A COMMON VARIANT OF THE FTO GENE IS ASSOCIATED NOT ONLY WITH INCREASED ADIPOSITY BUT ALSO ELEVATED BLOOD PRESSURE IN FRENCH-CANADIANS

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Pausova: FTO and hypertension


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ABSTRACT

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, here 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 magnetic resonance imaging, 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.

Key words: hypertension, obesity, sympathetic nervous system, vasomotor tone, and genetic association.
INTRODUCTION

A genome-wide association study of 4,862 subjects from the Wellcome Trust Case Control Consortium/U.K. 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 paper, 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).

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)(14). The paraventricular (PVH) and dorsomedial (DMH) nuclei of the hypothalamus, which show particularly high FTO expression (10), are key modulators of sympathetic outflow to the circulatory system (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 the present 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 elevated BP and increased 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 (15-17). In addition, a replication study was carried
out in an independent sample of 298 adults originating from 69 hypertensive families who were recruited from the same French-Canadian founder population (18).

METHODS

Study populations

**Adolescents:** Caucasian adolescents (n=485), aged 12–18 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 (SYS) (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 (19). In brief, all subjects are recruited via local high schools. At the time of analysis, about 5000 adolescents were informed about the SYS 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 illness (e.g. autism, schizophrenia), mental retardation (IQ<70), or MRI contraindications. An equal number of willing and eligible non-exposed subjects, matched to the exposed ones by maternal education (as a proxy of socio-economic status [SES]) 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 non-exposed subjects to the exposed ones by maternal education, overrepresentation of families with lower SES. In addition, the SYS is family based, focused primarily on recruitment of sib-pairs. The cohort investigated here included 17 unrelated individuals, 188 sib-pairs, 28 sib-ships of 3 siblings, and 2 sib-ships of 4 siblings were included.

**Adults:** An independent sample of Caucasian adults (n=298), aged 18 - 71 years, were recruited as members of 69 hypertensive families from the same French-Canadian founder population.
as the above adolescent sample. The families were selected on the basis of having 2 siblings with hypertension (onset at 55 years of age) and dyslipidemia. Hypertension was defined as having diastolic BP (DBP) 90 mm Hg (or higher) on 2 occasions or currently taking antihypertensive medication, with hypertension being documented in medical records. Dyslipidemia was defined as having serum levels of total cholesterol 5.2 mmol/L or HDL 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 <60 years of age are manifestations of a distinct familial syndrome (i.e., "familial dyslipidemic hypertension") (20). Additional selection criteria were the absence of: (1) secondary hypertension, (2) DBP 110 mm Hg (or higher) on BP-lowering medication, (3) gross obesity (body mass index [BMI] >35 kg/m²), (4) diabetes mellitus (fasting blood glucose >6 mmol/L or use of insulin or oral hypoglycemic agents), (5) renal dysfunction (serum creatinine >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.

French-Canadian founder population: Importantly for the genetic component of the present studies, the Saguenay-Lac St. Jean population is one of the largest population isolates in North America (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 5,200 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 (15), and limited allelic diversity exists among patients with these disorders (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.
Quantitative phenotyping

Adolescents

Body-fat quantity and distribution: Measurements included weight, height and waist circumference, multi-frequency bio-impedance analysis to estimate total body-fat mass (Xitron Technologies, Inc., San Diego, CA, USA), and magnetic resonance imaging (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 (21). Subjects were asked to refrain from caffeine, alcohol, and vigorous activity 24 h before the measurements.

Biochemical analyses: A fasting blood sample was drawn between 0800-0900 h. Serum levels of glucose, insulin, triglycerides, total cholesterol, and HDL-cholesterol were measured. We calculated the homeostasis model assessment (HOMA), an index of insulin resistance (22).

Cardiovascular measurements: All subjects underwent a 52-min cardiovascular protocol, conducted in a hospital setting on Saturdays between 0800-1200 h. 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 (23). Throughout the protocol, a non-invasive hemodynamic monitor, Finometer™ (FMS Finapres, Amsterdam, The Netherlands), was employed to record continuously 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.

Sympathetic modulation of vasomotor tone: Power spectral analysis of DBP was used to estimate non-invasively the sympathetic modulation of vasomotor tone and systemic vascular resistance (24-26). The analysis was performed in 2-min periods with 50% overlaps, using a sliding window routine, over the entire 52-min protocol. For each period, beat-to-beat time series of DBP were interpolated using a piecewise cubic-spline method, re-sampled 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, Mathworks, Inc.). Low-frequency spectral power of DBP (LF_{DBP}) was determined by integrating the power spectrum between 0.04–0.15 Hz. LF_{DBP} is thought to reflect mainly sympathetic modulation of vascular tone (24-26), but it may also be influenced by humoral and endothelial factors acting on the vasculature (26). Means of 2-min LF_{DBP} estimates during seven
different sections, i.e. supine (10 min), standing (10 min) and sitting (10 min) periods and means of 2-min LF\textsubscript{DHP} estimates during pre-stress (5 min), stress-test explanation (2 min), stress test (2 min) and stress-test recovery (10 min) periods were used for statistical analyses of posture and arithmetic stress tests, respectively.

**Questionnaires:** The subjects completed a questionnaire evaluating stages of pubertal development (27). Parents completed questionnaires including information on family income, which we used here as an index of socio-economic status (19).

**Adults**

**Body-fat quantity:** Measurements included weight and height and multi-frequency bio-impedance analysis to estimate total body-fat mass (Xitron Technologies, Inc., San Diego, CA, USA).

**BP measurements:** Outpatient measurements of BP were obtained with subjects seated quietly for at least 10 min. Three measurements were obtained in left arm, 2 min apart, with an automated BP monitor (Dinamap, Johnsons & Johnson Medical). The recorded BP value is an average of the 3 measurements. All personnel who measured BP were trained and certified by use of the Shared Care method (28).

**Genotyping**

**Adolescents:** 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 FRET quencher cassette oligos (KBioscience, Herts, UK). The call rate was 98% and the SNP was in Hardy-Weinberg equilibrium. The rs9939609 genotype distribution in the SYS cohort (AA=12.2%, AT=44.1%, and TT=43.7%) was similar to that reported in other population-based cohorts (1).

**Adults:** A SNP in intron 3 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 (29). 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 included means and standard errors for continuous variables, and proportions 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 multivariable mixed linear model in order to (i) account for clustering of observations within families, i.e. for the correlations of the outcomes between siblings, and (ii) adjust for potential confounders. The mixed linear model extends the conventional linear regression of continuous outcomes to correlated data (30). In all our mixed model analyses, the family clustering was accounted for by adding random intercepts, and the compound symmetry covariance structure of residuals was assumed to represent the within-family correlations (31). In addition, the mixed linear model analyses of longitudinal data handle well any randomly missing data, due to subjects' failure to complete some of the repeated outcome assessments (32). Finally, for each outcome, a preliminary analysis involved assessing the normality assumption, on which the statistical inference about the mixed linear model estimates relies. Then, the values of those outcomes for which the empirical distribution showed substantial positive skewness, i.e. IAF, SAF, insulin, HOMA, and triglycerides, were log transformed, using logarithm with basis of 2.0. For each of the log-transformed outcomes, the estimated effect of the *FTO*-risk genotype (AA versus AT or TT) was converted into the adjusted relative increase (in %), calculated as \([2^{\beta}-1]*100\%\), where \(\beta\) is the estimated regression coefficient. Using the above general mixed model approach, two different types of models were used 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 multivariable 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, while 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 IAF was repeated with an additional adjustment for SAF to examine whether the FTO-risk genotype predisposes to preferentially intra-abdominal obesity.

The mixed models analyses of repeated measures of SBP, DBP and LF<sub>DBP</sub> required a more complex approach. Firstly, 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 (31).

Second analytical complexity was due the fact that repeated measures corresponded to different experimental conditions, so that the resulting time series was composed of seven different “sections” (supine, standing, sitting, pre-stress, stress-test explanation, stress test, and stress-test recovery). A priori considerations suggested that the values of SBP, DBP and LF<sub>DBP</sub> could systematically differ between some “sections”. Therefore, the mixed models for each of the three repeated-over-time 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 above), we repeated the analyses of SBP and LF<sub>DBP</sub> 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 between the seven sections. Therefore, in preliminary analyses of each repeated-measures outcome, we have expanded the multivariable mixed model by including a series of two-way interactions between the FTO genotype and each of the section indicators. Then, an “omnibus” Wald-like test, on six degrees of freedom (df), was employed to test the significance of the joint effect of the
six 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 seven 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 seven sections.

**Adults:** The same statistical approach was employed 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 in order (i) to account for clustering of observations within families and (ii) to 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 diastolic BP (DBP) 90 mm Hg (or higher) on 2 occasions or currently taking antihypertensive medication, with the disease being documented in medical records, we employed Generalized Estimating Equations (GEE) 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 genotypes from the final mixed multivariable model as the estimated difference, with 95% confidence interval, in the mean values of a given outcome between subjects with AA versus AT or TT FTO genotype for rs9939609, 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, i.e. 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 two reasons (33). First, many of the outcomes considered in our analyses are inherently correlated with each other (e.g. six measures of adiposity and two measures of insulin resistance), implying that the corresponding tests, even within the same sample, are definitely not independent. Second, two tests of the same association in two 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 two tests are expected to be quite similar. Moreover, the joint probability of p-values from two independent samples falling below a conventional cut-off of 0.05 due to sampling error alone is extremely small. For all these reasons, we have decided to assess an overall pattern of the results of different tests (33) 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 un-corrected $\alpha=0.05$ level. Second, separately for each of the two 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) (34). This allowed us to assess to what extent the observed overall pattern of results is consistent, or not, with the overall null hypothesis, i.e. no association between FTO and outcomes tested.

RESULTS

Descriptive characteristics of the adolescent sample

In the present 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 (non-risk) genotypes is reported together with 95% confidence interval and a 2-tailed p-value.

**Body-fat quantity and distribution**: Even after accounting for family clustering and potentially confounding variables (sex, age, puberty 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 non-risk genotypes were clinically highly relevant, as body weight was higher by more than 4 kg and BMI by 1.5 kg/m². 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 abdominal fat (by 44%). Note that, because both outcomes were log-transformed, these % increases were calculated from the respective regression coefficients (β) shown in Tables 2 and 3, using the formula [2^β-1]*100%; e.g. for intra-abdominal fat: [2^{0.46-1}]*100%=[1.38-1]*100%=38%. The relationship between the *FTO*-risk genotype and intra-abdominal fat became completely non-significant after adjusting for subcutaneous abdominal fat, indicating that the relationships between *FTO* and these two 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 two groups were observed in plasma triglycerides, total cholesterol, and HDL-
cholesterol, although the variation in plasma triglycerides was in the predicted direction (i.e., 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 three repeated-measure outcomes.

Blood pressure: Analyses of data pooled across all seven “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 non-risk 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 non-risk 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 non-significant (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).

In contrast to SBP, the interaction between different “sections” of DBP time-series and the FTO-risk genotype was non-significant (p=0.47), indicating that the FTO effect on DBP was similar across all seven experimental conditions. While 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 seven “sections”, was 1.22 mm Hg and did not reach statistical significance (p=0.23, Figure 1).
**Sympathetic modulation of vasomotor tone:** The interaction between different “sections” of the protocol and the FTO-risk genotype was completely non-significant for LF\(_{\text{DBP}}\) (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 seven “sections”. These analyses showed that the risk variant of FTO is associated with a mean LF\(_{\text{DBP}}\) increase of about 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 LF\(_{\text{DBP}}\), 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). While the adjustment for BMI reduced the mean difference in SBP to about 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 between 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).

**Blood pressure:** 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 mm Hg (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 mm Hg (p=0.003) and 2.9 mm Hg (p=0.03), respectively. Finally, 86 out of 298
individuals were treated with anti-hypertensive medication during the time of BP measurement. When these individuals were excluded, the differences in SBP and DBP remained significant (6.1 mm Hg [p=0.01] and 3.0 mm Hg [p=0.04], respectively).

**Hypertension:** Consistent with the quantitative analyses of BP, Generalized Estimating Equations (GEE) 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 (OR=1.9 [1.0 to 3.7], p<0.05; Table 5).

**DISCUSSION**

The results of the present 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 two-fold outcome: (1) it replicates the *FTO* associations with adiposity and insulin resistance, and (2) it identifies novel *FTO* associations with both elevated BP and increased sympathetic vasomotor tone; the latter may be a mechanism that contributes to BP elevation. The fact that the *FTO* association with BP was identified in an adolescent sample (age 12 – 18 years) and confirmed in an independent adult sample (age 18 – 71 years), suggests a possibility that individuals at risk for hypertension may be recognized early during development of the disease.

The relationships we observed between *FTO* and adiposity measures were similar or even more pronounced than to those reported previously in a study of over 40,000 participants (1). There, the authors estimated that adult homozygotes of the risk allele weigh approximately 3 kg more (4% of body weight in a 75-kg individual) than homozygotes of the non-risk allele (1). In the current study assessed in adolescence and thus earlier during progression of obesity, this difference was already more than 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 magnetic resonance imaging. These analyses showed...
that, although the risk genotype compared with the non-risk genotypes 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 the present study, we identified novel associations between the FTO-risk genotype and both elevated BP and increased LF_{DBP}, 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 more than 6 mm Hg during an arithmetic stress test. Likewise, LF_{DBP} 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 appear 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 (OR=1.9 [1.0-3.7]). 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 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 PVH and DMH (14, 35), which are two regions of the hypothalamus that showed particularly high FTO expression (10). The PVH is thought to exert tonic influences under basal conditions (36), whereas the DMH is mainly involved in the regulation of phasic responses during stress (14, 35). FTO encodes 2-oxoglutarate-dependent nucleic-acid demethylase that is evolutionary 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 (AAMI) (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 AAMI requirements (40, 41).

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 due to 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 FTO genotype. With a total of 32 tests, we would expect one to two statistically significant effects at p<0.05 due to chance alone and not more than one result with p<0.01. In contrast, our analyses yielded p<0.05 for 24 out of the total of 32 tests, and among the 24 with p<0.05, 11 tests showed p<0.01. This clearly indicates that
a vast majority of 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.
ACKNOWLEDGEMENT

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CONFLICT OF INTEREST DISCLOSURES

None.
REFERENCES


8. S:green


11. Speakman JR, Rance KA, Johnstone AM 2008 Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. Obesity (Silver Spring) 16:1961-1965


15. De Braekeleer M 1991 Hereditary disorders in Saguenay-Lac-St-Jean (Quebec, Canada). Hum Hered 41:141-146
31. Waterman C, Laird NM, Ware JM 1989 Blood-lead concentration and cognitive development. JASA 84:33-41
<table>
<thead>
<tr>
<th>Table 1. Descriptive characteristics of adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Males/females</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>Puberty stage (1 – 5)</td>
</tr>
<tr>
<td>Family income (CDNS)</td>
</tr>
<tr>
<td>PEMCS (y/n)</td>
</tr>
</tbody>
</table>

Data are shown as means (standard errors). *Estimated differences (95% confidence intervals and p-values) between AA homozygotes and both AT heterozygotes and TT homozygotes were tested with mixed-model regression analyses, adjusting for family clustering. PEMCS: Prenatal exposure to maternal cigarette smoking. † Analyzed with chi-square test.
<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AT</th>
<th>TT</th>
<th>Difference (95% CI)*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=59)</td>
<td>(n=214)</td>
<td>(n=212)</td>
<td></td>
<td></td>
</tr>
<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 – 7.0)</td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.4 (0.5)</td>
<td>21.3 (0.2)</td>
<td>20.7 (0.2)</td>
<td>1.5 (0.4 – 2.6)</td>
<td><strong>0.007</strong></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 – 4.5)</td>
<td><strong>0.02</strong></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 – 5.7)</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Intra-abdominal fat (cm³)</td>
<td>292 (243)</td>
<td>222 (126)</td>
<td>210 (128)</td>
<td>0.5 (0.1 – 0.8) †</td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>Subcutaneous fat (cm³)</td>
<td>1271 (1030)</td>
<td>1045 (537)</td>
<td>961 (541)</td>
<td>0.5 (0.2 – 0.8) †</td>
<td><strong>0.0008</strong></td>
</tr>
</tbody>
</table>

Data are shown as unadjusted means (standard errors). *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>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AT</th>
<th>TT</th>
<th>Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=59)</td>
<td>(n=214)</td>
<td>(n=212)</td>
<td>AA vs. AT or TT (95% CI) *</td>
<td></td>
</tr>
<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 – 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 – 0.46) †</td>
<td><strong>0.007</strong></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 – 0.49) †</td>
<td><strong>0.008</strong></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 – 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 – 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 – 0.20)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Data are shown as unadjusted means (standard errors). * 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). HOMA: homeostasis model assessment and HDL: high-density lipoprotein. † The difference between AA homozygotes and both AT heterozygotes and TT homozygotes was estimated for a log-transformed value.
### Table 4. Blood pressure (SBP and DBP) and an index of sympathetic vasomotor tone (LF\textsubscript{DBP}) in adolescents

<table>
<thead>
<tr>
<th>Repeated-measure outcome</th>
<th>Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA vs. AT or TT 95% CI *</td>
<td></td>
</tr>
<tr>
<td>SBP, entire protocol (mm Hg)</td>
<td>4.4 (1.6 – 7.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>SBP, supine section (mm Hg)</td>
<td>4.1 (1.0 – 7.2)</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP, standing section (mm Hg)</td>
<td>4.0 (0.2 – 7.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>SBP, sitting section (mm Hg)</td>
<td>4.6 (1.0 – 8.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP, pre-stress section (mm Hg)</td>
<td>4.2 (0.6 – 7.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>SBP, stress-test explanation section (mm Hg)</td>
<td>2.5 (-1.5 – 6.4)</td>
<td>0.22</td>
</tr>
<tr>
<td>SBP, stress-test section (mm Hg)</td>
<td>6.4 (1.5 – 11.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP, stress-test recovery section (mm Hg)</td>
<td>4.9 (1.2 – 8.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>DBP, entire protocol (mm Hg)</td>
<td>1.2 (0.8 – 3.2)</td>
<td>0.23</td>
</tr>
<tr>
<td>LF\textsubscript{DBP}, entire protocol (mm Hg\textsuperscript{2})</td>
<td>632 (332 – 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 time-points for SBP and DBP and all 7 time-points for LF\textsubscript{DBP}. 

26
Table 5. Descriptive characteristics, body-fat quantity and blood pressure in the adult sample

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=29)</td>
<td>(n=121)</td>
<td>(n=148)</td>
<td></td>
<td>CC or CT vs. TT</td>
<td>(95% CI) *</td>
</tr>
<tr>
<td>Males/females</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 (years)</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>
</tbody>
</table>

**Descriptive characteristics**

**Outcome variables**

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>Difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<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>BMI (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 (y/n)</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 means (standard errors). * 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 and age, and when appropriate (weight and fat mass) height. † Analyzed with chi-square statistic. ¶ Analyzed with Generalized Estimating Equations (GEE) 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.
FIGURE LEGENDS

Figure 1. Systolic and diastolic blood pressures according to FTO genotypes in adolescents

One-minute means of beat-to-beat systolic blood pressure (SBP) and diastolic blood pressure (DBP) during a 52-minute cardiovascular protocol, including a posture test (minutes 1-30) and arithmetic stress test (minutes 31-52), are shown. The above 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).

Figure 2. Low-frequency power of DBP variability according to FTO genotypes in adolescents

Index of sympathetic vasomotor tone (low-frequency power of DBP [LFDBP]) is presented for 7 periods. For a posture test, these were supine (10 min), standing (10 min), sitting (10 min) periods; for an arithmetic stress test, they were pre-test (5 min), explanation (2 min), test (2 min) and recovery (10 min) periods. The data were 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 data were analyzed with mixed-model regression analysis.
Figure 1.
Figure 2.

Low-frequency power of DBP variability

A/A
A/T
T/T

FTO: p<0.0001
Section: p<0.0001
FTO*Section: p=0.86

Cardiovascular Genetics

JOURNAL OF THE AMERICAN HEART ASSOCIATION
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