Role of Tumor Necrosis Factor-α Gene Locus in Obesity and Obesity-Associated Hypertension in French Canadians


Abstract—Obesity represents a serious risk factor for the development of cardiovascular diseases, including hypertension. Segregation studies suggest that obesity and obesity-associated hypertension may share some genetic determinants. The results of the present candidate gene investigation suggest that in hypertensive pedigrees of French-Canadian origin, one such determinant is the tumor necrosis factor (TNF-α) gene locus. Gender-pooled quantitative sib-pair analysis demonstrated a significant effect of the gene locus on 3 global and 7 regional measures of obesity ($P=0.05$ to 0.0004). Gender-separate quantitative sib-pair analyses showed that the impact of the locus on obesity is most significant in the abdominal region in men and in the thigh region in women. Furthermore, the haplotype relative-risk test demonstrated a significant association between the TNF-α gene locus and both obesity ($P=0.006$) and obesity-associated hypertension ($P=0.02$). These effects were most significant in individuals with nonmorbid obesity. In conclusion, the results of linkage and association analyses suggest that in hypertensive pedigrees of French-Canadian origin, the TNF-α gene locus contributes to the determination of obesity and obesity-associated hypertension. In addition, the data indicate that gender modifies the effect of the locus on the regional distribution of body fat. (Hypertension. 2000;36:14-19.)

Key Words: tumor necrosis factor • hypertension, obesity • obesity • genes

Obesity is a leading risk factor for the development of essential hypertension. It has been estimated in the Framingham Heart Study that for each 4.5 kg of weight gain, there is an accompanying increase of 4 mm Hg in systolic blood pressure in both men and women. Several mechanisms have been implicated in the pathogenesis of obesity-associated hypertension, including insulin resistance, salt sensitivity, and activation of the sympathetic nervous system.

The pathogenesis of both obesity and hypertension is complex, characterized by the involvement of several genes and environmental factors. Genetic analyses suggest that some of the genes that determine obesity may also contribute to the development of obesity-associated hypertension. One such gene may be that coding for tumor necrosis factor (TNF-α).

TNF-α is a proinflammatory cytokine that, in addition to its role in the immune response and cancer, is involved in the development and phenotypic expression of obesity. It has been suggested that TNF-α functions as an adipostatic factor that is induced by increasing obesity to limit its further progression. This hypothesis is based on a large body of research that demonstrates TNF-α expression is heightened in obesity and that TNF-α exerts antiadipogenic effects. Thus, the exposure of adipose tissue and cells to TNF-α in vitro dramatically suppresses the gene expression of key enzymes involved in fatty acid uptake and lipogenesis. In addition, TNF-α inhibits differentiation and stimulates apoptosis of adipocytes.

In obesity, some of these antiadipogenic effects may be mediated by TNF-α–induced insulin resistance. The complete absence of TNF-α or of both of its receptors results in a significant improvement in insulin sensitivity in mice with dietary, hypothalamic, or genetic obesity. In obese humans, elevated TNF-α expression in adipose and muscle tissues is positively correlated with the level of fasting hyperinsulinemia.

The role of TNF-α has not been studied in hypertension as extensively as in obesity and insulin resistance. It has been demonstrated that TNF-α increases the production of endothelin and angiotensinogen. In addition, as described, TNF-α has been related to the development of obesity-associated insulin resistance, which is one of the proposed mechanisms of obesity-associated hypertension.

The goal of the present study was to investigate whether the TNF-α gene locus is involved in the determination of obesity and obesity-associated hypertension in hypertensive pedigrees of French-Canadian origin.

Methods

Fifty pedigrees of French descent were selected from a genetically isolated population of the Chicoutimi/Lac St Jean region in the

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In addition, affected status was characterized by the absence of (1) secondary hypertension, (2) diastolic blood pressure of $110 \text{ mm Hg}$ while on blood pressure–lowering medication, (3) gross obesity (body mass index [BMI] of $27 \text{ kg/m}^2$), (4) diabetes mellitus (fasting blood glucose of $6.6 \text{ mmol/L}$ or the use of insulin or oral hypoglycemic agents), (5) renal dysfunction (serum creatinine of $>180 \text{ mmol/L}$), (6) liver disease, (7) malignancy, (8) pregnancy, and (9) substance abuse, including alcohol. Furthermore, to ensure genetic homogeneity, only sib pairs with both parents of Catholic French-Canadian origin were selected. Once an affected sib pair was identified, other family members, including siblings, parents, children, and aunts and uncles, were also included. This collection included a total of 262 individuals. The study was approved by the local ethics committee, and the subjects gave their informed consent.

Canadian province of Quebec. The pedigrees were ascertained through “hypertension-affected” sib pairs. “Affected” status was defined by the presence of (1) early-onset essential hypertension, (2) a candidate gene approach was used here, we chose conservative unweighted option for sib-pair analysis. Given the fact that a candidate gene approach was used here, we chose $P<0.05$ as our initial criterion for linkage.

Quantitative sib-pair analysis was conducted with use of the SIBPAL computer program (Version 2.8, S.A.G.E. package; Department of Epidemiology and Biostatistics, Case Western Reserve University). This nonparametric linkage analysis is based on regression of the squared trait difference on the proportion of marker alleles shared between 2 siblings. One-tailed Student’s $t$ test is used to test the significance of the regression. Before linkage analyses, all variables were adjusted for significant covariates, such as age, gender, and height, by means of linear regression. The allele frequencies of DNA markers were estimated in the total sample of individuals ($n=262$). To control for possible bias, we used the more conservative unweighted option for sib-pair analysis. Given the fact that a candidate gene approach was used here, we chose $P<0.05$ as our initial criterion for linkage.

The power to detect linkage with quantitative sib-pair analysis is concentrated in sib pairs either concordant or discordant for high or low values of the trait, or both. Therefore, only individuals with either “low” ($\leq 22 \text{ kg/m}^2$) or “high” ($\geq 27 \text{ kg/m}^2$) BMI were selected for quantitative sib-pair analyses (Figure 1). These individuals created a total of 152 sib pairs, including 102 sib pairs concordant for high BMI, 8 sib pairs concordant for low BMI, and 42 sib pairs discordant for low and high BMI. BMI values of 22 and 27 kg/m$^2$ were chosen because it has been demonstrated that individuals with BMI of $\leq 22 \text{ kg/m}^2$ rarely have metabolic conditions, such as insulin resistance, and that Canadian adults with BMI of $\geq 27 \text{ kg/m}^2$ have nearly twice the prevalence of hypertension as those with BMI of $<27 \text{ kg/m}^2$.

Association analysis was performed with use of the TRANSMIT program (Version 2.3; D. Clayton, MRC Biostatistics Unit). This program tests for associations between a genetic marker and disease by examining the transmission of multipoint haplotypes from parents to affected offspring. The $\chi^2$ statistic is used to compare multipoint haplotype frequencies observed in affected offspring with those expected under mendelian transmission. The TRANSMIT program can also be used when parental genotypes are unknown; in this case, data from unaffected siblings are used.

### Table 1. Obesity-Related Phenotypes in Individuals Selected for Sib-Pair Analysis

<table>
<thead>
<tr>
<th>Obesity Measure</th>
<th>All ($n=140$)</th>
<th>Men ($n=59$)</th>
<th>Women ($n=81$)</th>
<th>Gender Difference ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global obesity measure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>28.5±5.4</td>
<td>28.7±4.0</td>
<td>28.3±6.2</td>
<td>NS</td>
</tr>
<tr>
<td>Body fat (skinfolds), %</td>
<td>36.6±7.6</td>
<td>33.2±5.9</td>
<td>40.3±7.5</td>
<td>9.2×10$^{-7}$</td>
</tr>
<tr>
<td>Body fat (bioimpedance), %</td>
<td>31.8±12.2</td>
<td>24.1±5.9</td>
<td>39.2±12.2</td>
<td>1.2×10$^{-11}$</td>
</tr>
<tr>
<td>Regional obesity measure: circumference, cm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper arm</td>
<td>33.2±4.7</td>
<td>34.2±3.6</td>
<td>32.5±5.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Waist</td>
<td>94.9±14.7</td>
<td>99.9±10.5</td>
<td>91.4±16.2</td>
<td>0.0003</td>
</tr>
<tr>
<td>Hip</td>
<td>102.8±9.9</td>
<td>100.8±6.1</td>
<td>104.1±11.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Waist/hip</td>
<td>0.92±0.10</td>
<td>0.99±0.07</td>
<td>0.87±0.09</td>
<td>8.6×10$^{-15}$</td>
</tr>
<tr>
<td>Proximal thigh</td>
<td>58.8±7.1</td>
<td>57.9±5.4</td>
<td>59.5±8.1</td>
<td>NS</td>
</tr>
<tr>
<td>Middle thigh</td>
<td>53.3±6.4</td>
<td>53.7±5.0</td>
<td>53.1±7.2</td>
<td>NS</td>
</tr>
<tr>
<td>Distal thigh</td>
<td>40.9±5.2</td>
<td>40.9±3.9</td>
<td>40.9±6.0</td>
<td>NS</td>
</tr>
<tr>
<td>Regional obesity measure: skinfold, mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps</td>
<td>28.6±13.6</td>
<td>20.6±9.3</td>
<td>36.6±12.6</td>
<td>4.9×10$^{-11}$</td>
</tr>
<tr>
<td>Biceps</td>
<td>18.2±11.1</td>
<td>12.1±6.7</td>
<td>24.2±11.3</td>
<td>2.1×10$^{-9}$</td>
</tr>
<tr>
<td>Subscapular</td>
<td>27.6±11.7</td>
<td>25.1±8.9</td>
<td>30.1±13.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Suprailiac</td>
<td>27.3±11.9</td>
<td>25.6±11.3</td>
<td>29.1±12.3</td>
<td>NS</td>
</tr>
<tr>
<td>Thigh</td>
<td>32.2±16.3</td>
<td>20.9±9.3</td>
<td>43.9±13.5</td>
<td>3.0×10$^{-16}$</td>
</tr>
</tbody>
</table>

*Essential hypertension with onset at the age of $\leq 55$ years. Values are mean±SD.
Results
Quantitative sib-pair analysis (SIBPAL) demonstrated significant effects of the TNF-α gene locus on all global and most regional measures of obesity (Figure 2). With respect to global measures of obesity, the most significant result was observed for BMI ($t = -2.74$, $P = 0.004$) and less significant data were obtained for TBF derived from skinfold measurements ($t = -1.79$, $P = 0.04$) and TBF determined through bioimpedance ($t = -1.63$, $P = 0.05$) (Figure 2). In regard to the regional obesity measures, the TNF-α gene locus demonstrated the most significant impact on thigh circumferences ($t = -3.10$ to $-3.47$, $P = 0.001$ to 0.0004). Upper arm, waist, and hip circumferences were also linked to the locus but less significantly. Among skinfold measurements, thigh skinfold was the only one that reached statistical significance ($t = -2.55$, $P = 0.007$) (Figure 2).

To confine this obesity-related effect of the locus closer to the TNF-α gene, an association-based analysis (TRANSMIT) was conducted. This analysis demonstrated that marker haplotype frequencies observed in affected offspring (BMI $\geq 27$ kg/m$^2$) differ significantly from those expected under mendelian transmission ($\chi^2 = 6.25$, 3 df, $P = 0.1$), suggesting that the TNF-α gene locus is not involved in the pathogenesis of obesity-associated hypertension. However, on the basis of the known actions and the presumed role of TNF-α in obesity and hypertension, the TNF-α gene is not likely to be involved in the development of hypertension in subjects with severe obesity. Therefore, we carried out association analysis, with affected status being assigned to hypertensive individuals with nonmorbid obesity (BMI 27 to 35 kg/m$^2$). This analysis revealed a significant association between the TNF-α gene locus and obesity-associated hypertension ($\chi^2 = 9.6$, 3 df, $P = 0.02$) (Table 2). Furthermore, the selection of only individuals with nonmorbid obesity (BMI 27 to 35 kg/m$^2$) as affected offspring also increased the significance of the association between the locus and obesity ($\chi^2 = 12.6$, 3 df, $P = 0.006$) (Table 2). In this analysis, the haplotype 1.2.10 was significantly associated with obesity ($\chi^2 = 4.57$, 1 df, $P = 0.03$). These results suggest that the TNF-α gene locus is a significant determinant of both obesity and obesity-associated hypertension and that this effect is limited mainly to individuals with nonmorbid obesity.

Furthermore, descriptive statistics on obesity-related phenotypes demonstrate that men and women differ in most measures of regional body fat distribution (Table 1). The average values of all skinfold measurements were found to be higher in women than in men, with the most significant difference being observed in the thigh skinfold ($P = 3.0 \times 10^{-16}$). In contrast, most of the circumference measures were greater in men than in women. Among them, the most significant difference was noticed in waist circumference ($P = 0.0003$) and the waist/hip ratio ($P = 8.6 \times 10^{-15}$). To further explore the issue of gender, we performed gender-separate quantitative sib-pair analyses. They showed that in male sib pairs, the TNF-α gene locus exerts the most significant effects on waist circumference ($t = -1.58$, $P = 0.06$), the waist/hip ratio ($t = -1.77$, $P = 0.04$), and suprailiac skinfold ($t = -2.87$, $P = 0.004$). In contrast, in female sib pairs, the locus has the most significant impact on upper thigh circumference ($t = -3.02$, $P = 0.002$), middle thigh circumference ($t = -3.00$, $P = 0.002$), and thigh skinfold ($t = -2.39$, $P = 0.02$).
The results of the present linkage and association analysis suggest that in hypertensive pedigrees of French-Canadian origin, the TNF-α gene locus is involved in the pathogenesis of obesity and obesity-associated hypertension. Although these results do not provide direct evidence for the involvement of the TNF-α gene, in consideration of the known actions of TNF-α, this gene represents the best candidate within the chromosomal region.

Discussion

The effect of the TNF-α gene locus on human obesity alone has been reported previously. A significant relationship between the gene locus and various global measures of adiposity was observed in populations as diverse as Pima Indians and European whites. These studies were performed in families or groups of unrelated individuals with obesity, non-insulin-dependent diabetes mellitus, or ischemic heart disease. The results of the current investigation extend the previous observations in that they demonstrate the effect of the locus in pedigrees with hypertension.

A role of the TNF-α gene locus in obesity-associated hypertension has not been previously demonstrated. However, it has been observed in an isolated Native Canadian population that a positive correlation exists between serum TNF-α concentration and both systolic blood pressure and insulin resistance in subjects with a wide range of adiposity. Furthermore, TNF-α has been implicated in the development of endothelial dysfunction. In vascular smooth muscle cells, TNF-α was shown to stimulate the production of a potent vasoconstrictor, endothelin-1. Consistent with this in vitro finding, significant positive correlations were found between serum TNF-α and serum endothelin-1 levels in patients with android obesity. Moreover, in spontaneously hypertensive rats (SHR), several studies have reported that TNF-α synthesis and secretion in response to lipopolysaccharide stimulation are increased in comparison with normotensive controls. This effect was most marked in adipose tissue and was associated with increased angiotensinogen gene expression. In addition, the body temperature response to lipopolysaccharide differs between SHR and its normotensive control, and it has been demonstrated that this response is, at least in part,
The TNF-α gene locus was determined to be involved in the pathogenesis of obesity and obesity-associated hypertension. In the present study, the effect of the TNF-α gene locus on both obesity and obesity-associated hypertension was found to be most significant in nonmorbidly obese individuals. This finding is consistent with the proposed actions of TNF-α in obesity. Enhanced activity of the cytokine due to the development of obesity is, on one hand, predicted to contribute to the development of hypertension but is, on the other hand, expected to limit the progression of obesity.

Gender-separate linkage analyses indicate that the TNF-α gene locus influences regional accumulation of fat, most significantly in the abdominal region in men and in the thigh region in women. Such a gender-specific effect of the TNF-α gene could be the result of a gender difference in the regional expression of either the gene itself or any other element involved in the cascade of events that lead from activation of the gene to its action in the target tissue. At present, the only element in the TNF-α cascade that is known to have gender-specific regional effects is lipoprotein lipase (LPL). This enzyme normally promotes lipid accumulation in adipose cells. A significant proportion of the antiadipogenic effects of TNF-α are mediated through the inhibition of LPL. Arner et al showed that both the mRNA level and the enzyme activity of LPL are higher in abdominal than in thigh adipose cells in men and vice versa in women. Notably, these gender-specific regional differences of LPL closely parallel those of the TNF-α gene effect on body fat accumulation observed in the present study, suggesting a possibility that LPL may be involved in determination of the gender-specific regional effects of the TNF-α gene.

In conclusion, the results of linkage and association analyses suggest that in hypertensive pedigrees of French-Canadian origin, the TNF-α gene locus contributes to the pathogenesis of obesity and obesity-associated hypertension.

**Figure 3.** Gender-separate quantitative linkage analysis: an effect of the TNF-α gene locus on global and regional obesity measures. Results obtained with a CA-dinucleotide repeat polymorphism are shown. They are presented as follows: negative T-values (providing evidence for linkage) are shown as black bars, and the lengths of the bars are proportionate to the values. In cases of statistically significant results, probability values are also shown as numbers above the bars. Positive T values or T values equal to zero (providing evidence against linkage) are indicated by a χ sign.
Furthermore, the results also indicate that the locus influences regional body fat distribution differently in men and women.

Acknowledgments

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