Diabetologia (2004) 47:559–565
DOI 10.1007/s00125-003-1319-x

Diabetologia

# Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans

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## Abstract

*Aims/hypothesis.* Resistin is thought to be an important link between obesity and insulin resistance. It has been suggested that genetic polymorphism in the promoter of resistin gene is a determinant of resistin mRNA expression and possibly associated with obesity and insulin resistance. In this study, we investigated the association between the genotype of resistin promoter and its plasma concentrations.
*Methods.* We examined g.-537A>C and g.-420C>G polymorphisms in the resistin promoter and measured plasma resistin concentrations in Korean subjects with or without Type 2 diabetes. We also did haplotype-based promoter activity assays and the gel electrophoretic mobility shift assay.
*Results.* The –420G and the –537A alleles, which were in linkage disequilibrium, were associated with higher plasma resistin concentrations. Individuals with haplotype A-G (–537A and –420G) had significantly higher plasma resistin concentrations than the others. Haplotype A-G had modestly increased promoter activity compared to the other haplotypes. Electrophoretic mobility shift assay showed that the –420G allele is specific for binding of nuclear proteins from adipocytes and monocytes. However, none of the two polymorphisms were associated with Type 2 diabetes or obesity in our study subjects.
*Conclusions/interpretation.* Polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. [Diabetologia (2004) 47:559–565]

**Keywords** Resistin · Plasma concentrations · Promoter · Genotype · Diabetes

Resistin belongs to a novel family of cystein-rich C-terminal domain proteins called resistin-like molecules, which are identical to those found in inflammatory zone family [1, 2, 3]. It has been reported that serum concentrations of resistin are markedly increased in obese mice and are decreased by treatment with thiazolidinediones [4]. Moreover, obese mice given an anti-resistin antibody had increased insulin-stimulated glucose uptake, whereas the treatment of normal mice with recombinant resistin impaired insulin action [4]. Resistin therefore could link obesity with insulin resistance and diabetes in mouse models. However, subsequent studies in rodent models have produced disparate findings on the role of resistin in obesity and insulin resistance [5, 6, 7].

In humans, the expression of resistin in adipocytes is much lower than that seen in rodents and does not differ between normal, insulin-resistant or Type 2 diabetic patients [8, 9, 10]. Little is known about the relationship between circulating resistin concentrations

Received: 27 August 2003 / Revised: 7 November 2003
Published online: 23 January 2004


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*Abbreviations:* HOMA-IR, homeostasis model assessment of insulin resistance · EMSA, electrophoretic mobility shift assay · OR, odds ratio

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and obesity and other metabolic parameters in humans. It has, however, been recently reported that plasma resistin concentrations are higher in patients with Type 2 diabetes than in non-diabetic subjects, but the mechanism of increased plasma resistin concentrations and their relation to insulin resistance in humans is still unclear [11].

The putative role of resistin in the pathogenesis of human obesity or diabetes led to genetic studies in different populations. Several genetic association studies in humans had contradictory results, which can be divided into three categories: (i) an association with obesity but not with diabetes [12, 13]; (ii) an association with insulin sensitivity but not with obesity or diabetes [14, 15]; and (iii) no association with obesity or with diabetes [16, 17, 18]. Interestingly, variations in the promoter region of the resistin gene were associated with obesity or insulin resistance [12, 15]. It has also been reported that the g.-420C>G polymorphism in the resistin promoter is the major determinant of the mRNA expression resistin and is associated with insulin resistance, and possibly also with cellular oxidative stress [19]. In addition, ADD1/SREBP1c and C/EBPα play a discrete role in the regulation of the resistin gene expression by binding its promoter region [20]. Thus by regulating circulating resistin concentrations, the promoter activity of human resistin gene could be important in the pathogenesis of obesity or diabetes.

## Subjects and methods

*Subjects.* We studied 411 unrelated patients with Type 2 diabetes (197 men, 214 women) and 173 non-diabetic subjects (60 men, 113 women). All subjects had Korean ethnicity. Type 2 diabetes was diagnosed according to World Health Organization criteria [21]. Selection of the non-diabetic control subjects was according to the following criteria: no diabetes among first-degree relatives, a fasting plasma glucose concentration of less than 6.1 mmol/l and an $HbA_{1c}$ value of less than 5.8%. The Institutional Review Board of the Clinical Research Institute in Seoul National University Hospital approved the study protocol, and written informed consent was obtained from each subject. This study was carried out in accordance with the Declaration of Helsinki as revised in 2000 (http://www.wma.net/e/policy/17cnote.pdf ).

In patients and control subjects blood pressure, height, weight, and the circumferences of the waist and hip were measured. Fasting plasma glucose, total cholesterol, triglyceride and HDL-cholesterol concentrations were measured enzymatically using an autoanalyzer (Hitachi 747, Hitachi, Tokyo, Japan). $HbA_{1c}$ was measured by high performance liquid chromatography using the Bio-Rad Variant II system (Bio-Rad Laboratories, Hercules, Calif., USA). Plasma insulin concentrations were measured by radioimmunoassay (BioSource, Nivelles, Belgium). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as described previously [22]. The clinical characteristics of the study populations are shown in Table 1. Compared to the non-diabetic control subjects the Type 2 diabetic patients were younger and had higher values in BMI, waist circumference, WHR, fasting plasma glucose concentrations, fasting plasma insulin concentrations, HOMA-IR, and triglyceride concentrations.

*Genotyping by single base extension and electrophoresis.* We examined two common polymorphisms (g.-537A>C and g.-420C>G) of human resistin gene. Nucleotide position numbering was based on GenBank accession number AF205952 and nomenclature for genetic variation followed a recent consensus [23]. Accordingly, the nucleotide +1 is the A of the ATG-translation initiation codon and the nucleotide 5′ to +1 is numbered −1. For genotyping of both alleles, amplifying and extension primers were designed for single base extension. The sequences of primer sets were; 5′-TGCCCAGACTGGAGTGCAG-3′ (forward primer), 5′-GCACACGAATTCCTGCACC-3′ (reverse primer), and 5′-TGACCAGTCTCTGGACATGA-3′ (extension primer) for g.-420C>G; and 5′-TGCCCAGACTGGAGTGCAG-3′ (forward primer), 5′-GCACACGAATTCCTGCACC-3′ (reverse primer), and 5′-AGGGTGGCTGATGCAAACA-3′ (extension primer) for g.-537A>C. Primer extension reactions were done with SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, Calif., USA).

**Table 1.** Clinical characteristics of study subjects

| | Control subjects ($n$=173) | Diabetic patients ($n$=411) | $p$ value |
|---|---|---|---|
| Sex (men:women) | 60:113 | 197:214 | 0.003 |
| Age (years) | 65±4 | 59±10 | <0.001 |
| BMI (kg/m$^2$) | 23.4±3.0 | 24.4±3.0 | <0.001 |
| Waist (cm) | 82.9±8.3 | 87.7±8.0 | <0.001 |
| Hip (cm) | 94.4±6.1 | 96.9±6.2 | <0.001 |
| WHR | 0.88±0.05 | 0.90±0.05 | <0.001 |
| Systolic blood pressure (mmHg) | 140 (90–198) | 139 (90–199) | NS |
| Diastolic blood pressure (mmHg) | 84 (51–120) | 82 (60–118) | NS |
| Fasting plasma glucose (mmol/l) | 5.1±0.5 | 8.7±2.6 | <0.001 |
| Plasma insulin (pmol/l) | 49.2 (12.0–142.8) | 63.0 (16.2–372.6) | <0.001 |
| HOMA-IR | 1.86 (0.48–5.37) | 4.87 (0.90–27.16) | <0.001 |
| Cholesterol (mmol/l) | 5.3±0.9 | 5.2±0.9 | NS |
| Triglyceride (mmol/l) | 1.4 (0.5–5.6) | 1.6 (0.4–11.6) | 0.001 |
| HDL-cholesterol (mmol/l) | 1.2±0.3 | 1.2±0.3 | 0.031 |

Data are given as means ± SD in cases of normal distribution, otherwise as median (range). HOMA-IR, homeostasis model assessment of insulin resistance

To clean up the primer extension reaction, one unit of shrimp alkaline phosphatase was added to the reaction mixture and the mixture was incubated for 1 h at 37°C, followed by 15 min at 72°C for enzyme inactivation. The DNA samples containing extension products and Genescan 120 Liz size standard solution were added to Hi-Di formamide (Applied Biosystems) according to the recommendations of the manufacturer. The mixture was incubated for 5 min at 95°C , followed by 5 min on ice, after which electrophoresis was done using ABI Prism 3100 Genetic Analyzer. The results were analysed using the program of ABI Prism GeneScan and Genotyper (Applied Biosystems).

*Plasma resistin concentrations.* Plasma resistin concentrations were measured by a sandwich ELISA method using monoclonal antibody as described previously [11]. Plasma samples were diluted 1:10 before the assay. All samples were assayed in duplicate. The lower limit of detection with the resistin assay was 100 pg/ml. The inter- and intra-assay coefficients of variation were 5.3% and 5.4% respectively.

*Plasmids for luciferase reporter assay.* To generate the luciferase reporter constructs Res(A-C)-luc and Res(C-G)-luc, the promoter region between −746 bp and −211 bp from the translation start site of resistin gene was amplified from genomic DNA of homozygous individuals: A/A(−537)-C/C(−420) and C/C(−537)-G/G(−420). Two primers, 5′-CCGCTCGAGTGTCATTCTCACCCAGAG-3′ (forward primer) and 5′-CCCAAGCTTCGGTGGGCTCAGCTAACC-3′ (reverse primer), were used for the PCR amplification. The amplified fragments were inserted into *Xho*I and *Hind*III sites of the pGL2-basic vector (Promega, Madison, Wis., USA). For the construction of Res(A/G)-luc, the corresponding sequences of Res(A-C)-luc were modified using the QuickChange Site-directed Mutagenesis Kit (Stratagene, La Jolla, Calif., USA). Primers used for the mutagenesis of C(−420) to G(−420) were: 5′-CTGGACATGAAGAGGGAGGCCCTGTTGG-3′ (forward primer) and 5′-CCAACAGGGCCTCCCTCTTCATGTCCAG-3′ (reverse primer). DNA sequences of each construct were confirmed by sequencing of the plasmids.

*Cell culture and transient transfection.* 3T3-L1 cells were cultured in DMEM supplemented with 10% calf serum. Cells were transfected at 50 to 70% confluency in six-well plates using LipofectAMINE Plus (Invitrogen, Carlsbad, Calif., USA). Transfection was done with 1 µg of DNA per well: 0.9 µg of reporter plasmids and 0.1 µg of pCMV-β-galactosidase. After cells with DNA mixture had been incubated for 1 h, the medium was changed to DMEM supplemented with 10% calf serum. Cells were harvested 40 h after transfection. Luciferase activity was measured according to the manufacturer's instructions using Luciferase Assay System (Promega). Luciferase activity was normalised to the β-galactosidase activity obtained from the same preparation of lysate.

*Gel electrophoretic mobility shift assay (EMSA).* Nuclear proteins were prepared from 3T3-L1 adipocytes differentiated for 10 days or from THP-1 monocytes [24]. Double-stranded oligonucleotides for the resistin gene containing −420G (CTGGACATGAAGA**G**GGAGGCCCTGTTGG) and −537A (GAGGCATTAATTTTGTC**A**TGTTTGCATCAGCCAC) were prepared using the Klenow fragment of DNA polymerase (Ambion, Austin, Tex., USA). The [α-$^{32}$P] CTP-labelled probe (25000 cpm) was incubated for 15 min at room temperature with nuclear proteins (5 or 10 µg) in 10 mmol/l Hepes, pH 7.9, 50 mmol/l KCl, 0.1 mmol/l EDTA, 0.25 mmol/l dithiothreitol, 0.1 mg/ml poly(dIdC), 0.01% Nonidet P-40, 10% glycerol. Competitor oligonucleotides were added at 100- to 500-fold molar excesses. Sequences of the competitors are −420C (CTGGACATGAAGA**C**GGAGGCCCTGTTGG) and −537C (GAGGCATTAATTTTGTC**C**TGTTTGCATCAGCCAC). Reaction mixtures were loaded on 6% acrylamide gel and subjected to electrophoresis. Complexes were visualised by exposure to X-ray films (Kodak, Rochester, N.Y., USA).

*Statistical analysis.* All continuous variables are expressed as means ± SD or median with range. Allele frequencies were estimated by the gene-counting method, and the chi square test was used to examine Hardy-Weinberg equilibrium. Haplotypes and their frequencies were computed using a recently developed algorithm [25]. Odds ratios (OR) and 95% CIs were assessed by logistic regression model. Student's *t* tests, ANOVA, the Kruskal-Wallis test with post hoc Dunn's test, and multiple linear regression analyses were done where appropriate. To approximate a normal distribution, blood pressure, HOMA-IR, insulin, triglyceride, and plasma resistin concentrations were log-transformed before analysis. To estimate the percentage of variation in plasma resistin concentrations attributable to each polymorphism, the measured genotype approach was applied [26]. A $p$ value of less than 0.05 was considered statistically significant.

## Results

*Genotypes and their association with diabetes and obesity.* Both g.-420C>G and g.-537A>C polymorphisms of resistin promoter were in Hardy-Weinberg equilibrium among diabetic and non-diabetic subjects respectively. The allele frequency of −420G was 0.303 for non-diabetic subjects and 0.330 for Type 2 diabetic patients ($p$=NS). The allele frequency of −537C

**Table 2.** Genotype frequencies of the g.-420C>G and g.-537A>C polymorphisms

| | Control subjects | | | Diabetic patients | | | $p$ value |
|---|---|---|---|---|---|---|---|
| g.-420C>G | C/C | C/G | G/G | C/C | C/G | G/G | |
| *n* | 89 | 63 | 21 | 194 | 163 | 54 | NA |
| Frequency (%) | (51.5) | (36.4) | (12.1) | (47.2) | (39.7) | (13.1) | NS |
| g.-537A>C | A/A | A/C | C/C | A/A | A/C | C/C | |
| *n* | 151 | 22 | 0 | 348 | 62 | 1 | NA |
| Frequency (%) | (87.3) | (12.7) | NA | (84.7) | (15.1) | (0.2) | NS |

The chi square test was used for the statistical analysis. NA, not applicable

**Table 3.** Plasma resistin concentrations according to genotypes or haplotypes

| | Plasma resistin concentration (ng/ml) | | | |
|---|---|---|---|---|
| | Non-diabetic | *n* | Diabetic | *n* |
| g.-537A>C | | | | |
| A/A | 4.74 (0.14–35.42) | 151 | 5.76 (0.14–36.75) | 348 |
| A/C | 3.47 (0.13–17.93) | 22 | 4.50 (0.21–23.6) | 62 |
| *p* value between groups | NS | | 0.019 | |
| g.-420C>G | | | | |
| C/C | 2.71 (0.14–35.42) | 89 | 3.77 (0.14–36.75)[b] | 194 |
| C/G | 6.06 (0.13–32.46) | 63 | 7.00 (0.13–36.75) | 163 |
| G/G | 10.26 (2.29–35.42)[a] | 21 | 9.77 (0.77–35.42) | 54 |
| *p* value between groups | <0.001 | | <0.001 | |
| Haplotype | | | | |
| A-C | 3.56 (0.14–35.42) | 242 | 4.57 (0.20–36.75) | 551 |
| A-G | 9.46 (0.16–35.42)[a] | 82 | 8.92 (0.86–36.75) | 207 |
| C-G | 3.47 (0.13–17.93) | 22 | 4.37 (0.21–23.68)[b] | 64 |
| *p* value between groups | <0.001 | | <0.001 | |
| Haplotype combination[C] | | | | |
| A-G/A-G | 9.83 (2.29–35.42) | 16 | 10.50 (0.86–21.58) | 32 |
| A-G/ X | 8.89 (0.16–32.46) | 50 | 8.14 (0.14–36.75) | 143 |
| X/X | 2.45 (0.13–35.43)[b] | 107 | 3.75 (0.13–31.99)[b] | 236 |
| *p* value between groups | <0.001 | | <0.001 | |

Data are given as median (range). Data were log-transformed for statistical analysis. *p* values are for Student's *t* test (g.-537A>C) and ANOVA (all the others)

[a] The *p* value was higher than the others by post hoc test ($p<0.05$)
[b] The *p* value was lower than the others by post hoc test ($p<0.05$)
[c] The X denotes haplotypes other than A-G

was 0.064 for non-diabetic subjects and 0.078 for Type 2 diabetic patients (*p*=NS). No difference in genotype frequencies was seen between Type 2 diabetic and control subjects (Table 2). In a logistic regression model including age and sex, neither the g.-537A>C polymorphism (OR=1.321, 95% CI: 0.765 to 2.281) nor the g.-420C>G polymorphism (OR=1.239, 95% CI: 0.851 to 1.804) contributed to Type 2 diabetes. After inferred haplotype reconstruction there were three haplotypes: A-C (−537A and −420C), A-G (−537A and −420G) and C-G (−537C and −420G). The frequencies of haplotypes A-C, A-G and C-G were 0.679, 0.247, and 0.074 respectively. The two loci were in linkage disequilibrium (Lewontin's D′=1 and $r^2$=0.169). No difference in haplotype frequencies was observed between non-diabetic and diabetic subjects. There was no association between promoter genotypes and obesity defined by a BMI of more than 25, 28 or 30 kg/m$^2$ respectively.

*Genotypes and plasma resistin concentrations.* For the locus −420, the plasma resistin concentrations increased in rising order from C/C to C/G and G/G (Table 3). Individuals with −537A/A had higher plasma resistin values than those with −537A/C (Table 3). Thus, −420G and −537A alleles could be responsible for the higher plasma resistin values. Individuals with haplotype A-G, which consists of −537A and −420G, had significantly higher plasma resistin values than the others ($p<0.001$) (Table 3). The genotypes in −420 and -537 loci remained significant in determining plasma resistin concentrations even after adjustment for diabetes or not, sex, age, BMI, fasting glucose, HOMA-IR, and triglyceride concentrations ($p<0.001$ and $p=0.010$ respectively). The estimated variance attributable to −420 or −537 locus in plasma resistin concentrations was 9.68% and 1.33% respectively.

*Genotypes and clinical parameters.* For the genotypes of both polymorphisms, non-diabetic and diabetic subjects had no differences in blood pressure, BMI, fasting plasma glucose and HOMA-IR. Clinical characteristics according to the resistin promoter haplotypes are shown in Table 4. Although haplotype A-G was associated with higher plasma resistin concentrations, there were no differences in other clinical criteria, including HOMA-IR (Table 4).

*Promoter activity.* In the three plasmid constructs generated to examine the effects of each haplotype on promoter activity, a significant difference in promoter activity was observed in all three haplotypes ($p=0.0085$ by Kruskal-Wallis test), and haplotype A-G had a 1.38-fold higher luciferase activity than haplotype A-C ($p<0.05$ by Dunn's test).

**Table 4.** Clinical characteristics according to the haplotypes

| Haplotype [(–537)-(–420)] | Non-diabetic subjects | | | | Diabetic patients | | | |
|---|---|---|---|---|---|---|---|---|
| | A-C | A-G | C-G | *p* value | A-C | A-G | C-G | *p* value |
| *n* | 242 | 82 | 22 | NA | 551 | 207 | 64 | NA |
| Age (years) | 65±4 | 66±5 | 66±4 | NS | 60±10 | 59±10 | 61±9 | NS |
| BMI (kg/m$^2$) | 23.5±3.0 | 23.3±2.9 | 22.8±2.7 | NS | 24.5±2.9 | 24.4±3.1 | 24.4±3.3 | NS |
| Waist (cm) | 82.9±8.7 | 83.0±7.4 | 82.2±6.9 | NS | 87.8±8.1 | 87.4±7.9 | 88.0±7.8 | NS |
| Hip (cm) | 94.2±6.3 | 94.8±5.6 | 94.0±5.3 | NS | 97.1±6.2 | 96.7±6.3 | 96.8±5.9 | NS |
| WHR | 0.88±0.06 | 0.88±0.05 | 0.88±0.05 | NS | 0.91±0.06 | 0.90±0.06 | 0.91±0.06 | NS |
| Systolic BP (mmHg) | 140 (90–198) | 140 (99–182) | 136 (90–180) | NS | 135 (97–199) | 130 (90–190) | 138 (90–180) | NS |
| Diastolic BP (mmHg) | 85 (51–120) | 85 (60–110) | 80 (60–100) | NS | 80 (60–113) | 80 (62–110) | 80 (60–118) | NS |
| Fasting glucose (mmol/l) | 5.1±0.6 | 5.2±0.6[a] | 4.9±0.6 | 0.006[d] | 8.8±2.6 | 8.6±2.4 | 8.7±3.1 | NS |
| Plasma insulin (pmol/l) | 49.4 (15.8–142.8) | 49.7 (12.0–142.6) | 40.2 (23.8–80.6) | NS | 62.4 (16.2–372.6) | 63.0 (16.2–208.6) | 74.4 (43.2–372.6)[b] | 0.005[d] |
| HOMA-IR | 1.86 (0.53–4.34) | 2.01 (0.48–5.37) | 1.55 (0.82–3.42) | NS | 4.58 (0.90–27.16) | 4.80 (1.09–18.03) | 6.57 (2.80–27.16)[c] | 0.017[d] |
| Cholesterol (mmol/l) | 5.3±0.9 | 5.2±0.9 | 5.3±0.9 | NS | 5.2±1.0 | 5.2±0.9 | 5.0±0.9 | NS |
| Triglyceride (mmol/l) | 1.4 (0.5–5.6) | 1.3 (0.6–5.6) | 1.4 (0.5–2.7) | NS | 1.6 (0.4–11.6) | 1.6 (0.4–8.5) | 1.4 (0.5–8.1) | NS |
| HDL-cholesterol (mmol/l) | 1.2±0.3 | 1.2±0.3 | 1.2±0.3 | NS | 1.2±0.3 | 1.2±0.3 | 1.3±0.3 | NS |
| Resistin (ng/ml) | 3.56 (0.14–35.42) | 9.46 (0.16–35.42)[a] | 3.47 (0.13–17.93) | <0.001 | 4.57 (0.20–36.75) | 8.92 (0.86–36.75)[c] | 4.37 (0.21–23.68) | <0.001 |

Data are given as means ± SD in case of normal distribution, otherwise as median (range). *p* values are for one-way ANOVA

[a] $p<0.05$ vs A-C and C-G

[b] $p<0.05$ vs A-C and A-G

[c] $p<0.05$ vs A-C

[d] These *p* values became insignificant when adjusted for multiple testing by the Bonferroni method

NA, not applicable; HOMA-IR, homeostasis model assessment of insulin resistance

*EMSA*. To determine whether there is specific nuclear protein binding on –420G or –537A, we did the gel EMSA using oligonucleotides containing –420G or –537A as probes. Competitor –420G competed with the probe for nuclear proteins from adipocytes and monocytes (band 1 and band 2), but competitor –420C did not (Fig. 1a). Competitor –537A and competitor –537C competed with the probe in a similar manner (Fig. 1b).

## Discussion

In this study, we showed that common genetic polymorphisms in the promoter of resistin gene are major determinants in regulating plasma concentrations of resistin in humans. It was reported that the –420G/G homozygotes (originally referred to as –180G/G homozygotes because the "1" upstream of the transcription start site was counted as nucleotide "–1") had significantly higher expression of resistin mRNA than heterozygotes [19], but the investigators could not find out whether the promoter polymorphism was associated with circulating concentrations. Our study confirmed that the promoter genotypes of resistin gene are associated with plasma concentrations and estimated the variance attributable to g.-420C>G polymorphism in plasma resistin concentrations to be 9.68%.

It has been speculated that np –420 is a putative Sp-1 binding site [19], whereas np –537 is a putative AP1 binding site [12]. In this study, the haplotype A-G had a modestly higher promoter activity than the other haplotypes. We also found that the competitor –420G competed with the probe for nuclear proteins from adipocytes and monocytes, but competitor –420C did not, which suggests that these bindings are specific to –420G. These findings are consistent with a previous report showing that the –420G/G homozygote construct had higher luciferase activity than the –420C/C homozygote construct in 3T3-L1 adipocytes [19]. In contrast, in our study, the variants at –537 did not affect the binding of nuclear proteins. These results are consistent with the findings that g.–420 C>G variant contributed more to the variance of plasma resistin concentrations than g.–537A>C variant. As the subjects with haplotype A-G had higher plasma resistin concentrations than the subjects with haplotype C-G, it is possible that a small difference in protein binding affinity between –537A and –537C affects resistin promoter activity or that another genetic variation affecting plasma resistin concentrations is linked to –537 variant.

It has been reported that g.-420C>G and g.-537A>C polymorphisms could be associated with obesity [12]. However, no concrete evidence of associations between g.-420C>G polymorphism and obesity or diabetes has been found [13, 14, 19]. We also were un-

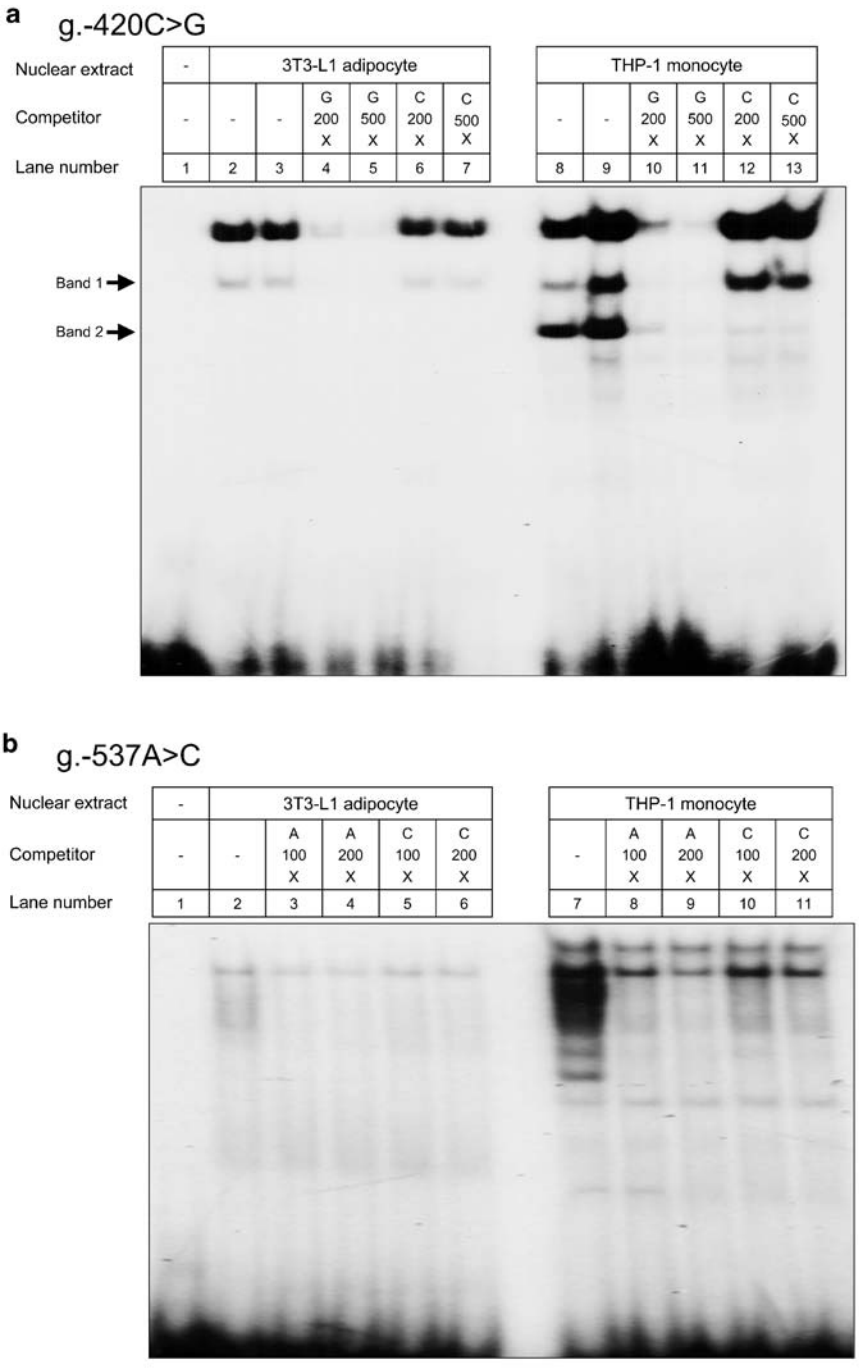


**Fig. 1a, b.** Binding of nuclear proteins to –420 and –537 regions of the resistin gene. The probe (**a**), –433 to –406 bp of the resistin gene containing –420G, was labelled as described in Materials and Methods. Lane 1: probe without nuclear proteins; lane 2: nuclear proteins (5 µg) of 3T3-L1; lanes 3–7: nuclear proteins (10 µg) of 3T3-L1; lane 8: nuclear proteins (5 µg) of THP-1; lanes 9–13: nuclear proteins (10 µg) of THP-1. Competitors were added to the probe at 200- or 500-fold molar excesses. Oligonucleotide (**b**), -520 to –553 bp of the resistin gene containing –537A, was labelled and incubated with 10 µg of nuclear proteins. Competitors were added at 100-, or 200-fold molar excesses

able to observe any association between g.-420C>G and g.-537A>C polymorphisms and Type 2 diabetes or obesity. This problem remains unexplained. It is, however, possible that different gene to environment interactions lead to different effects of polymorphisms in different populations. Although Koreans have a different diet and different degrees of obesity than white or Western populations [27], we did not examine these interactions in this study. In addition, the number of control subjects was rather small, which could negatively affect the statistical power.

It was thought that resistin might link obesity with insulin resistance [4], but subsequent studies have produced disparate findings on the role of resistin in obe-

sity and insulin resistance [5, 6, 7, 8, 9, 10]. Recently, it was shown that administration of recombinant resistin impaired hepatic insulin sensitivity, but did not affect peripheral glucose disposal in rats [28]. These findings suggest that resistin could be a marker of hepatic rather than peripheral insulin resistance. In our study, the subjects with higher plasma resistin concentrations did not have higher HOMA-IR values. However, we did not measure hepatic and peripheral insulin sensitivity separately, and this could be a limitation of the current study.

In conclusion, we showed that common polymorphisms in the promoter region of resistin are major determinants of circulating resistin concentrations in humans.

*Acknowledgements*. This study was supported by a grant from the Korea Health 21 R & D Project, Ministry of Health and Welfare, Republic of Korea (00-PJ3-PG6-GN07–001).

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