**CLINICAL STUDY**

**Testosterone, androgen receptor gene CAG repeat length, mood and behaviour in adolescent males**

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**Abstract**

**Objectives:** Androgen activity has been implicated in a range of traits and behaviours that have well-documented sex differences. However, the results of the studies on the relationship between testosterone and these traits and behaviours are inconsistent. This study has analyzed i) whether CAG repeat length, a presumed modulator of androgen receptor sensitivity, is associated with sex-dimorphic traits and behaviours (aggressive and non-aggressive risk-taking (ART and NART), dominance, depressive symptoms and self-esteem), and ii) whether CAG repeat length interacts with free testosterone (FT) with respect to these traits and behaviours.

**Design and methods:** Data obtained from a group of adolescent boys ($n = 301$; mean age: 14.4 years) were analyzed using multivariate general linear modelling (SPSS, Chicago, IL, USA 15.0).

**Results:** We found no direct correlation between CAG repeat length and dependent variables. We found significant interactions between CAG repeat length and testosterone, indicating that FT was more positively related to ART and NART with a shorter repeat length, and that an inverse association of FT with depressive symptoms and a positive association with self-esteem were stronger in boys with a longer CAG repeat length.

**Conclusion:** Our findings indicate the importance of studying FT and CAG repeat length simultaneously with respect to sex-dimorphic traits, taking into account the possible interactions between the two.

**Introduction**

Sex differences in human behaviour, personality and tendency to emotional states are well documented, and research on sex differences has found that boys/men engage in more dominance-seeking behaviour (1–3), are more involved in risk-taking (4) and are less prone to depression or low self-esteem than girls/women (5). It is assumed that androgens, in particular testosterone, may play a role in the aetiology of aggression (6–8), non-aggressive forms of adolescent risk-taking (9, 10) and, at least in hypogonadal men, mood-related variables such as depression or self-esteem (11–13). However, results have been inconsistent, with most studies finding at best small or moderate associations with androgens, often in clinical or atypical populations, e.g. hypogonadal men/boys.

Despite the recognition that serum levels of testosterone are only one aspect of the androgen cascade that plays a role in the aetiology of sex-dimorphic traits and behaviours (14, 15), a limited number of studies have analyzed the role of other factors (among them, genetic factors) that may modify testosterone action with respect to behaviour. Androgens bind to intracellular receptors, and as such, these intracellular receptors moderate androgen-related changes in gene expression. The androgen receptor (AR) gene is located on the X chromosome at XQ11–12 and is highly polymorphic, and research has identified at least 200 mutations in patients with androgen insensitivity syndrome (16). More subtle modulations of the transcriptional activity induced by the AR have been assigned to a polyglutamine stretch of variable length within the N-terminal domain of the receptor encoded by a polymorphic CAG repeat in the first exon of the AR gene. In a normal population, this triplet is repeated 9–37 times (17, 18). Reduction in androgen gene expression is thought to be proportional to the number of CAG repeats over the range of normal alleles, with the shorter alleles showing the greatest activity (16). Studies indicating that a lower number of CAG repeats is associated with androgen-dependent conditions, e.g. prostate cancer (19), benign prostate hyperplasia (20) and higher incidence of alopecia (21), support this finding. However, not all studies have found such associations (18, 22).

If both androgen serum levels and AR sensitivity are aspects of the androgen cascade, it may be important to assess both serum levels of testosterone and CAG repeat length, and to analyze the possible interactions between
the two. Some studies have already documented these interactions. It was found, for example, that the AR gene CAG polymorphism contributed modestly to the between-subject variation in testosterone action on body composition in community-dwelling elderly individuals (23). In addition, it has been shown that the CAG repeat polymorphism modulates responses induced by testosterone replacement therapy in hypogonadal men (24, 25).

It has been proposed that CAG repeat length may be relevant with respect to explaining androgen-related behaviours as well. Several studies in humans have found that the presence of shorter alleles of the AR gene was related to attention deficit hyperactivity disorder (ADHD), conduct disorder and oppositional defiant disorder (26) and to violent criminal behaviour (27). Others found that the relationship with criminal activity was small at best (28). Evidence of a relationship between CAG repeat length and personality traits has been inconsistent as well, with some studies finding that AR was related to personality traits such as Eysenck’s psychoticism (29), while others found no such association (30) or found tendencies for an inverse association (31) in boys.

Studies on the existence of interactions between CAG repeat length and testosterone with respect to sex-dimorphic behaviour or traits, however, are scarce. One study of middle-aged men showed depressive symptoms to be inversely associated with total testosterone (TT) in men with shorter CAG repeat lengths, but not in men with moderate and longer CAG repeat lengths (32).

In this study we have analyzed, in a sample of adolescent boys of Caucasian origin, the interactions between testosterone and CAG repeat length polymorphism as a modulator of AR sensitivity with respect to adolescent risk-taking and aggression, dominance, symptoms of depressive symptoms and self-esteem. These traits and behaviours were selected because they have well-documented sex differences, and because evidence exists on the relationship between testosterone and CAG repeat length polymorphism as a modulator of AR sensitivity with respect to adolescent risk-taking and aggression, dominance, symptoms of depressive symptoms and self-esteem. The sensitivity for TE2 was 10 ng/dl; the intra-assay coefficient of variation (CV) fell between 4.6 and 10.1%, and the inter-assay CV fell between 5.2 and 11.7%. The sensitivity for SHBG was 0.7 nmol/l; the intra-assay CV fell between 2.6 and 8.5%, and the inter-assay CV fell between 3.4 and 9.6%. The sensitivity for TE2 was 0.2 ng/dl; the intra-assay CV was 3% and the inter-assay CV was 8.5%.

Free testosterone (FT) and free oestradiol (FE2) were calculated from total hormone SHBG and albumin concentrations by means of validated equations derived from the mass action law (33). We used FT in the analyses rather than TT, as it is the unbound testosterone that binds to the ARs.

CAG repeat length. Genomic DNA was extracted from EDTA-treated blood using a commercial kit (Qiagen Midikit, Qiagen Inc). We used PCR to amplify exon 1 of the AR gene with primers 5'-AGCCTTGAGTCTCCTTCT-3' (sense) and 5'-CTGCCTTACAAACTCTTTCGGC-3' (antisense). After ethanol precipitation, the amplified fragment was directly sequenced on an ABI Prism 310 sequencer (ABI Prism, Perkin-Elmer Applied Biosystems, Foster City, CA, USA) using a BigDye Terminator Cycle Sequencing Reaction kit (ABI Prism, Perkin-Elmer Applied Biosystems). Fragment length was determined by running the GeneScan-400HD Analysis Software (ABI Prism, Perkin-Elmer Applied Biosystems).
Dependent variables. Adolescent risk-taking. Aggressive risk-taking (ART) and non-aggressive risk-taking (NART) scales were developed and validated by confirmatory factor analysis as part of the ADORISK project (full scales included in 10).

To measure ART, we asked our respondents to rate their experience, with six items on a 5-point scale. Items included actual physical aggression (I was involved in fighting at school), threats of violence (I threatened someone with violence) or verbal aggression (I insulted a teacher to his/her face). Cronbach’s α for this scale was 0.79, with the corrected item-total correlations between 0.38 and 0.65. To measure NART, we asked our respondents to rate their experience, with 21 items on a 5-point scale. Items included, e.g. ‘I smoked a joint’, ‘I took a train, tram or bus without paying’ and ‘I drank five glasses of alcohol in one evening’. Cronbach’s α for this scale was 0.88, with the corrected item-total correlations ranging between 0.38 and 0.66.

Dominance. To measure dominance, respondents were asked to complete the California Psychological Inventory-Dominance (CPI-D) scale. The CPI-D scale (34) consists of 11 items to be rated on a 5-point scale. Items include ‘I try to surpass others’ accomplishments’, ‘I want to control the conversation’ and ‘I put people under pressure’. Cronbach’s α for this scale was 0.83, with the corrected item-total correlations ranging between 0.38 and 0.61. The sum of the item scores was used.

Depressive symptoms. To measure depressive symptoms, we used the Centre for Epidemiologic Studies-Depression (CES-D) questionnaire (35). The CES-D questionnaire is an often-used and validated instrument (36–38) that consists of 20 items to be rated on a 4-point scale. The Dutch translation of this scale (39) has good reliability and validity. Cronbach’s α was 0.88, with the corrected item-total correlations ranging between 0.35 and 0.69. The sum of the item scores was used.

Control variables. Pubertal development. Pubertal development (PD) was measured by the Tanner stage (42) and assessed by a physician. Scores were assessed based on both pubic hair and pubis development, using a scale of 1 (prepubertal) to 5 (adult). Scores were totalled, resulting in an index of 2–10.

Age. Age was measured as ‘years completed at the time of the research’. Age is especially important, as previous analyses have indicated that the effect of testosterone on risk-taking (non-aggressive and aggressive forms) is more pronounced in older boys than in younger boys; therefore, age X testosterone interactions were included in the analyses.

Ethnicity. Ethnic differences have been reported in average CAG repeat lengths. In our sample, 18 individuals were not of European Caucasian origin (operationalized as having at least one parent of non-European ancestry). This group is too small and too diverse to allow for separate analyses. For this reason, and because differences in cultural background could affect the dependent variables, our study, like other studies (43), removed individuals of non-Caucasian ancestry (n=18) from the analyses.

<table>
<thead>
<tr>
<th>Table 1 Characteristics of the sample (n=283).</th>
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<tbody>
<tr>
<td>Mean</td>
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<tr>
<td>------</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>PD</td>
</tr>
<tr>
<td>TT</td>
</tr>
<tr>
<td>TE2</td>
</tr>
<tr>
<td>SHBG</td>
</tr>
<tr>
<td>FT (ng/dl)</td>
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<tr>
<td>FE2</td>
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<tr>
<td>CAG RL</td>
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<tr>
<td>Body fat (%)</td>
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<tr>
<td>NART</td>
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<tr>
<td>ART</td>
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<tr>
<td>Dominance</td>
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<tr>
<td>CES-D</td>
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<tr>
<td>Self-esteem</td>
</tr>
</tbody>
</table>

PD, pubertal development; TT, total testosterone; FT, free testosterone; TE2, total oestradiol; FE2, free oestradiol; CAG RL, CAG repeat length; NART, non-aggressive risk-taking; ART, aggressive risk-taking; CES-D, depressive symptoms.

**Note:** Reference values as indicated by commercial immunoassay kits.

*Zitzmann & Nieschlag (2003).*
Body fat percentage. Body fat may be related to CAG repeat length, and hormonal values may be associated with body fat (44, 45). Body fat percentage was measured by a nurse using leg-to-leg bioimpedance equipment (TBF-300, Tanita Arlington Heights, IL, USA).

Lifestyle factors. SHBG and unbound, bioactive testosterone may be influenced by lifestyle factors such as smoking and alcohol consumption (46) and, as such, their relationship with risk-taking and/or any of the mood-related dependent variable may be modified. Smoking and regular alcohol consumption are items that are included in the NART scale, and are as such strongly related to this scale. These variables cannot be easily controlled for as this would strongly increase multicollinearity. However, we controlled for alcohol consumption during the day prior to the study as a binary variable, with ‘0’ indicating ‘no consumption’ and ‘1’ indicating ‘consumption’ of alcohol.

Analyses

To analyze the relationships between independent and dependent variables, we used multivariate general linear modelling. This technique analyzes the relationship of each predictor variable, and the dependent variables, controlling for all other predictor variables, but also controls for the relationships among the multiple dependent variables.

Analyses were done using three models. In the first model, independent variables without interactions were introduced. In the second model, interaction terms were introduced. To avoid high levels of multicollinearity, independent variables were standardized before product terms were calculated. The effect of FE2 was controlled for only in the third model to avoid a potential problem of multicollinearity: FT and FE2 are strongly associated, and this may reduce the possibility of differentiating statistically between the effects of FT and FE2. Although variance inflation factor (VIF) scores in the analyses that include FE2 are moderate and do not exceed 3.05, multicollinearity may inflate the s.e.m. and affect the levels of significance. As such, differences between the results of the second and the third models should be interpreted with care. For this reason, we used an analysis in which FT was regressed on FE2 and the standardized residuals were calculated. These residuals – that reflect the variability in FE2 once FT is controlled for – were then substituted for FE2 in the analyses presented in the third model. The results of this additional analysis were compared with the results obtained using the third model.

Although CAG repeat length was used as a continuous variable in the analysis, to depict interaction effects, it was categorized by taking the mean repeat length (21.69)±0.5 s.d. (3.03), resulting in short (≤20; n = 94), medium (> 20 and ≤23; n = 115) and long (> 23; n = 71) CAG repeat lengths. Because using
Table 3 Multivariate general linear modelling of independent variables and interaction terms on NART, ART, dominance, depressive symptoms and self-esteem.

<table>
<thead>
<tr>
<th>Model</th>
<th>Intercept</th>
<th>PD</th>
<th>FT</th>
<th>Age</th>
<th>Body fat</th>
<th>Alcohol consumption</th>
<th>CAG repeat length</th>
<th>Age×FT</th>
<th>CAG repeats×FT</th>
<th>Adj. R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>NART</td>
<td>58.82</td>
<td>0.52</td>
<td>2.18</td>
<td>2.73</td>
<td>0.10</td>
<td>-8.58</td>
<td>0.248</td>
<td>2.28</td>
<td>-1.56</td>
<td>0.16</td>
</tr>
<tr>
<td>ART</td>
<td>53.20</td>
<td>0.43</td>
<td>1.73</td>
<td>2.18</td>
<td>0.11</td>
<td>-7.57</td>
<td>0.266</td>
<td>2.12</td>
<td>-1.55</td>
<td>0.20</td>
</tr>
<tr>
<td>Dominance</td>
<td>54.07</td>
<td>0.26</td>
<td>1.06</td>
<td>2.09</td>
<td>0.08</td>
<td>-1.40</td>
<td>0.232</td>
<td>2.12</td>
<td>-0.70</td>
<td>0.01</td>
</tr>
<tr>
<td>Depressive symptoms</td>
<td>12.35</td>
<td>0.04</td>
<td>0.24</td>
<td>0.13</td>
<td>0.02</td>
<td>-0.82</td>
<td>0.049</td>
<td>0.24</td>
<td>0.65</td>
<td>0.01</td>
</tr>
<tr>
<td>Self-esteem</td>
<td>11.28</td>
<td>0.12</td>
<td>0.14</td>
<td>0.03</td>
<td>0.02</td>
<td>-0.32</td>
<td>0.049</td>
<td>0.23</td>
<td>0.65</td>
<td>0.01</td>
</tr>
</tbody>
</table>

| NART  | 34.88 | 0.43 | 0.25 | 0.13 | 0.03 | -0.82 | 0.049 | 0.24 | 0.65 | 0.01 |
| ART   | 33.85 | 0.12 | 0.14 | 0.03 | 0.02 | -0.32 | 0.049 | 0.23 | 0.65 | 0.01 |
| Dominance | 33.65 | 0.12 | 0.14 | 0.03 | 0.02 | -0.32 | 0.049 | 0.23 | 0.65 | 0.01 |
| Depressive symptoms | 33.65 | 0.12 | 0.14 | 0.03 | 0.02 | -0.32 | 0.049 | 0.23 | 0.65 | 0.01 |
| Self-esteem | 33.65 | 0.12 | 0.14 | 0.03 | 0.02 | -0.32 | 0.049 | 0.23 | 0.65 | 0.01 |

| NART  | 41.52 | 0.43 | 0.25 | 0.13 | 0.03 | -0.82 | 0.049 | 0.24 | 0.65 | 0.01 |
| ART   | 41.72 | 0.12 | 0.14 | 0.03 | 0.02 | -0.32 | 0.049 | 0.23 | 0.65 | 0.01 |
| Dominance | 42.07 | 0.12 | 0.14 | 0.03 | 0.02 | -0.32 | 0.049 | 0.23 | 0.65 | 0.01 |
| Depressive symptoms | 42.07 | 0.12 | 0.14 | 0.03 | 0.02 | -0.32 | 0.049 | 0.23 | 0.65 | 0.01 |
| Self-esteem | 42.07 | 0.12 | 0.14 | 0.03 | 0.02 | -0.32 | 0.049 | 0.23 | 0.65 | 0.01 |

1 S.D. would have resulted in the ‘short’ category having an insufficient number of cases \((n = 35)\) to analyze, 0.5 S.D. was used. Cut-off values used were similar to those used by others (44). Alternative classifications using the 33th and 66th percentiles did not yield visualizations that were substantially different from those presented in this paper.

Missing values were present with respect to PD \((n = 11)\), CAG repeats \((1)\), NART \((n = 2)\), dominance \((n = 1)\), CES-D \((n = 1)\) and self-esteem \((n = 1)\).

**Results**

**Univariate analyses**

Table 1 shows the results of univariate analyses. The mean age was 14.4 years (S.D. = 0.74). The mean PD was 8.38 (S.D. = 1.9), and the average mean for TT, SHBG and FT was within the normal range for adult men. The mean CAG repeat length was comparable to what other studies on individuals of Caucasian origin found (14). In total, 9.4% of the participants (data not shown in Table 1) reported to have drunk alcohol on the day prior to the study.

**Bivariate analyses**

Table 2 shows the results of bivariate analyses. FT was related to NART \((r = 0.23; P < 0.001)\) and self-esteem \((r = 0.13; P < 0.05)\), but not to dominance or ART. CAG repeat length was unrelated to NART, ART or dominance. FT was related to age \((r = 0.23; P < 0.001)\) and PD \((r = 0.61; P < 0.001)\). PD was related to self-esteem \((r = 0.16; P < 0.01)\), FE2 was related to FT \((r = 0.79; P < 0.001)\) and NART \((r = 0.24; P < 0.01)\). Body fat was related to FT \((r = 0.18; P < 0.01)\) and FE2, \((r = 0.30; P < 0.001)\). Alcohol consumption on the day prior to the study was related to NART \((r = 0.25; P < 0.001)\) and ART \((r = 0.13; P < 0.05)\).

**Multivariate analyses**

Table 3 shows the results of the multivariate analyses. In the first step, the main effect of the independent variables was assessed. NART was related to age \((B = 2.73; P < 0.001)\) and FT \((B = 2.18; P < 0.012)\), but not to any of the other dependent variables. CAG repeat length was not associated with any of the dependent variables.

In the second step, we tested whether CAG repeat length moderated the relationship between FT and dependent variables. Significant interaction effects were found with respect to NART \((B = 1.56; P < 0.0033)\) and ART \((B = 0.45; P < 0.035)\), indicating that the effect of FT on NART and ART was more pronounced in boys with shorter CAG repeat lengths. While in the strictest sense, no interaction effect between FT and CAG repeat length was found with respect to dominance, Fig. 3 hints that a relationship between FT and dominance may exist in the short CAG repeat length group, but not in the other groups. Using the categorization outlined in Methods section, a multivariate linear regression (not shown in tables) in the short CAG repeat length category, controlling for age and PD, confirmed an effect of FT \((B = 1.62; P > 0.028)\).
that was absent in the medium (B = 0.02; P < 0.969) and the long (B = -0.15; P < 0.892) CAG repeat length categories. However, the effect of FT may not be strong enough when using CAG repeat length as a continuous variable to be translated into an interaction effect.

In addition, significant interaction effects were found between FT and CAG repeat length with respect to depressive symptoms (B = -1.11; P < 0.038) and self-esteem (B = 0.98; P < 0.041), indicating that in boys with long CAG repeat lengths, FT was more strongly and negatively associated with depressive symptoms and more strongly and positively associated with self-esteem. Figures 1–5 depict the interaction effects.

As reported in an earlier study (10), a significant interaction was found between age and FT with respect to NART (B = 2.28; P < 0.001) and ART (B = 0.42; P < 0.029), indicating that the effect of FT on ART and NART was more pronounced in older boys. No other significant interactions were found.

In the third step, the analyses were presented, controlling for FE2. FE2 was not related to any of the dependent variables. While the direct effect of FT with respect to NART was attenuated and was no longer significant (B = 1.06; P < 0.339), the interaction between CAG repeat length and FT with respect to NART, ART, depressive symptoms and self-esteem remained significant. Replacement of FE2 with the standardized residuals obtained from regressing FT on FE2 (see Methods) did not result in substantially different results in this model, except for the finding that the direct effect of FT on NART remained significant (B = 1.87; P < 0.031; data not shown).

**Conclusion and discussion**

In this paper, we analyzed the relationship between AR sensitivity and testosterone in a sample of adolescent boys. The mean CAG repeat length was 21.76, and is comparable to the number of CAG repeats in healthy Caucasians as reported in other studies (14).

In multivariate analyses, FT was related to NART but not to any of the other dependent variables. Unlike some studies (26, 27, 29), but consistent with other studies (28, 30), we found that CAG repeat length in itself – at least in adolescent boys – was not a direct correlate of behaviour or other traits. However, our analyses do not indicate that CAG repeat length is irrelevant with respect to sex-dimorphic traits and behaviours. We found interactions between FT and CAG repeat length, showing that FT is more strongly related to non-aggressive and aggressive risk-taking in boys with shorter CAG repeat lengths. We also found interactions between FT and CAG repeat length with respect to depressive symptoms and self-esteem, indicating that FT is negatively associated with depressive symptoms and self-esteem in boys with longer CAG repeat lengths. In addition, we found that FT is significantly related to dominance in boys with short CAG repeat lengths, but not to that in boys with medium or long CAG repeat lengths. FE2 and body fat were unrelated to any of the dependent variables. Alcohol consumption on the day prior to the study was related to NART and ART, but not to FE2 or FT.

Our findings are among the first to document this theoretically relevant interaction. These results and previous observations (14, 15, 23–25, 32) support the
concept that serum testosterone and indicators of AR sensitivity should be studied simultaneously with respect to sex-dimorphic traits and behaviours.

The finding that androgens affect mood mostly in hypogonadal males and rarely in eugonadal males may be consistent with our results that indicate that the effect of FT on depressive symptoms and self-esteem mood was stronger in boys with long CAG repeat lengths. Another study (32), however, found that in middle-aged men, depression was significantly and inversely associated with TT in men with shorter CAG repeat lengths, but not with that in men with moderate and longer CAG repeat lengths. A direct comparison between the results presented here and the results from that study is complicated, as the study populations are markedly different in age and average testosterone levels.

There is no theory at hand to explain our finding that the interaction effects with respect to depressive symptoms and self-esteem are different from the interaction effects we found with respect to the behavioural variables. While FT may affect mood-related and behavioural variables differently depending on AR sensitivity, further research should analyse the complex dynamics between CAG repeat length and FT (including potential compensatory mechanisms between the two) to explain this finding.

Several factors may further hinder the interpretation of the results. First, the study design is cross-sectional: as a result, conclusions only about associations and not about causation can be drawn. Secondly, CAG repeat length is not the only genetic factor involved in AR sensitivity: e.g. the polyglycine tract encoded by a polymorphic GGN repeat in exon 1 of the AR might have some modulatory effects (48, 49): a number of co-activators and co-repressors might further have major, tissue-specific modulating effects on AR trans-activation (16, 50). In addition, further research should try to control i) for lifestyle-related factors such as smoking, diet and physical activity; and ii) for other non-hormone-related factors such as the social environment in which adolescents act, as both may change the relationships between hormones and mood-related or behavioural outcomes.

The variance explained by the interaction between testosterone and CAG repeat length with respect to depressive symptoms and self-esteem is limited. It may not represent a mechanism that contributes much to the variability in depressive symptoms or self-esteem in a normal population of adolescent boys. The literature suggests that in this respect, the effect of testosterone administration on mood may be present in hypogonadal males, but may be less present in eugonadal males (50). In eugonadal males, other individual traits or characteristics of the social environment in which adolescents act may be much more important with respect to depressive symptoms and/or self-esteem.

In summary, in a sample of adolescent boys, using a multivariate design, we found i) no direct relationship between CAG repeat length and risk-taking or dominance, ii) interactions between testosterone and CAG repeat length with respect to NART, ART, depressive symptoms and self-esteem.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References
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