

Heritability of reproductive hormones in adult male twins

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BACKGROUND: Proper functioning of the male reproductive axis depends on complex feedback systems between several hormones. In this study, the genetic contribution of various endocrine components of the hypothalamic–pituitary–testicular axis is evaluated and previously observed differences in FSH and inhibin B levels between mono- (MZ) and dizygotic (DZ) twins are re-investigated. **METHODS:** Inhibin B, FSH, LH, sex hormone-binding globulin (SHBG) and testosterone levels were assayed in 128 adult males (20 MZ twin pairs, 7 single MZ twins, 10 DZ twin pairs, 27 single DZ twins and 34 siblings of twins, constituting 10 sibling pairs), aged 15.6–68.7 years. Hormone levels were compared across zygosity groups and heritability estimates were obtained using maximum likelihood variance component analysis. **RESULTS:** Heritability estimates ranged from 56% (testosterone) to 81% (inhibin B and SHBG). For LH and FSH, the heritability was estimated at 68% and 80% respectively. No mean differences in hormone levels were observed across groups. **CONCLUSIONS:** All measured hormones are highly heritable. A difference in the FSH-inhibin B feedback system between DZ twin males and MZ twin males could not be confirmed.

Keywords: heritability; hormones; male; reproductive; twins

Introduction

The testis is the target organ of the hypothalamic–pituitary–gonadal axis. GnRH secreted by the hypothalamic pulse generator is released in a pulsatile fashion into the portal vascular system, through which the hypothalamus communicates with the pituitary. This pulsatile secretion is essential for the release of FSH and LH by the anterior pituitary (Veldhuis *et al.*, 1983). The main functions of the human testis are the production of hormones and the regulation of spermatogenesis. Pituitary-released FSH stimulates testicular Sertoli cells to produce inhibin B, which in adult males has been shown to be a serum marker of Sertoli cell function **c.q.** spermatogenesis (Jensen *et al.*, 1997; Pierik *et al.*, 1998; Andersson and Skakkebaek, 2001; Andersson *et al.*, 2003). FSH release and synthesis consist of both a GnRH dependent and an autonomous component which is inhibited by inhibin B (Jenner *et al.*, 1985; Moore *et al.*, 1994; Andersson and Skakkebaek, 2001). Recently, Sutcliffe *et al.* (2006) compared reproductive hormones in monozygotic (MZ) versus dizygotic (DZ) male twins and reported elevated inhibin B levels in DZ twins. No such difference for FSH was found. This led to the suggestion that the FSH-inhibin B endocrine feedback axis is functioning differently in male DZ twins. However, as regular siblings were not included in these analyses it is as yet unclear

whether DZ twins have significantly higher inhibin B levels than usual or whether MZ twins have significantly lower inhibin B levels.

LH interacts with the testicular Leydig cells to enhance the conversion of cholesterol to testosterone. Both dihydrotestosterone, a testosterone metabolite, and estradiol play a role in the feedback system regulating LH secretion (Santen, 1975; Hayes *et al.*, 2001). Testosterone inhibits LH release directly (Sheckter *et al.*, 1989) by altering the pulse amplitude and frequency (Valk *et al.*, 1980; Shupnik, 1996) of the hypothalamic-stimulated release of LH from the pituitary (Santen, 1975; Matsumoto and Bremner, 1984).

In 1996, Harris *et al.* first stated that testosterone level are highly heritable especially in young adult males, with an estimated 66% of the variance due to genetic components (Harris *et al.*, 1998; Hoekstra *et al.*, 2006). Plasma hormone levels reflect the quantity of hormone available for binding to their target tissues. Biological activity is determined by several factors, e.g. sensitivity of the target tissue defined by hormone-receptor interaction (Wilson *et al.*, 1981; Meikle *et al.*, 1986). Serum hormone concentrations are subject to regulation at the genetic level as well as having environmental influences. Genetic effects might occur somewhere along the hypothalamic–pituitary–testicular axis, i.e. the hypothalamic

GnRH pulse generator, the anterior pituitary, the gonadal receptor or the hormonal feedback system.

The aim of this current study was to evaluate the genetic contribution of the various endocrine components of the hypothalamic–pituitary–testicular axis. Therefore, a sample of adult male twins and their siblings was assayed for plasma hormones including inhibin B, LH, FSH, sex hormone-binding globulin (SHBG) and testosterone. Males are chosen as study subjects because they provide a relatively stable endocrine environment compared to females, due to the absence of a monthly cycle.

Furthermore, we aimed to verify the earlier reported difference between MZ and DZ twins with regard to inhibin B secretion. To test for the possibility that twins differ from singleton offspring, siblings of the MZ and DZ twins were included in the analyses as well.

Subjects and Methods

This study is part of a larger study on the genetics of adult brain function (Posthuma *et al.*, 2005). Forty-seven MZ males (20 complete pairs and 7 single MZ twin males), 47 male DZ twins (10 complete pairs and 27 single DZ males) and 34 brothers of these twins (17 brothers of a MZ and 17 brothers of a DZ twin, making up 10 complete sibling pairs) were included in the study. Twins and their siblings were invited to the lab where fasting blood samples were taken before 11:00 am to determine inhibin B, FSH, LH, SHBG and testosterone levels. The participants age ranged 15.6–68.7 years (mean \pm SD: 38.1 \pm 12.7) and had no apparent endocrine disease. Zygosity was determined by typing a series of DNA polymorphisms. The study was approved by the Medical Ethical committee of the VU medical center and all participants signed an informed consent.

Assays

All hormone measurements were done in duplicate and within the same series. Family members were randomly distributed within the different batches.

Inhibin B

An immunometric assay (Serotec Limited, Oxford, UK) was used to measure inhibin B in serum. According to the manufacturer the lower detection level for inhibin is 15 IU/L. The inter-assay coefficients of variation (CVs) were <16% and the intra-assay CVs were <11%.

Testosterone

The plasma concentrations of testosterone were measured using a radioimmunoassay (Coat-a-count, DPC, Los Angeles, USA). The inter-assay CVs were all 20% or less and the intra-assay CVs were <9%.

Follicle stimulating hormone

FSH was measured using an AxSYM random access immunoassay analyzer (Abbott Laboratories, Abbott Park, IL, USA) with a microparticle enzyme immunoassay reagent kit. According to the manufacturer, the lower detection limit for

FSH is 0.09 IU/l. The inter-assay CVs and intra-assay CVs were both <6%.

Luteinizing hormone

An immunometric assay (Delfia, Wallac Turku Finland) was used to measure LH in serum. According to the manufacturer the lower detection level for LH was 0.3 IU/l. The inter-assay CVs were <9% and the intra-assay CVs were <5%.

Sex hormone-binding protein

An immunoradiometric assay (Orion Diagnostica, Espoo, Finland) was used for the determination of SHBG levels in plasma. The inter-assay CVs were 8% or less and the intra-assay CVs were all 4%.

Statistical analysis

For FSH, LH and SHBG the data were positively skewed and were logarithmically transformed to normalize the distributions. The Mx package (Neale, 2003) was used to conduct the genetic analyses and to test whether the hormonal levels of the MZ twins, DZ twins and siblings differed significantly from each other. The fact that the participants can not be regarded as independent observations (i.e. are related) is accounted for in all these analyses. The data were first summarized into correlations for MZ and DZ twins and sibling pairs. These correlations between MZ twins and DZ twin sibling pairs can be used to obtain rough estimates of the proportional contribution of additive genetic influences and dominant genetic influences to the variation in the levels of the hormones under study, i.e. the so called Falconer heritability estimates.

Next, the variation in hormone levels was decomposed into additive genetic variation (A), variation due to genetic dominance (D), shared environmental variation (C) or non-shared environmental variation (E) (Boomsma *et al.*, 2002; Posthuma *et al.*, 2003). Shared environmental variation, by definition, includes all environmental sources of variation that twins and siblings from the same family share, while non-shared environmental variation refers to the environmental variation, i.e. unique to an individual and, i.e. typically not shared with other family members. In DZ twins and sibpairs, similarity in shared environmental influences is 100%, while similarity in additive genetic influences is 50% and similarity in genetic dominance effects is 25%. In MZ twin pairs, similarities of additive genetic effects, genetic dominance effects and shared environmental influences are 100% (Neale and Cardon, 1992; Posthuma *et al.*, 2003).

There is, by definition, no similarity in non-shared environmental influences between family members irrespective of their precise relationship. Effects for genetic dominance and shared environment are confounded in the classical twin model and cannot be modelled simultaneously. Disentangling the contributions of dominance and shared environment requires additional data from, e.g. twins reared apart, half-sibs, or non-biological relatives reared together (Posthuma *et al.*, 2003). As such additional data were not available in the present study, the analyses were confined to ACE- or ADE models, respectively. When the observed correlation between DZ twins is about half the size of those observed in MZ

twins, dominance effects are assumed absent and an ACE model is deemed most suitable. However, when the observed correlation between DZ twins is substantially smaller than half the correlation observed between MZ twins, dominance effects are likely to be present and an ADE model is deemed most suitable. The expectation for the total variance given an ACE model is $A+C+E$, while the expectation for the covariance between MZ twins is $A+C$, and the expectation for DZ twins/sibpairs is half $A+C$. The expectation for the total variance given an ADE model is $A+D+E$, while the expectation for the covariance between MZ twins is $A+D$ and the expectation for DZ twins/sibpairs half $A+D$. Heritability is calculated as the proportional contribution of genetic variation to the total, observed variation (i.e. A in the ACE model and $A+D$ in the ADE model). Estimates of parameters were obtained in the Mx package by normal theory maximum likelihood. Whether additive genetic (A), dominance effects (D) and shared environmental (C) influences contribute significantly to the total variance, was tested by constraining these parameters at zero and comparing the fit of the resulting model to the fit of the model without these constraints using likelihood ratio tests (Posthuma *et al.*, 2003). In addition, Akaike's information criterion (AIC) was used to differentiate between alternative models. The model with the smallest AIC is regarded as the best fitting model (Schermelleh-Engel *et al.*, 2006). As hormone levels (especially FSH) might be influenced by age of the participant or age of the participant's mother at time of birth, both age measures were regressed out in all analyses.

Results

Group characteristics are summarized as the mean (\pm SD) of age, inhibin B, testosterone, FSH, LH and SHBG levels for MZ twins, DZ twins and singleton brothers (Table 1).

Hormone comparison

The mean age between the groups did not differ significantly [$\chi^2(3) < 1$, ns]. Comparison of serum inhibin B levels revealed no significant differences between DZ twins and their siblings, MZ twins and their siblings or between MZ and DZ families [for all tests $\chi^2(1) < 1$, ns]. Correction for age of the participants and maternal age at time of birth of the twin did not alter these outcomes [for all tests $\chi^2(2) < 1$, ns]. Correction of inhibin B levels for LH, SHBG and testosterone did not

change the results either [DZ twins versus their siblings: $\chi^2(1) = 1.72$ (ns); MZ twins versus their siblings: $\chi^2(1) < 1$ (ns) and offspring DZ families versus offspring MZ families: $\chi^2(1) < 1$ (ns)]. Likewise, the FSH levels of DZ twins did not differ from that of their siblings [$\chi^2(1) = 1.77$, ns], nor did the FSH levels of MZ twins differ from their siblings [$\chi^2(1) < 1$, ns]. FSH levels between offspring from DZ and MZ families were comparable [$\chi^2(1) = 3.37$, ns]. Again, correction for age of the participants and maternal age at time of birth of the twin and correction for levels of LH, SHBG and testosterone did not change these results [DZ twins versus their siblings: $\chi^2(1) = 1.07$ (ns); MZ twins versus their siblings: $\chi^2(1) < 1$ (ns) and offspring DZ families versus offspring MZ families: $\chi^2(1) = 1.07$ (ns)].

As expected, no mean differences between MZ and DZ twins and their siblings were found for testosterone levels [DZ twins versus their siblings: $\chi^2(1) = 1.38$ (ns); MZ twins versus their siblings: $\chi^2(1) < 1$ (ns) and offspring DZ families versus offspring MZ families: $\chi^2(1) = 1.45$ (ns)], for LH levels [DZ twins versus their siblings: $\chi^2(1) = 1.65$ (ns); MZ twins versus their siblings: $\chi^2(1) = 2.20$ (ns) and offspring DZ families versus offspring MZ families: $\chi^2(1) = 1.75$ (ns)] and for SHBG levels [for all tests: $\chi^2(1) < 1$ (ns)] before and after correction for age of the participants and maternal age at time of birth of the twin.

Genetic analysis

The DZ twin correlations did not differ significantly from twin-sib or sib-sib correlations and these correlations were constrained to be equal in all further analyses. The twin correlations and the Falconer heritability estimate for LH, FSH, inhibin B, testosterone and SHBG are listed in Table 2.

For all reproductive hormone measurements, the MZ correlation exceeded the DZ twin correlation, suggesting genetic influences on hormonal levels. As the DZ correlations for inhibin B and testosterone were considerably smaller than half the MZ correlations, ADE models were deemed suitable for these hormones. For the other hormones ACE models were fitted. In all following analyses, age of the participant and age of the mother at time of birth of the participants is regressed out. Table 3 shows the results of variance component modelling for all reproductive hormones. For FSH and SHBG, shared environmental influences were not important, i.e. the parameters representing the shared environmental

Table 1: The mean (\pm SD) of age and reproductive hormone levels for MZ and DZ twins and their siblings

Characteristics	MZ ($n = 47$)	DZ ($n = 47$)	MZ sibs ($n = 17$)	DZ sibs ($n = 17$)
	Mean (\pm SD)	Mean (\pm SD)	Mean (\pm SD)	Mean (\pm SD)
Age (years)	37.7 (11.3)	37.5 (12.8)	42.3 (15.9)	36.6 (12.8)
Inhibin B (ng/l)	201.8 (58.5)	208.9 (70.9)	207.2 (83.8)	194.1 (42.9)
Testosterone (nmol/L)	18.5 (5.1)	18.9 (4.1)	18.5 (5.3)	17.5 (4.9)
FSH (IU/l)	4.3 (2)	4.1 (2.3)	4.3 (1.4)	2.9 (1.6)
LH (IU/l)	4.1 (1.5)	4.1 (1.7)	4.5 (1.4)	3.3 (1.4)
SHBG (nmol/l)	36.4 (15.1)	34.7 (14.8)	33.6 (1.3)	31.1 (1.6)

n , number of individuals. MZ twins (20 complete pairs and 7 single twins), DZ twins (10 complete pairs and 27 single twins).

Table 2: MZ and the DZ+sibs twin correlations [95% confidence interval (CI)] for all reproductive hormones

	r_{MZ} (95% CI)	$r_{DZ+sibs}$ (95% CI)	Broad sense heritability
Inhibin B	0.82 (0.62–0.90)	0.15 (–0.11–0.42)	0.82
Testosterone	0.58 (0.27–0.76)	0.20 (–0.11–0.48)	0.58
FSH	0.79 (0.56–0.89)	0.48 (0.12–0.70)	0.62
LH	0.68 (0.36–0.83)	0.36 (–0.01–0.62)	0.64
SHBG	0.81 (0.63–0.90)	0.41 (0.12–0.63)	0.80

r_{MZ} = correlation between MZ twins (based on 20 complete pairs); r_{DZ+sib} = correlation between DZ twins and regular siblings (based on 10 complete DZ pairs and 10 complete sib-pairs). Broad sense heritability is calculated using the formula: $2(r_{MZ} - r_{DZ+sib})$. If the r_{MZ} is more than twice the r_{DZ+sib} dominance is likely to be present and the broad sense heritability is calculated using the formula: $(4r_{DZ+sib} - r_{MZ}) + 2(r_{MZ} - 2r_{DZ+sib})$.

Table 3: Fit results from models where the variance of hormonal levels is decomposed into additive genetic variance (A), shared environmental variance (C) or genetic dominance variance (D) and non-shared environmental variance (E)

	Tested model	Saturated model	<i>P</i> -value	AIC	A	C	D	E
Inhibin B	ADE	SAT	0.874	–8.187	0.00		0.82	0.18
	AE	ADE	0.06	1.448	0.81		–	0.19
	E	ADE	<0.001	15.320	–		–	1
Testosterone	ADE	SAT	0.287	–3.797	0.22		0.35	0.42
	AE	ADE	0.574	–1.684	0.56		–	0.44
	E	ADE	0.003	7.763	–		–	1
FSH	ACE	SAT	0.958	–8.943	0.63	0.16		0.21
	AE	ACE	0.602	–1.728	0.80	–		0.20
	CE	ACE	0.027	2.894	–	0.59		0.41
LH	E	ACE	<<0.001	17.973	–			1
	ACE	SAT	0.688	–6.925	0.66	0.03		0.32
	AE	ACE	0.933	–1.993	0.68	–		0.32
SHBG	CE	ACE	0.091	0.862	–	0.48		0.52
	E	ACE	<0.001	10.508	–	–		1
	ACE	SAT	0.951	–8.863	0.80	0.01		0.19
SHBG	AE	ACE	0.964	–1.998	0.81	–		0.19
	CE	ACE	0.002	7.644	–	0.53		0.47
	E	ACE	<<0.001	25.745	–	–		1

The ACE models were all tested against a saturated model in which the variance was not decomposed into A, C and E (i.e. the null model). In the AE model, the shared environmental effect (C) is fixed to zero. In the CE model, the additive genetic effect (A) is fixed to zero. In the E model, both A and C are fixed to zero. The fit of the AE model, the CE model and the E model were compared to the fit of the ACE model. The ADE models were all tested against a saturated model in which the variance was not decomposed into A, D and E (i.e. the ‘null model’). In the AE model, the genetic dominance effect (D) is fixed to zero. In the E model, both A and D are fixed to zero. The fit of the AE model and the E model were compared to the fit of the ADE model. A significant *P*-value ($P < 0.05$) denotes a significant deterioration of the fit. Best fit models are in bold. AIC with lower values indicating better fit. These analyses are based on 20 complete MZ twin pairs, 10 complete DZ twin pairs, 10 complete sibling pairs and 48 single subjects.

influences were not significantly different from zero. Constraining the additive genetic effects to zero did however result in significant deterioration of the model fit. For LH, both the AE model (shared environmental effects fixed to zero, $p = 0.933$) and the CE model (additive genetic effects fixed to zero, $p = 0.091$) were statistically acceptable. The AE model, however, is the preferred model as it fits the data better (largest *P*-value and lowest AIC). For FSH, LH and SHBG variation in hormonal levels could thus be attributed to additive genetic influences (A) and unique environmental influences (E). Heritability estimates for LH, FSH and SHBG were 68, 80 and 81%, respectively. For both inhibin B and testosterone, the AE model (genetic dominance effects constrained to zero) was tenable, i.e. the fit was not statistically worse than the ADE model. Note that the DE models are not tested as such models are biologically implausible. Heritability estimates for inhibin B and testosterone were 81 and 56%, respectively.

Discussion

The results of this study indicate that all measured reproductive hormones are highly heritable, with heritability estimates ranging from 56% (testosterone) to 81% (inhibin B and SHBG). This high heritability does not imply that environmental factors are unimportant but it indicates that within the population, genetic factors are responsible for the majority of the variation. For testosterone, but especially inhibin B, the broad-sense heritability seems driven for a large part by non-additive genetic or dominance influences. However, our sample is relatively small and larger samples are needed to allow a more accurate estimate of these influences and for sufficient power to discriminate between additive and non-additive genetic influences. Our data confirm and extend the findings by Storgaard *et al.* (2006) who recently published a heritability estimate for inhibin B of 79%. In 1997, Meikle *et al.* (1997) reported a 74% heritability for testicular volumes of male twins between 25 and 75 years old. Most of

the testicular volume in adult males is determined by Sertoli cell mass, and inhibin B is known to be a serum marker for the competence of Sertoli cells c.q. spermatogenesis (Pierik *et al.*, 1998). With regard to plain comparison of hormone levels between twins, Sutcliffe *et al.* (2006) reported elevated inhibin B levels in DZ adult male twins when compared with MZ twins across an age range of 20–68 years old. In our data, we found no significant difference in inhibin B or FSH levels between MZ and DZ twins and their siblings (age range 15–69 years old), or for any of the other hormones under study. Correction for possible confounding effects of age of the participants and maternal age at time of birth of her twin did not alter these findings; neither did correction of inhibin B and FSH levels for LH, SHBG and testosterone. Close inspection of the means of inhibin B in Table 1 shows that the lowest inhibin B levels are observed for the youngest group (siblings of DZ twins). Yet, inhibin B levels did not differ significantly between any of the groups as the variation within groups was rather large and analyses with age as a (linearly related) covariate did not alter these results. It is however possible that the relation between age and inhibin B is non-linear. We did not test for non-linear effects of age on hormone levels in the present study because of the small sample size, but the possibility merits further study.

The analysis of variance (ANOVA) analysis used by Sutcliffe *et al.* (2006) to compare hormone levels between the twins does not account for the dependency of the observations. As twins and siblings are related (i.e. share genes and environment) they should not be analysed as independent samples (Rebollo *et al.*, 2006). We therefore used the Mx package to analyse the twin hormone levels as this software package is designed to deal with family data. Nevertheless, when we analysed our data using the ANOVA that does not account for dependency of the data, we did not observe significant mean differences in inhibin B levels between MZ and DZ twins (data not shown). The difference between our results and the results presented by Sutcliffe *et al.* (2006) is therefore not due to the different statistical methods used. It is however possible that our study lacked the power to detect a mean difference in inhibin B as our sample was rather small. The power to detect substantial mean differences (e.g. mean difference of 0.5 or 1 SD, corresponding to Cohen's *d* effect size of 0.5 or 1, respectively, would have been mediocre to good (0.55–0.90) [Cohen's *d* is calculated as $(M_1 - M_2) / \sigma_{\text{pooled}}$, where M_1 is the mean of the first group, M_2 is the mean of the second group and σ_{pooled} is the average of the SD in the first and the second group]). However, the observed effect size in the present study was much smaller than that (0.11) and also much smaller than the that of Sutcliffe *et al.* (0.35). Furthermore, both Sutcliffe's and our study lack information on familial DZ twinning. Previous studies have shown that mothers of hereditary DZ twins (familial twinning) are known to have elevated follicular phase FSH levels resulting in multiple follicle growth and therefore an increased chance of having DZ twins (Martin *et al.*, 1991; Lambalk *et al.*, 1998a,b). As elevated follicular phase FSH levels are said to be inherited autosomally, increased FSH levels are expected in all offspring of these mothers. Differences in outcomes between our study and

the one done by Sutcliffe *et al.* (2006), may therefore be due to the ratio of hereditary DZ twins participating in the studies. Therefore, inclusion of information on familial twinning would be recommended for future studies on the heritability and mean levels of reproductive hormones.

In the late 1970s, Meikle *et al.* (1982) studied the contribution of genetic factors on the plasma levels of sex-steroids in siblings. They found less variation in testosterone within groups of brothers than among non-brothers. Within their twin studies (age 20–60 years), heritability estimates ranged from 34% for free (unbound) testosterone to 26% for plasma testosterone (Meikle *et al.*, 1986). A high familial resemblance in testosterone levels of around 70% in both black and white adults (Hong *et al.*, 2001) and 66% in adolescent male twins (age 14–21 years) was reported by Harris and colleagues (1998). Genetic variations accounted for 57% (maximum likelihood estimate) and 32% (Falconer heritability estimate) of the plasma testosterone concentrations reported in a twin study with a narrow age range of 59–70 years (Ring *et al.*, 2005). In a recent twin study (age 20–45 years), a heritability of 46% was shown (Storgaard *et al.*, 2006). Circulating in the blood, the sex steroids estradiol and testosterone are bound to the protein carrier SHBG. The heritability of SHBG measured in siblings ranged 31%–54% in blacks (Jaquish *et al.*, 1997) and was estimated at 64% in whites (An *et al.*, 2001). Meikle *et al.* (1997) reported a heritability estimate of 62% based on their twin studies (age 25–75 years), whereas estimates of 32% (by Falconer method) and 68% (based on maximum likelihood) were reported by Ring *et al.* (2005). Our results for testosterone are equal to those reported by Ring *et al.*, while the heritability estimates for SHBG in the present study are much higher than those reported by others. These differences in heritability estimates could partially be explained by the different statistical methods used. In classical twin studies, the intraclass twin pair correlation was used to estimate the heritability (Falconer). In recent research, however, model fitting and comparison using the maximum likelihood is the preferred methodology. Another possible explanation for the variation in heritability estimates concerns the age range of the research samples. Although aging affects hormonal concentrations in men, there are no divergent effects on hormonal concentrations between MZ and DZ twins (Meikle *et al.*, 1997). In the present study, the age range is indeed large but the mean age (\pm SD) showed no significant difference between the groups. Therefore, aging affects the data in a similar manner for MZ twins, DZ twins and their siblings. Furthermore, age of the participant and age of the mother at time of birth of the participant were regressed out in all genetic analyses.

For LH and FSH, a wide variety of heritability estimates is reported in 20–60-year old male twins, varying from 28% to almost 100% for FSH (Meikle *et al.*, 1986, 1997; Wang *et al.*, 2003; Storgaard *et al.*, 2006) and from 50% to over 70% for LH (Meikle *et al.*, 1986; 1997; Bishop *et al.*, 1988; Wang *et al.*, 2004; Storgaard *et al.*, 2006). The serum LH and FSH concentrations display pulsatile and diurnal variation. These factors, together with the short serum half-life of the gonadotrophins, contribute to a substantial intra-individual variability. Despite this, the heritability estimates found in

our study, 68% for LH and 80% for FSH, are high and well within the range estimated by others.

The objective of this study was to explore the genetic variation at the various levels in the regulation of gonadal function, i.e. the hypothalamic pulse generator, the anterior pituitary, the gonad and the feedback system. The hypothalamic pulse generator synchronizes the activity of groups of GnRH neurons to produce a meaningful secretory output. Recent *in vitro* animal studies indicate that GnRH cells contain 'molecular clocks', which may be coupled to the fundamental mechanism of pulsatile GnRH secretion. Disruption of the normal molecular clock gene expression leads to decreased pulse frequency and increased pulse amplitude (Balsalobre, 2002; Chappell *et al.*, 2003). The variation in frequency and amplitude of the GnRH pulses in relation to the overall serum hormone levels has to be marginal considering the high heritability estimates found here, despite the large sampling time span. Earlier studies indicate that levels of various other hormones that are known to have a biorhythm, such as cortisol (Bartels *et al.*, 2003a,b) and growth hormone (Mendlewicz *et al.*, 1999), are also highly heritable. This led us to believe that the GnRH pulse patterns might be heritable. GnRH interacts with high-affinity receptors in cell membranes of gonadotrophs in the anterior pituitary, resulting in gene transcription of gonadotropin subunits and biosynthesis of LH and FSH. The gonadotropin gene transcription is modulated by the frequency and magnitude of the GnRH pulses and also by age and sex of the individual (Savoy-Moore and Swartz, 1987; Kaiser *et al.*, 1997; Karges *et al.*, 2003). Considering the high heritability observed for gonadotropins, the GnRH receptor is also a candidate for regulation of the genetic variance. FSH and LH act on the testis, through binding to their receptors, to promote spermatogenesis and secretion of testosterone and inhibin B. Testosterone and inhibin B act at the pituitary level to alter numbers of GnRH receptors and have hormone-specific effects on gene transcription. Testosterone also influences the hypothalamic pulse generator by altering gonadotropin pulse amplitude and frequency (Valk *et al.*, 1980; Shupnik, 1996). The high heritability estimates observed for testosterone and inhibin B suggest that both the testicular receptor sensitivity and the hormonal feedback system could be responsible for the small variation found between twins.

In summary, the results of this study show high heritability estimates for inhibin B, FSH, LH, testosterone and SHBG in male twins and their siblings. Most likely, the heritability of these hormones is regulated at multiple levels, including the hypothalamic pulse generator, the pituitary GnRH receptor, the hormonal feedback system and the gonadal receptors. Furthermore, the significant difference in FSH and inhibin B levels between MZ and DZ twins, as observed in previous data, was not replicated in the present study.

References

- An P, Rice T, Gagnon J, Borecki IB, Rankinen T, Gu C, Leon AS, Skinner JS, Wilmore JH, Bouchard C *et al.* Population differences in the pattern of familial aggregation for sex hormone-binding globulin and its response to exercise training: the HERITAGE family study. *Am J Hum Biol* 2001;**13**:832–837.
- Andersson AM, Carlén E, Petersen JH, Skakkebaek NE. Variation in levels of serum inhibin B, testosterone, estradiol, luteinizing hormone, follicle-stimulating hormone, and sex hormone-binding globulin in monthly samples from healthy men during a 17-month period: possible effects of seasons. *J Clin Endocrinol Metab* 2003;**88**:932–937.
- Andersson AM, Skakkebaek NE. Serum inhibin B levels during male childhood and puberty. *Mol Cell Endocrinol* 2001;**180**:103–107.
- Balsalobre A. Clock genes in mammalian peripheral tissues. *Cell Tissue Res* 2002;**309**:193–199.
- Bartels M, de Geus EJ, Kirschbaum C, Sluyter F, Boomsma DI. Heritability of daytime cortisol levels in children. *Behav Genet* 2003a;**33**:421–433.
- Bartels M, van den BM, Sluyter F, Boomsma DI, de Geus EJ. Heritability of cortisol levels: review and simultaneous analysis of twin studies. *Psychoneuroendocrinology* 2003b;**28**:121–137.
- Bishop DT, Meikle AW, Slattery ML, Stringham JD, Ford MH, West DW. The effect of nutritional factors on sex hormone levels in male twins. *Genet Epidemiol* 1988;**5**:43–59.
- Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. *Nat Rev Genet* 2002;**3**:872–882.
- Chappell PE, White RS, Mellon PL. Circadian gene expression regulates pulsatile gonadotropin-releasing hormone (GnRH) secretory patterns in the hypothalamic GnRH-secreting GT1-7 cell line. *J Neurosci* 2003;**23**:11202–11213.
- Harris JA, Vernon PA, Boomsma DI. The heritability of testosterone: a study of Dutch adolescent twins and their parents. *Behav Genet* 1998;**28**:165–171.
- Hayes FJ, DeCruz S, Seminara SB, Boepple PA, Crowley WF Jr. Differential regulation of gonadotropin secretion by testosterone in the human male: absence of a negative feedback effect of testosterone on follicle-stimulating hormone secretion. *J Clin Endocrinol Metab* 2001;**86**:53–58.
- Hoekstra RA, Bartels M, Boomsma DI. Heritability of testosterone levels in 12-year-old twins and its relation to pubertal development. *Twin Res Hum Genet* 2006;**9**:558–565.
- Hong Y, Gagnon J, Rice T, Perusse L, Leon AS, Skinner JS, Wilmore JH, Bouchard C, Rao DC. Familial resemblance for free androgens and androgen glucuronides in sedentary black and white individuals: the HERITAGE Family Study. Health, Risk Factors, Exercise Training and Genetics. *J Endocrinol* 2001;**170**:485–492.
- Jaquish CE, Blangero J, Haffner SM, Stern MP, MacCluer JW. Quantitative genetics of serum sex hormone-binding globulin levels in participants in the San Antonio Family Heart Study. *Metabolism* 1997;**46**:988–991.
- Jenner AA, de Koning J, Tijssen AM, van Rees GP. Divergent effects on LH and FSH synthesis and release from intact female rat pituitary glands *in vitro* by methylxanthines, cyclic AMP derivatives and sodium fluoride. *Acta Endocrinol (Copenh)* 1985;**109**:315–319.
- Jensen TK, Andersson AM, Hjollund NH, Scheike T, Kolstad H, Giwercman A, Henriksen TB, Ernst E, Bonde JP, Olsen J *et al.* Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J Clin Endocrinol Metab* 1997;**82**:4059–4063.
- Kaiser UB, Jakubowiak A, Steinberger A, Chin WW. Differential effects of gonadotropin-releasing hormone (GnRH) pulse frequency on gonadotropin subunit and GnRH receptor messenger ribonucleic acid levels *in vitro*. *Endocrinology* 1997;**138**:1224–1231.
- Karges B, Karges W, de Roux N. Clinical and molecular genetics of the human GnRH receptor. *Hum Reprod Update* 2003;**9**:523–530.
- Lambalk CB, Boomsma DI, De Boer L, de Koning CH, Schoute E, Popp-Snijders C, Schoemaker J. Increased levels and pulsatility of follicle-stimulating hormone in mothers of hereditary dizygotic twins. *J Clin Endocrinol Metab* 1998a;**83**:481–486.
- Lambalk CB, de Koning CH, Braat DD. The endocrinology of dizygotic twinning in the human. *Mol Cell Endocrinol* 1998b;**145**:97–102.
- Martin NG, Shanley S, Butt K, Osborne J, O'Brien G. Excessive follicular recruitment and growth in mothers of spontaneous dizygotic twins. *Acta Genet Med Gemellol (Roma)* 1991;**40**:291–301.
- Matsumoto AM, Bremner WJ. Modulation of pulsatile gonadotropin secretion by testosterone in man. *J Clin Endocrinol Metab* 1984;**58**:609–614.
- Meikle AW, Bishop DT, Stringham JD, West DW. Quantitating genetic and nongenetic factors that determine plasma sex steroid variation in normal male twins. *Metabolism* 1986;**35**:1090–1095.
- Meikle AW, Stanish WM, Taylor N, Edwards CQ, Bishop CT. Familial effects on plasma sex-steroid content in man: testosterone, estradiol and Sex-hormone-binding globulin. *Metabolism* 1982;**31**:6–9.

- Meikle AW, Stephenson RA, Lewis CM, Wiebke GA, Middleton RG. Age, genetic, and nongenetic factors influencing variation in serum sex steroids and zonal volumes of the prostate and benign prostatic hyperplasia in twins. *Prostate* 1997;**33**:105–111.
- Mendlewicz J, Linkowski P, Kerkhofs M, Leproult R, Copinschi G, Van Cauter E. Genetic control of 24-hour growth hormone secretion in man: a twin study. *J Clin Endocrinol Metab* 1999;**84**:856–862.
- Moore A, Krummen LA, Mather JP. Inhibins, activins, their binding proteins and receptors: interactions underlying paracrine activity in the testis. *Mol Cell Endocrinol* 1994;**100**:81–86.
- Neale MC. A finite mixture distribution model for data collected from twins. *Twin Res* 2003;**6**:235–239.
- Neale MC, Cardon LR. Methodology for genetic studies in twins and families. Dordrecht, Kluwer, 1992. The Netherlands.
- Pierik FH, Vreeburg JT, Stijnen T, de Jong FH, Weber RF. Serum inhibin B as a marker of spermatogenesis. *J Clin Endocrinol Metab* 1998;**83**:3110–3114.
- Posthuma D, Beem AL, de Geus EJ, van Baal GC, von Hjelmborg JB, Iachine I, Boomsma DI. Theory and practice in quantitative genetics. *Twin Res* 2003;**6**:361–376.
- Human cytokine response to ex vivo amyloid-beta stimulation is mediated by genetic factors. *Twin Res Hum Genet* 2005;**8**:132–137.
- Rebollo I, de Moor MH, Dolan CV, Boomsma DI. Phenotypic factor analysis of family data: correction of the bias due to dependency. *Twin Res Hum Genet* 2006;**9**:367–376.
- Ring HZ, Lessov CN, Reed T, Marcus R, Holloway L, Swan GE, Carmelli D. Heritability of plasma sex hormones and hormone binding globulin in adult male twins. *J Clin Endocrinol Metab* 2005;**90**:3653–3658.
- Santen RJ. Is aromatization of testosterone to estradiol required for inhibition of luteinizing hormone secretion in men? *J Clin Invest* 1975;**56**:1555–1563.
- Savoy-Moore RT, Swartz KH. Several GnRH stimulation frequencies differentially release FSH and LH from isolated, perfused rat anterior pituitary cells. *Adv Exp Med Biol* 1987;**219**:641–645.
- Schermelleh-Engel K, Moosbrugger H, Muller H. Evaluating the fit of structural equation models: test of significance and descriptive goodness-of-fit measures. *Methods Psychol Res* 2006;**8**:23–74.
- Sheckter CB, Matsumoto AM, Bremner WJ. Testosterone administration inhibits gonadotropin secretion by an effect directly on the human pituitary. *J Clin Endocrinol Metab* 1989;**68**:397–401.
- Shupnik MA. Gonadotropin gene modulation by steroids and gonadotropin-releasing hormone. *Biol Reprod* 1996;**54**:279–286.
- Storgaard L, Bonde JP, Ernst E, Andersen CY, Spano M, Christensen K, Petersen HC, Olsen J. Genetic and Environmental Correlates of Semen Quality: A Twin Study. *Epidemiology* 1996;**17**:674–681.
- Sutcliffe A, Spoudeas HA, Nair D, Bouloux P, Oliver T, Sambrook P, Bannister W, Lambalk CB, Spector T. Comparison of serum FSH and Inhibin B levels between adult male dizygotic and monozygotic twins. *Hum Reprod* 2006;**21**:447–450.
- Valk TW, Corley KP, Kelch RP, Marshall JC. Hypogonadotropic hypogonadism: hormonal responses to low dose pulsatile administration of gonadotropin-releasing hormone. *J Clin Endocrinol Metab* 1980;**51**:730–738.
- Veldhuis JD, Rogol AD, Johnson ML. Endogenous opiates modulate the pulsatile secretion of biologically active luteinizing hormone in man. *J Clin Invest* 1983;**72**:2031–2040.
- Wang JX, Kwan M, Davies MJ, Kirby C, Judd S, Norman RJ. Risk of multiple pregnancy when infertility is treated with ovulation induction by gonadotropins. *Fertil Steril* 2003;**80**:664–665.
- Wang W, Ji C, Peng Z, Yang Y, Chen T, Li H, Zhan X, Wang Y, Hu Y. Genetic analysis of gonadotropin-gonadal axis in boys: a twin study. *Zhonghua Nan Ke Xue* 2004;**10**:250–252.
- Wilson JD, George FW, Griffin JE. The hormonal control of sexual development. *Science* 1981;**211**:1278–1284.

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