A genetic polymorphism of the peroxisome proliferator-activated receptor $\gamma$ gene influences plasma leptin levels in obese humans

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Peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) is a transcription factor implicated in adipocyte differentiation, lipid and glucose metabolism. A polymorphism corresponding to a silent C$\rightarrow$T substitution was detected in exon 6 of the PPAR$\gamma$ gene. We analysed the relationships between this genetic polymorphism and various markers of the obesity phenotype (body weight, body mass index, waist:hip ratio and plasma leptin levels) in a representative sample of 820 men and women living in northern France. The frequencies of the C and T alleles were 0.860 and 0.140 respectively. In the whole sample no association of the polymorphism with the markers tested was observed but a statistically significant interaction (P < 0.03) existed between this polymorphism and body mass index for plasma leptin levels. This result suggested that the impact of the PPAR$\gamma$ gene polymorphism on plasma leptin levels differed according to the BMI of the subjects. Indeed, obese subjects (BMI > 30 kg/m$^2$) bearing at least one T allele (CT + TT) had higher plasma leptin levels than subjects who did not (35.0 $\pm$ 17.4 ng/ml versus 28.3 $\pm$ 14.8 ng/ml respectively; P < 0.001). This effect existed in both genders, despite the higher plasma leptin levels observed in women. The plasma leptin level increase was not associated with elevation of body mass index, even though these two variables were highly correlated. Thus for a given leptin level the BMI was relatively lower in obese subjects carrying at least one T allele than in obese CC homozygotes. Our results show that in obese subjects variability within the PPAR$\gamma$ gene locus is associated with circulating leptin levels and may modify the relationship between leptin levels and adipose tissue mass.

INTRODUCTION

Obesity is a complex metabolic disorder with strong genetic components (1). Candidate genes are numerous and may involve both structural and regulatory proteins from various tissues. Peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) is a member of the nuclear hormone receptor superfamily and is secreted in adipose tissue. PPAR$\gamma$ controls adipocyte differentiation (2–4) and regulates lipid and glucose homeostasis (reviewed in 5,6). For instance, in animal models and in in vitro cell culture systems PPAR$\gamma$ enhances expression of genes such as lipoprotein lipase (7), aP2 (8), phosphoenolpyruvate carboxykinase (9) and acylCoA synthase (10), while it down-regulates expression of the leptin gene (11–14). Recently thiazolidinediones were shown to bind PPAR$\gamma$ with high affinity, offering a clue to the biological efficiency of these antidiabetic drugs (3,15). Therefore, because of its pleiotropic role in glucose and lipid metabolism, PPAR$\gamma$ is clearly a candidate gene for regulation of adipose tissue metabolism in humans. The PPAR$\gamma$ gene has been cloned and characterized (16). Recently we identified a frequent polymorphism located in exon 6. This polymorphism results from a silent C$\rightarrow$T substitution at nt 161 (CT exon 6 PPAR$\gamma$). Because of the link between PPAR$\gamma$ and adipocyte metabolism, we hypothesized that variability at the genetic locus of PPAR$\gamma$ might be associated with variability of adipose tissue phenotype. We used the CT exon 6 PPAR$\gamma$ polymorphism as a marker to detect possible relationships between PPAR$\gamma$ and several clinical and biological variables related to adipose tissue mass, such as leptin. Leptin, a 167 amino acid protein secreted by adipocytes (17), exerts some of its effects in the brain (arcuate nucleus), where specific receptors have been identified (18). Basal concentrations of leptin in the bloodstream

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are proportional to adipose tissue mass and hence leptin level is a good marker of adipose tissue size (19–22).

RESULTS

In the 1154 subjects characterized, including 585 men and 569 women, the genotypic distribution of the CT exon 6 PPARγ polymorphism was in Hardy–Weinberg equilibrium (CC, 73.9%; CT, 24.5%; TT, 1.6%). Allelic frequencies were 0.867 for the wild-type C allele and 0.133 for the rare T allele. The genotypic and allelic frequencies were similar in men and women. Because of interference by a number of metabolic disorders and treatments with blood variables, individuals treated for dyslipidemia, hypertension or diabetes mellitus were excluded from the analyses. This resulted in a sample of 820 individuals (404 men, 416 women). The allelic and genotypic frequencies of this subsample were not different from those of the entire group (0.860 and 0.140 for the C and T alleles respectively). In order to increase the statistical power of the analyses the sample was divided in two subgroups: subjects bearing at least one T allele (CT+TT) and subjects without the T allele (CC). After adjustment for confounding factors (age, gender, alcohol consumption and smoking) no association between the CT exon 6 PPARγ polymorphism and any of the anthropometric and biological variables was found. There was no statistically significant interaction between the CT exon 6 PPARγ polymorphism and gender or age for any variables. The distributions of these variables according to 10 year age classes for the whole and for the non-treated subjects are presented in Table 1.

Since PPARγ plays an essential role in adipocyte differentiation, we analysed whether the relationships between the CT PPARγ polymorphism and biological variables could be influenced by BMI. Indeed, there was a statistically significant interaction ($P < 0.03$) between the CT exon 6 PPARγ polymorphism and BMI (introduced in the model as a quantitative variable) for plasma leptin levels (Fig. 1). Thus we examined the effect of the polymorphism on plasma leptin levels in obese and non-obese subjects separately, according to the WHO criteria of obesity (BMI $> 30$ kg/m$^2$) (23; Table 2). The CT exon 6 PPARγ allelic frequencies were similar in obese (C, 0.849; T, 0.151) and non-obese individuals (C, 0.862; T, 0.138).

### Table 1. Distribution of the anthropometric and biological variables according to 10 year age classes

|                | Whole population (n = 1154) | Non-treated subjects (n = 820) |
|----------------|-----------------------------|-------------------------------|
|                | 35–44 years (n = 388)       | 35–44 years (n = 340)         |
|                | 45–54 years (n = 386)       | 45–54 years (n = 280)         |
|                | 55–64 years (n = 380)       | 55–64 years (n = 200)         |
| **Weight (kg)**| 73.5 ± 15.5                 | 72.4 ± 15.2                   |
|                | 74.7 ± 15.2                 | 72.5 ± 14.3                   |
|                | 74.9 ± 14.9                 | 72.0 ± 14.6                   |
| **P value**    | 0.31                        | 0.9                           |
| **Waist:hip ratio** | 0.86 ± 0.09                 | 0.85 ± 0.09                   |
|                | 0.89 ± 0.09                 | 0.87 ± 0.09                   |
|                | 0.91 ± 0.10                 | 0.89 ± 0.10                   |
| **BMI (kg/m$^2$)** | 25.6 ± 4.7                  | 25.3 ± 4.6                    |
|                | 26.8 ± 5.2                  | 25.9 ± 4.4                    |
|                | 27.4 ± 4.9                  | 26.1 ± 4.2                    |
| **Leptin (ng/ml)$^a$** | 14.6 ± 13.02                | 14.24 ± 12.96                 |
|                | 16.53 ± 13.14               | 15.28 ± 12.04                 |
|                | 18.33 ± 13.7                | 16.41 ± 12.73                 |
| **Leptin:BMI ratio$^b$** | 0.54 ± 0.41                 | 0.53 ± 0.41                   |
|                | 0.59 ± 0.40                 | 0.57 ± 0.40                   |
|                | 0.64 ± 0.43                 | 0.60 ± 0.43                   |
| **Glucose (mmol/l)$^b$** | 5.27 ± 1.27                 | 5.09 ± 0.68                   |
|                | 5.55 ± 1.42                 | 5.29 ± 0.80                   |
|                | 5.85 ± 1.78                 | 5.43 ± 1.26                   |
| **Insulin (µU/ml)$^b$** | 15.42 ± 10.72               | 13.88 ± 7.14                  |
|                | 15.97 ± 10.58               | 14.03 ± 7.77                  |
|                | 16.70 ± 10.82               | 14.40 ± 8.39                  |
| **Total cholesterol (mmol/l)$^b$** | 5.61 ± 0.97                 | 5.58 ± 0.97                   |
|                | 5.92 ± 1.03                 | 5.90 ± 1.05                   |
|                | 6.17 ± 1.16                 | 6.13 ± 1.02                   |
| **Triglycerides (mmol/l)$^b$** | 1.41 ± 1.29                 | 1.37 ± 1.31                   |
|                | 1.49 ± 1.39                 | 1.39 ± 1.35                   |
|                | 1.38 ± 0.89                 | 1.24 ± 0.84                   |
| **Systolic blood pressure (mm Hg)$^b$** | 124.7 ± 14.8                | 123.6 ± 14.6                  |
|                | 134.4 ± 19.2                | 131.0 ± 17.5                  |
|                | 141.9 ± 20.0                | 137.9 ± 19.3                  |
| **Diastolic blood pressure (mm Hg)$^b$** | 80.1 ± 10.7                 | 79.4 ± 10.6                   |
|                | 84.6 ± 12.0                 | 82.6 ± 11.3                   |
|                | 84.3 ± 11.3                 | 83.0 ± 11.4                   |

$^a$Test for linear trends.

$^b$The statistical tests were calculated on log transformed values.

### Table 2. Effect of the CT PPARγ polymorphism in obese and non-obese subjects

| Non-obese subjects (BMI ≤ 30 kg/m$^2$; n = 703) | Obese subjects (BMI > 30 kg/m$^2$; n = 116) |
|-----------------------------------------------|---------------------------------------------|
| CC (n = 520)                                  | CC (n = 82)                                  |
| $^{CT+TT}$ (n = 183)                          | $^{CT+TT}$ (n = 34)                          |
| **Weight (kg)**                               | 68.3 ± 11.9                                 |
|                                               | 70.4 ± 11.5                                 |
| **P**                                        | ns                                          |
| **Waist:hip ratio**                           | 0.86 ± 0.09                                 |
|                                               | 0.87 ± 0.09                                 |
| **BMI (kg/m$^2$)**                            | 24.2 ± 2.9                                  |
|                                               | 24.6 ± 2.7                                  |
| **P**                                        | ns                                          |
| **Leptin (ng/ml)$^a$**                        | 12.9 ± 10.3                                 |
|                                               | 11.8 ± 9.3                                  |
| **P**                                        | 0.001                                       |
| **Leptin:BMI ratio$^b$**                      | 0.52 ± 0.39                                 |
|                                               | 0.47 ± 0.36                                 |
| **P**                                        | 0.007                                       |
| **Glucose (mmol/l)$^b$**                      | 5.15 ± 0.66                                 |
|                                               | 5.17 ± 0.71                                 |
| **P**                                        | ns                                          |
| **Insulin (µU/ml)$^b$**                       | 13.1 ± 7.86                                 |
|                                               | 13.5 ± 6.89                                 |
| **P**                                        | 0.93                                         |
| **Total cholesterol (mmol/l)$^b$**            | 5.77 ± 1.02                                 |
|                                               | 5.95 ± 1.05                                 |
| **P**                                        | 0.92                                         |
| **Triglycerides (mmol/l)$^b$**                | 1.34 ± 1.99                                 |
|                                               | 1.48 ± 2.12                                 |
| **P**                                        | 0.97                                         |
| **Systolic blood pressure (mm Hg)$^b$**       | 128.5 ± 17.9                                |
|                                               | 128.5 ± 16.2                                |
| **P**                                        | ns                                          |
| **Diastolic blood pressure (mm Hg)$^b$**      | 80.2 ± 10.9                                 |
|                                               | 80.8 ± 10.4                                 |
| **P**                                        | ns                                          |

Obesity was defined according to the WHO criteria (BMI > 30 kg/m$^2$; 23). Data are means ± sd.

$^a$The statistical tests were calculated on log transformed values. For leptin the statistical test was adjusted for age, gender, BML, plasma insulin levels, alcohol consumption and smoking. For the leptin:BMI ratio the statistical test was adjusted for age, gender, plasma insulin levels, alcohol consumption and smoking.
Interaction between the C/T PPAR γ polymorphism and BMI for plasma leptin levels. Regression lines of log transformed values of leptin versus BMI and leptin versus BMI (on a smaller scale) were drawn for CC subjects (crosses, continuous line) and CT+TT subjects (squares, broken line).

As expected, obese subjects (n = 116) had higher plasma leptin levels than non-obese subjects (n = 703) (30.2 ± 15.8 ng/ml versus 12.6 ± 10.0 ng/ml respectively; P < 0.0001). In obese subjects multivariate analysis adjusted for confounding factors (age, gender, BMI, plasma insulin levels, alcohol consumption and smoking) revealed a statistically significant difference in leptin levels between genotypes (35.0 ± 17.4 ng/ml for CT+TT versus 28.3 ± 14.8 ng/ml for CC; P < 0.001) (Fig. 2). There were no statistically significant differences for BMI (Fig. 2) (34.0 ± 3.6 ng/ml for CT+TT versus 34.0 ± 3.2 ng/ml for CC), waist:hip ratio or body weight between CC subjects and T allele bearers. Moreover, the leptin:BMI ratio was statistically different according to genotype (1.02 ± 0.48 for CT+TT versus 0.82 ± 0.42 for CC; P < 0.007) (Table 2). There were no other statistically significant differences in clinical and biological variables according to the CT exon 6 PPAR γ polymorphism. No statistically significant difference was found in the non-obese group.

Finally, since plasma leptin levels differ significantly between men and women, we compared association of the C/T exon 6 PPAR γ polymorphism in obese men (n = 51) and women (n = 65) separately (Fig. 3). As expected, in our population women exhibited higher leptin levels than men (21.9 ± 13.3 ng/ml versus 8.0 ± 6.6 ng/ml respectively; P < 0.0001). Both obese men and women bearing at least one T allele had increased plasma leptin levels as compared with their obese CC counterparts (19.6 ± 9.1 ng/ml for CT+TT versus 16.5 ± 8.5 ng/ml for CC in men; P < 0.016; 45.7 ± 13.1 ng/ml for CT+TT versus 38.0 ± 11.6 ng/ml for CC in women; P < 0.015).

**DISCUSSION**

In a representative sample of a northern French population the frequencies of the C and T alleles of the C/T PPAR γ polymorphism were 0.867 and 0.133 respectively. These frequencies were similar in obese (BMI > 30 kg/m2) and non-obese subjects. Although levels of circulating leptin are higher in women than in men, this effect was similar in both genders. The plasma leptin level increase observed in obese T allele bearers was not associated with a concomitant elevation of body mass index,
Gene–environment interactions were previously described in serum leptin levels was detected in obese subjects only. Similar mass.

modify the relationships between leptin levels and adipose tissue subjects is associated with circulating leptin levels and may results show that variability within the resulting in a greater leptin:BMI ratio in these subjects. These findings suggest that the relationship was not dependent on the leptin concentration but was linked to elevation of body mass index. These results may correspond to gene–environment interactions occurring between the PPARγ gene and environmental factors associated with obesity.

In obese T allele bearers the observed increase in leptin levels was not associated with an increase in BMI, body weight or waist:hip ratio, resulting in a higher leptin:BMI ratio than in obese non-bearers. Thus for a given leptin level the BMI was relatively lower in obese T allele carriers than in obese CC homozygotes. Therefore, it appears that the T allele of the C/T exon 6 PPARγ polymorphism may confer an advantage because overweight subjects carrying the T allele are less obese than would be expected from their plasma leptin levels. Overall, this finding may suggest that the association between leptin level and adipose tissue mass may be modulated by a mutation in linkage disequilibrium with the C/T exon 6 PPARγ polymorphism.

Little is known about the factors that regulate circulating leptin levels. An earlier genetic study looked for genes involved in quantitative variation of leptin levels in Mexican Americans (27). In this study a candidate locus on chromosome 2 (but not on chromosome 3, where the PPARγ gene is located) was associated with leptin level variability. Several hypotheses may explain the lack of association between genetic markers on chromosome 3 and circulating leptin levels in the latter study. Firstly, the markers used in the analysis were spaced an average of 20 cM apart, which may be too large to detect a linkage disequilibrium on chromosome 3. Secondly, the distribution of genetic polymorphisms differs substantially between Mexican Americans and European Caucasians. Therefore, it is conceivable that some mutations may not exist in Mexican Americans but only in Caucasians. Finally, since the effect reported in our study was not found in the whole population but only in the obese subjects, the linkage might be undetectable in the whole population.

Experimental evidence suggests that PPARγ has a direct effect on leptin gene transcription. For instance, in animal models as well as in cell culture studies the pharmacological activation of PPARγ by thiazolidinediones results in down-regulation of leptin gene expression (11–14). These findings have not yet been demonstrated in humans (28). In our study the association between the C/T PPARγ polymorphism and circulating leptin levels in obese subjects could support these experimental observations. However, since the C/T exon 6 polymorphism is silent, this suggests that this polymorphism is in linkage disequilibrium with a functional mutation in the PPARγ locus or in a nearby genetic locus.

In conclusion, we report in obese subjects a significant association between a C/T exon 6 polymorphism of the PPARγ transcription factor and circulating leptin levels. Indeed, obese subjects bearing at least one T allele (CT+TT) had higher plasma leptin levels than subjects who did not (CC). This circulating leptin level increase was not associated with elevation of body mass index. These findings suggest that in obese subjects variability within the PPARγ gene locus is associated with
circulating leptin levels and may modify the relationship between leptin level and adipose tissue mass. Further epidemiological and genetic studies, particularly family and association studies, of the PPARγ gene locus and nearby polymorphisms are needed to improve our understanding of the complex regulatory mechanisms governing leptin expression by adipocytes and their importance in obesity.

MATERIALS AND METHODS

Population study

Within the framework of the WHO MONICA (Multinational MONItoring of trends and determinants of CArdiovascular diseases; 29,30) project in 1995–1996 we constituted a representative sample of 1195 men (n = 601) and women (n = 594) living in the urban community of Lille (northern France), aged 35–65 years. This study was randomly sampled from the electoral rolls and stratified on gender and 10 year age groups (overall response 70%). This study was approved by the Ethics Committee of the Centre Hospitalier et Universitaire de Lille. Each individual signed an informed consent. A detailed questionnaire was filled out including alcohol consumption and smoking estimations and personal medical history. BMI, waist:hip ratio and blood pressure were measured. A blood sample was drawn after a 10 h fast from 1170 participants (590 men, 580 women).

Biological measurements

Glucose was measured by the glucose oxidase method (DuPont Dimension). Plasma insulin was measured by radioimmunoassay (Blinsaline, ERIA Pasteur). Serum total cholesterol and triglyceride levels were measured by enzymatic methods (DuPont Dimension). Plasma leptin levels were measured by radioimmunoassay (Human Leptin RIA Kit, Wak-Chemie Medical GmBH, Germany).

Genetic analysis

Genomic DNA was extracted from white blood cells as previously described (31). DNA amplification was performed using the polymerase chain reaction (PCR) for 1154 subjects (25 refused blood sampling; 16 could not be genotyped for technical reasons). The primers used to amplify part of exon 6 of the PPARγ gene were derived from the genomic sequence of the PPARγ locus (16) and use of these primers gave a PCR product of 200 bp. The C/T exon 6 PPARγ polymorphism was detected using PmlI digestion followed by 3% agarose gel electrophoresis. To achieve this, 10 µl PCR product was digested using PmlI at 37 °C overnight, giving two fragments of 120 and 80 bp. The most common allele has a C at nt 161, while the variant allele has a T.

Statistical analysis

Complete results were obtained for 1154 subjects. Statistical analyses were performed with the SAS statistical software, v.6.11 (SAS Institute Inc., Cary, NC). Obesity was defined according to the WHO criteria (BMI > 30 kg/m²) (23). A body builder with a BMI of 30.8 kg/m² was excluded from this group. Genotypic and allelic distributions according to gender and BMI subgroup were compared with Pearson χ² statistical tests. The effect of the C/T exon 6 PPARγ polymorphism on quantitative variables was tested with a multivariate analysis of covariance using a general linear model. Because of interference by diseases and therapeutic treatments with blood variables, diabetic subjects and individuals under hypcholesterolemic and hypotensive treatment were excluded from these analyses (n = 334). The C/T exon 6 PPARγ genotype was introduced as a dichotomous variable in the analyses: subjects carrying at least one T allele versus subjects carrying no T allele. Interactions between genotypes and covariates were tested. Data for triglycerides, insulin, glucose and leptin were log transformed to normalize their distributions. Statistical significance was considered at the P < 0.05 level and Bonferroni approximation for multiple comparisons was used.

ABBREVIATIONS

PPARγ, peroxisome proliferator-activated receptor γ; BMI, body mass index (weight, kg/height, m²).

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