Functional Variation in the Androgen-Receptor Gene Is Associated With Visceral Adiposity and Blood Pressure in Male Adolescents

Zdenka Pausova, Michal Abrahamowicz, Amel Mahboubi, Catriona Syme, Gabriel T. Leonard, Michel Perron, Louis Richer, Suzanne Veillette, Daniel Gaudet, Tomas Paus

Abstract—Intra-abdominal accumulation of fat is a hallmark of male body-fat distribution and a major risk factor for hypertension. Sympathoactivation may be one of the mechanisms linking intra-abdominal obesity to hypertension. The aim of the present study was to investigate whether a functional variation in the androgen-receptor gene (AR, a variable number of CAG repeats in exon 1) is associated with intra-abdominal adiposity, sympathetic modulation of vasomotor tone, and blood pressure in adolescent boys but not girls. We studied 223 boys and 259 girls (age 12 to 18 years) from a French-Canadian founder population. Intra-abdominal fat and subcutaneous-abdominal fat were quantified with an MRI. Blood pressure was recorded beat-to-beat during an hour-long protocol including physical and mental challenges, and these blood pressure time series were used to assess sympathetic modulation of vasomotor tone by power spectral analysis. The results showed that boys with a “low” versus “intermediate” or “high” CAG-repeat number in AR demonstrated higher intra-abdominal fat (by 28% and 48%, respectively) but not subcutaneous-abdominal fat. These intra-abdominal fat differences remained significant after adjusting for serum levels of sex hormones and subcutaneous-abdominal fat. Furthermore, boys with low versus intermediate or high CAG-repeat numbers also showed higher blood pressure, with the differences being most pronounced during mental stress (8.0 and 8.5 mm Hg, respectively) and higher sympathetic modulation of vasomotor tone. As expected, no such differences were seen among girls. In adolescent boys, low CAG-repeat numbers in AR may be a genetic risk factor for intra-abdominal obesity and hypertension; sympathoactivation may be an underlying link between the 2 conditions. (Hypertension. 2010;55:706-714.)

Key Words: visceral obesity ▪ hypertension ▪ sympathoactivation ▪ androgen-receptor gene ▪ adolescence

Body-fat distribution exhibits a striking sexual dimorphism. Males tend to develop android obesity, with a greater intra-abdominal accumulation of fat, whereas females present with gynoid obesity, with predominantly gluteofemoral deposition of fat.1 Because intra-abdominal obesity is a major risk factor for cardiovascular morbidity and mortality, the sex difference in body-fat distribution parallels closely that in blood pressure (BP), with males having higher BP than females throughout their reproductive age.2 Mechanisms underlying this relationship are not very clear but may include increased sympathetic outflow to the vasculature and kidneys.3,4

The sexual dimorphism in body-fat distribution develops during adolescence. A biological hallmark of male adolescence is the elevated secretion of testosterone. It exerts its androgenic effects by both genomic and nongenomic mechanisms; the former is mediated through an intracellularly located androgen receptor (AR), which is a ligand-activated nuclear receptor that regulates target-gene expression at the transcriptional level.5 The AR is encoded by a single gene located on Xq11-12 and contains a functional polymorphism that consists of a variable number of CAG repeats determining a polyglutamine stretch of a variable length.5 Pathological elongation of the polyglutamine stretch (>40 repeats) underlies X-linked Kennedy disease associated with hypoandrogenic traits,6 and even within a normal range, a greater length of the stretch results in lesser transcriptional activity of the receptor in vitro and is associated with diminished androgenicity in vivo, characterized by reduced spermatogenesis and smaller seminal vesicles.7-9 Although intra-abdominal accumulation of fat is a characteristic feature of male body-fat distribution, its relationship with the functional AR polymorphism has not been studied. On the basis of previous studies demonstrating that AR with a “lower” versus “higher” CAG-repeat number encodes a more potent receptor,9,10 we hypothesized that a lower versus
Table 1. Basic Characteristics According to Sex-Specific Tertiles of CAG-Repeat No. in the Androgen-Receptor Gene

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Unadjusted Mean ± SE</th>
<th>Low – Intermediate Tertile</th>
<th>Low – High Tertile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Tertile of CAG Repeats</td>
<td>Intermediate Tertile of CAG Repeats</td>
<td>High Tertile of CAG Repeats</td>
</tr>
<tr>
<td>Boys</td>
<td>Age, y</td>
<td>14.4 ± 0.2</td>
<td>14.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Puberty, 1 to 5°</td>
<td>3.4 ± 0.1</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Height, cm</td>
<td>166.3 ± 1.0</td>
<td>169.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Income, CAN$†</td>
<td>53 041 ± 2679</td>
<td>53 986 ± 2775</td>
</tr>
<tr>
<td></td>
<td>PEMCS, no/yes*</td>
<td>40/34</td>
<td>33/37</td>
</tr>
<tr>
<td>Girls</td>
<td>Age, y</td>
<td>14.3 ± 0.2</td>
<td>14.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Puberty, 1 to 5°</td>
<td>4.1 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>Height, cm</td>
<td>160.5 ± 1.1</td>
<td>160.2 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Income, CAN$†</td>
<td>52 647 ± 2795</td>
<td>54 115 ± 2352</td>
</tr>
<tr>
<td></td>
<td>PEMCS, no/yes*</td>
<td>34/36</td>
<td>51/46</td>
</tr>
</tbody>
</table>

Adjusted means ± SEs are shown for groups of individuals with sex-specific tertiles of the CAG-repeat No. in the androgen-receptor gene. Differences (95% CIs) among groups with sex-specific tertiles were evaluated with multiple linear model separately in boys and girls.

*Data were analyzed with logistic regression.
†Data were log-transformed and show Canadian dollars (CANS).

higher CAG-repeat number will be associated with greater intra-abdominal adiposity and that this relationship will be present only in boys. Furthermore, because intra-abdominal adiposity is a major risk factor for sympathoactivation and, at least partly related to this, for hypertension, we also predicted that, in boys only, a lower versus higher number of CAG repeats in AR will be associated with greater sympathetic modulation of vasomotor tone and higher BP. The AR genotype was using sex-specific tertiles of the empirical distribution of CAG-repeat number in AR.

Materials and Methods

Study Population

White adolescents (n = 482), aged 12 to 18 years, were recruited from a founder population living in the Saguenay-Lac St. Jean region of Quebec, as part of the Saguenay Youth Study (SYS). It is expected that, in founder populations, fewer gene variants contribute to the determination of a complex trait, such as intra-abdominal adiposity. Consequently, the prevalence of several recessive disorders is higher in the Saguenay-Lac St. Jean region than in other populations, and limited allelic diversity exists among patients with these disorders. The Saguenay Youth Study is an ongoing investigation of the long-term consequences of prenatal exposure to maternal cigarette smoking (PEMCS) on cardiovascular and metabolic health and on brain and behavior in adolescence. Recruitment and selection criteria have been described previously. The investigated cohort is representative of the French-Canadian adolescent population, except for the higher proportion of subjects with PEMCS (by design 50% in our sample versus 25% in the general population) and, associated with this fact and the matching of the nonexposed subjects to the exposed ones, overrepresentation of families with lower socioeconomic status. The cohort investigated here included 25 unrelated individuals, 195 sibling pairs, 21 sibships of 3 siblings, and 1 sibship of 4 siblings. Written consent of the parents and assent of the adolescents were obtained before the commencement of data collection. The research ethics committee of the Chicoutimi Hospital approved the study protocol. The current sample consists of subjects recruited and tested between November 2003 and June 2007.

Quantitative Phenotyping

Body-Fat Quantity and Distribution

Measurements included weight, height, and waist circumference, as well as MRI of the abdomen. A 10-mm-thick axial T1-weighted image acquired at the level of the umbilicus was segmented into intra-abdominal fat (IAF) and subcutaneous-abdominal fat (SAF), as described previously.

Biochemical Analyses

A fasting blood sample was drawn between 8:00 AM and 9:00 AM. Serum total testosterone (T) was measured with radioimmunoassay (Testosterone RIA DSL-4000, Diagnostic Systems Laboratory Inc). Serum estradiol (E2) and sex hormone-binding globulin (SHBG) were assayed at the Clinical Biochemistry Department of the Hôtel-Dieu Hospital (Montreal, Quebec, Canada). Serum free T and E2 that are not bound to SHBG and albumin and are thought to represent the physiologically active fractions of these hormones were calculated as described previously.

Cardiovascular Measurements

All of the subjects underwent a 52-minute cardiovascular protocol, conducted in a hospital setting on Saturdays between 8:00 AM and 12:00 PM. The protocol included a resting period, changes in posture from supine to standing and from standing to sitting, and an arithmetic stress test. Throughout the protocol, a noninvasive hemodynamic monitor, Finometer (FMS Finapres), was used to record continuous finger blood flow. The Finometer derives beat-to-beat brachial systolic and diastolic BPs (SBP and DBP, respectively) from the reconstructed and level-corrected finger blood flow waveform. It is a reliable device for tracking BP in adults and children older than 6 years of age.

Sympathetic Modulation of Vasomotor Tone

Power spectral analysis of DBP was used to estimate noninvasively the sympathetic modulation of vasomotor tone. The analysis was performed over the entire 52-minute protocol in 2-minute periods with 50% overlaps, using a sliding window routine. For each period, beat-to-beat time series of DBP were interpolated using a piecewise cubic-spline method, resampled at a frequency of 5 Hz and detrended before being transformed by a 1024-point fast Fourier transform, using standard Matlab functions (Matlab 7.3.0, Math-
works, Inc. Low-frequency spectral power of DBP (LF\textsubscript{DBP}) was determined by integrating the power spectrum between 0.04 and 0.15 Hz. LF\textsubscript{DBP} is thought to reflect mainly sympathetic modulation of vascular tone\textsuperscript{19-21} although it may also be influenced by humoral and endothelial factors acting on the vasculature\textsuperscript{22}. Means of 2-minute LF\textsubscript{DBP} estimates during 7 different sections, that is, supine (10 minutes), standing (10 minutes), sitting (10 minutes), prestress (5 minutes), stress-test explanation (2 minutes), stress test (2 minutes), and stress-test recovery (10 minutes), were used for statistical analyses.

**Questionnaires**
The subjects completed a questionnaire evaluating stages of pubertal development\textsuperscript{23}. Parents completed a questionnaire on family income, which we used here as an index of socioeconomic status\textsuperscript{24}.

**Genotyping**
The CAG-repeat polymorphism in AR was genotyped with forward and reverse primers (\textsuperscript{5}-GACCTACCGAGGAGCTTTCCT-\textsuperscript{3}' and \textsuperscript{5}-GCTTGAAGGGTGTCTCC-\textsuperscript{3}', respectively) at the Montreal Genome Centre. In both boys and girls, CAG-repeat number varied within a normal range (boys: n=9 to 32; girls: n=11 to 31), and the mean was 22. In our analyses, the AR genotype was defined by sex-specific tertiles of the empirical distribution of CAG-repeat number in AR. Because AR is located on the X chromosome, boys have only 1 copy of the gene, whereas girls have 2 copies of the gene, with only one of them expressed and the other randomly inactivated in uterus\textsuperscript{25}. Therefore, not knowing which of the 2 copies is expressed in girls, we used a mean of the 2 alleles of the polymorphism, as done previously\textsuperscript{26}.

**Statistical Methods**
Descriptive statistics used to characterize the study population included means and SEs for continuous variables and proportions for categorical variables. Our main analyses focused on estimating putative associations between the AR genotype (sex-specific tertiles of the empirical distribution of CAG-repeat number in AR) and outcomes (IAF, SAF, waist circumference, body weight, SBP, and LF\textsubscript{DBP}). These analyses relied on the multivariable mixed linear model to account for clustering of observations within families, that is, correlation of outcomes between siblings, and to adjust for potential confounders. The mixed linear model extends the conventional linear regression of continuous outcomes to correlated data\textsuperscript{27} and handles well any randomly missing data\textsuperscript{28}. Family clustering was accounted for by adding random intercepts, and compound symmetry covariance structure of residuals was assumed to represent the structure of the within-subject residuals, which implies that measurements that are closer in time correlate more strongly\textsuperscript{29}. Second, we assessed the normality assumption on which the statistical inference about the mixed linear model estimates relies. The values of outcomes for which the empirical distribution showed substantial positive skewness, namely, IAF, SAF, and LF\textsubscript{DBP}, were log transformed. All of the mixed-model analyses were carried out separately in boys and girls because of the sex-specific role of testosterone in human physiology\textsuperscript{30}. Using the above general mixed-model approach, 2 different types of models were used for single-valued outcomes and repeated-measures outcomes.

For each single-valued continuous outcome (IAF, SAF, waist circumference, and body weight), the multivariable mixed model estimated the fixed effect of the AR genotype, while adjusting for a set of a priori selected potential confounders. Age, height, and family income were modeled as continuous covariates. Family income was log-transformed to account for its distribution being highly skewed to higher socioeconomic status. Puberty stage was represented by a set of dummy variables, with the highest stage 5 as the reference category, whereas PEMCS was analyzed as a binary variable (exposed versus nonexposed). In addition, to assess to what extent the effect of the AR genotype on IAF depended on its effect on SAF and serum free T and E\textsubscript{2}, we repeated the analyses for IAF with additional statistical adjustments for these variables.

The mixed-model analyses of repeated measures of SBP and LF\textsubscript{DBP} required a more complex approach. First, in addition to accounting for potential confounders, these analyses had to account for the interdependence of repeated outcome measures for the same subject. This was achieved by specifying time as a repeated factor in the mixed model and assuming autoregressive order-1 covariance structure of the within-subject residuals, which implies that measurements that are closer in time correlate more strongly\textsuperscript{31}. Second, we had to account for the fact that repeated measures corresponded with different experimental conditions (7 different “sections,” namely, supine, standing, sitting, pretest, test explanation, math test, and test recovery). A priori physiological considerations suggested that SBP and LF\textsubscript{DBP} could systematically differ between sections. Therefore, the mixed models for each of the 2 repeated-overtime measures included, as independent variables, the binary indicators of each section, in addition to aforementioned subjects’ potential confounders considered in the analyses of single-valued outcomes.

Furthermore, we considered a possibility that the putative effects of the AR genotype might differ among the 7 sections. Therefore, in preliminary analyses of each repeated-measures outcome, we expanded the multivariable mixed model by including a series of 2-way interactions between the AR genotype, represented by the 2 indica-
tors of, respectively, intermediate and high versus low tertiles of the number of CAG repeats, and each of the 6 section indicators (with “supine” section as the reference). Then, an “omnibus” Wald-like test, on 12 degrees of freedom, was used to test the significance of the joint effect of the 12 interaction terms. If the omnibus test yielded a 2-tailed \( P \) value \( < 0.05 \), this was considered as evidence of significant differences between the section-specific effects of the \( AR \) genotype on a given outcome. In such a case, the final analysis of that outcome was stratified by sections, and, using the same general mixed-model approach, separate adjusted effects of the \( AR \) genotype were estimated for each of the 7 sections corresponding with different experimental conditions. In contrast, if the 12-degrees-of-freedom omnibus interaction test yielded \( P \) value \( > 0.05 \), then we concluded that there was no evidence that the association between the \( AR \) genotype and a given repeated-measures outcome depended on the experimental condition. In such situations, the genotype-section interactions were excluded from the final multivariable mixed model, and a single adjusted effect of the \( AR \) genotype was estimated by pooling repeated outcome measures across the 7 sections, while still adjusting for the main effects of sections.

For both types of outcomes, we reported the adjusted effect of the \( AR \) genotype from the final mixed multivariable model as the estimated differences, with 95% CIs, in the mean values of a given outcome between subjects with a low versus intermediate or high number of CAG repeats in \( AR \). A 2-tailed mixed model–based Wald-like test was used to test the significance of this adjusted difference, that is, of the independent association between the \( AR \) genotype and a given outcome.

**Results**

**Basic Characteristics of the Studied Cohort**

Boys (\( n = 223 \)) compared with girls (\( n = 259 \)) did not differ significantly by age, but they were taller and at an earlier stage of sexual maturation (Table 1); the latter is consistent with a known delay in sexual maturation in boys versus girls. Among the 259 girls studied, there were 40 who were taking oral contraception. The 2 sexes also did not differ by family income (an index of socioeconomic status) and the proportion of individuals with PEMCS (a potentially confounding factor related to ascertainment; Table 1).

Sex-specific tertiles of the empirical distribution of CAG-repeat number were as follows: for boys: low was \( \leq 20 \) CAG repeats, intermediate was 21 to 23 CAG repeats, and high was \( \geq 24 \) CAG repeats; for girls, low was \( \leq 20 \) CAG repeats, intermediate was 21 to 22 CAG repeats, and high was \( \geq 23 \) CAG repeats. In both boys and girls, individuals with low, intermediate, and high tertiles of CAG-repeat number in \( AR \) did not differ...
in any of the potential confounders, namely, age, puberty stage, height, family income, or PEMCS (Table 1).

Sexual Dimorphism in Body-Fat Distribution

Although boys and girls showed similar IAF, boys compared with girls demonstrated significantly less SAF, by 43% (Figure 1A). In both sexes, IAF correlated strongly with SAF (boys: $r^2=0.69$, $P<0.001$; girls: $r^2=0.60$, $P<0.001$; Figure 1B). For any given quantity of SAF, boys compared with girls tended to have more IAF, thus demonstrating their greater predisposition for visceral obesity (Figure 1B), as observed previously in adults. Genetically determined functional differences in the AR activity may depend on serum androgen and, because of androgen aromatization, on serum estrogen. $^5$ $^30$ In the present study, the IAF differences between boys with low and those with intermediate or high CAG-repeat number remained virtually unchanged after additional adjusting for serum free T; IAF, IAF adjusted additionally for serum free E2; waist, waist circumference; BW, body weight.

Data show variables that were analyzed with log-transformed values.

**Table 2. Adiposity According to Sex-Specific Tertiles of CAG-Repeat No. in the Androgen-Receptor Gene**

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Unadjusted Mean ± SE</th>
<th>Low—Intermediate Tertile</th>
<th>Low—High Tertile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Tertile of CAG repeats</td>
<td>Intermediate Tertile of CAG repeats</td>
<td>High Tertile of CAG repeats</td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IAF, cm$^3$</td>
<td>32.3 ± 3.3</td>
<td>24.2 ± 3.4</td>
<td>21.9 ± 3.2</td>
</tr>
<tr>
<td>IAF$_{SAF}$, cm$^3$</td>
<td>32.3 ± 3.3</td>
<td>24.2 ± 3.4</td>
<td>21.9 ± 3.2</td>
</tr>
<tr>
<td>IAF$_{T}$, cm$^3$</td>
<td>32.3 ± 3.3</td>
<td>24.2 ± 3.4</td>
<td>21.9 ± 3.2</td>
</tr>
<tr>
<td>IAF$_{T-2}$, cm$^3$</td>
<td>32.3 ± 3.3</td>
<td>24.2 ± 3.4</td>
<td>21.9 ± 3.2</td>
</tr>
<tr>
<td>SAF, cm$^3$</td>
<td>101.9 ± 10.5</td>
<td>94.5 ± 10.9</td>
<td>78.9 ± 10.4</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>1.87 ± 0.01</td>
<td>1.87 ± 0.01</td>
<td>1.85 ± 0.01</td>
</tr>
<tr>
<td>BW, kg</td>
<td>60.2 ± 1.3</td>
<td>59.3 ± 1.4</td>
<td>58.5 ± 1.2</td>
</tr>
</tbody>
</table>

Unadjusted means ± SEs are shown for groups of individuals with sex-specific tertiles of the CAG-repeat No. in the androgen-receptor gene. Differences among the tertile groups were evaluated with mixed-model analysis separately in boys and girls, while adjusting for family clustering and 5 potential confounders (age, height, puberty stage, family income, and PEMCS). The adjusted differences are shown together with their 95% CIs. IAFFT indicates IAF adjusted additionally for serum free T; IAF$_{T}$, IAF adjusted additionally for serum free E2; waist, waist circumference; BW, body weight.

Data show variables that were analyzed with log-transformed values.
be assigned to the same tertile, that is, “tertile homozygotes” (n=106); in this subset, P values of the differences between low and intermediate or high tertile ranged from 0.54 to 0.88.

Functional Variation in AR, BP, and Sympathetic Modulation of Vasomotor Tone

Because IAF accumulation is a major risk factor for hypertension, and at least part of this relationship may be mediated by sympathoactivation,31 we examined whether boys with a low, intermediate, or high number of CAG repeats differ in SBP and in LFDBP, an index of sympathetically modulated vasomotor tone. The results showed that, similar to IAF, boys with low CAG-repeat number demonstrated significantly higher SBP than those with intermediate (mean adjusted difference of 4.8 mm Hg; P=0.02) or high (mean adjusted difference of 3.4 mm Hg; P=0.02) CAG-repeat number (Figure 3B). The differences varied across different sections (tertiles-by-sections interaction: P=0.009), but in all of the sections, the low tertile had higher mean values than the 2 other tertiles, with the adjusted mean differences between the low and intermediate tertiles ranging from 1.5 mm Hg (P=0.59) to 8.0 mm Hg (P=0.02), and those between the low and high tertiles varying from 1.7 mm Hg (P=0.42) to 8.5 mm Hg (P=0.01; Figure 3B). Similar to SBP, boys with a low number of CAG repeats compared with boys with an intermediate or high number of repeats showed significantly higher LFDBP (P=0.0005; Figure 3B). These differences did not significantly vary across sections (tertiles-by-sections interaction: P=0.08; Figure 3B).

As predicted, no statistically significant differences across female adolescents with a low, intermediate, or high number of CAG repeats were identified for either SBP or LFDBP with P values for pairwise comparisons of different tertiles ranging from 0.11 to 0.94 (Figure S1). Furthermore, this lack of association between tertiles of CAG repeats and both SBP and LFDBP in girls was consistent across all of the protocol sections (tertiles-by-sections interaction: P=0.49 and P=0.78, respectively).

Discussion

The results of the present study suggest that, in adolescent boys but not girls, a low CAG-repeat number in the coding region of AR is associated with greater intra-abdominal adiposity and higher BP, and higher sympathetic modulation of vasomotor tone may be one of the underlying links between the 2 conditions.

To our knowledge, the current study is the first to identify a functional genetic variant that may increase intra-abdominal adiposity, which is a characteristic feature of android body-fat distribution. As such, our finding is consistent with previous research demonstrating that the same variant (ie, low CAG-repeat number in AR) is associated with other features of androgenicity, such as higher spermatogenesis or larger seminal vesicles.8,32 Similar to our investigation, in vitro transactivation studies showed that constructs with 20 CAG repeats are more potent than constructs with 31 CAG repeats.8

We observed a significant association between the AR variation with intra-abdominal adiposity but not with subcutaneous-abdominal adiposity, waist circumference, or body weight.

Figure 3. Boys, CAG-repeat tertiles in AR and IAF adjusted for SAF, SBP, and an index of sympathetic modulation of vasomotor tone. Mean±SE of (A) IAF, (B) SBP, and an index of sympathetic modulation of vasomotor tone (LFDBP) are presented for groups of males with low, intermediate, and high tertiles of CAG-repeat number (<20, 21 to 23, and ≥24 CAG repeats, respectively). SBP and LFDBP are shown for 7 periods of an hour-long protocol, defined by various experimental conditions (ie, supine, standing, sitting, pre-test, test explanation, math test, and test recovery). All of the variables shown were adjusted for 5 potential confounders (age, height, puberty stage, family income, and PEMCS). IAF was additionally adjusted for SAF. Male-specific tertile differences in IAF, SBP, and LFDBP were assessed with mixed-model analysis, adjusting for the above-specified potential confounders.
sympathetic tone contributes to hypertension\textsuperscript{42} and that, sympathoadrenal activity.\textsuperscript{43} In addition, the quantity of IAF, similar to BP, men compared with women have greater tonic their reproductive lives.\textsuperscript{36} Androgens may play a key role in

Furthermore, the association with intra-abdominal adiposity remained significant even after additional adjustment for subcutaneous-abdominal adiposity. These results suggest that, at least in adolescence, AR may play a role in determining intra-abdominal and not whole-body adiposity. This is in line with the fact that whole-body adiposity does not increase in boys but only in girls during adolescence.\textsuperscript{33} It is also in line with most previous research carried out in adults, demonstrating no significant relationships between the AR variation and whole-body adiposity.\textsuperscript{30,34,35}

Men compared with women have higher BP throughout their reproductive lives.\textsuperscript{36} Androgens may play a key role in this sex dimorphism. In rats, for example, males compared with females also show higher BP, and this difference is reduced by removal of the testes in the males (reviewed in Reference 37). In the present study, boys with a low versus intermediate or high CAG-repeat number demonstrated not only preferential IAF accumulation but also elevated BP and augmented sympathetically modulated vasomotor tone. These results suggest that, in males, low CAG-repeat number in AR may contribute to BP elevation through, at least in part, the activation of sympathetic vasomotor tone. This activation may be mediated by testosterone either directly, through its effect in the central and peripheral nervous systems,\textsuperscript{38} or indirectly, via its effects on intra-abdominal adiposity. Although the mechanisms linking intra-abdominal adiposity to sympathetic activation are not completely understood, the higher lipid turnover in intra-abdominal rather than subcutaneous fat\textsuperscript{39} may result in greater hepatic delivery of free fatty acids that, in turn, may increase sympathoadrenal activity and BP through afferent vagal signals to the brain.\textsuperscript{40,41} Consistent with this possibility, previous research suggests that elevated sympathetic tone contributes to hypertension\textsuperscript{42} and that, similar to BP, men compared with women have greater tonic sympathoadrenal activity.\textsuperscript{43} In addition, the quantity of IAF, but not that of SAF, correlates closely with muscle sympathetic nervous activity,\textsuperscript{4,31} and intra-abdominal adiposity is a risk factor for elevated BP in adolescent boys but not girls.\textsuperscript{3}

The relationship between the AR variation and BP has been examined previously in older men, but these studies were confounded by including participants treated with antihypertensive medication.\textsuperscript{30,35,34} Consistent with our results, however, it has been shown in healthy adult men that a low CAG-repeat number in AR is associated with lower flow-mediated vasodilation\textsuperscript{45}, which may also contribute to the development of hypertension.\textsuperscript{46}

Our results suggest a threshold rather than a dose response of CAG-repeat number not only on intra-abdominal adiposity that is independent of subcutaneous abdominal adiposity but also on BP and sympathetic modulation of vasomotor tone. More specifically, boys with a CAG-repeat number $\leq 20$ compared with the rest of the group demonstrated significantly higher values of all of these 3 main outcomes. This threshold response of CAG-repeat number is analogous to that in the Kennedy syndrome in which the AR CAG-repeat number $>40$ is causal to spinobulbar muscular atrophy.\textsuperscript{6}

The current study has certain limitations. We used an indirect method to assess sympathetic modulation of vasomotor tone, that is, power spectral analysis of cardiovascular variability. Direct methods, such as muscle sympathetic nerve activity recording and ganglionic blockade, are invasive and, as such, not suitable for population-based studies of adolescents. Importantly, power spectral analysis of cardiovascular variability has been validated against these methods.\textsuperscript{20}

Finally, as in any study that involves testing multiple associations, the risk for type I error has to be assessed carefully. Our analyses involved testing 6 main outcomes (4 adiposity measures, SBP, and LF\textsubscript{DBP}). Because they are interrelated, as described above, the focus should be on the overall pattern of the results of all 6 tests, rather than on their
individual $P$ values. From this perspective, the fact that, in boys, we found 3 of 6 associations significant at the 2-tailed 0.05 level (IAF, SBP, and $LFD_{DHB}$) provides very strong evidence in support of our a priori hypotheses. Indeed, assuming independence of all 6 of the tests, the joint probability of obtaining 3 of 6 $P$ values <0.05 by chance alone is as low as 0.0036. Furthermore, all of the differences between tertiles in boys were in the a priori expected direction (Figures 2 and 3 and Table 2), which further reduces the risk of these results being attributable to chance. In contrast, as expected, in girls, all 6 of the tests yielded definitely nonsignificant results, although the analyses in girls (n = 259) had actually slightly higher power than those in boys (n = 223). All of these arguments indicate that it is very unlikely that our results in boys are because of chance and, thus, support our a priori overarching hypothesis.

**Perspectives**

Our results suggest that a low number of CAG repeats in the coding region of AR may predispose adolescent boys (but not girls) to intra-abdominal obesity and hypertension. Because the relationships are apparent already during adolescence, this genotype may represent a risk factor for preclinical cardiovascular disease.

**Acknowledgments**

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**Disclosures**

None.

**References**

4. Pausova et al. Androgen-Receptor Gene, Visceral Fat, and BP 713


FUNCTIONAL VARIATION IN THE ANDROGEN-RECEPTOR GENE IS ASSOCIATED WITH VISCERAL ADIPOSITY AND BLOOD PRESSURE IN MALE ADOLESCENTS

Short title: Androgen receptor, visceral obesity and hypertension

Zdenka Pausova MD1,2 *, Michal Abrahamowicz PhD3, Amel Mahboubi PhD3, Catriona Syme MSc1, Gabriel T. Leonard PhD4, Michel Perron PhD5, Louis Richer PhD6, Suzanne Veillette PhD5, Daniel Gaudet MD, PhD7, and Tomas Paus MD, PhD1,4

1Brain & Body Centre, University of Nottingham, Nottingham, United Kingdom;  
2Research Centre-CHUM, Montréal, Canada;  
3McGill University, Montréal, Canada;  
4Montreal Neurological Institute, McGill University, Montreal, Canada;  
5Groupe ECOBEs, Jonquière, Canada;  
6Université du Québec à Chicoutimi, Canada;  
7Community Genomic Centre, Université de Montréal, Chicoutimi, Canada;

*Corresponding author.
Figure S1. Females: CAG-repeat terciles in AR and intra-abdominal fat adjusted for subcutaneous-abdominal fat, systolic blood pressure and an index of sympathetic modulation of vasomotor tone

Means ± standard errors of (A) intra-abdominal fat (IAF), (B) systolic blood pressure (SBP) and an index of sympathetic modulation of vasomotor tone (low-frequency power of DBP (LF$_{DBP}$) are presented for groups of males with low, intermediate, and high terciles of CAG-repeat number (≤20, 21-22 and ≥23 CAG repeats, respectively). SBP and LF$_{DBP}$ are shown for seven periods of an hour-long protocol, defined by various experimental conditions (i.e. supine, standing, sitting, pre-test, test explanation, math test, and test recovery). All variables shown were adjusted for five potential confounders (age, height, puberty stage, family income, and prenatal exposure to maternal cigarette smoking). IAF was additionally adjusted for subcutaneous-abdominal fat. Female-specific tercile differences in IAF, SBP, and LF$_{DBP}$ were assessed with mixed-model analysis, adjusting for the above-specified potential confounders.
Figure S1.