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The Heritability of Testosterone: A Study of Dutch Adolescent Twins and Their Parents

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The heritability of total plasma testosterone levels, determined from blood samples, was examined in 160 adolescent twin pairs and their parents. Subjects were tested as part of a larger study of cardiovascular risk factors, conducted in Amsterdam. Each subject provided a sample of blood which was assayed to measure testosterone concentrations. Correlations of testosterone in monozygotic twins were higher than in dizygotic twins. No resemblance was found between testosterone values in fathers and those in their children and a moderate correlation was seen between mothers and their daughters. The lack of resemblance between family members of opposite sex suggests that different genetic factors influence plasma testosterone concentrations in men and women. In adolescent men, approximately 60% of the variance in testosterone levels is heritable. The lack of father–son resemblance suggests that different genetic factors may be expressed in adolescence and adulthood. In women, 40% of the variance in testosterone levels is heritable, both in adolescence and in adulthood.

KEY WORDS: Testosterone; genetics; twins; families; sex differences.

INTRODUCTION

Since humans are diverse social beings, exposed to a variety of differing experiences, it may not be surprising that sex steroid hormones do not have a strong relationship with behavior, but a relationship does appear to be consistently found for certain sexually dimorphic behaviors. For example, the gonadal sex hormone testosterone has been suggested to be positively related to human aggression and possible delinquency and criminality, following reviews of the testosterone-aggression literature [(Archer, 1991, 1994a, b; see also Dent, 1983; Rose, 1978); see review by Albert *et al.* (1993) for a negative conclusion relating testosterone and aggression]. Dabbs and Morris (1990) and Booth and Osgood (1993) found that plasma testosterone was positively related to antisocial activities, alcoholism, and drug abuse in a sample of over 4000 male military veterans. Dabbs *et al.* (1988) reported that salivary testosterone concentrations were higher in female prisoners who were convicted of unprovoked aggressive crimes than in females who had committed an aggressive act as a means of defense. In contrast, Gladue (1991) found a negative relationship between testosterone and aggression in women.

Recently, Harris *et al.* (1996) found that salivary testosterone was positively related to self-report aggression and negatively related to prosocial personality characteristics, such as nurturance and empathy, in both male and female university students. These findings suggest that testosterone may be related to socially important variables, such as aggression and prosocial personality dimensions,

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which themselves have been reported to have a genetic component (Rushton *et al.*, 1986) and may further suggest that both genetic and hormonal influences may contribute to sexually dimorphic behaviors.

Some reviews of the testosterone literature have suggested that testosterone may not have a direct effect on behavior but that the relationship may be reciprocal (see Archer, 1994b). This position, however, is contrasted by findings of a causal nature of hormones on behavior. For example, Beatty (1992) reviewed numerous studies on rodents and suggested that testosterone may influence aggressive behavior either by direct action on androgen receptors in the brain or through the aromatization or conversion of testosterone to estradiol. These suggestions are based, in part, on findings that in mice, castrated males show lower levels of fighting behavior, which is restored following androgen replacement. Aggressive behavior in male mice has also been found to increase in puberty, coinciding with the increase of androgens at this stage in development. Prenatal exposure to hormones may also influence aggressive behavior in mice. Research reviewed by Beatty has suggested that male mice that have developed prenatally between two other males will be more aggressive as an adult than will a male mouse which developed between two female mice. This finding suggests that the prenatal exposure to androgens may organize the structure of the brain in such a way as to increase the sensitivity to, or activational effect of, gonadal hormones.

Manipulations of gonadal hormones may also influence human behavior. For example, in an investigation of human transsexuals, Van Goozen and colleagues (1994, 1995) reported that anger and aggression proneness increased when female-to-male transsexuals were administered androgens orally, suggesting that the increase in androgen level increased aggressive behavior.

Testosterone has been found to decrease with age in adult men (Dabbs, 1990; Deslypere and Vermeulen, 1984; Persky *et al.* 1971). Julian and McKenry (1989) have suggested that a lower level of testosterone in middle-aged men contributes to good marital relations and to androgynous behaviors and that this finding supports the concept of a sex convergence in older adults. Gray *et al.* (1991) also found that in middle-aged men, testosterone was negatively correlated with a self-report anger-in scale (turning aggressive feelings inward), which may suggest that men higher in testosterone are more likely to turn their anger outward toward other people, behaviors which may not be conducive to a high quality of interpersonal relationships. These results suggest that testosterone may have an influencing effect on behavior, at least in men, over the life span.

The possibility that testosterone concentrations may have a genetic component has been investigated in two studies which measured plasma testosterone in adult male twins. Meikle *et al.* (1988) reported that genetic factors accounted for approximately 85% of the variance in testosterone production rate and for approximately 4% of the variance in testosterone clearance rate. In another study, Meikle *et al.* (1987) found that approximately 34% of the variance in free (or unbound) testosterone was due to genetic factors and that genetic factors accounted for 26% of the variance in plasma (or bound) testosterone. One limitation of these studies is the absence of women as subjects. The present study examines the possible heritability of total plasma testosterone levels collected from male and female twin pairs as well as from their parents. These analyses are beneficial in increasing the understanding of the etiology of individual differences in testosterone levels during adolescence in both men and women. In addition, by including the parents of twins, it is also possible to study the inheritance across generations.

METHOD

Participants

This study is part of a larger project in which cardiovascular risk factors were studied in 160 adolescent twin pairs and their parents (see Boomsma *et al.*, 1993a, b, 1994, 1996). Addresses of twins (between 14 and 21 years of age) living in Amsterdam and neighboring cities were obtained from City Council population registries. Twins still living with both their biological parents were contacted by letter. A family was included in the study if the twins and both parents were willing to participate. In addition, a small number of families who heard of the study from other twins also volunteered.

Zygosity was determined by typing blood and DNA polymorphisms. There were 35 monozygotic (MZ) female pairs (average age, 16.0 years; SD =

2.2 years), 35 MZ male pairs (average age, 16.6 years; SD = 1.8 years), 30 dizygotic (DZ) female pairs (average age, 17.7 years; SD = 2.0 years), 31 DZ male pairs (average age, 17.2 years; SD = 1.7 years), and 29 DZ opposite-sex pairs (average age, 16.4 years; SD = 1.8 years). The average age of the fathers was 48.1 years (SD = 6.3 years) and the average age of the mothers was 45.6 years (SD = 5.9 years). For 24 fathers, 24 mothers, 15 sons, and 16 daughters, no testosterone data were available. The main reason for missing data was that the plasma sample was too small for assaying. Data from women (16 mothers and 23 daughters) who used oral contraceptives were discarded from the analyses because oral contraceptives can suppress endogenous testosterone production (Alexander *et al.*, 1990; Bancroft *et al.*, 1980).

Procedure

Fasting blood samples were taken between 0830 hours and 1030 hours. Because all samples were collected in the morning, this procedure helps to control for the circadian rhythm in testosterone, in which testosterone is higher in the morning and lowest in the evening (Riad-Fahmy *et al.*, 1983). Blood was collected under standardized conditions by venipuncture, using Becton–Dickinson Vacutainers containing sodium–EDTA. Plasma was separated from cells after centrifugation at 3000 rpm for 10 min at 4°C. The samples were stored at −20°C and thawed immediately before use. Total plasma testosterone (bound and unbound) levels were obtained using a commercially available Coat-a-Count ^{125}I radioimmunoassay (RIA) kit (Diagnostic Products, Los Angeles, CA). All samples were assayed in duplicate, and the amount of testosterone is expressed as nanograms per milliliter. The sensitivity of the assays ranged from 0.1 to 0.2 ng/ml. Figure 1 shows the distribution of testosterone levels and lists the mean testosterone values for family members (fathers, mothers, sons, daughters). This figure demonstrates that there is a moderate degree of variability in testosterone levels for both men and women. Data were log-transformed before statistical analysis.

Statistical Analysis

Genetic models specified variation in testosterone levels to be due to genotype and environment. Sources of variation considered were G, additive genetic variation, and E, a random environmental deviation that is not shared by family members. Their influence on the phenotype is given by parameters h and e. The proportion of variance due to each source is the square of these parameters. Figure 2 provides an illustration of the path diagram used to estimate additive genetic and specific environmental variances for the family members (a_f and e_f represent additive genetic and environmental variances, respectively, for fathers, and a_m and e_m represent additive genetic and environmental variances, respectively, for mothers). To account for possible sex or generation differences in genetic architecture, estimates for h and e were allowed to differ in magnitude between males and females and between parents and offspring. The correlation between genetic influences expressed in family members of opposite sex (i.e., DZ male–female twin, father–daughter, and mother–son pairs) was also estimated as a free parameter (r_g). Parameters h, e, and r_g and mean values for testosterone in each sex and generation were estimated by maximum-likelihood, using the computer program Mx (Neale, 1997).

Model fitting was carried out on the raw data in the five family groupings (i.e., families of MZ male and female twins and families of DZ male, female, and opposite-sex twins). With missing data in some families, as for female twins and mothers who used oral contraceptives, the data cannot be summarized into dispersion matrices, but the model has to be fitted directly to the raw data. Mx allows the likelihood of each pedigree to be calculated separately and maximized over all available pedigrees.

The likelihood of the genetic model described above was compared to the likelihood of a model which, for all five sex × zygosity groups specified a 4 × 4 covariance matrix and a 4 × 1 vector of mean testosterone concentrations. Goodness-of-fit of submodels was assessed by likelihood-ratio χ^2 tests. Submodels were compared by subtracting $-2\cdot$log-likelihood for the full model from that for a reduced model. This difference is distributed as a χ^2. The degrees of freedom (df) for this test are equal to the difference between the number of estimated parameters in the full model and that in the submodel.

RESULTS

Table I lists the correlations of testosterone concentrations with their confidence intervals

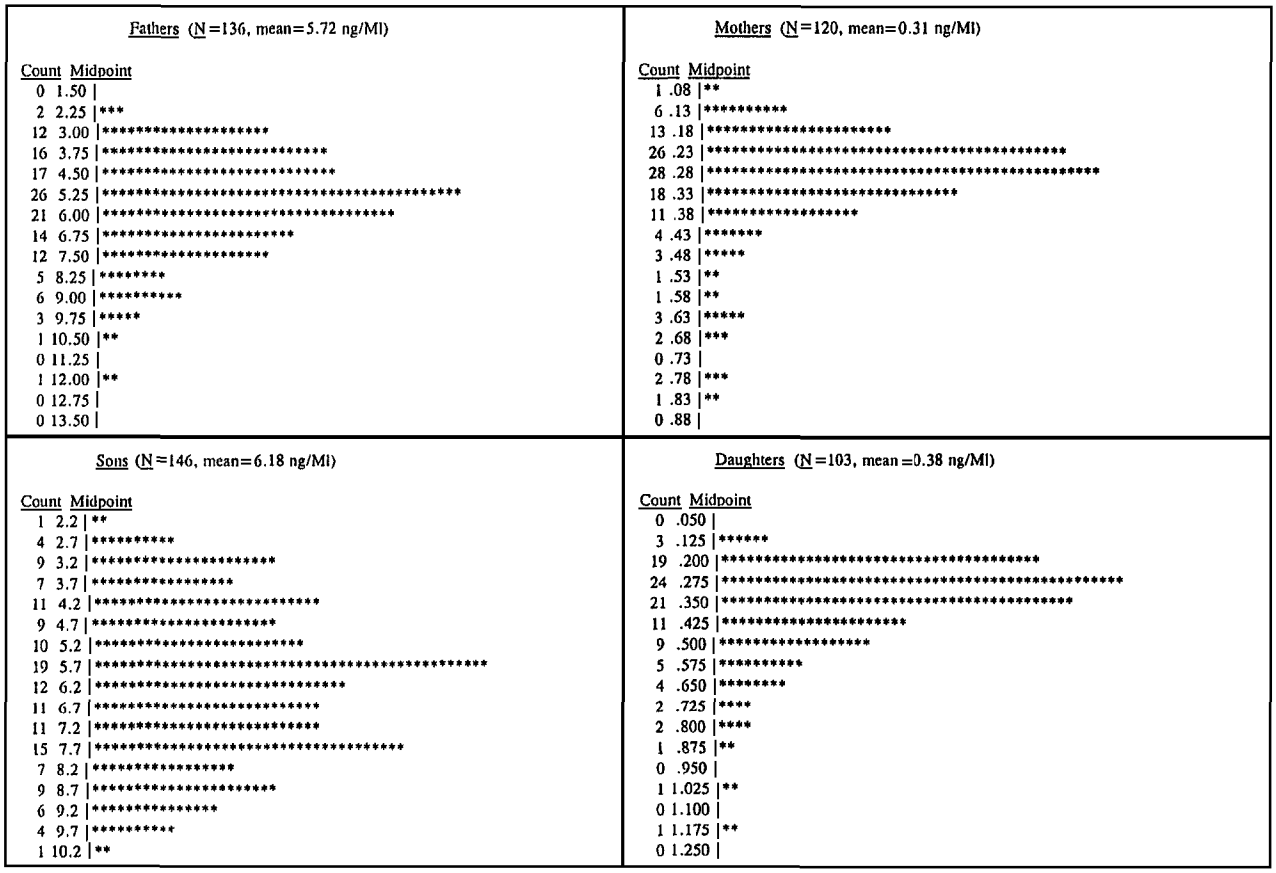

Fig. 1. Distribution and mean values for (untransformed) testosterone data in fathers, mothers, sons, and daughters.

(Neale & Miller, 1997) in twin pairs, in spouses and in parents and their children. The correlations for MZ twins (around 0.6) are higher than for other family members. For males the DZ correlation (0.34) is half the MZ correlation, indicating that additive genetic effects influence variation in testosterone levels. For DZ females, however, the correlation is estimated as zero. This could indicate genetic dominance in females. However, the observation that the mother–daughter correlation is 0.20 makes the hypothesis of genetic dominance in females unlikely. The zero correlation in opposite-sex DZ twins suggests that different genetic effects may influence variation in testosterone levels in males and females. This suggestion is supported by the lack of resemblance in mother–son and father–daughter pairs. The lack of resemblance between fathers and sons could indicate either that the heritability of testosterone is zero in adult men or that different genes are expressed in adulthood and during adolescence. As there is no way of distinguishing between these two alternatives in this data set, we have simply fixed the genetic correlation between fathers and sons at 0.5.

Table II gives the results of model fitting to the log-transformed testosterone data. Model 1 was the model used to obtain maximum-likelihood (ML) estimates of correlations, as presented in Table I. Models 2 and 3 test generation differences in means. This test of generation effects on testosterone means was nonsignificant in men [$\chi^2(1) = 3.62$] and significant in women [$\chi^2(1) = 12.76$], indicating that daughters have higher testosterone levels than their mothers. Models 4–7 examine causes of familial resemblance. The -2·log-likelihood for the full AE model with sex and generation differences in path coefficients and the genetic correlation between opposite-sex family members es-

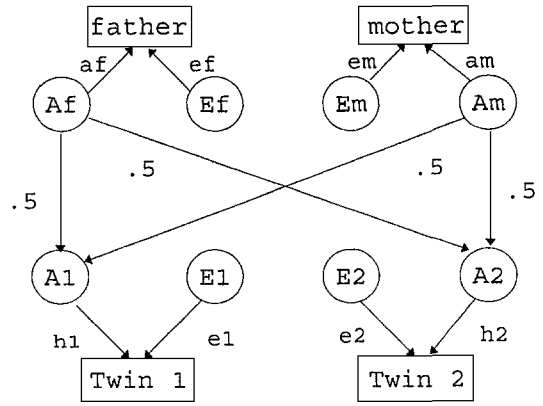

Fig. 2. Path diagram of genetic (*A*) and environmental (*E*) sources of variance for family members.

Table I. Maximum-Likelihood Estimates of Familial Correlations and 90% Confidence Interval Values

	ML correlation estimate	Confidence interval
MZ males	0.66	0.44–0.79
DZ males	0.34	0.02–0.57
MZ females	0.60	0.21–0.78
DZ females	−0.01	−0.39–0.34
DZ opposite sex	0.02	−0.27–0.31
Spouses	0.02	−0.13–0.18
Father–son	0.04	−0.12–0.19
Father–daughter	−0.11	−0.29–0.07
Mother–son	0.02	−0.15–0.19
Mother–daughter	0.20	0.00–0.38

timated as a free parameter was −449.191. This model fits the data as well as the first model that specified 10 correlations between family members [$\chi^2(4) = 2.46$]. The chi-squared test of whether the zero estimate for the genetic correlation between opposite-sex family members was significantly different from 0.5 (model 5) indicated a significantly worse fit of the model if this correlation was fixed at 0.5. The −2·log-likelihood for the AE model with equal heritabilities for mothers and daughters (model 6) was not worse than that for the model with generation differences. For fathers and sons the heritabilities could not be constrained to be equal without significant loss in fit.

The model fitting thus indicated that the influence of genetic factors was larger in young-adult males than it was in young-adult females, that genetic factors that influence testosterone levels differ in males and females, that the same factors are expressed in mothers and daughters, and that the heritability in women is the same in adolescence and adulthood. The heritability in young-adult males was estimated at 66%. A heritability of 41% was observed in females. The genetic correlation of opposite-sex family members was estimated as zero.

DISCUSSION

The heritability of total plasma testosterone concentrations was assessed in male and female twin pairs and their parents. The results suggest that testosterone concentrations are heritable in young-adult men, with 66% of the variance due to genetic effects. In adolescent and adult women, genetic effects were found to account for 41% of the variance in testosterone concentrations. The lack of resemblance of DZ opposite-sex twins suggested that different genetic factors influence testosterone levels in men and women. This suggestion is supported by the finding of no resemblance in testosterone concentrations between fathers and daughters and between mothers and sons. The ML estimate of correlation between genetic effects expressed in males and females was zero.

Two earlier studies in adult male twins found a high heritability of individual differences in testosterone levels later in life. Meikle *et al.* (1987, 1988) found that testosterone production rates and bound (plasma) and free (unbound) testosterone concentrations had a genetic component. The absence of a correlation between fathers and sons in the present study suggests that different genetic mechanisms may influence plasma testosterone concentrations during the life span. To test formally the assumption that different genes are expressed in the two generations, longitudinal data from genetically informative subjects are needed, or a heritability estimate from male twins that have the same age as the fathers in this study. We are currently trying to collect data on testosterone levels in middle-aged male twins, so that it will become possible to address this issue further.

No evidence for shared family environmental influences was found in our study, although the sample consisted entirely of parents and children living in the same household. This result was supported by the absence of any correlation between testosterone values of spouses.

Table II. Model Fitting to Testosterone Data from Twin Families[a]

	Model	−2log-likelihood	Number of estimated parameters	χ^2	df
1.	Separate means, father, mother, son, daughter; separate SD, father, mother, son, daughter (10 correlations)	−451.651	18	—	—
2.	Equal means, father–son (tested against model 1)	−448.030	17	3.621	1 (NS)
3.	Equal means, mother–daughter (tested against model 1)	−438.890	17	12.761	1
4.	AE, genetic correlation of opposite-sex family members free, different parameter estimates for father, mother, son, daughter (tested against model 1)	−449.191	13	2.460	4 (NS)
5.	AE, as 4, genetic correlation of opposite-sex family members fixed at 0.5 (tested against model 4)	−444.506	12	4.685	1
6.	AE, as 4, equal heritability in mothers and daughters (tested against model 4)	−446.948	11	2.243	2 (NS)
7.	AE, as 6, equal heritability in fathers and sons (tested against model 6)	−438.068	9	8.880	2

[a] Models 2 and 3 test generation differences in means and models 4–7 examine causes of familial resemblance (using log-transformed data).

This is the first study to find that plasma testosterone concentrations are heritable in women, an area of research not studied previously. The finding that plasma testosterone concentrations are heritable in men and women has important implications since testosterone may be related to socially important variables such as aggression and criminality, as reviewed above. Because testosterone has been found to have a similar pattern of relationships with aggression and prosocial personality characteristics in both men and women (Harris *et al.*, 1996), an area that needs further exploration is the examination of the genetic architecture of the covariance between gonadal hormones and behavior. In particular, if entirely different genes are involved in testosterone levels for men and women, then there may not be a genetic covariance between testosterone and behavior. Our results suggest either that any phenotypic correlations among testosterone and personality, such as aggression, may be due to environmental factors, or that a model of phenotypic causation, including reciprocal interactions, applies.

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