Gender Diagnosticity and Androgen Receptor Gene CAG Repeat Sequence

John C. Loehlin1, Erik G. Jönsson2, J. Petter Gustavsson2, Martin Schalling3, Sarah E. Medland4, Grant W. Montgomery4, and Nicholas G. Martin4

1Department of Psychology, University of Texas, Austin, TX, United States of America
2Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden
3Department of Molecular Medicine, Karolinska Institute, Stockholm, Sweden
4Queensland Institute of Medical Research, Brisbane, Australia

The gender diagnosticity (GD) approach of Lippa (1995) was used to evaluate the relationship of within-sex differences in psychological masculinity–femininity to a genetic characteristic, the length of a repeated CAG sequence in the X-linked androgen receptor (AR) gene. Previously assessed adult samples in Australia and Sweden were used for this purpose. A weak relationship (correlations in the range .11 to .14) was obtained in both countries. Additional data from adolescent twins from Australia (12-, 14-, 16-year-olds) did not confirm such a relationship at those ages, especially for males. The fact that this sample consisted of twins permitted two kinds of within-pair comparisons: (1) Did the dizygotic twin who had the longer AR sequence have the higher GD score? (2) Was one twin’s GD score more highly correlated with the other twin’s AR score in MZ than in DZ pairs? The answer in both cases was negative. Clarification of these relationships will require large samples and measurements at additional ages.

Does a genetic characteristic, the length of a repeated sequence of CAG triplets on the X-linked androgen receptor gene (AR), predict variations in the psychological trait of masculinity–femininity (MF) within the two sexes? Previous work based on samples in Australia and Sweden who had been administered personality tests and AR assessments (Jonsson et al., 2001; Loehlin et al., 1999; Loehlin et al., 2003) has suggested that such a relationship is very weak at best, but the question still arises: ‘Does AR predict MF at all?’ The literature is littered with failed attempts to link specific genes to specific behavioral characteristics, despite ample evidence from twin, adoption, and family studies that genetic predispositions play an important role in the development of most behavioral traits (Plomin et al., 1997).

The initial study in Australia (Loehlin et al., 1999) suggested that one aspect of psychological MF, the preference for intimacy versus aloofness in interpersonal relationships, was modestly associated with the length of the AR repeat sequence in women (correlation of .13). The direction of the association—more repeats going with more feminine scores on the trait—seemed theoretically appropriate. Shorter AR sequences have been associated with more effective transcriptional activation activity (Chamberlain et al., 1994) and many androgen-related characteristics, such as higher sperm concentrations (Tut et al., 1997; von Eckardstein et al., 2001), pattern baldness (Ellis et al., 2001; Sawaya & Shalita, 1998), an elevated risk of prostate cancer (e.g., Giovannucci et al., 1997; Irvine et al., 1995), and a lower ratio of the length of the index finger to the length of the ring finger (Manning et al., 2003). All of the above would lead us to expect longer CAG sequences to go with more feminine characteristics. However, an analysis of the data from the Swedish study, via a US sample that had been given items from the questionnaires used in both Sweden and Australia, failed to replicate such an association (Loehlin et al., 2003).

One recent perspective on psychological masculinity–femininity, that of gender diagnosticity (Lippa, 1995; Lippa & Connelly, 1990), suggests that we may have gone about this in the wrong way. Instead of assuming that the trait MF should be measured by the same items across samples, Lippa says that we should assess MF within samples in terms of response to those items which in that sample discriminate males from females. Thus gender diagnosticity (GD) is not a fixed trait, but one that may vary with age, eras and cultures.

Two methods of scoring GD have been in common use. A traditional method dating back to Terman and Miles (1936) is to select a set of items for which males and females differ in their endorsement frequencies, and score each individual based on his or her tendency to answer these items in a masculine or

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Address for correspondence: J. C. Loehlin, University of Texas, Psychology Department, 1 University Station A8000, Austin, TX 78712–0187, USA. E-mail: loehlin@psy.utexas.edu
feminine direction. However, Lippa and Connelly (1990) have proposed the alternative method of carrying out two-group discriminant analyses on subsets of items, using males and females as the criterion groups, and for each such subset to assign to each individual in the sample the probability (as calculated by the discriminant analysis program) that someone responding in this way is male or female. These probabilities are then averaged over all the item subsets to constitute the individual’s GD score. In the present study, GD scores derived in both these ways were correlated with AR scores in the Swedish and Australian samples. As reported in a separate paper (Loehlin et al., 2004), the choice between methods is probably not very critical, in the sense that scales derived in the two ways in the present samples tend to be highly correlated (rs mostly in the .80s and .90s).

Some time following the initial analyses of the adult Australian and Swedish samples, an additional sample of Australian adolescents became available, for whom AR and personality questionnaire data had both been obtained. These were predominantly twins for whom personality measures were available at ages 12, 14, and 16 years—some at all three ages, some at two of the three, and some at a single age. Some siblings of the twins were also tested, and those within a year of these ages were included in the present study. These samples permit us to examine whether results from the adults generalize to adolescent ages when masculine and feminine characteristics are presumably undergoing rapid development and change.

**Method**

**Samples**
The samples and data sets are briefly described in Table 1. They consisted of Swedish adults (mean age 43.1 years) from the Stockholm area tested in person, and adult Australian twins (mean age 42.2 years) tested by mail. In Australia, one twin from each of 294 female monozygotic pairs had been assessed for AR repeats for another study (Spurde et al., 2000); since MZ twins are genetically identical, this implies 588 women with known AR scores. In the Swedish study, all participants received the AR assessment. The Australian adolescents were tested in person. The androgen receptor polymorphism was genotyped by the Australian Genome Research Facility (Ewen et al., 2000). The number of repeats ranged from 13 to 29 in both males (M 21.04, SD 2.69) and females (M 21.47, SD 2.84).

**Questionnaires**
The Swedish subjects were administered the 135-item Karolinska Scales of Personality (KSP; Schalling et al., 1987). The Australian adult twins received a 56-item version of the Eysenck Personality Questionnaire (EPQ; Eysenck et al., 1985) and a 54-item version of Cloninger’s Tridimensional Personality Questionnaire (TPQ; Cloninger et al., 1991). The Australian adolescents received the Junior Eysenck Personality Questionnaire (JEPQ; Eysenck & Eysenck, 1975). Each inventory contains scales purporting to measure several personality or temperament dimensions, but we treat them here as simply collections of items, some more often endorsed by males than females or the reverse.

**Derivation of Scales**
The procedure for deriving the discriminant-based GD scales followed Lippa’s. Each item set was divided into 10- or 11-item subsets. The probability that an individual would be diagnosed as male or female was calculated for each subset by the program SPSS Discriminant (SPSS, 1990). These probabilities were then averaged to yield an overall GD score for the individual.

In the contrasted groups procedure, all items were correlated with sex, and those with correlations above a threshold were selected to form the scale. For the Swedish sample, the threshold used was an absolute correlation of .135, which corresponded to the .01 level of significance for a sample size of 363. For the Australian samples, an arbitrary threshold correlation of .100 was used for item selection. (With the large samples, smaller correlations than this would be nominal statistically significant, but not very useful for a scale.)

**Results**

**GD and AR Sequence Length in Adults**

Is psychological femininity related to AR sequence length in adults? Table 2 provides the evidence.

The results suggest that the relationship originally detected in the Australian sample for one aspect of femininity holds for the broader GD measure, and that this relationship is replicated for both men and women.
in the Swedish sample. With the smaller samples of Swedish men and women, correlations of this magnitude are only marginally different from zero, by two-tailed test — the \( p \) values range from .070 to .105. Nevertheless, quantitatively the replication is good. In the larger Australian sample, the \( r \)s are clearly statistically significant, although obtaining exact \( p \) values would require taking into account the twin structure of the data. If the two twins are assumed to be completely redundant, the two-tailed \( p \) values would be .03 and .04; if the twins are completely independent, .002 and .004. Presumably, the true probabilities lie somewhere in between these limits. In any case, it is clear that the relationship between AR and MF is quantitatively a weak one. Within a given sex, knowing AR scores would only predict 1% to 2% of the individual variation in masculinity–femininity.

**GD and Sequence Length in Adolescents**

Does the same relationship hold for the 12- to 16-year-olds in the Australian sample? The answer, shown in Table 3, is largely negative. The correlations are all low, and only in the case of the girls at ages 12 and 14 are they even in the same direction as those among the adults. In particular, the girls at age 16, whom one might expect a priori to be more like adult women than girls of 12 or 14, are, if anything, less like them. Could this last result somehow reflect differential selection in the group tested at age 16, or the nature of the specific GD scale derived at this age?

The answers to these questions are given in Table 4, and they are both ‘No’.

As shown in Table 4, the results remain very similar when we confine ourselves to the group of adolescents who were measured at all three ages, or (in the third column) to measurements on a scale of GD items common to the three specific age scales. The bottom panel of Table 4 addresses another possibility. Some studies have found stronger relations to AR repeats in women based on just the longer of their two alleles. The center panel of Table 4 shows correlations with the mean repeat length for the two X-chromosomes. The bottom panel shows correlations with just the longer of the two alleles. The correlations with the longer allele do not appear to be notably different from those with the mean of the two alleles.

**Within-Twin-Pair Analyses**

Because the adolescent sample consists largely of twins, we can see if there is any association between AR and GD scores within twin pairs. This permits comparisons in which many factors that vary between families are controlled. The first such comparison asks the question: In dizygotic same-sex pairs, is the twin with the longer AR sequence the more feminine of the two?

The first column of Table 5 provides the answer, and it is again ‘No’. Although the numbers of pairs are not large enough for any of the correlations to differ significantly from zero, the largely negative correlations in this better-controlled comparison certainly do not suggest a positive relationship between femininity and length of AR gene repeat sequence at these ages.

In the right-hand columns of Table 5 another within-pair comparison is made to investigate if there may be a genetic link between AR and GD. Here we
Table 5
Comparisons of GD and AR Within-Twin Pairs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Correlation of AR and GD differences</th>
<th>Cross-twin correlations of AR and GD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DZ pairs</td>
<td>MZ</td>
</tr>
<tr>
<td>Boys, age 12</td>
<td>-.12 (66)</td>
<td>-.35 (32)</td>
</tr>
<tr>
<td>age 14</td>
<td>-.12 (66)</td>
<td>-.28 (31)</td>
</tr>
<tr>
<td>age 16</td>
<td>-.15 (46)</td>
<td>-.19 (26)</td>
</tr>
<tr>
<td>Girls, age 12</td>
<td>.09 (58)</td>
<td>.20 (35)</td>
</tr>
<tr>
<td>age 14</td>
<td>-.11 (63)</td>
<td>.13 (36)</td>
</tr>
<tr>
<td>age 16</td>
<td>-.22 (51)</td>
<td>-.15 (30)</td>
</tr>
</tbody>
</table>

Note: Ns in parentheses. MZ — monozygotic; DZ — dizygotic; GD—gender diagnosticity (discriminant version); AR — length of androgen receptor gene repeat sequence: for boys, on their one X-chromosome; for girls, the mean for their two X-chromosomes.

examine cross-twin correlations. Is the correlation between AR in one twin and GD in the other higher for monozygotic (MZ) than for dizygotic (DZ) pairs? If there is a gene-based association between the two, we would expect this to be the case. The correlations are again based on fairly small numbers of pairs, and again, none reach conventional levels of statistical significance. But once more, the suggestion is that if there is any kind of genetic link between AR gene sequence length and gender diagnosticity in this age group, it is in the wrong direction. With the possible exception of the 12-year-old girls, there is little hint of positive MZ correlations that are higher than positive DZ correlations.

Discussion

The approach to the assessment of within-sex masculinity–femininity via Lippa’s gender diagnosticity yielded consistent evidence in adults of both sexes for a weak association between the length of a repeated sequence on the AR gene and psychological femininity. The association, as evidenced by correlations in the .11 to .14 range, would account for only a small fraction of the roughly 45% of the total within-sex variance in MF that is accounted for by genes (Loehlin et al., 2004). Many other genes presumably affect the relative masculinity or femininity of members of each sex, as do environmental factors—although apparently not those environmental factors that are shared by family members.

The results for the Australian adolescents seriously cloud this simple picture. Instead of correlations in the range .11 to .14, we observe correlations in the range −.07 to .10, more often negative than positive. In particular, the correlations for the boys, although low, suggest that more masculine attitudes and behavior tend to be associated with longer, rather than shorter AR CAG sequences. Moreover, it is the 12- and 14-year-old girls who tend to show correlations in the direction of those of the adult Australian and Swedish women, not the 16-year-old girls whom one might expect to be closer to adult status. Comparisons restricted to thrice-tested individuals and to common sets of items and comparisons made within twin pairs or to just the longer allele did not alter this picture.

On the whole, then, the adolescents fail to confirm the adult within-sex association between longer AR sequences and more feminine attitudes. Why? One possible source of difference between adolescents and adults is a shift in the content of GD scales with age. At age 12, the differences between girls and boys emphasize good behavior and social conformity (Loehlin et al., 2004), but this component diminishes in importance with age, while sex differences in emotional instability and maladjustment increasingly characterize GD. With respect to these trends, the 16-year-olds are shifting toward adult characteristics, and thus these changes would not explain the detailed pattern of the AR-GD results. Beyond this we can only speculate. It is possible, for example, that the sheer impact of changing hormone levels at adolescent ages may swamp any effects of the AR gene. Studies covering a wider range of ages would help clarify such an interpretation. Or it may be that the groups studied here, for whatever reasons, constitute unrepresentative samples. If so, repetition of the comparisons in other samples will eventually straighten matters out. Finally, it is possible that we are dealing with the very common situation in this field of a failure to replicate weak associations when tested in new populations. Eventually, samples will be large and representative enough to let us give confident ‘Yes’ or ‘No’ answers concerning weak associations between particular genes and behavioral traits. That day has not yet arrived.

Some other recent efforts to link personality to AR gene sequence length should be noted. Longer CAG sequences tended to be associated with masculine traits in 42-year-old Swedish women, but not with traits identified as feminine (Baghaei et al., 2003). These results are at odds with the findings of the present paper in another group of Swedish women, for which longer CAG sequences tended to go with femininity. Comings and his colleagues (1999) have reported a tendency of short CAG and GGC AR sequences to go with such externalizing disorders as ADHD, conduct disorder, and oppositional-defiant disorder in a population of Tourette syndrome probands and controls. On the whole this seems consistent with the typical association between short sequences and androgen-related characteristics.

We should also note that the relation of the AR gene to testosterone levels is not necessarily a straightforward one. For example, the distribution of AR gene CAG sequence lengths does not differ notably between males and females, although testosterone levels do, both during prenatal development and after puberty. Shorter CAG sequences are associated with more rapid declines in testosterone as men age (Krithivas et al., 1999). As
noted in the introduction, a variety of androgen-related conditions have been found to be associated with short AR CAG sequence lengths, but levels of free serum testosterone in young men have been reported to be correlated positively, not negatively, with CAG sequence length (Giwercman et al., 2004). This could conceivably relate to the unexpected direction of correlation of CAG sequence lengths with GD in the Australian adolescent males. The more typical negative relationship has, however, been reported for women: levels of total testosterone were found to be lower in individuals with long CAG sequences in the sample of 42-year-old Swedish women previously mentioned (Westberg et al., 2001). Complex regulatory relationships may occur among variables in this area, and simple pictures are not always to be expected.

In addition, we should keep in mind that psychological masculinity and femininity are complex traits, and that it would be surprising if AR gene CAG sequence lengths were to prove to be equally related to all aspects of them. For example, males of heterosexual orientation, compared to males of heterosexual orientation, tend to show feminine characteristics in some respects although not in others (e.g., Loehlin & McFadden, 2003). GD measures (based on occupational and avocational preferences) show large average differences between homosexual and heterosexual men (Lippa, 2000). Thus it becomes of some interest in the present context to ask what the AR genes of homosexuals look like. At least one study has compared the CAG sequence lengths of heterosexual and homosexual men (Macke et al., 1993). They found no difference.

In summary, although long CAG sequences on the AR gene sometimes seem to be associated with psychological femininity and short sequences with masculinity, this is not uniformly the case. The present study raises doubts about adolescents, and other studies have reported mixed findings for adults. Because the relationships are weak at best, large samples at different ages will be required to clarify them.

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References


