) to the central Melbourne laboratory on dry ice in eight batches. Large shipments were sent from Melbourne to London on dry ice for analysis.

Laboratory methods

Samples were stored at −20 C for 2–6 wk at the Royal Marsden Hospital, London before assay, and all immunoassays were performed in duplicate. Sisterships within families were assayed in the same batch (a run of samples made using the same lot number calibrators and reagents on the same day). The reported coefficients of variation were based on matrix comparable quality control materials.

E_2 was measured by RIA after ether extraction (16). The within- and between-batch coefficients of variation were 6.2 and 12.0%, respectively, at a concentration of 30 pmol/liter. Blinded within-batch replicates gave coefficients of variation from 4–14.3% (16). Our published data from application of this assay in split samples from postmenopausal women showed excellent correlations ($r = 0.94$) with those from tandem mass spectrometry (17). The lowest limit of detection was 3.0 pmol/liter.

Total T was measured using a solid-phase RIA kit (Diagnostic Products Corp., Los Angeles, CA). The within- and between-batch coefficients of variation were 9.0 and 11.0%, respectively, at a concentration of 2.6 nmol/liter. The lowest limit of detection was 0.14 nmol/liter.

IGF-I was measured using a two-site immunoradiometric assay kit (Diagnostic Systems Laboratories, Webster, TX). The within- and between-batch coefficients of variation were 1.5 and 3.7%, respectively, at a concentration of 34 nmol/liter. The lowest limit of detection was 1.3 nmol/liter.

SHBG was measured by a solid-phase chemiluminescent immunometric assay using an IMMULITE 1000 autoanalyzer (Diagnostic Products). The within-batch coefficient of variation was 4.1% at a concentration of 64 nmol/liter, whereas the between-batch coefficient of variation was 8.0% at a concentration of 35 nmol/liter. The lowest limit of detection was 0.2 nmol/liter.

Prolactin was measured by a solid-phase two-site chemiluminescent immunometric assay using an IMMULITE 1000 autoanalyzer (Diagnostic Products). The within-batch coefficient of variation was 6.8% at a concentration of 170 mIU/liter, whereas the between-batch coefficient of variation was 9.6% at a concentration of 299 mIU/liter. The lowest limit of detection was 11 mIU/liter.

Statistical methods

The variance and covariance structure was analyzed in two ways. First, descriptive models involved fitting the residual variance (σ^2) and separate correlation coefficients for MZ (ρ_{MZ}), DZ (ρ_{DZ}), and sister (ρ_{Ss}) pairs or for DZ and sister pairs combined (ρ_{DZS}). Note that sister pairs could include one twin. Second, variance components models were fitted. The residual variance was partitioned into three components of variance: σ_a^2, representing the effects of additive genetic factors; σ_c^2, representing the effects of environmental (*i.e.* nongenetic) factors that are common to sisterships within the same family; and σ_e^2, representing person-specific environmental factors, including measurement error. Note that $\sigma^2 = \sigma_a^2 + \sigma_c^2 + \sigma_e^2$ is the residual variance after adjusting the mean for covariates.

Under the assumption that the effect of nongenetic factors common to sisterships and specific to the measure of interest are the same for all sister pairs, the correlation between pairs is $(\sigma_a^2 + \sigma_c^2)/\sigma^2$ for MZ pairs and $(0.5\sigma_a^2 + \sigma_c^2)/\sigma^2$ for DZ and sister pairs (18). This includes the assumption of the classic twin model, in which σ_c^2 is assumed to be independent of zygosity. Under this model, the proportion of residual variance attributed to additive genetic factors (heritability) is $h^2 = \sigma_a^2/\sigma^2$ whereas $c^2 = \sigma_c^2/\sigma^2$ is the proportion of residual variance attributed to nongenetic effects common to sisterships.

We modeled the extent to which relatives being measured in the same batch might explain familial correlations by conducting analyses in which an additional variance component, σ_b^2, was included, assuming that the correlation between relatives measured in the same batch was increased by σ_b^2/σ^2.

We fitted mixed-effects linear models under maximum likelihood theory assuming multivariate normality using the statistical package FISHER (19–21). Estimation of parameters and SE and statistical inference were based on standard asymptotic likelihood theory. Tests of means were based on Student's *t* test and ANOVA. All quoted *P* values are nominal and two sided, and following convention, values greater than 0.05 were not considered statistically significant.

Statistical analysis under maximum likelihood theory makes optimal use of the data by taking into consideration all sister pairings and allows for testing of differences between correlation and other estimates based on the likelihood ratio test. In particular, whether ρ_{MZ} is greater than ρ_{DZ}, or for that matter ρ_{Ss}, is a test of the hypothesis that there are no genetic factors influencing variation. On the other hand, the finding that ρ_{MZ} is greater than ρ_{DZ} or ρ_{Ss} is consistent with, but does not prove that, genetic factors exist under the model assumptions. Whether ρ_{DZ} is greater than ρ_{Ss} is a test of whether the common sistership effects are greater within (DZ) pairs than within sister pairs.

We conducted various tests of the model assumptions and identification of potentially influential women and sisterships (outliers) (19). As a consequence, we found that the distributions of the residuals did not differ substantially from multivariate normal for IGF-I, for E_2, SHBG, and prolactin concentrations when log transformed, and for T concentrations when square-root transformed.

Results

A total of 645 women, comprising 182 MZ pairs, 107 DZ pairs, and 67 nontwin sisters, provided blood samples. There were 299 sisterships of which 242 were twin pairs alone (153 MZ, 89 DZ), 41 were a twin pair and one sister (26 MZ, 15 DZ), five were a twin pair and two sisters (three MZ, two DZ), five were two sisters (no twins), one was two sets of DZ pairs, and six were women with no sisters. This gave a total of 117 nontwin sister pairings, although these were not necessarily independent of either each other or the twin pairs. Participants were excluded if BMI was missing (two women) or if blood measurement failed. In addition, for analyses involving E_2 only, 49 women were excluded because their recorded E_2 level was higher than 150 pmol/liter, highly inconsistent with menopausal status. For the other hormones, statistical analysis was performed both with and without these individuals in the dataset, and the results did not differ substantially.

Table 1 summarizes subject characteristics and mean serum concentrations by relationship and zygosity. The mean age was approximately 61 yr (range 43–78), and the time since menopause was on average about 14 yr (range 1–44). Serum concentrations of E_2, T, SHBG, IGF-I, and prolactin were below the level of detection for two, 129, zero, zero, and one subject, respectively. In this instance, the corresponding minimum detection level was used. There were no differences in means between MZ twins, DZ twins, and nontwin sisters for any characteristic or hormone level (all $P > 0.2$). Figure 1 shows plots of the unadjusted serum concentrations for MZ and DZ pairs.

Table 2 shows, for each measure, estimates of the residual variance and of the MZ, DZ, and sister pair correlations. It also shows the correlation for DZ and sister pairs combined and *P* values for comparison of the latter with the correlation for MZ pairs, a test of the null hypothesis that genetic factors do not influence residual variation.

For log E_2, the mean was negatively associated with age ($P = 0.01$) and positively associated with BMI ($P < 0.0001$) and time since menopause ($P = 0.05$). The residual correlation for MZ pairs was 0.54 [95% confidence interval (CI) 0.44–0.64]. Although this was numerically greater than the 0.44 (95% CI 0.26–0.62) for DZ pairs, the difference was not significant ($P = 0.3$). The correlation for sister pairs was 0.51 (95% CI 0.31–0.71), similar to that for DZ pairs, and when pooled, it was 0.46 (95% CI 0.32–0.60) and also not different from the MZ pair correlation ($P = 0.3$). There was therefore no evidence that genetic factors influence variation in E_2.

For T, the square-root transformed mean was not associated with age and was positively associated with BMI ($P = 0.05$). The residual correlation for MZ pairs of 0.63 (95% CI 0.55–0.71) was greater than that for the DZ and sister pairs [0.44 (95% CI 0.32–0.56), $P < 0.007$]. There-

Table 1. Characteristics of female MZ and DZ twins and their sisters

Characteristic	MZ twin (n = 364)	DZ twin (n = 214)	Sisters (n = 67)	*P* value
Age (yr)	60.7 (6.6)	60.9 (7.0)	62.1 (5.5)	0.3
BMI (kg/m^2)	26.4 (4.9)	26.7 (5.1)	27.9 (5.9)	0.09
Parity (% parous)	87.6	87.4	92.5	0.5
No. of live births	2.5 (1.5)	2.6 (1.5)	2.9 (1.5)	0.1
Age at first birth (yr)	24.7 (4.6)	25.1 (4.5)	23.8 (4.4)	0.2
Oral contraceptive use (% ever)	81.6%	84.1%	77.6%	0.5
Time since menopause (yr)	13.8 (8.8)	14.4 (8.3)	14.5 (8.9)	0.7
HRT use (% ever)	45.6	43.9	52.2	0.5
E_2 (pmol/liter)	19.0 (7.9–52.0)	19.0 (8.4–54.0)	16.0 (9.8–43.0)	
T (nmol/liter)	0.54 (0.14–1.17)	0.49 (0.14–1.05)	0.54 (0.14–1.14)	
SHBG (nmol/liter)	48.0 (26.0–85.0)	48.0 (27.0–79.0)	44.0 (28.0–78.0)	
IGF-I (nmol/liter)	32.0 (17.0–52.0)	34.0 (20.0–50.0)	30.0 (17.0–48.0)	
Prolactin (mIU/liter)	137.5 (79.0–258.0)	132.0 (76.0–241.0)	131.0 (88.0–235.0)	
Transformed				
Log E_2	2.9 (0.7)	3.0 (0.7)	2.8 (0.6)	0.3
Square root T	0.74 (0.28)	0.70 (0.25)	0.71 (0.27)	0.2
Log SHBG	3.9 (0.4)	3.8 (0.4)	3.8 (0.4)	0.8
Log prolactin	4.9 (0.5)	4.9 (0.5)	5.0 (0.5)	0.9

Results are shown as means and SD or proportions or medians and 10th–90th percentiles, where applicable. *P* values testing differences in means and proportions were calculated via ANOVA and χ^2 tests, respectively, on two degrees of freedom.

fore, there was evidence consistent with genetic factors influencing some variation in T.

For log SHBG, the mean was positively associated with age ($P = 0.0009$) and negatively associated with BMI ($P < 0.0001$) and time since menopause ($P = 0.05$). The residual correlation for MZ pairs was 0.70 (95% CI 0.64–0.76), almost twice the 0.39 (95% CI 0.23–0.55) for DZ and the 0.46 (95% CI 0.26–0.66) for sister pairs (all $P < 0.005$). The approximately 2:1 ratio of these correlations is consistent with familial correlations being due to additive effects of one or more variants in one or more genes.

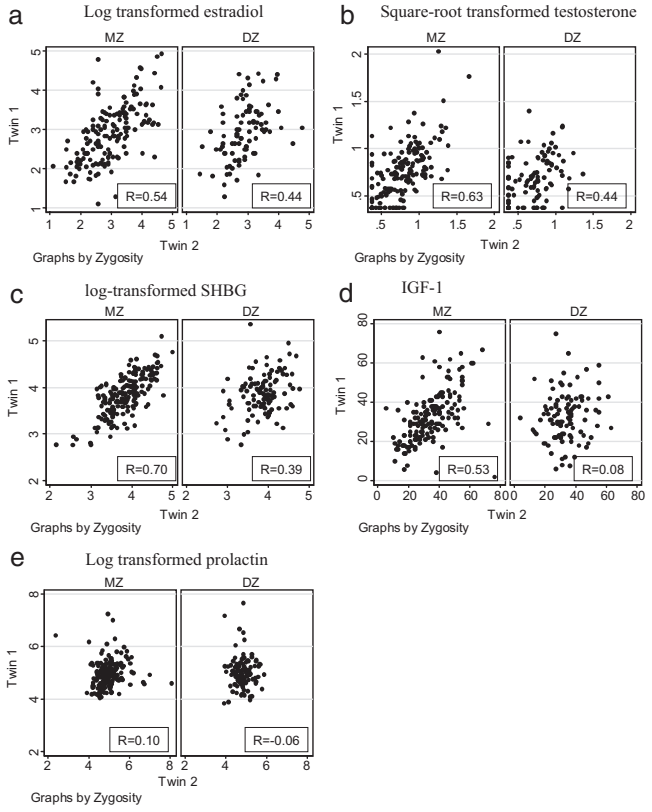

FIG. 1. Correlations in serum concentrations of sex steroid hormones and other mitogens for MZ and DZ pairs of Australian twins.

For IGF-I, the mean was negatively associated with age ($P = 0.001$). The residual correlation for MZ pairs of 0.53 (95% CI 0.43–0.63) was greater than the 0.08 (95% CI −0.14–0.3) for DZ and the 0.32 (95% CI 0.16–0.48) for sister pairs and more than twice the pooled estimate for DZ and sister pairs of 0.22 (95% CI 0.08–0.36) (all $P < 0.01$). (The latter two correlations did not differ; $P = 0.08$.) These correlations are consistent with variation being due at least in part to additive genetic factors.

For log prolactin, the mean was not associated with any of the measured covariates. There was no evidence of within-pair correlation for either MZ, DZ, or sister pairs, with estimates of 0.10 (95% CI −0.06–0.24), −0.06 (95% CI −0.24–0.12), and 0.12 (95% CI −0.10–0.34), respectively, or for all pairs combined for which the correlation was 0.004 (95% CI −0.14–0.14). The MZ pairs correlation was no different to the DZ and sister pair correlations (all $P > 0.1$). Thus, there was no evidence that familial factors explain variation in prolactin.

Table 3 shows the results of fitting the full model involving additive genetic, common sistership, and individual specific components of variance. For every measure, the estimates of A and C were highly negatively correlated (around −0.9). The results of fitting other specific models are included below.

For log E_2, the common sistership factor was significant ($P = 0.008$), and once fitted, the additive genetic factor

Table 2. Estimates for the residual variance and MZ, DZ, and sib pair correlations (and their 95% CI) for each of the measures adjusted for age at blood collection and BMI

	Residual variance	MZ correlation	DZ correlation	Sib correlation	DZ/sib correlation	*P* value (MZ *vs.* DZ/sib)	Age	BMI
Log E_2 ($n = 594$)[a]	0.41 (0.35–0.47)	0.54 (0.44–0.64)	0.44 (0.26–0.62)	0.51 (0.31–0.71)	0.46 (0.32–0.60)	0.3	−0.016 (−0.028–0.028)	0.056 (0.046–0.066)
Square root T ($n = 622$)	0.07 (0.06–0.08)	0.63 (0.55–0.71)	0.44 (0.28–0.6)	0.44 (0.28–0.60)	0.44 (0.32–0.56)	0.007	0.0002 (−0.0038–0.0042)	0.004 (0.0–0.008)
Log SHBG ($n = 643$)[a]	0.16 (0.14–0.18)	0.70 (0.64–0.76)	0.39 (0.23–0.55)	0.46 (0.26–0.66)	0.41 (0.27–0.55)	<0.0001	0.010 (0.004–0.016)	−0.034 (−0.04 to −0.028)
IGF-I ($n = 610$)	158.65 (139.52–177.78)	0.53 (0.43–0.63)	0.08 (−0.14–0.3)	0.32 (0.16–0.48)	0.22 (0.08–0.36)	0.0002	−0.28 (−0.46 to −0.10)	−0.05 (−0.25–0.15)
Log prolactin ($n = 642$)	0.28 (0.24–0.32)	0.10 (−0.06–0.24)	−0.06 (−0.24–0.12)	0.12 (−0.10–0.34)	0.004 (−0.14–0.14)	0.3	−0.01 (−0.016 to −0.004)	0.001 (−0.007–0.009)

[a] Estimates are also adjusted for time since menopause.

was not significant ($P = 0.4$). Under the full model, the heritability was 14% with a wide 95% CI from −17–45%. The common sistership component explained 39% (95% CI 12–66%) of residual variance when the additive component was included.

For log T, the additive genetic factor was significant ($P = 0.005$), and once fitted, the common sistership factor was marginally significant ($P = 0.07$). The additive genetic component explained 39% (95% CI 12–66%) of residual variance after adjustment for common sistership factors, which explained 24% (95% CI −2–50%) of variance.

For log SHBG, the additive genetic factor was highly significant, and once fitted, the common sistership factor was negligible and not significant ($P = 0.3$). The additive genetic component explained 56% (95% CI 29–83%) of residual variance when the common sistership factor was constrained to be nonnegative.

For IGF-I, the additive genetic factor was highly significant, and once fitted, the common sistership factor was estimated to be negative and not significant ($P = 0.6$). The additive genetic component explained 56% (95% CI 29–83%) of residual variance when the common sistership component was constrained to be nonnegative.

For log prolactin, both the additive genetic and common sistership factors were not significantly different from zero ($P = 0.4$ and $P = 0.6$, respectively).

The component of variance for batch effects was not significant for any measure, although for IGF-I, the evidence was marginal ($P = 0.05$) and explained 39.82/158.65 = 25% of residual variance. If a batch component of variance was fitted, all the correlations reduced: from 0.53 to 0.44 (SE 0.10) for MZ pairs, from 0.08 to −0.22 (0.24) for DZ pairs, from 0.32 to 0.23 (0.12) for sister pairs, and from 0.22 to 0.12 (0.08) for DZ and sister pairs. The evidence for a genetic component of variance for IGF-I, based on differences in correlations between MZ and non-MZ pairs, remained.

Discussion

Clear, but not necessarily the same, patterns emerged for the twin and sister correlations of the various serum concentrations. The data for E_2 were inconsistent with genetic factors causing variation because there were no differences between the MZ and non-MZ pair correlations. On the other hand, for SHBG and IGF-I, the MZ pair correlation was close to twice the non-MZ pair correlations, and the DZ and sister pair correlations were similar. This is consistent with the prediction of an additive genetic model, so that virtually all the familial correlations might be due to genetic factors. For T, an intermediate situation existed. Although there was evidence to support a role for genetic

Table 3. Estimates of components of variance and proportion of residual variance attributed to genetic factors (h^2) and shared environmental factors (c^2) after adjusting for covariates as in Table 2

	σ_a^2	σ_c^2	σ_e^2	h^2	c^2
Log E_2 (n = 594)	0.058 (0.065)	0.16 (0.06)	0.19 (0.02)	0.14 (0.16)	0.39 (0.14)
Square root T (n = 622)	0.028 (0.010)	0.018 (0.010)	0.027 (0.003)	0.39 (0.14)	0.24 (0.13)
Log SHBG (n = 643)	0.089 (0.021)	0.021 (0.020)	0.047 (0.005)	0.56 (0.14)	0.13 (0.13)
IGF-I (n = 610)	97.57 (27.88)	−13.11 (23.41)	74.90 (7.98)	0.61 (0.17)	−0.08 (0.15)
Log prolactin (n = 642)	0.050 (0.056)	−0.024 (0.044)	0.25 (0.02)	0.18 (0.20)	−0.09 (0.16)

SE are shown in *parentheses*.

factors, there was also marginal evidence of a role for common sistership factors. The standard approach to modeling twin data for these datasets would first eliminate a role for common sistership effects, then attribute all familial correlations to genetic factors, and consequently claim higher heritabilities despite the weak, at best, evidence against the null hypothesis of no genetic effect.

There do not appear to be any other published reports of familial correlations in these measures for females alone. Ring and colleagues (8) measured several sex hormones for 134 MZ and 132 DZ adult white male twin pairs aged 59–70 yr. They do not appear to have transformed the measures, so analyses could be unduly influenced by extreme values. Nevertheless, they found for these elderly men, as did we for postmenopausal women, that there were substantial familial correlations (of unadjusted values) for E_2, T, and SHBG with MZ correlations from 0.40–0.68 and DZ correlations from 0.39–0.58 (all SE, although unreported, were around 0.10). There were also fixed effects of age and BMI on the means of all measures. For age- and BMI-adjusted E_2, the MZ and DZ correlations were 0.69 and 0.56, respectively. It can be determined from their published ACE and CE model fit diagnostics that these were not different (χ_1^2 =3.69; P = 0.05). That is, similar to our finding for postmenopausal women, there were high correlations in E_2 that did not differ according to genetic relationship.

In that study of males, the MZ correlation for age- and BMI-adjusted T was 0.56 compared with 0.34 for DZ pairs, and these correlations were different (χ_1^2 = 10.58 − 4.36 = 6.22; P = 0.01). For age- and BMI-adjusted SHBG, the MZ correlation of 0.67 and the DZ correlation of 0.41 were also different (χ_1^2 = 18.27 − 7.88 = 10.39; P = 0.001). These correlations, and their relationships to one another, are similar to those we observed for postmenopausal women.

Kuijper and colleagues (9) also measured T and SHBG for 128 adult males (20 MZ twin pairs, seven single MZ twins, 10 DZ twin pairs, 27 single DZ twins, and 34 brothers of twins, constituting 10 sibling pairs) aged 16–69 yr. For T, the MZ correlation of 0.58 (95% CI 0.10–0.81) and DZ brother correlation of 0.20 (95% CI −0.28–0.59) were not significantly different (P = 0.2; note the published CI are incorrect). Therefore, there was no evidence for a genetic component of variance and the authors' conclusion that the heritability was 56% is not justified. For SHBG, the respective MZ and DZ brother correlations of 0.81 (95% CI 0.64–0.92) and 0.41 (95% CI −0.04–0.73) were marginally different (P = 0.04). A study of 248 pairs of male and female Swedish twins found, for IGF-I, the combined MZ (97 pairs) and DZ (151 pairs) correlations were 0.61 (95% CI 0.47–0.72) and 0.38 (95% CI 0.24–0.51), respectively (P = 0.02) (11). These two studies, when properly analyzed, provide evidence for males (as did we for females) that there could be genetic components of variation in both SHBG and IGF-I.

There was no evidence for familial determinants of prolactin, so variance in this measure appears to be solely dependent on individual-specific factors. This perhaps reflects the sensitivity of prolactin concentrations to stress (22), circadian rhythm, fasting status, and other factors that we have not measured. A study of only 10 MZ and 10 DZ male twin pairs claimed that genetic factors partly determine both the basal daytime concentrations of prolactin and the temporal organization of prolactin secretion over a 24-h cycle for normal young men. However, this remains unresolved given that there was no formal evaluation of whether there were differences between MZ and DZ pair correlations (10).

The highly correlated concentrations of E_2, and to a lesser extent T, later in life for MZ, DZ, and nontwin pairs cannot all, or even partly, be due to an underlying genetic predisposition. It could be that there are environmental effects in addition to those adjusted for that are shared by twins and sisters while cohabiting and these effects on these two serum concentrations persist into postmenopausal life. Another potential explanation is that there are parental, if not prenatal, influences common to sisterships that have a substantial and lasting influence on steroidogenesis. There is also increasing evidence from several sources to support the hypothesis that there is a relationship between the growth and development of the fetus and health in later years, and this might be reflected in the

correlations in these two serum concentrations between sisters and twins (23–27). Review of the current literature leads to various possible mechanisms, but all are speculative at the present time.

Similarly, for the measures for which there appears to be no evidence for factors common to sisterships influencing postmenopausal serum concentrations, there could still be effects that we did not have power to detect. Note also that the models we have fitted assume that genetic and shared environmental factors act independently, so we cannot exclude the existence of interactions between these factors in their effects on postmenopausal serum concentrations.

In summary, this study provides new evidence about the extent, and likely causes, of familial correlations in postmenopausal female hormone and mitogen concentrations that is not inconsistent with the limited published findings for males of a similar age. Pursuit of the genetic components of the substantial familial causes of variation of SHBG and IGF-I , using for example genome-wide association and linkage studies, is not contraindicated. However, for E_2 and to a lesser extent T, there is evidence that a substantial proportion of the familial variance could be due to nongenetic factors. Larger studies might be required to find their genetic causes of variation. Prolactin does not appear to have detectable familial causes of variation, at least not as measured by these studies. Circulating hormone concentrations are known risk factors for the development of various cancers. The findings of this study, particularly for E_2 and T suggest that, with a greater depth of knowledge, it might be possible to modify the familial risk of some diseases by making adjustments to environmental elements, some of which might originate in the womb.

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References

1. **Key T, Appleby P, Barnes I, Reeves G** 2002 Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 94:606–616
2. **Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, Stanczyk FZ, Stephenson Jr HE, Falk RT, Miller R, Schatzkin A, Allen DS, Fentiman IS, Key TJ, Wang DY, Dowsett M, Thomas HV, Hankinson SE, Toniolo P, Akhmedkhanov A, Koenig K, Shore RE, Zeleniuch-Jacquotte A, Berrino F, Muti P, Micheli A, Krogh V, Sieri S, Pala V, Venturelli E, Secreto G, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE, Cauley JA, Kuller LH, Cummings SR, Helzlsouer KJ, Alberg AJ, Bush TL, Comstock GW, Gordon GB, Miller SR, Longcope C** 2003 Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. J Natl Cancer Inst 95:1218–1226
3. **Dunning AM, Dowsett M, Healey CS, Tee L, Luben RN, Folkerd E, Novik KL, Kelemen L, Ogata S, Pharoah PD, Easton DF, Day NE, Ponder BA** 2004 Polymorphisms associated with circulating sex hormone levels in postmenopausal women. J Natl Cancer Inst 96:936–945
4. **Santen RJ, Boyd NF, Chlebowski RT, Cummings S, Cuzick J, Dowsett M, Easton D, Forbes JF, Key T, Hankinson SE, Howell A, Ingle J** 2007 Critical assessment of new risk factors for breast cancer: considerations for development of an improved risk prediction model. Endocr Relat Cancer 14:169–187
5. **Allen NE, Roddam AW, Allen DS, Fentiman IS, Dos Santos Silva I, Peto J, Holly JM, Key TJ** 2005 A prospective study of serum insulin-like growth factor-I (IGF-I), IGF-II, IGF-binding protein-3 and breast cancer risk. Br J Cancer 92:1283–1287
6. **dos Santos Silva I, Johnson N, De Stavola B, Torres-Mejía G, Fletcher O, Allen DS, Allen NE, Key TJ, Fentiman IS, Holly JM, Peto J** 2006 The insulin-like growth factor system and mammographic features in premenopausal and postmenopausal women. Cancer Epidemiol Biomarkers Prev 15:449–455
7. **Tworoger SS, Eliassen AH, Sluss P, Hankinson SE** 2007 A prospective study of plasma prolactin concentrations and risk of premenopausal and postmenopausal breast cancer. J Clin Oncol 25:1482–1488
8. **Ring HZ, Lessov CN, Reed T, Marcus R, Holloway L, Swan GE, Carmelli D** 2005 Heritability of plasma sex hormones and hormone binding globulin in adult male twins. J Clin Endocrinol Metab 90:3653–3658
9. **Kuijper EA, Lambalk CB, Boomsma DI, van der Sluis S, Blankenstein MA, de Geus EJ, Posthuma D** 2007 Heritability of reproductive hormones in adult male twins. Hum Reprod 22:2153–2159
10. **Linkowski P, Spiegel K, Kerkhofs M, L'Hermite-Balériaux M, Van Onderbergen A, Leproult R, Mendlewicz J, Van Cauter E** 1998 Genetic and environmental influences on prolactin secretion during wake and during sleep. Am J Physiol 274:E909–E919
11. **Hong Y, Pedersen NL, Brismar K, Hall K, de Faire U** 1996 Quantitative genetic analyses of insulin-like growth factor I (IGF-I), IGF-binding protein-1, and insulin levels in middle-aged and elderly twins. J Clin Endocrinol Metab 81:1791–1797
12. **Stone J, Gurrin LC, Byrnes GB, Schroen CJ, Treloar SA, Padilla EJ, Dite GS, Southey MC, Hayes VM, Hopper JL** 2007 Mammographic density and candidate gene variants: a twins and sisters study. Cancer Epidemiol Biomarkers Prev 16:1479–1484
13. **Goldsmith HH** 1991 A zygosity questionnaire for young twins: a research note. Behav Genet 21:257–269
14. **Spitz E, Moutier R, Reed T, Busnel MC, Marchaland C, Roubertoux PL, Carlier M** 1996 Comparative diagnoses of twin zygosity by SSLP variant analysis, questionnaire, and dermatoglyphic analysis. Behav Genet 26:55–63
15. **Torgersen S** 1979 The determination of twin zygosity by means of a mailed questionnaire. Acta Genet Med Gemellol (Roma) 28:225–236
16. **Dowsett M, Goss PE, Powles TJ, Hutchinson G, Brodie AM,**

Jeffcoate SL, Coombes RC 1987 Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer: optimization of therapeutic dose and route. Cancer Res 47:1957–1961
17. **Lee JS, Ettinger B, Stanczyk FZ, Vittinghoff E, Hanes V, Cauley JA, Chandler W, Settlage J, Beattie MS, Folkerd E, Dowsett M, Grady D, Cummings SR** 2006 Comparison of methods to measure low serum estradiol levels in postmenopausal women. J Clin Endocrinol Metab 91:3791–3797
18. **Fisher R** 1918 The correlation between relatives on the supposition of Mendelian inheritance. Trans R Soc Edinburgh 52:399–433
19. **Hopper JL, Mathews JD** 1982 Extensions to multivariate normal models for pedigree analysis. Ann Hum Genet 46:373–383
20. **Lange K, Boehnke M** 1983 Extensions to pedigree analysis. IV. Covariance components models for multivariate traits. Am J Med Genet 14:513–524
21. **Lange K, Boehnke M, Weeks D** 1987 Programs for pedigree analysis. Los Angeles: Department of Biomathematics, University of California
22. **Jeffcoate WJ, Lincoln NB, Selby C, Herbert M** 1986 Correlation between anxiety and serum prolactin in humans. J Psychosom Res 30:217–222
23. **Eisner JR, Dumesic DA, Kemnitz JW, Colman RJ, Abbott DH** 2003 Increased adiposity in female rhesus monkeys exposed to androgen excess during early gestation. Obes Res 11:279–286
24. **Gluckman PD, Hanson MA, Cooper C, Thornburg KL** 2008 Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 359:61–73
25. **MacKenzie SM, Huda SS, Sattar N, Fraser R, Connell JM, Davies E** 2008 Depot-specific steroidogenic gene transcription in human adipose tissue. Clin Endocrinol (Oxf) 69:848–854
26. **Xita N, Tsatsoulis A** 2006 Fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. J Clin Endocrinol Metab 91:1660–1666
27. **Trichopoulos D** 1990 Hypothesis: does breast cancer originate in utero? Lancet 335:939–940