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Association between the A–2518G polymorphism in the monocyte chemoattractant protein-1 gene and insulin resistance and Type 2 diabetes mellitus

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Abstract

Aims/hypothesis. The molecular mechanisms of obesity-related insulin resistance are incompletely understood. Macrophages accumulate in adipose tissue of obese individuals. In obesity, monocyte chemoattractant protein-1 (MCP-1), a key chemokine in the process of macrophage accumulation, is overexpressed in adipose tissue. *MCP-1* is an insulin-responsive gene that continues to respond to exogenous insulin in insulin-resistant adipocytes and mice. MCP-1 decreases insulin-stimulated glucose uptake into adipocytes. The A–2518G polymorphism in the distal regulatory region of *MCP-1* may regulate gene expression. The aim of this study was to investigate the impact of this gene polymorphism on insulin resistance.

Methods. We genotyped the Ludwigshafen Risk and Cardiovascular Health (LURIC) cohort ($n=3307$). Insulin resistance, estimated by homeostasis model assessment, and Type 2 diabetes were diagnosed in 803 and 635 patients respectively.

Results. Univariate analysis revealed that plasma MCP-1 levels were significantly and positively cor-

related with WHR ($p=0.011$), insulin resistance ($p=0.0097$) and diabetes ($p<0.0001$). Presence of the *MCP-1* G–2518 allele was associated with decreased plasma MCP-1 ($p=0.017$), a decreased prevalence of insulin resistance (odds ratio [OR]=0.82, 95% CI: 0.70–0.97, $p=0.021$) and a decreased prevalence of diabetes (OR=0.80, 95% CI: 0.67–0.96, $p=0.014$). In multivariate analysis, the G allele retained statistical significance as a negative predictor of insulin resistance (OR=0.78, 95% CI: 0.65–0.93, $p=0.0060$) and diabetes (OR=0.80, 95% CI: 0.66–0.96, $p=0.018$).

Conclusions/interpretation. In a large cohort of Caucasians, the *MCP-1* G–2518 gene variant was significantly and negatively correlated with plasma MCP-1 levels and the prevalence of insulin resistance and Type 2 diabetes. These results add to recent evidence supporting a role for MCP-1 in pathologies associated with hyperinsulinaemia.

Keywords Diabetes · Insulin resistance · Macrophage · Monocyte chemoattractant protein-1 · Polymorphism

Introduction

The molecular mechanisms that link obesity and insulin resistance are incompletely understood [1, 2, 3]. Obesity, insulin resistance and diabetes mellitus share a

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Abbreviations: CAD, coronary artery disease · HOMA_{IR}, homeostasis model assessment of insulin resistance · MCP-1, monocyte chemoattractant protein-1 · MIP-1 α , macrophage inflammatory protein-1 α · NF- κ B, nuclear factor- κ B · PAI-1, plasminogen-activator inhibitor-1

chronic, low-grade inflammatory component [4, 5, 6, 7], as reflected by increased expression of proinflammatory cytokines such as TNF- α [8], IL-6 [9], and monocyte chemoattractant protein-1 (MCP-1) [6, 7, 10, 11]. Adipose tissue functions as an endocrine organ that secretes a number of biologically active proteins, including TNF- α [8], IL-6 [6, 12], IL-8 [13], C-reactive protein, inducible nitric oxide synthase [6], TGF- β 1, plasminogen-activator inhibitor-1 (PAI-1) [14], adiponectin [15], resistin [16], leptin [13, 17], macrophage inflammatory protein-1 α (MIP-1 α) [7, 13] and MCP-1 [6, 7, 10, 11, 17]. Obesity alters adipose tissue endocrine function and leads to the increased secretion of proinflammatory cytokines (so-called “adipokines”), hormones and fatty acids [6, 7, 10]. In turn, TNF- α , IL-1 β , and other adipokines stimulate MCP-1 production and release by human differentiated adipocytes [13].

MCP-1, which is a member of the chemokine family, plays a key role in the recruitment of monocytes/macrophages to inflammatory foci [18]. High glucose directly up-regulates *MCP-1* expression in vascular endothelial cells and monocytes [19, 20]. MCP-1 mRNA is dramatically overexpressed (increased by a factor of seven) in white adipose tissue of genetically obese (*ob/ob*) mice compared with lean control mice [10]. In these mice, the highest level of MCP-1 expression is in subcutaneous adipose tissue, with the liver showing <10% and the kidney and lung showing 1–2% of the level of MCP-1 expression in adipose tissue [10]. Insulin induces the production and secretion of substantial amounts of MCP-1 in insulin-resistant adipocytes and insulin-resistant obese mice [10]. *MCP-1* is similar to other genes such as *PAI-1* and *SREBP-1c* in that it continues to respond to exogenous insulin in insulin-resistant states [10, 21]. Moreover, MCP-1 inhibits (by approximately 30%) insulin-stimulated glucose uptake into mouse adipocytes [10]. Transcriptional profiling experiments have shown that a number of inflammation and macrophage-enriched or specific genes, such as *MCP-1*, *MIP-1 α* , *ADAM8*, *MAC-1*, *F4/80* and *CD68*, are up-regulated in white adipose tissue of mice with genetic or diet-induced obesity [6, 7]. Of the 100 genes whose expression was most significantly correlated with body mass, 30% encoded proteins that are characteristic of macrophages. In human subcutaneous adipose tissue, both BMI and average adipocyte size were significant predictors of macrophage accumulation [6]. In bone marrow transplant experiments in irradiated mice, 85% of the macrophages that accumulated in adipose tissue after placing the mice on a high-fat diet were of bone marrow origin [6]. In addition, in *Csf1^{op/op}* mice lacking macrophages, the presence of cells expressing the macrophage marker F4/80 in adipose tissue was greatly reduced compared with that in wild-type mice [6]. These findings suggest new models involving macrophage infiltration into adipose tissue in obesity-related insulin resistance.

Genetic differences in chemokine expression may contribute to differences in the severity of inflammatory processes between individuals. Substantial inter-individual variability in MCP-1 production in response to proinflammatory cytokines has previously been described [22, 23]. Human *MCP-1* transcription is under the control of two distinct regions within the 5'-flanking sequence of the gene. The distal region contains two NF- κ B binding sites and is essential for cytokine-dependent regulation, while the proximal region contains a GC box that may regulate tissue-specific expression [24]. A common A→G polymorphism located at position -2518 in the distal regulatory region regulates MCP-1 expression. This was demonstrated by transfection experiments in A172 and 293T cells using reporter gene constructs containing the distal regulatory region of the *MCP-1* gene with either variant at position -2518 [22, 25]. Moreover, MCP-1 expression in isolated, cytokine-stimulated human peripheral blood mononuclear cells [22, 25] and hepatic cells [26], and plasma MCP-1 levels in patients with lupus nephritis support the assumption that the gene polymorphism regulates MCP-1 expression at the transcriptional level [25]. Case-control gene association studies have reported statistical associations between the polymorphism and lupus nephritis [25], coronary artery disease (CAD) [27] and asthma [28]. The aim of the present study was to test the hypothesis that the *MCP-1* A-2518G polymorphism influences plasma MCP-1 levels and the risk of developing insulin resistance and Type 2 diabetes.

Subjects and methods

Study population and clinical definitions. We genotyped the Ludwigshafen Risk and Cardiovascular Health (LURIC) cohort of 3316 individuals who underwent coronary angiography because of chest pain or non-invasive tests consistent with myocardial ischaemia [29]. All individuals were Caucasians born in Germany to parents of German ancestry. Coronary artery disease was defined angiographically by a $\geq 20\%$ reduction in luminal diameter in one or more major epicardial coronary arteries or a 10% to 19% reduction in three or more (out of 15) coronary artery segments. Genetic testing of this cohort was approved by the appropriate institutional ethics committee and informed consent was obtained from the patients. After excluding nine individuals with Type 1 diabetes, the study population included 3307 individuals. Insulin resistance was estimated by homeostasis model assessment (HOMA_{IR}) [30] in the 2798 individuals who were not receiving oral hypoglycaemic drugs or insulin treatment. The threshold for insulin resistance was defined as the 90th percentile of HOMA_{IR} distribution in individuals ($n=350$) with no markers of the metabolic syndrome [30]. Type 2 diabetes was defined according to the new criteria of the American Diabetes Association (ADA) and the provisional criteria of the World Health Organization (WHO) [31, 32, 33]. Unlike in a previous study of the same cohort [34], subjects with a single test showing a high blood glucose level, in the absence of a confirmatory test, were not assigned an unequivocal diabetes status. Older age was defined by the 50th percentile of the study population.

Assessment of plasma MCP-1 levels. Plasma MCP-1 was measured in a subgroup of 940 consecutive patients (starting from recruitment number 1001) using the Quantikine Human MCP-1 Immunoassay (R&D Systems, Abingdon, UK). There were no significant differences ($p>0.1$) between this subgroup and the study population as a whole with respect to insulin resistance or diabetes distribution. Blood samples were collected, processed and stored according to the manufacturer's instructions. The sensitivity of the kit is approximately 0.6 pmol/l, which is well below the lowest concentration measured in the present study (2.9 pmol/l).

Genotyping. Genotyping of the *MCP-1 A-2518G* polymorphism was carried out by RFLP-PCR as described previously [22]. Data are incomplete due to missing or equivocal results in five subjects.

Statistical analysis. Data are shown as frequencies in percent (numbers of subjects) or median values (25–75 percentiles). The JMP program (Version 5; SAS Institute, Cary, N.C., USA) was used for statistical analysis. Frequencies were compared by the Cochran–Armitage trend test. Due to skewed distribution, plasma MCP-1 data were analysed by non-parametric tests (Wilcoxon and Kruskal–Wallis rank-sum tests). The gene counting method estimated allele frequencies, and the chi square test was used to test for deviations of genotype frequencies from Hardy–Weinberg equilibrium. The independent contributions of clinical and genetic characteristics (age, insulin resistance, diabetes, obesity, WHR, HDL cholesterol, triglycerides, hypertension, CAD and *MCP-1* polymorphism) to MCP-1 plasma levels (entered as the dependent variable) were assessed by multivariate analysis (standard least square). The independent contributions of the *MCP-1* polymorphism to insulin resistance and diabetes were determined by nominal logistic analysis, with insulin resistance or diabetes entered as the dependent variable, and age, sex, obesity, hypertension, HDL cholesterol, triglycerides and WHR entered as independent variables. In a subanalysis, diabetes was entered as a three-class-dependent variable (encoded as no diabetes = 0, non-insulin-treated diabetes = 1, insulin-treated diabetes = 2). A p value (two-tailed) of less than 0.05 was considered to be statistically significant.

Results

Clinical characteristics, insulin resistance and diabetes. In total, 29% (803 of 2798) of individuals who were not on pharmacological oral hypoglycaemic treatment had HOMA_{IR} index values higher than or equal to 2.96, indicating insulin resistance. Type 2 diabetes was diagnosed in 20% (635 of 3205) of subjects. Among the diabetic patients, 25% ($n=161$) were on no oral hypoglycaemic drugs, 44% ($n=278$) were on oral hypoglycaemic drugs and 31% ($n=196$) were on insulin. Coronary angiography showed coronary artery stenoses (lumen reduction $\geq 50\%$) in 69% of patients, minor lesions (lumen reduction $<50\%$) in 14% and smooth arteries in 17%. Table 1 shows the clinical characteristics of the study population subdivided according to insulin sensitivity or diabetes status, and the results of univariate analysis of the clinical characteristics in these subsets.

Genotype frequencies. In the population as a whole, the frequencies of the genotypes of the *A-2518G* polymorphism were: 1757 (53%) AA homozygous, 1303 (39%) GA heterozygous and 242 (7%) GG homozygous. The genotype frequencies were in Hardy–Weinberg equilibrium in the population as a whole and in cases and controls defined by insulin sensitivity or diabetes status. No differences were found between *G* allele carriers (GA or GG genotype) and non-carriers (AA genotype) with respect to age, sex, obesity, WHR, hypertension, triglycerides or HDL cholesterol between subsets (data not shown).

Associations between plasma MCP-1 levels and clinical and genetic data. In univariate analysis, plasma MCP-1 levels were significantly and positively correlated with age, CAD and markers of the metabolic syndrome (insulin resistance, Type 2 diabetes, hypertension, obesity, WHR, high triglycerides and low HDL cholesterol; Table 2). In addition, plasma

Table 1. Associations between clinical characteristics and insulin resistance and diabetes

Characteristic	Insulin-sensitive patients ($n=1995$) ^a	Insulin-resistant patients ^k ($n=803$) ^b	p value	Non-diabetic patients ($n=2570$) ^c	Diabetic patients ($n=635$) ^d	p value
Older age ^e	50.1 (422)	52.6 (999)	0.24	48.9 (1256)	70.2 (446)	<0.0001
Sex (males)	68.7 (1370)	74.2 (596)	0.0037	70.3 (1806)	67.7 (430)	0.21
Arterial hypertension ^f	53.6 (1070)	64.9 (521)	<0.0001	54.6 (1404)	72.3 (459)	<0.0001
High triglycerides ^g	39.2 (782)	60.8 (488)	<0.0001	42.8 (1100)	61.4 (389)	<0.0001
Low HDL cholesterol ^h	47.12 (940)	61.6 (494)	<0.0001	50.0 (1285)	66.8 (424)	<0.0001
Obesity ⁱ	15.2 (304)	38.7 (311)	<0.0001	20.2 (518)	33.20 (211)	<0.0001
WHR ^j	72.9 (1433)	81.3 (644)	<0.0001	74.1 (1875)	83.9 (533)	<0.0001

Values shown are % (n). ^a Missing data for WHR ($n=30$); ^b missing data for triglycerides ($n=1$), HDL cholesterol ($n=1$) and WHR ($n=11$); ^c missing data for HDL cholesterol ($n=1$) and WHR ($n=41$); ^d missing data for triglycerides ($n=1$); ^e older age, ≥ 62.7 years; ^f hypertension, supine systolic blood pressure

≥ 140 mm Hg and/or diastolic pressure ≥ 90 mm Hg; ^g high triglycerides, ≥ 150 mg/ml; ^h low HDL cholesterol, <35 mg/ml in males and <45 mg/ml in females; ⁱ obesity, BMI ≥ 30 kg/m²; ^j high WHR, >0.95 in males and >0.80 in females; ^k insulin resistance, HOMA_{IR} index ≥ 2.96

Table 2. Associations between plasma MCP-1 levels and clinical characteristics

Group	n	Plasma MCP-1 (pmol/l)	p value
Younger	419	16.3 (13.7–19.9)	<0.0001
Older	521	19.2 (15.6–23.6)	
No CAD	170	16.8 (14.1–20.2)	0.005
CAD	738	17.9 (14.8–23.6)	
Insulin-sensitive	573	17.1 (14.4–20.9)	0.0097
Insulin-resistant	211	18.2 (15.1–22.2)	
Non-diabetic	729	17.2 (14.4–21.0)	<0.0001
Diabetic	177	20.3 (15.9–25.9)	
Normotensive	409	17.2 (14.3–21.0)	0.016
Hypertensive	531	18.2 (14.9–22.6)	
Normal triglycerides	496	17.1 (14.1–21.0)	0.0010
High triglycerides	444	18.5 (15.1–23.0)	
Normal HDL cholesterol	440	17.1 (14.0–20.7)	<0.0001
Low HDL cholesterol	499	18.5 (15.2–23.3)	
Lean	690	17.5 (14.5–21.7)	0.042
Obese	250	18.5 (15.3–22.3)	
Normal WHR	231	16.9 (14.0–20.8)	0.011
High WHR	708	18.0 (14.8–22.4)	

Values are medians (25–75 percentiles). Missing data for CAD ($n=32$), diabetes status (absence of confirmatory test; $n=34$), HDL cholesterol ($n=1$) and WHR ($n=1$)

Table 3. Associations between the MCP-1 polymorphism and plasma MCP-1 levels, insulin resistance and diabetes

Group	Genotype			p value	G allele carriers	p value
	AA	AG	GG			
Plasma MCP-1 (pmol/l) ^a	18.2 (15.2–22.4)	17.4 (14.4–21.4)	17.1 (13.7–21.5)	0.043	17.2 (14.4–21.4)	0.017
Insulin-sensitive	51.1 (1018)	41.4 (826)	7.5 (150)	0.020	49.0 (976)	0.021
Insulin-resistant	55.9 (448)	37.9 (304)	6.2 (50)	0.053	44.1 (354)	0.014
Non-diabetic	52.0 (1335)	40.6 (1043)	7.4 (190)		48.0 (1233)	
Diabetic	57.4 (363)	35.1 (222)	7.4 (47)		42.6 (269)	

Values are % (n) unless otherwise indicated. ^a Values are medians (25–75 percentiles)

MCP-1 levels were negatively correlated with the presence of the G allele at position –2518 of MCP-1 ($p=0.043$ and $p=0.017$ for genotype and G allele carrier frequencies respectively; Table 3). In multivariate analysis, age and HDL cholesterol remained associated with plasma MCP-1 ($p<0.0001$ and $p=0.002$ respectively), with a trend towards statistical significance for the MCP-1 polymorphism ($p=0.06$), whereas all other associations lost statistical significance ($p>0.1$).

Association between the MCP-1 A-2518G polymorphism and insulin resistance. The frequencies of the MCP-1 genotypes were significantly different between insulin-sensitive and insulin-resistant subjects ($p=0.020$), as were the frequencies of G allele carriers ($p=0.021$; Table 3). In multivariate analysis (Table 4), both genotype and G allele carrier frequencies retained significant associations with insulin resistance ($\chi^2=7.56$, $p=0.023$ and $\chi^2=7.55$, $p=0.006$ respectively).

Association between the MCP-1 A-2518G polymorphism and Type 2 diabetes. Presence of the G allele was associated with a decreased prevalence of Type 2 diabetes ($p=0.014$; Table 3). In multivariate analysis, genotype and G allele carrier frequencies were significantly associated with a decreased prevalence of diabetes ($\chi^2=7.03$, $p=0.030$ and $\chi^2=5.62$, $p=0.018$, respectively; Table 5). The decrease in the frequency of G allele carriers was more marked in the subgroup of insulin-treated patients than in the subgroup of non-insulin-treated diabetic patients (39% and 44% respectively, vs 48% in non-diabetic individuals; $\chi^2=7.76$, $p=0.021$ by three-class analysis).

Association between the MCP-1 A-2518G polymorphism and obesity-related insulin resistance. A subgroup analysis revealed that, among obese patients, the insulin-sensitive subgroup contained a higher proportion of MCP-1 G-2518 carriers than the insulin-resistant subgroup (52.5% vs 43.9%; $p=0.04$). Similarly, obese individuals who did not develop Type 2 dia-

Table 4. Nominal logistic fit, with insulin resistance entered as the dependent variable

Source of variation	Genotype analysis				G allele carriers analysis				
	df	r ²	χ ²	p value	df	r ²	χ ²	p value	OR (95% CI)
All variables	9	0.09	301.28	<0.0001	8	0.09	301.27	<0.0001	
Older age	1		7.60	0.0058	1		7.62	0.0058	1.29 (1.08–1.54)
Male sex	1		17.77	<0.0001	1		17.83	<0.0001	1.56 (1.27–1.92)
Hypertension	1		5.52	0.019	1		5.51	0.019	1.25 (1.04–1.50)
High triglycerides	1		52.99	<0.0001	1		53.06	<0.0001	1.96 (1.64–2.35)
Low HDL cholesterol	1		21.99	<0.0001	1		21.97	<0.0001	1.55 (1.29–1.86)
Obesity	1		116.19	<0.0001	1		116.19	<0.0001	3.00 (2.45–3.66)
High WHR	1		5.01	0.025	1		5.02	0.025	1.29 (1.03–1.62)
<i>MCP-1 A–2518G</i> polymorphism	2		7.56	0.023	1		7.55	0.0060	0.78 (0.65–0.93)

In this multivariate model, $n=2753$ due to incomplete data. *df*: 2 for genotype three-class analysis (encoded as $AA=0$, $AG=1$, $GG=2$), 1 for *G* allele carrier two-class analysis (encoded as $AA=0$, $AG+GG=1$). OR, odds ratio

Table 5. Nominal logistic fit, with Type 2 diabetes entered as the dependent variable

Source of variation	Genotype analysis				G allele carriers analysis				
	df	r ²	χ ²	p value	df	r ²	χ ²	p value	OR (95% CI)
All variables	9	0.09	291.73	<0.0001	8	0.09	290.26	<0.0001	
Older age	1		88.33	<0.0001	1		88.81	<0.0001	2.60 (2.13–3.17)
Male sex	1		2.08	0.15	1		2.15	0.14	1.17 (0.95–1.44)
Hypertension	1		26.50	<0.0001	1		26.45	<0.0001	1.70 (1.39–2.08)
High triglycerides	1		43.45	<0.0001	1		42.80	<0.0001	1.90 (1.57–2.30)
Low HDL cholesterol	1		28.88	<0.0001	1		28.86	<0.0001	1.71 (1.41–2.09)
Obesity	1		21.69	<0.0001	1		21.49	<0.0001	1.63 (1.32–2.00)
High WHR	1		5.13	0.024	1		5.08	0.024	1.33 (1.03–1.71)
<i>MCP-1 A–2518G</i> polymorphism	2		7.03	0.030	1		5.62	0.018	0.80 (0.66–0.96)

In this multivariate model, $n=3247$ due to incomplete data. *df*: 2 for genotype three-class analysis (encoded as $AA=0$, $AG=1$, $GG=2$), 1 for *G* allele carrier two-class analysis (encoded as $AA=0$, $AG+GG=1$). OR, odds ratio

betes were more likely to be *G* allele carriers than those who became diabetic (48.5% vs 40.5%; $p=0.06$). Plasma MCP-1 levels were significantly increased in obese diabetic patients compared with those in obese non-diabetic patients (21.4 vs 17.6 pmol/l; $p<0.05$).

Discussion

There is increasing evidence that MCP-1, a potent chemoattractant of monocytes/macrophages, participates in metabolic activities that involve regulation of adipose tissue functions, inhibition of insulin-stimulated glucose uptake into adipocytes [10] and accumulation of macrophages in adipose tissue in obese humans [6]. In search of genetic factors that affect insulin resistance, we investigated the role of the common *MCP-1 A–2518G* polymorphism, which may regulate MCP-1 expression at the transcriptional level [22, 25, 26]. In a large cohort of Caucasians with a high prevalence of cardiovascular risk factors, we found decreased plasma MCP-1 levels and a decreased preva-

lence of insulin resistance and Type 2 diabetes in carriers of the *G* allele compared with subjects homozygous for the frequent *A* allele. A protective role for this genetic variant is supported by the finding that, among obese patients, the frequency of *G* allele carriers was increased in those with preserved insulin sensitivity. In multivariate analysis, the *G* allele remained significantly and negatively correlated with insulin resistance and diabetes after correction for risk factors identified by univariate analysis (including CAD). The decrease in *G* allele frequency was more pronounced among insulin-treated patients than among non-insulin-treated diabetic patients, suggesting that the role of the gene polymorphism may become more manifest at later stages of the disease.

Our findings of decreased plasma MCP-1 levels in carriers of the *G* allele are in agreement with data in patients with lupus nephritis [25]. In the present study, plasma MCP-1 levels were significantly and positively correlated with markers of the metabolic syndrome, such as insulin resistance, Type 2 diabetes, hypertension, obesity, WHR, high triglycerides and low HDL

cholesterol, according to univariate analysis. These results are consistent with previous data in patients with Type IIa and IIb hyperlipoproteinaemia [35]. Moreover, we observed increased plasma MCP-1 levels in patients with Type 2 diabetes, corroborating previous data from the Orbofiban in Patients With Unstable Coronary Syndromes—Thrombolysis In Myocardial Infarction (OPUS-TIMI) 16 trial and from a cohort of women with glucose intolerance [36, 37]. Conversely, another study showed that insulin infusion results in decreased plasma MCP-1 levels in obese subjects [38].

Macrophage accumulation in human subcutaneous adipose tissue increases with increasing obesity and adipocyte size [6]. In obesity, infiltrating macrophages account for almost all TNF- α expression and a significant proportion of the expression of other inflammatory mediators in adipose tissue [6]. Interestingly, the epicardial adipose tissue of patients with CAD produces increased amounts of MCP-1, TNF- α , IL-1 β , and IL-6 compared with epicardial adipose tissue of subjects without CAD, suggesting that regional adipose tissue may be a source of atherogenic proinflammatory molecules [39].

The molecular mechanism by which the polymorphism regulates *MCP-1* gene transcription is unclear. Although it is located in the distal regulatory region of the gene that contains two NF- κ B binding sites essential for cytokine-stimulated gene transcription [24], the polymorphism does not alter the sequence of the NF- κ B sites. Electrophoretic mobility shift assays have suggested that the binding site for an as yet unidentified transcription factor might be altered [22]. We propose that decreased MCP-1 expression in carriers of the G allele may prevent the pathogenic vicious circle by which MCP-1 expression promotes macrophage accumulation in adipose tissue, TNF- α release by macrophages, TNF- α -stimulated MCP-1 expression and MCP-1-mediated antagonism of insulin activities in this tissue. Similarly, a single-nucleotide polymorphism in the promoter of the gene encoding resistin, an adipocyte-secreted hormone, has been linked to insulin resistance in humans [40].

In conclusion, we have reported, for the first time, that the *MCP-1* G-2518 allele is significantly and negatively correlated with circulating MCP-1 levels, insulin resistance and Type 2 diabetes. Replication of the reported genetic associations in independent cohorts are needed to validate the role of the polymorphism in the development of insulin resistance.

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