**Brief Genetics Report**

**Human Resistin Gene, Obesity, and Type 2 Diabetes**

Mutation Analysis and Population Study

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The hormone resistin has been suggested to link obesity to type 2 diabetes by modulating steps in the insulin-signaling pathway and inducing insulin resistance. Thus, the resistin gene represents a potential candidate for the etiology of insulin resistance and type 2 diabetes. In this study, we analyzed the coding sequence of the three exons of the resistin gene, together with its 5′ regulatory region and 3′ untranslated region (UTR), by single-strand conformation polymorphism (SSCP) in 58 type 2 diabetic subjects, 59 obese subjects, and 60 normal subjects. Only one sequence variant was detected in the resistin gene. Sequencing of this variant revealed the presence of a single nucleotide substitution (SNP) in the 3′-UTR of exon 3 (G1326C). Because 3′-UTR SNPs have been shown to affect gene expression, we examined the frequency of this SNP in 591 subjects (198 obese subjects, 207 diabetic subjects, and 186 control subjects) by PCR amplification and BseRI digestion. No significant association was found between the G1326C variant and diabetes and obesity. Comparison of clinical and metabolic parameters between G1326C carriers and noncarriers again showed no significant difference. In conclusion, our data suggest that genetic defects of the resistin gene are unlikely to play a role in the etiology of these common disorders in our population. *Diabetes* 51:860–862, 2002

Common disorders such as type 2 diabetes, hypertension, and premature atherosclerosis recognize insulin resistance as the core pathogenic factor. Hyperinsulinemia and glucose intolerance are often present in obesity, and again defective insulin action is considered the primary factor. Familial transmission and variations in ethnic distribution suggest that insulin resistance is genetically determined. However, despite the strong evidence in favor of a genetic component, the defects responsible for insulin resistance are yet to be identified.

Very recently the newly discovered hormone resistin has been suggested to link obesity to diabetes (1). In animal models resistin is secreted specifically by adipocytes, and is increased markedly in both genetic and diet-induced obesity. Increased resistin secretion was correlated to impaired glucose tolerance and insulin action in mice, while thiazolidinedione treatment greatly downregulated resistin gene expression, and neutralization of the resistin protein enhanced blood glucose uptake and insulin sensitivity. It has been suggested that resistin antigenizes insulin, modulating one or more steps in the insulin-signaling pathway and possibly playing a role in the pathogenesis of insulin resistance. Thus, the resistin gene represents a potential candidate for the etiology of insulin resistance and type 2 diabetes, although the expression of the resistin gene in fat cells and adipose tissue from overweight subjects has been reported to be almost absent (2), making the question of the role of resistin in human obesity and diabetes controversial.

To establish whether genetic variations in the resistin gene might contribute to insulin resistance, the coding sequence of the three exons of the resistin gene, together with its 5′ regulatory region and 3′-UTR, was analyzed by SSCP (3) in type 2 diabetic patients and obese subjects. A total of 177 subjects were screened for sequence variants within the resistin gene. The group included 58 type 2 diabetic subjects, 59 obese subjects, and 60 normal control subjects. The cDNA sequence of the resistin gene (AF323081) was aligned with BLAST 2 to the draft sequence of human chromosome 19 clone CTD-3214H19 (AC008763) in order to determine intron/exon boundaries. The human resistin gene is encoded in a 1.3-kb segment on chromosome 19 (1), and the 476-nucleotide transcript is contained within three exons. Three primer pairs were used to amplify the three exons, the 5′ regulatory region, and the 3′-UTR (Table 1). Only one sequence variant was detected in exon 3 of the resistin gene. This variant was found in 2 of 58 diabetic subjects (2 homozygous) and in 4 of 59 obese subjects (2 homozygous and 2 heterozygous); it was found in none of the control subjects. Sequencing of the variant revealed the presence of an SNP substitution in the 3′-UTR of exon 3 (G1326C), 60 bp 3′ from the stop codon and 8 bp 5′ from the AATAAA polyadenilation signal.
It has been shown that SNPs in 3'-UTR of genes can affect gene expression (4,5), and it is possible that this G1326C variant in the 3'-UTR of the resistin gene might have an influence on resistin gene expression. Therefore, we examined the frequency of this SNP in obese, diabetic, and normal subjects.

A total of 591 subjects (198 obese subjects, 207 diabetic subjects, and 186 control subjects) were studied for the presence of the G1326C variant of the resistin gene by PCR amplification and BseRI restriction enzyme digestion.

The clinical characteristics of the study subjects are shown in Table 2. The obese subjects were significantly younger than the diabetic and control subjects. BMI was significantly different among the three groups (P < 0.0001), as well as glucose concentrations (P < 0.0001). Obese subjects also showed higher fasting insulin (P < 0.0001) and a greater degree of insulin resistance, as measured by homeostasis model assessment (HOMAIR) (6), compared with control subjects (P < 0.0001) (these parameters were not calculated in diabetic subjects due to possible interference of treatment). Diabetic subjects also had significantly lower HDL cholesterol levels compared with obese subjects and control subjects (P < 0.001). Total triglycerides were different among all three groups (P < 0.001), whereas total cholesterol was not different.

Genotype distributions and allele frequencies in obese, diabetic, and control subjects are shown in Table 3. All groups were in Hardy-Weinberg equilibrium (P = NS by \( \chi^2 \) analysis). The frequency of the G1326C allele was not significantly different among all groups. To test whether the G1326C SNP could be associated with any of the obesity- or diabetes-related phenotypes, in particular indexes of insulin resistance, we compared clinical and metabolic parameters (BMI, blood glucose, insulin, HOMA\(_{IR} \), and lipid profile) between carriers and noncarriers of the G1326C SNP. Again, no significant difference was found (data not shown).

Resistin has been proposed to link obesity and diabetes; it has been shown to be increased in 3T3L1 adipocytes and in the genetic (db/db) and diet-induced obese mice. It has also been shown that thiazolidinediones are capable to reduce levels of resistin and to improve insulin sensitivity (1). Considering the role played by genetic factors in the etiology of insulin resistance, the possibility that genetic mutation in the resistin gene may be involved in the susceptibility to diabetes should be examined. In our Italian population, we found a G1326C SNP in the 3'-UTR of the resistin gene. However, this SNP did not associate with either diabetes and obesity or with obesity- and diabetes-related phenotypes. Two recent studies have questioned the role of resistin in insulin resistance (7,2). The first study in several animal models of obesity, including the db/db mice, showed that resistin expression is in fact reduced in experimental models of obesity and diabetes. In the second study, mRNA expression of the resistin gene was reported to be very low or absent in overweight insulin-resistant subjects, as well as insulin-sensitive subjects, suggesting that the resistin gene may not be the hormonal link to insulin resistance and obesity in humans. A third study (8) in isolated human adipocytes confirmed these results. In our study, only one SNP was found, without association with type 2 diabetes and/or obesity in these Italian subjects. Our data suggest that genetic defects of the resistin gene are unlikely to play a role in the etiology of these common disorders in our population.

### TABLE 1
Primer conditions for SSCP analysis of resistin gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Exon size (bp)</th>
<th>Product size (bp)</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Ta (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>118</td>
<td>420</td>
<td>ggtgcggagattggtag (222)</td>
<td>gccaggctcagttggtct (41)</td>
<td>56</td>
</tr>
<tr>
<td>2</td>
<td>78</td>
<td>160</td>
<td>aggacagtttgctcac (34)</td>
<td>gcgcttgacaaacgtct (11)</td>
<td>56</td>
</tr>
<tr>
<td>3</td>
<td>131</td>
<td>256</td>
<td>agatcgaaccttgtggt (11)</td>
<td>tcatacatcatcatcagg (72)</td>
<td>56</td>
</tr>
</tbody>
</table>

Number of nucleotides between the end of each PCR primer and the corresponding exon-intron boundary in parentheses. Ta, annealing temperature.

**RESEARCH DESIGN AND METHODS**

Patients. A total of 591 Caucasian subjects were studied. All subjects were recruited in the Lazio region of Italy, mostly from Rome and its surrounding towns. The 198 obese and the 207 diabetic subjects (mean duration of diabetes...
Resistin gene, obesity, and type 2 diabetes

TABLE 3
Genotype distributions and allele frequencies for resistin gene SNP in obese, diabetic, and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Genotypes</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GC</td>
</tr>
<tr>
<td>Obese subjects</td>
<td>198</td>
<td>191 (96.4%)</td>
</tr>
<tr>
<td>Diabetic subjects</td>
<td>207</td>
<td>204 (98.5%)</td>
</tr>
<tr>
<td>Lean subjects</td>
<td>186</td>
<td>183 (98.3%)</td>
</tr>
</tbody>
</table>

All comparisons are not significant.

11.5 ± 7.1 years) were consecutively recruited from the obesity and diabetic clinics of the Department of Clinical Sciences, University of Rome “La Sapienza.” The control group comprised 186 unrelated individuals randomly selected from a population of free-living individuals screened for coronary artery disease (CAD) risk factors. Exclusion criteria for control subjects were 1) the presence of a BMI >26 kg/m², 2) the presence of type 2 diabetes or of a first-degree relative with type 2 diabetes, and 3) the presence of CAD. CAD was excluded by use of the Rose questionnaire and electrocardiogram (Minnesota coding) (9). For obese, diabetic, and control subjects, a complete medical history was obtained by questionnaire. History taking included questions about smoking habits, history of hypertension and type 2 diabetes, and current medication used. All obese patients were selected on the basis of a BMI >28 kg/m². Exclusion criteria were the presence of type 2 diabetes or of a first-degree relative with type 2 diabetes. Diagnosis of type 2 diabetes was based on history of hypoglycemic treatment and/or confirmed fasting blood glucose >126 mg/dl (7 mmol/l) (10). Type 2 diabetic subjects were selected on the basis of at least 5 years from diagnosis without insulin treatment, a BMI >25 kg/m², an age of diagnosis >50 years, and absence of concomitant autoimmune disease.

Molecular genetic screening. Sequence variants of the resistin gene were analyzed with the PCR-SSCP technique as previously described (3). Briefly, primers were designed to include intron-exon boundaries, in order to evaluate mutations in splicing sites, and to include the 5’ regulatory region and the 3’-UTR (Table 1). To confirm the expected nucleotide sequence, the three PCR fragments corresponding to the three exons were analyzed by direct sequencing using the PRISM Dye Terminator Cycle Sequencing kit and an ABI 310 automated sequencer (Applied Biosystems, Milan, Italy) according to the manufacturer’s instructions.

After PCR amplification, DNA fragments were electrophoresed in 1 × MDE (Mutation Detection Enhancement) gel (FMC BioProducts, Rockland, Maine) and visualized with silver staining. All exons not showing SSCP variants with this method were electrophoresed in a 12% polyacrylamide gel in 1 × TBE buffer and run at room temperature at a constant of 25 mA for 14–16 h, with and without 5% glycerol. SSCP variants were investigated by direct sequencing as described above. The SSCP method is reported (11) to have a 90% sensitivity when used, as in this study, with two gel conditions; thus, it must be acknowledged that there is a 10% chance that unknown SNPs might have been missed. By use of Webcutter 2.0 (www.firstmarket.com/cutter/cut2.html), it was found that the G1326C variant in exon 3 of the resistin gene creates a Bse/R restriction site, resulting in the presence of two bands of 235 and 21 bp. Thus, restriction enzyme digestion of the 256-bp PCR fragment of exon 3 was carried out at 37°C for 1.5 h in 22-μl reactions containing 12 μl PCR product, 2.2 μl buffer, and 6 units of the Bse/R restriction enzyme. Fragments were analyzed on 4% high-resolution gel stained with ethidium bromide.

Statistical analysis. Categorical variables were compared by χ² or Fisher’s exact test. Differences between continuous variables were evaluated by two-tailed Student’s t test. Genotype distributions between the study groups were compared by 2 × 2 and 2 × 3 contingency tables and χ² analysis.

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REFERENCES