A Common Variant of the FTO Gene Is Associated With Not Only Increased Adiposity but Also Elevated Blood Pressure in French Canadians

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Background—FTO is the first gene established as contributing to common forms of obesity. The gene is highly expressed in the hypothalamus and is thought to mediate this effect through its influence on energy homeostasis. The hypothalamus, however, also regulates blood pressure (BP). Therefore, we investigated whether the FTO-risk variant is associated not only with increased adiposity but also with elevated BP and whether the latter may be mediated, in part, by increased sympathetic modulation of vasomotor tone.

Methods and Results—The primary study was carried out in 485 adolescents recruited from a French Canadian founder population who underwent detailed body-composition and cardiovascular phenotyping. Body fat was examined with MRI, bioimpedance, and anthropometry. BP was recorded beat to beat at rest and during physical and mental challenges. Sympathetic modulation of vasomotor tone was assessed with power spectral analysis of BP. We found that individuals with the FTO-risk genotype compared with those without it demonstrate greater adiposity, including the amount of intra-abdominal fat (by 38%). They also showed higher systolic BP throughout the entire protocol, with a maximum difference during a mental stress (6.4 [1.5 to 11.3] mm Hg). The difference in BP was accompanied by elevated index of sympathetic modulation of vasomotor tone. A replication in an independent sample of adults from the same founder population confirmed the association between FTO and BP.

Conclusions—These results suggest that, in a French Canadian founder population, FTO may increase not only risk for obesity, as demonstrated in other populations, but also for hypertension. The latter may be related, at least in part, to the regulation of sympathetic vasomotor tone. (Circ Cardiovasc Genet. 2009;2:260-269.)

Key Words: genetics ■ hypertension ■ obesity ■ sympathetic nervous system ■ genetic association

A genome-wide association study of 4862 subjects from the Wellcome Trust Case Control Consortium (United Kingdom) identified a common sequence variant in the FTO (fatso/fat mass and obesity associated) gene that predisposes its carriers to type 2 diabetes mellitus through an effect on body mass index (BMI).1 In the same article, the authors reported replication of the association between this variant and BMI in 13 cohorts with a total of 38 759 individuals.1 Associations of FTO with various obesity traits and insulin resistance/type 2 diabetes has since been replicated in numerous other studies.2-9 More recently, an association of FTO with obesity-related dyslipidemia has also been reported.6

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The mechanisms through which FTO may increase the risk for obesity and obesity-related metabolic abnormalities are still not clear. The gene encodes 2-oxoglutarate-dependent nucleic-acid demethylase, which is highly expressed in the hypothalamus where it may regulate gene transcription.10 The hypothalamus is a brain structure involved in the regulation of energy homeostasis. Recent evidence suggests that the FTO-risk variant may promote the development of obesity by increasing energy intake,11-13 perhaps through its influence on appetite-regulating regions of the hypothalamus.2
The hypothalamus, however, is also a powerful regulator of blood pressure (BP).\textsuperscript{14} The paraventricular and dorsomedial nuclei of the hypothalamus, which show particularly high FTO expression,\textsuperscript{10} are key modulators of sympathetic outflow to the circulatory system.\textsuperscript{14} Despite the known role of the hypothalamus in BP regulation, the role of FTO in BP and its regulation has not been studied.

The aim of this study was to investigate whether FTO is associated not only with increased adiposity, insulin resistance, and dyslipidemia, as shown previously in other populations, but also with increased BP and sympathetic modulation of vasomotor tone. This primary investigation was carried out in 485 adolescents recruited from a French Canadian population with a known founder effect in which fewer gene variants are expected to contribute to the determination of complex traits, such as BP and autonomic function.\textsuperscript{15–17} In addition, a replication study was carried out in 485 adolescents recruited from a French Canadian population with a known founder effect in which fewer gene variants are expected to contribute to the determination of complex traits, such as BP and autonomic function.\textsuperscript{15–17} In brief, all subjects are recruited via local high schools. At the time of analysis, \textasciitilde{}5000 adolescents were informed about the Saguenay Youth Study at both school and home. All who were exposed prenatally to maternal cigarette smoking were willing to participate and were free of any exclusion criteria were included in the study. The exclusion criteria were positive medical history of heart disease requiring surgery/medication, diabetes mellitus treated with insulin, meningitis, malignancy, severe mental ill (eg, autism, schizophrenia), mental retardation (intelligence quotient \textasciitilde{}70), or MRI contraindications. An equal number of willing and eligible nonexposed subjects, matched to the exposed ones by maternal education (as a proxy of socioeconomic status) and the school attended, were also included. With this ascertainment, the investigated cohort is representative of the French Canadian adolescent population, except for the higher proportion of subjects exposed prenatally to maternal cigarette smoking (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 by maternal education, overrepresentation of families with lower socioeconomic status. In addition, the Saguenay Youth Study is family based and focused primarily on recruitment of sib pairs. The cohort investigated here included 17 unrelated individuals, 188 sib pairs, 28 sibships of 3 siblings, and 2 sibships of 4 siblings.

\textbf{Methods}

\textbf{Study Populations}

\textbf{Adolescents}

White adolescents (n=485), aged 12 to 18 years, were recruited from a population with a founder effect living in the Saguenay-Lac St Jean region of Quebec, Canada, as part of the Saguenay Youth Study.\textsuperscript{19} This is an ongoing investigation of the long-term consequences of prenatal exposure to maternal cigarette smoking on cardiovascular and metabolic health and on brain and behavior in adolescence. Recruitment and selection criteria have been described previously.\textsuperscript{19} In brief, all subjects are recruited via local high schools. At the time of analysis, \textasciitilde{}5000 adolescents were informed about the Saguenay Youth Study at both school and home. All who were exposed prenatally to maternal cigarette smoking were willing to participate and were free of any exclusion criteria were included in the study. The exclusion criteria were positive medical history of heart disease requiring surgery/medication, diabetes mellitus treated with insulin, meningitis, malignancy, severe mental ill (eg, autism, schizophrenia), mental retardation (intelligence quotient \textasciitilde{}70), or MRI contraindications. An equal number of willing and eligible nonexposed subjects, matched to the exposed ones by maternal education (as a proxy of socioeconomic status) and the school attended, were also included. With this ascertainment, the investigated cohort is representative of the French Canadian adolescent population, except for the higher proportion of subjects exposed prenatally to maternal cigarette smoking (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 by maternal education, overrepresentation of families with lower socioeconomic status. In addition, the Saguenay Youth Study is family based and focused primarily on recruitment of sib pairs. The cohort investigated here included 17 unrelated individuals, 188 sib pairs, 28 sibships of 3 siblings, and 2 sibships of 4 siblings.

\textbf{Adults}

An independent sample of white adults (n=298), aged 18 to 71 years, were recruited as members of 69 hypertensive families from the same French Canadian founder population as the earlier adolescent sample. The families were selected on the basis of having 2 siblings, and 2 sibships of 4 siblings.

\textbf{Dyslipidemia was defined as having serum levels of total cholesterol 5.2 mmol/L or high-density lipoprotein cholesterol 0.9 mmol/L. Dyslipidemia was chosen as a selection criterion because of its potential role in the pathogenesis of essential hypertension; family-based studies in Utah suggest that dyslipidemia and hypertension with an onset at \textasciitilde{}60 years of age are manifestations of a distinct familial syndrome (ie, “familial dyslipidemic hypertension”).\textsuperscript{20} Additional selection criteria were the absence of (1) secondary hypertension, (2) DBP \textasciitilde{}110 mm Hg on BP-lowering medication, (3) gross obesity (BMI \textasciitilde{}35 kg/m\textsuperscript{2}), (4) diabetes mellitus (fasting blood glucose level \textasciitilde{}6 mmol/L or use of insulin or oral hypoglycemic agents), (5) renal dysfunction (serum creatinine \textasciitilde{}180 mmol/L), (6) liver disease, (7) malignancy, (8) pregnancy, and (9) substance abuse, including alcohol. Once 2 siblings in a family satisfied these selection criteria, other siblings, parents, uncles, aunts, and children, not necessarily hypertensive, were also enrolled in the study. The average size of the families studied was 4.3 individuals.

\textbf{French Canadian Founder Population}

Importantly, for the genetic component of these studies, the Saguenay-Lac St Jean population is one of the largest population isolates in North America.\textsuperscript{15–17} It originates from ancestors of French descent who migrated to this region in the early 19th Century. The population has experienced high intrinsic growth, from 5200 inhabitants in 1852 to 285 000 at present. Because of the founder effect, the prevalence of several recessive disorders is higher in the Saguenay-Lac St. Jean region than in other populations,\textsuperscript{18} and limited allelic diversity exists among patients with these disorders.\textsuperscript{16,17}

Written consent of the parents and assent of the adolescents (adolescent sample) and subjects (adult sample) were obtained before the commencement of data collection. The Research Ethics Committee of the Chicoutimi Hospital approved the study protocols.

\textbf{Quantitative Phenotyping}

\textbf{Adolescents}

\textbf{Body-Fat Quantity and Distribution}

Measurements included weight, height, waist circumference, multi-frequency bioimpedance analysis to estimate total body-fat mass (Xitron Technologies Inc, San Diego, Calif), and MRI of the abdomen. A 10-mm thick axial T1-weighted image at the level of the umbilicus was segmented into intra-abdominal and subcutaneous fat, as described previously.\textsuperscript{21} Subjects were asked to refrain from caffeine, alcohol, and vigorous activity 24 hours before the measurements.

\textbf{Biochemical Analyses}

A fasting blood sample was drawn between 8:00 and 9:00 AM. Serum levels of glucose, insulin, triglycerides, total cholesterol, and high-density lipoprotein cholesterol were measured. We calculated the homeostasis model assessment (HOMA), an index of insulin resistance.\textsuperscript{22}

\textbf{Cardiovascular Measurements}

All 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, as well as changes in posture from supine to standing and from standing to sitting, and an arithmetic stress test.\textsuperscript{23} Throughout the protocol, a noninvasive hemodynamic monitor, Finometer (FMS Finapres, Amsterdam, The Netherlands), was used to record continuously the finger blood flow. The Finometer derives beat-to-beat brachial systolic BP (SBP) and DBP from the reconstructed and level-corrected finger blood-flow waveform.

\textbf{Sympathetic Modulation of Vasomotor Tone}

Power spectral analysis of DBP was used to estimate noninvasively the sympathetic modulation of vasomotor tone and systemic vascular resistance.\textsuperscript{24–26} The analysis was performed in 2-minute periods with 50\% overlaps, using a sliding window routine, over the entire 52-minute protocol. 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.
A single-nucleotide polymorphism (SNP) in intron 1 of FTO (rs9939609, T/A) with the minor A allele was genotyped using KASPar. KASPar is a competitive allele specific PCR-based SNP fluorescent genotyping system that uses fluorescence resonance energy transfer quencher cassette oligos (KBioscience, Herts, United Kingdom). The call rate was 98% and the SNP was in Hardy-Weinberg equilibrium. The rs9939609 genotype distribution in the Saguenay Youth Study cohort (AA, 12.2%; AT, 44.1%; and TT, 43.7%) was similar to that reported in other population-based cohorts.1

Genotyping

Adolescents

A single-nucleotide polymorphism (SNP) in intron 1 of FTO (rs9302652, T/C) with the minor allele C was genotyped using the GeneChip Human Mapping 50K Array Xba240 (Affymetrix) at the Centre Hospitalier de l’Université de Montréal Research Centre, Montreal. This FTO SNP is a SNP on the array that is closest to the FTO SNP genotyped in adolescents. The rs9302652 genotype distribution in the adult cohort was following: CC, 9.7%; CT, 40.6%; and TT, 49.7%.

Statistical Methods

Adolescents

Descriptive statistics used to characterize the study population that included means and SEs for categorical variables. The main analyses focused on estimating putative associations between the FTO-risk genotype (AA versus AT or TT) and various outcomes. In all analyses, we relied on the general mixed-model approach, and separate adjusted effects of the putative associations between the FTO-risk genotype on selected outcomes may be mediated through its effect on obesity, we repeated the analyses for (a) single-valued outcomes and (b) repeated-measures outcomes.

For each single-valued continuous outcome (measures of adiposity and glucose and lipid metabolism), the multivariate mixed model estimated the fixed effect of the FTO genotype, while adjusting for the set of a priori selected covariates. Age, height (except in the model where height was the dependent variable), and family income were modeled as continuous covariates, and family income was log transformed to account for its distribution being highly skewed to the right. Puberty stage was represented by a set of dummy variables, with the highest stage 5 as the reference category, whereas sex and prenatal exposure to maternal smoking were analyzed as binary variables. In addition, to assess to what extent the effects of the FTO-risk genotype on selected outcomes may be mediated through its effect on obesity, we repeated the analyses for insulin, HOMA, and triglycerides, with an additional adjustment for BMI. Finally, the analysis for intraabdominal fat was repeated with an additional adjustment for subcutaneous abdominal fat to examine whether the FTO-risk genotype predisposes to preferentially intra-abdominal obesity.

The mixed-models analyses of repeated measures of SBP, DBP, and LFDBP required a more complex approach. First, in addition to accounting for sibling clustering within families, these analyses had to account for the interdependence of repeated outcome measures for the same subject. This was achieved by specifying the assessment of 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.

Second, analytic complexity was due to the fact that repeated measures corresponded to different experimental conditions, so that the resulting time series was composed of 7 different “sections” (supine, standing, sitting, prestress, stress-test estimation, stress test, and stress-test recovery). A priori considerations suggested that the values of SBP, DBP, and LFDBP could systematically differ between some sections. Therefore, the mixed models for each of the 3 repeated-overtime measures included the binary indicators of each section, in addition to aforementioned subjects’ characteristics considered in the analyses of single-valued outcomes, as independent variables. Finally, similar to selected single-valued outcomes (see earlier), we repeated the analyses of SBP and LFDBP with an additional adjustment for BMI to examine whether the effect of the FTO-risk genotype on these outcomes is dependent on its effect on obesity.

Furthermore, we considered a possibility that the putative effects of the FTO-risk genotype might also differ among the 7 sections. Therefore, in preliminary analyses of each repeated-measures outcome, we have expanded the multivariable mixed model by including a series of 2-way interactions between the FTO genotype and each of the section indicators. Then, an “omnibus” Wald-like test, on 6 df, was used to test the significance of the joint effect of the 6 interaction terms. If the omnibus test yielded a 2-tailed P value below 0.05, this was considered as an evidence of significant differences between the section-specific effects of the FTO genotype on a given outcome. In such a case, the final analyses of the particular outcome were stratified by sections, using the same general mixed-model approach, and separate adjusted effects of the
FTO genotype were estimated and tested for each of the 7 sections corresponding to different experimental conditions. In contrast, if the 6-df omnibus test yielded $P>0.05$, then we concluded that there was no evidence that the association between the FTO 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, which still adjusted for the main effects of sections, and a single-adjusted effect of the FTO genotype was estimated by pooling repeated outcome measures across the 7 sections.

**Adults**

The same statistical approach was used in the adult sample, with the main analyses focused on estimating putative associations between the rs9302625 SNP within FTO (CC or CT versus TT) and 4 quantitative single-valued outcomes, namely, body weight, body-fat mass, and SBP and DBP. In brief, we relied on the multivariable mixed linear model to (1) account for clustering of observations within families and (2) adjust for potential confounders. For body weight and body-fat mass, the potential confounders were sex, age, and height, and for BP, they were sex and age. To estimate a putative association between the rs9302625 SNP in FTO (CC or CT versus TT) and the binary outcome of hypertension, defined as having DBP $\geq$90 mm Hg on 2 occasions or currently taking antihypertensive medication, with the disease being documented in medical records, we used the generalized estimating equations extension of the multivariable logistic regression to account for family clustering and adjust for age and sex.

For both single-valued and repeated-measures outcomes and in both samples, we reported the adjusted effect of the FTO genotype from the final mixed multivariable model as the estimated difference, with 95% CI, in the mean values of a given outcome between subjects with AA versus AT or TT FTO genotype for rs9302625, and with CC or CT versus TT FTO genotype for rs9302625. A 2-tailed mixed-model–based Wald test was used to test the statistical significance of this adjusted difference (ie, of the independent association between the FTO-risk genotype and a given outcome).

While testing for associations between FTO and various outcomes, we had to account for multiple testing and a potential inflation of type I error. In our context, however, a conventional Bonferroni correction, which assumes independence of all tests, would not be appropriate for 2 reasons. First, many of the outcomes considered in our analyses are inherently correlated with each other (eg, 6 measures of adiposity and 2 measures of insulin resistance), implying that the corresponding tests, even within the same sample, are definitely not independent. Second, 2 tests of the same association in 2 independent samples are statistically independent only under the null hypothesis of no association. In contrast, if there is a true association, the results of the 2 tests are expected to be quite similar. Moreover, the joint probability of $P$ values from 2 independent samples falling below a conventional cutoff of 0.05 because of sampling error alone is extremely small. For all these reasons, we have decided to assess an overall pattern of the results of different tests rather than to apply a Bonferroni-type correction to individual tests. First, if a given association was significant ($P<0.05$) in both samples, we considered this association very unlikely to be due to chance, and thus statistically significant at the uncorrected $\alpha=0.05$ level. Second, separately for each of the 2 samples, we compared the number of associations between FTO and outcomes tested that were significant at $\alpha=0.05$ with the number of significant results that would be expected by chance alone (calculated by multiplying the total number of associations being tested by 0.05). This allowed us to assess to what extent the observed overall pattern of results is consistent, or not, with the overall null hypothesis (ie, no association between FTO and outcomes tested).

### Results

#### Descriptive Characteristics of the Adolescent Sample

In this study, a total of 485 adolescents were included. Individuals with the FTO-risk genotype (AA genotype) compared with the rest of the cohort did not differ significantly in any of the potentially confounding variables, including the proportion of males and females, age, height, puberty stage, the proportion of subjects exposed prenatally to maternal cigarette smoking, and family income (Table 1).

#### Single-Valued Outcomes in the Adolescent Sample

Tables 2 and 3 summarize the results of multivariable mixed-model analyses of 12 single-valued outcomes of body-fat quantity and distribution and glucose and lipid metabolism. For each outcome, adjusted difference in mean values between subjects with AA (risk) genotype and those with AT or TT (nonrisk) genotypes is reported together with 95% CI and a 2-tailed $P$ value.

#### Body-Fat Quantity and Distribution

Even after accounting for family clustering and potentially confounding variables (sex, age, pubertal stage, height, prenatal exposure to maternal cigarette smoking, and family income), subjects with the FTO-risk genotype when compared with the rest of the cohort demonstrated significantly higher body weight and BMI ($P<0.01$ for both). For these outcomes, the differences between the risk and nonrisk genotypes were clinically highly relevant, as body weight was higher by $\geq$4 kg and BMI by 1.5 kg/m$^2$. Subjects with the risk genotype also demonstrated higher fat mass (by 2.5 kg) and waist circumference (by 2.9 cm, $0.01<P<0.05$).

When assessing abdominal adiposity directly with MRI, the FTO-risk genotype was associated with higher amounts of both intra-abdominal fat (by 38%) and subcutaneous abdomi-
inal fat (by 44%). Note that, because both outcomes were log transformed, these percent increases were calculated from the respective regression coefficients ($\beta$) shown in Tables 2 and 3, using the formula $\left(2^{\beta} - 1\right) \times 100\%$; eg, for intra-abdominal fat: $\left(2^{0.40} - 1\right) \times 100\% = (1.38 \text{ to } 1) \times 100\% = 38\%$. The relationship between the FTO-risk genotype and intra-abdominal fat became completely nonsignificant after adjusting for subcutaneous abdominal fat, indicating that the relationships between FTO and these 2 strongly correlated outcomes were quite similar.

### Glucose and Lipid Metabolism

Subjects with the FTO-risk genotype compared with the rest of the cohort showed similar plasma glucose levels but higher plasma levels of fasting insulin (by 21%, $P = 0.007$) and HOMA index (by 21%, $P = 0.008$; Table 3). In contrast, no statistically significant differences between the 2 groups were observed in plasma triglycerides, total cholesterol, and high-density lipoprotein cholesterol, although the variation in plasma triglycerides was in the predicted direction (ie, higher by 4% in individuals with the risk genotype; Table 3). Similar to previous studies, the differences in fasting insulin and HOMA index lost their significance after the additional adjustment for BMI ($P = 0.09$ and 0.10, respectively), indicating that the association of the FTO-risk genotype with insulin resistance is mediated mostly through its effect on BMI, as suggested by others.1,6

### Repeated-Measure Outcomes in the Adolescent Sample

Table 4 summarizes the results of the multivariable mixed linear analyses of these 3 repeated-measure outcomes.

### BP

Analyses of data pooled across all 7 sections of the protocol demonstrated that SBP is significantly higher (4.4 mm Hg, $P = 0.0024$) in subjects with the FTO-risk genotype than in those with nonrisk genotypes (Table 4 and Figure 1). The omnibus test of interactions between sections and FTO, however, was statistically significant ($P = 0.01$), indicating that the association between the FTO-risk genotype and SBP varies across specific experimental conditions implemented in different sections. Accordingly, Table 4 reports adjusted differences in SBP between subjects with the risk and nonrisk genotypes separately for each section. For all sections, the risk variant of FTO was associated with higher SBP, and these differences were statistically significant ($P < 0.05$) in almost all experimental conditions. The only exception was the section of stress-test explanation during which the difference was only 2.5 mm Hg and was statistically nonsignificant ($P = 0.22$). For most other sections (and experimental conditions), adjusted mean SBP differences between subjects with and without the FTO-risk genotype varied between 4 and 5 mm Hg, but for the section of the stress-test, the difference reached 6.4 mm Hg (Figure 1).

### Table 2. Body-Fat Quantity and Distribution in Adolescents

<table>
<thead>
<tr>
<th></th>
<th>AA (n=59)</th>
<th>AT (n=214)</th>
<th>TT (n=212)</th>
<th>Difference (95% CI) for AA vs AT or TT*</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>58.9 (1.7)</td>
<td>57.1 (0.9)</td>
<td>56.6 (0.9)</td>
<td>4.1 (1.1 to 7.0)</td>
<td>0.007</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>22.4 (0.5)</td>
<td>21.3 (0.2)</td>
<td>20.7 (0.2)</td>
<td>1.5 (0.4 to 2.6)</td>
<td>0.007</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>13.0 (0.9)</td>
<td>11.5 (0.5)</td>
<td>11.2 (0.5)</td>
<td>2.5 (0.5 to 4.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>73.4 (1.2)</td>
<td>70.8 (0.6)</td>
<td>70.7 (0.6)</td>
<td>2.9 (0.2 to 5.7)</td>
<td>0.04</td>
</tr>
<tr>
<td>Intraabdominal fat, cm$^3$</td>
<td>29 (24)</td>
<td>22 (13)</td>
<td>21 (13)</td>
<td>0.5 (0.1 to 0.8)†</td>
<td>0.007</td>
</tr>
<tr>
<td>Subcutaneous fat, cm$^3$</td>
<td>127 (103)</td>
<td>105 (54)</td>
<td>96 (54)</td>
<td>0.5 (0.2 to 0.8)†</td>
<td>0.0008</td>
</tr>
</tbody>
</table>

Data are shown as unadjusted mean (SE).

*Estimated differences (95% CI and $P$ values) between AA homozygotes and both AT heterozygotes and TT homozygotes were tested with mixed-model regression analyses, adjusting for family clustering and potentially confounding variables (proportion of males and females, age, height, puberty stage, family income, and prenatal exposure to maternal cigarette smoking).

†The difference between AA homozygotes and both AT heterozygotes and TT homozygotes was estimated for a log-transformed value.

### Table 3. Glucose and Lipid Metabolism in Adolescents

<table>
<thead>
<tr>
<th></th>
<th>AA (n=59)</th>
<th>AT (n=214)</th>
<th>TT (n=212)</th>
<th>Difference (95% CI) for AA vs AT or TT*</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>4.77 (0.05)</td>
<td>4.72 (0.03)</td>
<td>4.69 (0.03)</td>
<td>0.07 (−0.05 to 0.19)</td>
<td>0.26</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>90.8 (4.6)</td>
<td>75.6 (2.4)</td>
<td>73.2 (2.4)</td>
<td>0.27 (0.07 to 0.46)†</td>
<td>0.007</td>
</tr>
<tr>
<td>HOMA index</td>
<td>2.79 (0.20)</td>
<td>2.39 (0.10)</td>
<td>2.23 (0.10)</td>
<td>0.28 (0.07 to 0.49)†</td>
<td>0.008</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.11 (0.05)</td>
<td>1.02 (0.03)</td>
<td>0.99 (0.03)</td>
<td>0.06 (−0.11 to 0.23)†</td>
<td>0.48</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.48 (0.04)</td>
<td>1.46 (0.02)</td>
<td>1.47 (0.02)</td>
<td>0.01 (−0.07 to 0.10)</td>
<td>0.74</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>4.18 (0.10)</td>
<td>4.22 (0.05)</td>
<td>4.18 (0.05)</td>
<td>−0.03 (−0.27 to 0.20)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Data are shown as unadjusted means (SEs). HDL indicates high-density lipoprotein.

*Estimated differences (95% CI and $P$ values) between AA homozygotes and both AT heterozygotes and TT homozygotes were tested with mixed-model regression analyses, adjusting for family clustering and potentially confounding variables (proportion of males and females, age, height, puberty stage, family income, and prenatal exposure to maternal cigarette smoking).

†The difference between AA homozygotes and both AT heterozygotes and TT homozygotes was estimated for a log-transformed value.
Although the adjustment for BMI reduced the mean difference with and without the risk genotype by 3.8 kg, the post hoc test showed that BMI was marginally significant, with $0.01<P<0.05$ (Table 5).

**BP**
Analyses of sitting BP showed statistically significant associations between *FTO* and BP. Specifically, SBP and DBP were higher in adults with CC or CT genotype than those with TT genotype by 7.5 (P=0.0008) and 3.5 mm Hg (P=0.01), respectively (Table 5). Similar to adolescents, analyses with additional adjustment for BMI reduced modestly the differences in SBP and DBP to 6.2 (P=0.003) and 2.9 mm Hg (P=0.03), respectively. Finally, 86 of 298 individuals were treated with antihypertensive medication during the time of BP measurement. When these individuals were excluded, the differences in SBP and DBP remained significant (6.1 [P=0.01] and 3.0 mm Hg [P=0.04], respectively).

**Hypertension**
Consistent with the quantitative analyses of BP, Generalized Estimating Equations analysis examining the proportion of individuals with hypertension, while adjusting for family clustering and potential confounders (age and sex), showed that the disease is more frequent among individuals with CC or CT genotype than among individuals with TT genotype (odds ratio, 1.9 [1.0 to 3.7], P<0.05; Table 5).

**Discussion**
The results of this study suggest that, in a French Canadian population isolate, *FTO* increases not only the risk for obesity and insulin resistance, as demonstrated previously in other populations, but also for hypertension. The results also suggest that the latter may be related, at least in part, to higher sympathetic modulation of vasomotor tone associated with this genotype. As such, the current study has a 2-fold outcome: (1) it replicates the *FTO* associations with adiposity and insulin resistance, and (2) it identifies novel *FTO* associations with increased BP and sympathetic vasomotor tone; the latter may be a mechanism that contributes to BP elevation. Because *FTO* association with BP was identified in an adolescent sample (age, 12 to 18 years) and confirmed in an independent adult sample (age, 18 to 71 years), suggests a possibility that individuals at risk for hypertension may be recognized early during development of the disease.

The relationships that we observed between *FTO* and adiposity measures were similar or even more pronounced than those reported previously in a study of close to 40,000 participants. There, the authors estimated that adult homozygotes of the risk allele weigh $\approx$3 kg more (4% of body weight in a 75-kg individual) than homozygotes of the nonrisk allele. In the current study assessed in adolescence and thus earlier during progression of obesity, this difference was already $\geq$4 kg (7% of body weight in a 57-kg adolescent).

To the best of our knowledge, the current study is the first to evaluate the relationship of *FTO* with abdominal adiposity measured directly with MRI. These analyses showed that, although the risk genotype compared with the nonrisk geno-

### Table 4. BP (SBP and DBP) and an Index of Sympathetic Vasomotor Tone (LFDBP) in Adolescents

<table>
<thead>
<tr>
<th>Repeated-Measure Outcome</th>
<th>Difference (95% CI) for AA vs AT or TT, mm Hg*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, entire protocol</td>
<td>4.4 (1.6 to 7.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>SBP, supine section</td>
<td>4.1 (1.0 to 7.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP, standing section</td>
<td>4.0 (0.2 to 7.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>SBP, sitting section</td>
<td>4.6 (1.0 to 8.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP, pre-stress section</td>
<td>4.2 (0.6 to 7.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>SBP, stress-test explanation section</td>
<td>2.5 (1.5 to 6.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP, stress-test section</td>
<td>6.4 (1.5 to 11.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP, stress-test recovery section</td>
<td>4.9 (1.2 to 8.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>DBP, entire protocol</td>
<td>1.2 (0.8 to 3.2)</td>
<td>0.23</td>
</tr>
<tr>
<td>LFDBP, entire protocol, mm Hg^2</td>
<td>632 (332 to 931)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Estimated differences (95% CI) between AA homozygotes and both AT heterozygotes and TT homozygotes were tested with mixed-model regression analyses, adjusting for family clustering and potentially confounding variables (proportion of males and females, age, height, puberty stage, family income, and prenatal exposure to maternal cigarette smoking). For these analyses, we used all 52 one-minute means of SBP and DBP and all 7 section means of LFDBP.

In contrast to SBP, the interaction between different sections of DBP time series and the *FTO*-risk genotype was nonsignificant (P=0.47), indicating that the *FTO* effect on DBP was similar across all 7 experimental conditions. Although there was also a trend for a higher DBP in adolescents with the risk variant, the overall adjusted difference between subjects with and without the variant, estimated by pooling data from all 7 sections was 1.22 mm Hg and did not reach statistical significance (P=0.23, Figure 1).

**Sympathetic Modulation of Vasomotor Tone**
The interaction among different sections of the protocol and the *FTO*-risk genotype was completely nonsignificant for LFDBP (P=0.86), indicating that the *FTO* effect was similar across all experimental conditions implemented in our protocol. Therefore, we estimated the overall adjusted difference between subjects with and without the risk genotype by pooling data from all the 7 sections. These analyses showed that the risk variant of *FTO* is associated with a mean LFDBP increase of $\approx$632 mm Hg^2 and that this difference is statistically highly significant even after adjusting for family clustering and potentially confounding variables (P<0.0001, Figure 2).

Analyses with additional adjustment for BMI demonstrated that, for LFDBP, the adjusted difference between adolescents with and without the *FTO*-risk genotype remained essentially unchanged (637.2 mm Hg^2) and highly significant (P<0.0001). Although the adjustment for BMI reduced the mean difference in SBP to $\approx$3.7 mm Hg, it remained significant (P=0.01).

**Descriptive Characteristics of the Adult Sample**
Adults with the rs9302652 genotypes did not statistically significantly differ by sex, age, or height (Table 5).

**Single-Valued Outcomes in the Adult Sample**

**Body-Fat Quantity**
Adults with CC or CT genotypes when compared with adults who have TT genotype demonstrated higher body weight, body-fat mass, and BMI, even after adjusting for family clustering and potential confounders (sex, age, and height [not included in case of BMI]). The differences among genotypes were clinically relevant (body weight was higher by 3.8 kg, BMI by 1.3 kg/m^2, and fat mass by 2.7 kg) and marginally significant, with 0.01<P<0.05 (Table 5).
types is associated with a greater amount of intra-abdominal fat (by 38%) the difference is proportionally similar to that in the amount of subcutaneous-abdominal fat, suggesting that the FTO-risk genotype predisposes to general (whole body) rather than preferentially intra-abdominal obesity. This finding supports previous observations made with DEXA.9

In this study, we identified novel associations between the FTO-risk genotype and both increased BP and LFDBP, an index of sympathetic modulation of vasomotor tone. SBP was higher in carriers of the risk genotype compared with the rest of the cohort throughout the protocol, with the difference reaching 6 mm Hg during an arithmetic stress test. Likewise, LFDBP was higher throughout most of the protocol, suggesting that the FTO-risk allele may increase BP via its influence on sympathetic vasomotor tone. Interestingly, unlike obesity-related insulin resistance, associations between FTO and both BP and the index of sympathetic vasomotor tone seem to be independent of the association between FTO and adiposity. Our analyses demonstrated that additional adjustment for BMI did not alter the difference between subjects with and without the FTO-risk genotype for the index of sympathetic modulation of vasomotor tone, and for SBP, it reduced the difference from 4.4 to 3.7 mm Hg, which remained significant. These results suggest that FTO may influence adiposity and BP/sympathetic vasomotor tone independently.

Importantly, we replicated the novel association between FTO and BP observed in adolescents in an independent sample of adults recruited from the same French Canadian founder population.18 In that sample, we showed that SBP and DBP were higher in individuals with the FTO CC/CT versus TT genotype by 7.5 and 3.5 mm Hg, respectively. Consistent with these BP differences, we also showed that the CC/CT- versus TT-genotype individuals are more likely to have hypertension (odds ratio, 1.9 [1.0 to 3.7]). Moreover, as in the adolescent sample, additional adjustment for BMI made a minimal impact on the BP differences between the genotypes, thus providing further support for the possibility.

**Figure 1.** SBP and DBP according to FTO genotypes in adolescents. One-minute means of beat-to-beat SBP and DBP during a 52-minute cardiovascular protocol, including a posture test (minutes 1 to 30) and arithmetic stress test (minutes 31 to 52), are shown. The earlier data are adjusted for potentially confounding variables, including proportion of males and females, age, height, puberty stage, family income, and prenatal exposure to maternal cigarette smoking. The 52 one-minute means of SBP and DBP were analyzed with mixed-model regression analyses. During the protocol, we saw unexpected BP increases during the collection of saliva samples, when subjects were chewing on a cotton ball (indicated by black arrows).
that FTO may influence BP and adiposity independently. Although FTO as such has not been investigated previously in the relationship to BP, it is of note that a genome-wide linkage study carried out previously identified a region of chromosome 16 including FTO as a region of suggestive linkage to BP.18

Indirect evidence exists suggesting that FTO may be involved in the regulation of sympathetic vasomotor tone. Thus, sympathetic vasomotor tone is powerfully modulated by the paraventricular and dorsomedial nuclei of the hypothalamus,14,35 which are 2 regions of the hypothalamus that showed particularly high FTO expression.10 The paraventricular nucleus of the hypothalamus is thought to exert tonic influences under basal conditions,36 whereas the dorsomedial nucleus of the hypothalamus is mainly involved in the regulation of phasic responses during stress.14,35

FTO encodes 2-oxoglutarate-dependent nucleic acid demethylase that is evolutionarily highly conserved, indicating its biological importance.37 Throughout the body, the highest expression of the gene was detected in the brain and, within the brain, in the hypothalamus.10 It has been proposed that the FTO demethylase may regulate gene transcription in the regions of high expression. This may involve not only genes of energy homeostasis, as suggested previously,10 but also genes of sympathetic modulation of vasomotor tone. This, however, requires further studies in experimental animals.

In the adolescent sample, we used Finometer to measure BP. In the past, precision of SBP measurements with this device exceeded the limits recommended by the American Association for the Advancement of Medical Instruments.38,39 But, more recently, the precision of SBP measurements has improved by the implementation of waveform filtering, level correction, and return-to-flow calibration, and it now meets the Association for the Advancement of Medical Instruments requirements.40,41

Table 5. Descriptive Characteristics, Body-Fat Quantity, and BP in the Adult Sample

<table>
<thead>
<tr>
<th></th>
<th>CC (n=29)</th>
<th>CT (n=121)</th>
<th>TT (n=148)</th>
<th>Difference (95% CI) for CC or CT vs TT*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descriptive characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>17/12</td>
<td>48/73</td>
<td>78/70</td>
<td>2.6†</td>
<td>0.11</td>
</tr>
<tr>
<td>Age, y</td>
<td>42.3 (2.2)</td>
<td>42.0 (1.1)</td>
<td>44.3 (1.0)</td>
<td>−2.2 (−5.3 to 0.9)</td>
<td>0.16</td>
</tr>
<tr>
<td>Height, cm</td>
<td>166.7 (1.9)</td>
<td>164.4 (0.9)</td>
<td>167.6 (0.8)</td>
<td>−0.9 (−3.3 to −1.6)</td>
<td>0.48</td>
</tr>
<tr>
<td>Outcome variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.4 (3.2)</td>
<td>74.4 (1.5)</td>
<td>73.8 (1.4)</td>
<td>3.8 (0.4 to 7.2)</td>
<td>0.03</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.4 (0.9)</td>
<td>27.3 (0.4)</td>
<td>26.1 (0.4)</td>
<td>1.3 (0.1 to 2.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>20.2 (1.8)</td>
<td>22.2 (0.9)</td>
<td>19.9 (0.8)</td>
<td>2.7 (0.4 to 5.0)</td>
<td>0.02</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>129.6 (3.3)</td>
<td>130.2 (1.6)</td>
<td>123.0 (1.5)</td>
<td>7.5 (3.5 to 11.5)</td>
<td>0.0008</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>85.8 (2.1)</td>
<td>83.8 (1.0)</td>
<td>81.4 (0.9)</td>
<td>3.5 (0.9 to 6.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypertension, yes/no</td>
<td>21/8</td>
<td>60/61</td>
<td>58/90</td>
<td>1.9 (1.0 to 3.7)‡</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Data are shown as unadjusted mean (SE).

*Estimated differences (95% CI and P values) between CC homozygotes or CT heterozygotes and TT homozygotes were tested with mixed-model regression analyses, adjusting for family clustering and potential confounders (sex, age, and, when appropriate, weight and fat mass) height.

†Analyzed with χ² statistic.

‡Analyzed with generalized estimating equations to account for the family clustering and potential confounders (age and sex), with odds ratio (95% CI) presented. Office SBP and DBP measurements were obtained while subjects were seated quietly for at least 10 minutes.
A potential limitation of the current study is our use of BP variability as an indirect measure of sympathetic vasomotor tone. Direct measures, such as muscle sympathetic nerve activity, are not suitable for population-based studies of adolescents. Importantly, cardiovascular variability has been validated against this method.24–26

Finally, we have tested the effect of the FTO-risk genotype on several outcomes and, thus, we have to deal with possible inflation of type I error because of multiple testing. Specifically, in both the original and replication studies, we have carried out 19 tests for different single-valued outcomes, 3 tests for the main effects on each of the repeated-measures outcomes, 3 tests of the interactions between the FTO genotype and sections, and 7 tests of section-specific effects of the FTO genotype. With a total of 32 tests, we would expect 1 to 2 statistically significant effects at P<0.05 because of chance alone and not >1 result with P<0.01. In contrast, our analyses yielded P<0.05 for 24 of the total of 32 tests, and among the 24 with P<0.05, 11 tests showed P<0.01. This clearly indicates that most statistically significant results reported in this study represents true rather than spurious effects of the FTO genotypes that would be the result of a sampling error or multiple testing. The replication of the main finding, i.e., an association between BP and FTO, in an independent (adult) sample supports further the true nature of our observations.

In summary, the current results suggest that, in a French Canadian population isolate, the FTO increases not only risk for obesity and insulin resistance, as demonstrated previously in other populations, but also for hypertension. The results also suggest that the latter may be related, at least in part, to higher sympathetic modulation of vasomotor tone associated with this genotype. As such, the current study replicated the FTO associations with adiposity and insulin resistance reported previously, and identified a novel association of the gene with BP and a potential underlying mechanism. The FTO association with BP was identified in an adolescent sample and confirmed in an independent adult sample, indicating that individuals at risk for hypertension may be recognized early during development of the disease when preventive measures may still be effective. We believe that the current study illustrates the value of genetic studies that focus on extensive, detailed phenotyping carried out in populations characterized by genetic and environmental homogeneity.

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Disclosures
None.

References


A Common Variant of the \textit{FTO} Gene Is Associated With Not Only Increased Adiposity but Also Elevated Blood Pressure in French Canadians

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