Meta-analysis on the *G*-308*A* Tumor Necrosis Factor α Gene Variant and Phenotypes Associated with the Metabolic Syndrome

Silvia C. Sookoian, Claudio González,† and Carlos J. Pirola**

Abstract

SOOKOIAN, SILVIA C., CLAUDIO GONZÁLEZ, AND CARLOS J. PIROLA. Meta-analysis on the *G*-308*A* tumor necrosis factor-α gene variant and phenotypes associated with the metabolic syndrome. *Obes Res.* 2005;13:2122–2131.

Objective: The *G*-308*A* tumor necrosis factor (TNF) α gene variant has been associated with obesity, insulin resistance, and hypertension. We performed a systematical review of the literature by means of a meta-analysis to assess the association of the *G*-308*A* TNFα polymorphism with the components of the metabolic syndrome.

Research Methods and Procedures: Studies were identified by searches of the literature for reports using the terms: diabetes, insulin resistance, hypertension, obesity or metabolic syndrome and TNF, variants or polymorphism or alleles, and Nco or −308. From 824 reports, we included 31 observational studies, case control and cohort at baseline, which analyzed the association between the TNFα polymorphism and one or more components of the metabolic syndrome. A fixed effect model was used to pool data from individual studies.

Results: Obesity [odds ratio, 1.23; 95% confidence interval (CI), 1.045 to 1.45; $p = 0.013$] in a total of 3562 individuals from eight homogeneous studies, systolic arterial blood pressure (standardized difference, 0.132; 95% CI, 0.016 to 0.25; $p < 0.03$) in a total of 1624 individuals from four homogeneous studies and plasma insulin levels (standardized difference, 0.095; 95% CI, 0.020 to 0.17; $p = 0.013$) in a total of 3720 subjects from 16 homogeneous studies were positively associated with the −308*A* variant.

Discussion: These results indicate that individuals who carried the −308*A* *TNF*α gene variant are at 23% risk of developing obesity compared with controls and showed significantly higher systolic arterial blood pressure and plasma insulin levels, supporting the hypothesis that the *TNF*α gene is involved in the pathogenesis of the metabolic syndrome.

Key words: genetics, hypertension, meta-analysis, tumor necrosis factor, metabolic syndrome

Introduction

The clustering of abdominal obesity, high triglycerides, low levels of high-density lipoprotein cholesterol, high blood pressure, and insulin resistance has been called the metabolic syndrome (1). This entity not only affects nearly one in four adults in western countries (2) but is also an increasingly major health problem in young populations (3,4) as well as a major risk factor for cardiovascular disease (5).

The etiology of the metabolic syndrome is complex, determined by the interplay of both genetic and environmental factors. Pathways leading to the clinical manifestations of the metabolic syndrome involve a number of metabolic risk factors, as well as mediators of inflammatory response such as the proinflammatory adipokine, tumor necrosis factor (TNF)[1] α (6).

Because the TNFα expression may be increased in adipose tissue of both rodent models of obesity and obese humans, TNFα was considered as a candidate gene for obesity (7). Afterward, a similar overproduction of TNFα in human fat cells was demonstrated in human obesity and insulin resistance (8,9).

Received for review March 17, 2005.
Accepted in final form September 27, 2005.
*Molecular Cardiology, Institute of Medical Research, A. Lanari, University of Buenos Aires, Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina; and †Pharmacology Department, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina.
Address correspondence to Carlos J. Pirola, Instituto de Investigaciones Medicas, A. Lanari, Cardiología Molecular, Av. Combatientes de Malvinas 3150, CF (1427), Buenos Aires. Argentina.
E-mail: cjpirola@lanari.fmed.uba.ar

[1] Nonstandard abbreviations: TNF, tumor necrosis factor, OR, odds ratio; SABP, systolic arterial blood pressure; WHR, waist-to-hip ratio; HOMA-IR, homeostasis model assessment; D, standardized difference.

The human *TNFα* gene has been localized at the chromosome 6p21.3 coding for a 157-amino acid polypeptide processed from a 233-amino acid precursor (10). Among other variants, the most characterized biallelic *TNFα* polymorphism involves a single base change, *G/A*−308, in the promoter region (11). In vitro experiments have suggested that the *G*-308*A* variant has a functional role because it is located within a consensus sequence of the transcription factor AP-2. Consequently, Wilson et al. (12) have demonstrated that the *G*-308*A* variant increases transcriptional activation of the *TNFα*.

Since then, many control studies have examined the association between the *G*-308*A* variant and the components of the metabolic syndrome. Some, but not all studies, have indicated a key role for the variant in the pathogenesis of obesity and obesity-associated insulin resistance (13–22).

Because meta-analysis is a reliable way to address the discrepancies in genetic association studies, we decided to evaluate the influence of the *G*-308*A TNFα* promoter gene variant on the occurrence of phenotypes associated with metabolic syndrome.

Research Methods and Procedures

Data Sources and Study Selection

For the electronic searches, published studies were found through PubMed at the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/query) and in Medline databases for the keywords [diabetes, insulin resistance, hypertension, obesity, or metabolic syndrome] and TNF and [gene variants or polymorphism or alleles] and [Nco or −308]. Reference lists in relevant publications were also examined. In addition, >6500 abstract citations on TNF from PubMed were revised using the program RefViz (Thomson, ISI Research Soft, Stamford, CT) by searching for the above-mentioned additional keywords in the abstract text. The literature search was done on studies up to November 2004. There were no language or country restrictions.

We evaluated 824 citations identifying 31 studies that met the selection criteria: population-based or hospital-based case-control or cross-sectional studies concerning one or more components of the metabolic syndrome, in which information about number of subjects in each category, sufficient data to calculate outcomes, and *G*-308*A* TNFα genotyping performed with a validated molecular method could be extracted. In the case of cohorts, we included variables before any intervention. All investigations analyzed in this meta-analysis have been carried out in accordance with the Declaration of Helsinki as declared by the authors of each study.

Data Collection

All odds ratios (ORs) were calculated against healthy control subjects. For each study, information was collected concerning the following characteristics of the subjects: demographic information (age, sex, ethnicity), clinical features [obesity, type 2 diabetes, hypertension, and systolic arterial blood pressure (SABP)], and phenotype measurements [BMI calculated as weight (kilograms)/height (meters) squared and waist circumference divided by the circumference of the hip to obtain the waist-to-hip ratio (WHR)]. Biochemical determinations such as fasting glucose and insulin, insulin resistance by homeostasis model assessment (HOMA-IR) (23), and plasma leptin concentration using any standard laboratory method were also analyzed. All quantitative variables had to be expressed as mean ± SD or SE, in which case it was converted to SD. In all of the studies included, obesity was defined as BMI ≥ 27 kg/m^2.

Because the frequency of the −308*A* allele was low particularly in Asian populations, a dominant model of inheritance grouping *GA* + *AA* genotypes was used to assess differences between genotypes.

Study Characteristics

Five studies were population-based (22,24–27), and 26 were hospital-based studies (13–15,20,21,28–48). Twenty studies included white subjects (13–15,20–22,24–29,32,34,35,37,41,44,47,48), two studies included Finnish population (40,43), four studies included Korean and Chinese subjects (38,39,45,46), three involved Japanese (31,33,36), one study included Arabian subjects (30), and one study included Brazilian subjects (42).

Genotyping for the *G*-308*A* polymorphism was carried out across studies using polymerase chain reaction-restriction fragment length polymorphism followed by digestion with the restriction enzyme NcoI in 28 studies, whereas in three studies, genotyping was performed by allele-specific oligonucleotides.

Statistical Analysis

For quantitative variables, effect stands for standardized difference (D), which is defined as the mean difference divided by the common within-group SD, and for dichotomic variables, effect stands for ORs. Summary OR and corresponding 95% CI were estimated by fixed and random effects meta-regression analysis. Fixed effect models using the Mantel-Haenszel method was used to summarize results, obtaining the corresponding pooled OR. We assessed heterogeneity by using Q statistics. For D, the Cohen test was used to summarize the results, and heterogeneity was evaluated with the Q statistic and the I2 statistic, a transformation of Q that estimates the percentage of the variation in effect sizes that is due to heterogeneity. All calculations were performed using the Comprehensive Meta-Analysis computer program (Biostat, Englewood, NJ).

Table 1. Summary estimates for the ORs and corresponding 95% CI limits (lower and upper effect) in hypertension and diabetes for the *G*-308*A* TNFα variant

Effect	Number of subjects	Case/controls	ORs	95% CI limits	Heterogeneity	*p*
Hypertension	1092	505/587	1.04	0.76 to 1.44	None	0.8
Diabetes	3202	1424/1778	1.043	0.851 to 1279	None	0.68

OR, odds ratio; CI, confidence interval; TNF, tumor necrosis factor.

In the case of heterogeneity, we identified study characteristics that stratify the studies into subsets with homogeneous effects. To check for publication bias, a funnel plot was drawn. A *p* value lower than 0.05 was considered statistically significant.

Results

Results from 31 studies were included in this meta-analysis. The overall frequency of the *GA + AA* genotypes for the *G*-308*A TNF*α variant was 0.18 in Chinese, 0.03 in Japanese, 0.45 in white, 0.36 in Finnish, and 0.23 in a Brazilian population, indicating a significant ($p < 0.001$) variation of the polymorphism across ethnic groups. Because the aim of this study was to evaluate the association between the *G*-308*A TNF*α variant with components of the metabolic syndrome, we described each topic separately.

Hypertension and SABP

We evaluated four studies (30,39,42,45), and no association between hypertension and the *G*-308*A TNF*α variant was found (Table 1). Conversely, as can be seen in Table 2, SABP data were available in four studies (15,25,33,48) encompassing 1624 individuals, and we observed a significant difference in SABP between *GG* homozygotes and *A* allele carriers; this difference represents an increase of 3.5 mm Hg SABP in *A* allele carriers over that observed in *GG* homozygotes. No significant heterogeneity was found. We further examined whether the association of the −308*A* variant with SABP did depend on ethnicity and age of the subjects, and we observed that the effect was only significant for studies with white subjects and in the group of patients between 21 and 40 years old (D, 0.149; 95% CI, 0.012 to 0.286; $p < 0.03$, $n = 1053$). From the funnel plot showed in Figure 1A, it seems that the there was no publication bias.

Table 2. Summary estimates for the standardized difference (D) in systolic arterial blood pressure between the two groups according to the *G*-308*A TNF*α variant (homozygotes *GG* and *A* allele carriers, *GA + AA*)

				Number of individuals			Effect			
Effect	Ethnicity	Citation	Age	Total	*GG*	*GA+AA*	D	Lower	Upper	*p*
	White	Wybranska 48	41 to 60	121	64	57	0.000	−0.361	0.361	1.000
	White	Hoffstedt 15	41 to 60	239	173	66	0.182	−0.103	0.468	0.210
	White	Hoffstedt 15	21 to 40	378	273	105	0.069	−0.157	0.295	0.549
	White	Nicaud 25	21 to 40	675	501	174	0.197	0.023	0.370	0.026
Fixed	White (4)			1413	1011	402	0.139	0.022	0.256	0.020
Random	White (4)			1413	1011	402	0.139	0.022	0.256	0.020
	Japanese	Hayakawa 33	41 to 60	211	206	5	−0.287	−1.180	0.606	0.527
Fixed	Japanese (1)			211	206	5	−0.287	−1.179	0.605	0.527
Random	Japanese (1)			211	206	5	−0.287	−1.179	0.605	0.527
Fixed	Combined (5)			1624	1217	407	0.132	0.016	0.247	0.026
Random	Combined (5)			1624	1217	407	0.132	0.016	0.247	0.026

Corresponding 95% CI limits (lower and upper effect) were estimated by fixed and random effects meta-regression analysis. Numbered references are indicated in the citation column. The studies were divided according to ethnicity, and the total number of combined studies is indicated in parentheses.

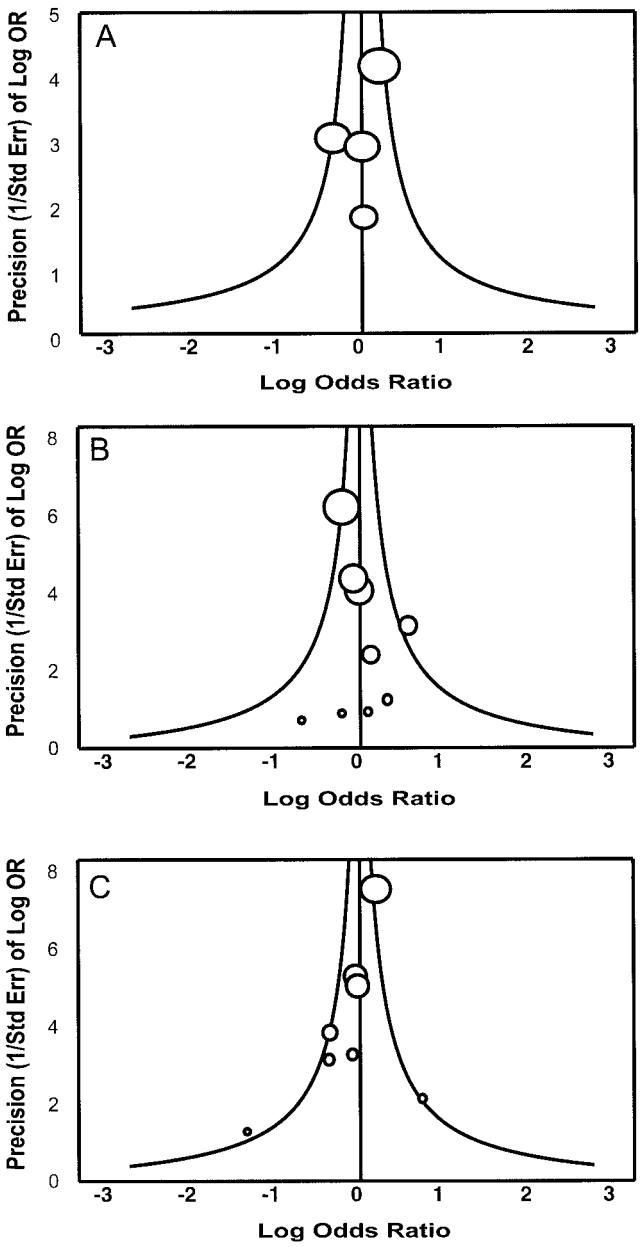


Figure 1: Funnel plots of precision by effect size for (A) hypertension, (B) type 2 diabetes, and (C) obesity.

Type 2 Diabetes: Fasting Insulin, Fasting Glucose, and HOMA-IR

We found nine studies (31,32,34,36–38,40,42,46) showing that there was no association between type 2 diabetes and the *G*-308*A TNF*α variant (Table 1). We did not identify heterogeneity in terms of statistical significance, and the funnel plot (Figure 1B) indicates no publication bias.

By contrast, we found from 14 homogeneous studies (13–15,20,22,25,27,31,33,36,41,44,45,48) that fasting insulin was significantly higher in *A* allele carriers with respect to *GG* homozygous subjects (Table 3). This difference roughly represents an increase of 24.2% in the *A* allele carrier group with respect to the *GG* homozygous group. When subjects were stratified by ethnicity, this difference was only significant in white individuals. On the other hand, there were two age groups in which the difference in fasting insulin showed statistical significance: individuals with an age under 20 years (D, 0.250; 95% CI, 0.028 to 0.471; $p = 0.027$; $n = 404$) and the group between 41 and 60 years old (D, 0.132; 95% CI, 0.002 to 0.263; $p = 0.046$; $n = 1337$). Conversely, fasting glucose was similar in both genotypes in 13 studies (13–15,20–22,25,31,33,36,41,44,45) without heterogeneity (Table 4).

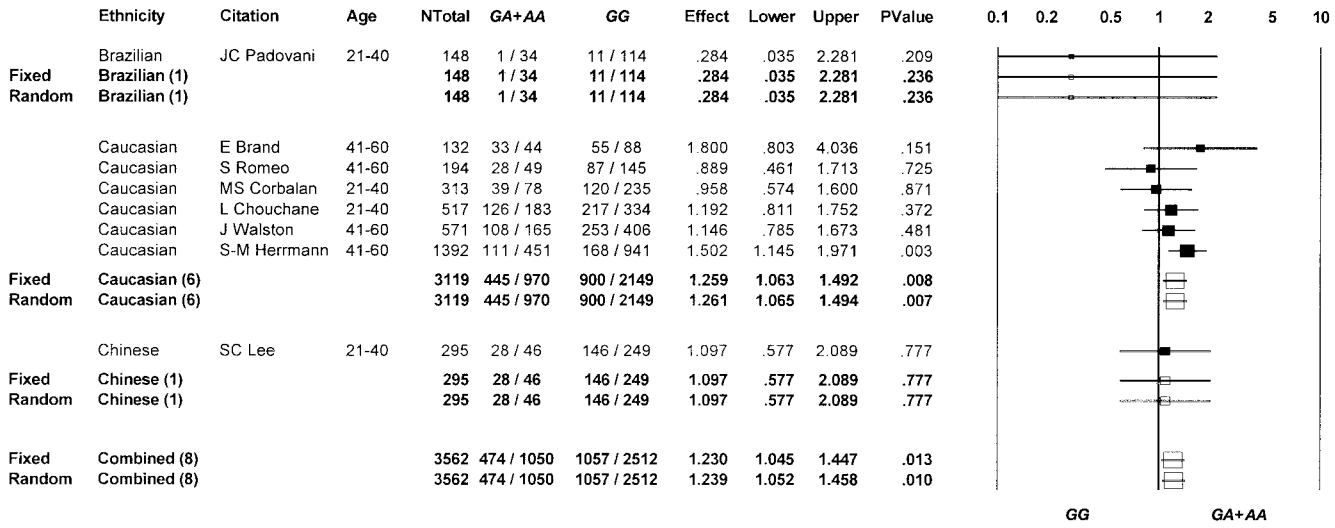
In six studies without heterogeneity (13,31,33,36,44,45), there was a trend that did not reach statistical significance toward a difference in the HOMA-IR between *GG* homozygous and *A* allele carriers (Table 4). However, when we analyzed the data between the 362 white individuals, a significant difference was observed showing that the HOMA-IR was higher in individuals who carried the −308*A TNF*α variant (D, 0.260; 95% CI, 0.036 to 0.484; $p = 0.023$; $n = 362$). In addition, there was also a significant difference in individuals with ages between 41 and 60 years (D, 0.178; 95% CI, −0.020 to 0.37; $p = 0.05$; $n = 961$).

Obesity, BMI, and WHR

Across eight studies (21,26,28,29,35,39,42,44), we found a significant association between the *G*-308*A TNF*α variant and obesity (Figure 2), with no heterogeneity between studies. Again, from the funnel plot depicted in Figure 1C, it can be observed that there appeared to be no publication bias. However, when we examined whether the association of the −308 *A* variant with obesity depends on ethnicity, we observed that the effect was only significant in white subjects (D, 1.25; 95% CI, 1.063 to 1.492; $p < 0.01$ in 3119 individuals). Furthermore, the difference was only found among individuals between 41 and 60 years old (D, 1.32; 95% CI, 1.09 to 1.63; $p = 0.005$; $n = 2289$).

BMI was found to be similar between genotypes (D, 0.058; 95% CI, −0.033 to 0.148; $p = 0.21$; $n = 5009$) from 18 studies that reported the data, although there was heterogeneity among them ($p = 0.031$), particularly in the white group (13–15,20–22,25–27,29,31,33,36,39,43–45,48). By subtracting two reports (21,44), heterogeneity was removed, and a significant association was demonstrated between the −308*A TNF*α allele and BMI (D, 0.074; 95% CI, 0.005 to 0.142; $n = 4647$; $p = 0.034$).

Plasma leptin levels seemed not to be different between genotypes, results analyzed from four heterogeneous studies (14,22,36,45) (Table 4). Finally, WHR was reported in 13 homogeneous studies (13,15,20–22,25,26,29,33,39,48), and no association was found between genotypes and WHR (Table 4).



	Ethnicity	Citation	Age	NTotal	*GA+AA*	*GG*	Effect	Lower	Upper	PValue
	Brazilian	JC Padovani	21-40	148	1 / 34	11 / 114	.284	.035	2.281	.209
Fixed	**Brazilian (1)**			**148**	**1 / 34**	**11 / 114**	**.284**	**.035**	**2.281**	**.236**
Random	**Brazilian (1)**			**148**	**1 / 34**	**11 / 114**	**.284**	**.035**	**2.281**	**.236**
	Caucasian	E Brand	41-60	132	33 / 44	55 / 88	1.800	.803	4.036	.151
	Caucasian	S Romeo	41-60	194	28 / 49	87 / 145	.889	.461	1.713	.725
	Caucasian	MS Corbalan	21-40	313	39 / 78	120 / 235	.958	.574	1.600	.871
	Caucasian	L Chouchane	21-40	517	126 / 183	217 / 334	1.192	.811	1.752	.372
	Caucasian	J Walston	41-60	571	108 / 165	253 / 406	1.146	.785	1.673	.481
	Caucasian	S-M Herrmann	41-60	1392	111 / 451	168 / 941	1.502	1.145	1.971	.003
Fixed	**Caucasian (6)**			**3119**	**445 / 970**	**900 / 2149**	**1.259**	**1.063**	**1.492**	**.008**
Random	**Caucasian (6)**			**3119**	**445 / 970**	**900 / 2149**	**1.261**	**1.065**	**1.494**	**.007**
	Chinese	SC Lee	21-40	295	28 / 46	146 / 249	1.097	.577	2.089	.777
Fixed	**Chinese (1)**			**295**	**28 / 46**	**146 / 249**	**1.097**	**.577**	**2.089**	**.777**
Random	**Chinese (1)**			**295**	**28 / 46**	**146 / 249**	**1.097**	**.577**	**2.089**	**.777**
Fixed	**Combined (8)**			**3562**	**474 / 1050**	**1057 / 2512**	**1.230**	**1.045**	**1.447**	**.013**
Random	**Combined (8)**			**3562**	**474 / 1050**	**1057 / 2512**	**1.239**	**1.052**	**1.458**	**.010**

Figure 2: Summary estimates for ORs (effect), the corresponding 95% CI limits (lower and upper), and significance (p) were estimated by fixed and random effects meta-regression analysis for obesity between the two groups according to the *G*-308*A* TNFα variant (homozygous *GG* and *A* allele carriers, *GA* + *AA*). The ratio under *GG* and *GA* + *AA* indicates proportion of case individuals with respect to the total in each genotyped group. The first author of the study is indicated under citation. In the graph, numbers indicate log of OR, filled squares represent the effect of individual studies, and empty squares express fixed combined effect and express random combined effect. The square size is proportional to the number of individuals involved in each study.

Discussion

Persistent difficulties in obtaining robust and replicable results in genetic association studies are almost certainly because genetic effects are small, requiring studies with many thousands of subjects to be detected (49,50). Case-control studies are the most widely applied strategy of association studies for characterizing the genetic contribution to common diseases, although this approach is prone to find spuriously associated gene variants with disease. This difficulty can be solved, at least in part, by doing a systematic review of the literature and, thereby, performing a meta-analysis. Then, the results of the present study may contribute to resolve the controversy concerning the association between the *G*-308*A TNF*α polymorphism and components of the metabolic syndrome because we found that the −308*A* allele may confer a risk of 1.23 for obesity and a significant increase of ~3.5 mm Hg in SABP. This conclusion arises from a total of 3562 individuals recruited from eight homogenous studies and 1624 individuals from four homogenous studies, respectively. Additionally, there was no evidence of publication bias for both outcomes. In addition, in 3720 subjects, there was also a positive association with insulin plasma levels in *A* allele carriers, and there was a trend of HOMA-IR to be higher in subjects carrying the −308*A* allele ($p < 0.07$, $n = 1083$ from six studies). It is worth mentioning that fasting insulin, hypertension, and obesity are strongly related phenotypes in which it is difficult to define which is the cause and which is the consequence. Unfortunately, most of the studies included in the meta-analysis are on single phenotypes, making impossible the adjustment of hypertension and fasting insulin by the presence of obesity. On the contrary, there was no significant association of *TNF*α genotypes with BMI, WHR, fasting glucose, plasma leptin levels, diabetes, and hypertension.

Age and ethnicity may have acted as important variables in determining association risk with obesity, SABP, and plasma insulin levels because all studies demonstrated that white *A* allele carriers had an excess risk for obesity and higher values of SABP, as well as higher plasma insulin levels. In the same way, the allele *A* was associated with obesity and higher insulin in individuals between 40 and 60 years but higher SABP in the group of 21 to 40 years. This may indicate that obesity and insulin resistance may either be related variables or have common causes acting later in life in which TNFα plays an important role. In contrast, the contributing role of TNFα to a higher SABP seemed to appear early in life. More studies are necessary to investigate whether the TNFα exerts its influence in SABP earlier and independently of its effect on insulin resistance and body weight.

Several issues should be discussed regarding our findings. First, we found a significant association between the *A* allele and SABP, showing that carriers of this allele had higher values of SABP. The increase in SABP of ~3.5 mm Hg may be an important risk factor for cardiovascular morbidity and mortality since it has been estimated that a 10 mm Hg increase in SABP is associated with a 40% higher risk for coronary heart disease (51). Although there were not enough studies to address the question of whether the

Table 3. Summary estimates for the standardized difference (D) in fasting plasma insulin levels between the two groups according to the *G*-308*A* TNFα variant (homozygotes *GG* and *A* allele carriers, *GA* + *AA*)

			Number of individuals				Effect			
Effect	**Ethnicity**	**Citation**	**Age**	**Total**	***GG***	***GA + AA***	**D**	**Lower**	**Upper**	***p***
	White	Fernandex-Real 14	21 to 40	38	23	15	0.848	0.147	1.549	0.015
	White	Wybranska 48	41 to 60	121	64	57	0.278	−0.084	0.641	0.129
	White	Dalziel 13	41 to 60	168	100	68	0.396	0.083	0.709	0.013
	White	Jaquet 27	0 to 20	171	125	46	0.375	0.032	0.718	0.031
	White	Romeo 44	41 to 60	194	145	49	0.129	−0.197	0.455	0.436
	White	Jaquet 27	0 to 20	233	172	61	0.156	−0.138	0.450	0.297
	White	Hoffstedt 15	41 to 60	239	173	66	−0.060	−0.345	0.225	0.678
	White	Morris 41		256	157	99	0.057	−0.196	0.309	0.660
	White	Rosmond 22	41 to 60	262	148	114	0.020	−0.226	0.265	0.874
	White	Rasmussen 20	21 to 40	325	214	111	0.092	−0.138	0.322	0.433
	White	Hoffstedt 15	21 to 40	378	273	105	0.140	−0.086	0.366	0.223
	White	Nicaud 25	21 to 40	614	453	161	−0.017	−0.197	0.163	0.852
Fixed	White (12)			2999	2047	952	0.116	0.038	0.194	0.004
Random	White (12)			2999	2047	952	0.132	0.036	0.227	0.007
	Chinese	Sheu 45	21 to 40	246	207	39	−0.334	−0.679	0.011	0.057
Fixed	Chinese (1)			246	207	39	−0.334	−0.677	0.010	0.057
Random	Chinese (1)			246	207	39	−0.334	−0.677	0.010	0.057
	Japanese	Ishii 36	21 to 40	122	118	4	−0.129	−1.136	0.877	0.799
	Japanese	Furuta 31	41 to 60	142	139	3	0.000	−1.154	1.154	1.000
	Japanese	Hayakawa 33	41 to 60	211	206	5	0.522	−0.372	1.416	0.250
Fixed	Japanese (3)			475	463	12	0.175	−0.400	0.750	0.549
Random	Japanese (3)			475	463	12	0.175	−0.400	0.750	0.549
Fixed	Combined (16)			3720	2717	1003	0.095	0.020	0.170	0.013
Random	Combined (16)			3720	2717	1003	0.111	0.011	0.210	0.030

Corresponding 95% CI limits (lower and upper effect) were estimated by fixed and random effects meta-regression analysis. Numbered references are indicated in the citation column. The studies were divided according to ethnicity, and the total number of combined studies is indicated in parentheses.

Table 4. Summary estimates for the standardized difference (D) and corresponding 95% CI limits (lower and upper effect) in fasting glucose, HOMA-IR, plasma leptin levels, and WHR for the *G*-308*A* TNFα variant

Effect	**Number of subjects**	**Standardized difference (D)**	**95% CI limits**	**Heterogeneity**	***p***
Fasting glucose	3830	−0.006	−0.81 to 0.07	None	0.88
HOMA-IR	1083	0.165	−0.013 to 0.343	None	0.069
Leptin levels	845	−0.024	0.207 to 0.159	None	0.79
WHR	3910	0.022	−0.05 to 0.09	None	0.55

CI, confidence interval; HOMA-IR, homeostasis model assessment; WHR, waist-to-hip ratio.

−308*A* allele is associated with high circulating TNFα protein levels as was previously reported (12), it may be speculated that high levels of plasma TNFα could be associated with vascular vasoconstriction. This speculation is consistent with the fact that TNFα induces the synthesis of endothelin-1, a potent vasoconstrictor (52). Furthermore, it was demonstrated in animal models that treatment with TNFα induced an increase in the circulating endothelin-1 levels, a finding associated with a striking coronary vasoconstriction in the heart (53). In addition, it was demonstrated that patients with uncomplicated mild essential hypertension showed elevated plasma intercellular adhesion molecule-1 and TNFα concentrations (54), suggesting that the metabolic syndrome is a low-grade, systemic inflammatory condition.

Second, we found that the *A* allele was associated with a 1.25-fold increase in fasting plasma insulin levels. TNFα may be an important mediator of insulin resistance in obesity and diabetes through its ability to decrease the tyrosine kinase activity of the insulin receptor (55) interfering with insulin action. The difference between HOMA-IR of homozygotes *GG* and −308*A* allele carriers, however, did not reach statistical significance, although a trend was observed. This may be likely due to a lack of power of the analysis because for a total of 1083 individuals, it was <20% for such small effect.

Third, obesity was positively associated with the −308*A* allele. It is noteworthy that the pathogenesis of both obesity and hypertension is complex, probably involving several genes and environmental factors. However, genetic analysis suggests that some of the genes that confer susceptibility to obesity may also contribute to the development of obesity-associated hypertension; one such gene may be *TNF*α (19). In this scenario, one may hypothesize that the higher production of TNFα linked to the −308*A* variant may induce adipose tissue development by increasing the total number of stromal-vascular and/or uncommitted cells within the tissue because Kras et al. (56) have reported that these cells may be recruited to become preadipocytes or may serve alternatively as infrastructure to support adipose tissue growth.

Furthermore, insulin has adipogenic activity by stimulating differentiation and accelerating lipid accumulation (57), and it is conceivable to assume that a cross-talk between TNFα and insulin signaling pathways may participate in the adipocyte differentiation. A note of caution, however, should be added because our analysis could not find an association between *TNF*α genotypes and BMI, although there was heterogeneity among the studies. It is difficult to reconcile the findings of a lack of association with the continuous variable, BMI, with the positive association with the dichotomous variable defined as a threshold. In this case, the total number of individuals studied was high (5009 individuals), and even in whites, the number seemed to be sufficiently high (3102 individuals) to have good statistical power. Then, the different outcomes probably are due to an excessively low cut-off for BMI in defining obesity (27 kg/m^2). When the individuals were distributed by age, in the group of 21 to 40 years old, there was a trend ($p < 0.067$, $n = 1851$) toward a significant difference for BMI between homozygous *GG* and −308*A* allele carriers, which is also difficult to explain considering that the association of the genotype with obesity was mainly in the 41- to 60-year-old group. Furthermore, because the −308*A* *TNF*α variant has been associated with BMI in non-morbidly obese but not in morbidly obese individuals (19), we tried to solve this discrepancy by subtracting from the meta-analysis three references that included morbidly obese subjects (mean BMI > 35 kg/m^2) (13,21,29) showing a similar result. Another possible explanation is that heterogeneity was more significant ($p = 0.007$) among studies where gender data were not reported; therefore, sex differences may be an important confounder factor. To explain heterogeneity, a step-wise subtraction of reports was performed. By subtracting two publications (21,44), heterogeneity was removed, and a significant association was demonstrated between the −308*A* *TNF*α allele and BMI in all remaining studies. Remarkably, these two studies encompassing 618 individuals showed a significant negative association between the −308*A* *TNF*α allele and BMI; one of them (21) included morbidly obese patients. Importantly, by subtracting the above-mentioned studies, heterogeneity was also removed in the groups of studies with no sex data available (data not shown).

In the same way, although with a small number of individuals studied, leptin was not different across genotypes. Then, further studies are necessary to solve this inconsistency, especially considering a recent study in which, in a step-wise multivariate regression analysis, BMI was significantly associated with plasma levels of circulating TNFα (58).

Finally, no publication bias was found regarding the ethnic backgrounds of the included individuals. However, when the population was stratified by ethnic groups, the positive association between the *A* variant and SABP, plasma insulin levels, and obesity was only observed in whites.

In conclusion, this report represents the first meta-analysis including all available evidence, to date, indicating that carriers of the −308*A* allele of *TNF*α are at a 23% risk of developing obesity and have higher levels of SABP and fasting insulin compared with non-carriers. However, we acknowledge that we have not corrected by multiple testing, even though 10 different phenotypes were analyzed, and some differences we observed are of small significance, which can be lost if multiple testing adjustments are applied. However, we did not consider this procedure appropriate because we tested specific null hypotheses regarding the

variables analyzed following a prevalent view widely held by epidemiologists (59). We also used a different combination of raw data for a particular phenotype.

These findings may have an important impact in public health because the prevalence of *A* allele carriers seems to be as high as 45% among whites. Although in the Asian population, the power of the meta-analysis was lower due to the small number of studies addressing this issue, the very low frequency of the −308*A TNF*α allele among Japanese and Chinese may contribute to the lack of statistical power to detect a modest effect of the *A* allele on obesity and SABP at a general population level. In any case, additional studies are required to solve this issue because it has been suggested recently that, although the variant frequency of different complex disease-contributing genes are very different among diverse ethnic groups, their genetic impact seems to be similar among them, and a particular gene variant carries a similar risk in individuals of different ethnic backgrounds (60).

We wish to add a final note of caution since the *G*-308*A TNF*α polymorphism may be a neutral marker associated with the true variant influencing the transcriptional activity of the gene. Moreover, *TNF*α is located downstream, very close to the *TNF*β gene, forming a cluster that seems to be regulated in concert (61), suggesting that these might be one of the few examples of polycistronic systems under the control of a unique upstream promoter in eukaryotes (62). Certainly, more experiments are necessary to explore this intriguing possibility.

Acknowledgments

This work was partially supported by Grants B119 (Universidad de Buenos Aires) and PICT 05-08719 (Agencia Nacional de Promoción Científica y Tecnológica). S.S. and C.J.P. belong to Consejo Nacional de Investigaciones Científicas y Técnicas.

References

1. **Reaven GM, Chen YD.** Role of insulin in regulation of lipoprotein metabolism in diabetes. *Diabetes Metab Rev*. 1988;4:639–52.
2. **Ford ES, Giles WH, Dietz WH.** Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA*. 2002;287: 356–9.
3. **Weiss R, Dziura J, Burgert TS, et al.** Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med*. 2004;350:2362–74.
4. **Porto PI, Garcia SI, Dieuzeide G, et al.** Clinical features of the metabolic syndrome in adolescents: minor role of the Trp64Arg beta3-adrenergic receptor gene variant. *Pediatr Res*. 2004;55:836–41.
5. **Isomaa B, Almgren P, Tuomi T, et al.** Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*. 2001;24:683–9.
6. **Sonnenberg GE, Krakower GR, Kissebah AH.** A novel pathway to the manifestations of metabolic syndrome. *Obes Res*. 2004;12:180–6.
7. **Hotamisligil GS, Shargill NS, Spiegelman BM.** Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. 1993;259:87–91.
8. **Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM.** Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest*. 1995;95:2409–15.
9. **Porter MH, Cutchins A, Fine JB, Bai Y, DiGirolamo M.** Effects of TNF-alpha on glucose metabolism and lipolysis in adipose tissue and isolated fat-cell preparations. *J Lab Clin Med*. 2002;139:140–6.
10. **Beutler B, Cerami A.** Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature*. 1986;320: 584–8.
11. **Wilson AG, di Giovine FS, Blakemore AI, Duff GW.** Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product. *Hum Mol Genet*. 1992;1:353.
12. **Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW.** Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA*. 1997;94:3195–9.
13. **Dalziel B, Gosby AK, Richman RM, Bryson JM, Caterson ID.** Association of the TNF-alpha −308 G/A promoter polymorphism with insulin resistance in obesity. *Obes Res*. 2002; 10:401–7.
14. **Fernandez-Real JM, Gutierrez C, Ricart W, et al.** The TNF-alpha gene Nco I polymorphism influences the relationship among insulin resistance, percent body fat, and increased serum leptin levels. *Diabetes*. 1997;46:1468–72.
15. **Hoffstedt J, Eriksson P, Hellstrom L, Rossner S, Ryden M, Arner P.** Excessive fat accumulation is associated with the TNF alpha-308 G/A promoter polymorphism in women but not in men. *Diabetologia*. 2000;43:117–20.
16. **Hotamisligil GS.** Mechanisms of TNF-alpha-induced insulin resistance. *Exp Clin Endocrinol Diabetes*. 1999;107:119–25.
17. **Kroeger KM, Carville KS, Abraham LJ.** The −308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Mol Immunol*. 1997;34:391–9.
18. **Norman RA, Bogardus C, Ravussin E.** Linkage between obesity and a marker near the tumor necrosis factor-alpha locus in Pima Indians. *J Clin Invest*. 1995;96:158–62.
19. **Pausova Z, Deslauriers B, Gaudet D, et al.** Role of tumor necrosis factor-alpha gene locus in obesity and obesity-associated hypertension in French Canadians. *Hypertension*. 2000; 36:14–9.
20. **Rasmussen SK, Urhammer SA, Jensen JN, et al.** The −238 and −308 G→A polymorphisms of the tumor necrosis factor alpha gene promoter are not associated with features of the insulin resistance syndrome or altered birth weight in Danish Caucasians. *J Clin Endocrinol Metab*. 2000;85:1731–4.
21. **Walston J, Seibert M, Yen CJ, Cheskin LJ, Andersen RE.** Tumor necrosis factor-alpha −238 and −308 polymorphisms do not associated with traits related to obesity and insulin resistance. *Diabetes*. 1999;48:2096–8.

22. **Rosmond R, Chagnon M, Bouchard C, Björntorp P.** *G*-308*A* polymorphism of the tumor necrosis factor alpha gene promoter and salivary cortisol secretion. *J Clin Endocrinol Metab*. 2001;86:2178–80.
23. **Matthews DR, Hosker JP, Rudenski AS, et al.** Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–9.
24. **da Silva B, Gapstur SM, Achenbach YH, et al.** Lack of association between the −308 polymorphism of the tumor necrosis factor-alpha gene and the insulin resistance syndrome. *J Investig Med*. 2000;48:236–44.
25. **Nicaud V, Raoux S, Poirier O, et al.** The TNF alpha/*G*-308*A* polymorphism influences insulin sensitivity in offspring of patients with coronary heart disease: the European Atherosclerosis Research Study II. *Atherosclerosis*. 2002;161:317–25.
26. **Brand E, Schorr U, Kunz I, et al.** Tumor necrosis factor-alpha-308 G/A polymorphism in obese Caucasians. *Int J Obes Relat Metab Disord*. 2001;25:581–5.
27. **Jaquet D, Tregouet DA, Godefroy T, et al.** Combined effects of genetic and environmental factors on insulin resistance associated with reduced fetal growth. *Diabetes*. 2002; 51:3473–8.
28. **Chouchane L, Danguir J, Beji C, et al.** Genetic variation in the stress protein hsp70-2 gene is highly associated with obesity. *Int J Obes Relat Metab Disord*. 2001;25:462–6.
29. **Corbalan MS, Marti A, Forga L, et al.** Influence of two polymorphisms of the tumoral necrosis factor-alpha gene on the obesity phenotype. *Diabetes Nutr Metab*. 2004;17:17–22.
30. **Frossard PM, Gupta A, Pravica V, et al.** A study of five human cytokine genes in human essential hypertension. *Mol Immunol*. 2002;38:969–76.
31. **Furuta M, Yano Y, Ito K, et al.** Relationship of the tumor necrosis factor-alpha −308 A/G promoter polymorphism with insulin sensitivity and abdominal fat distribution in Japanese patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract*. 2002;56:141–5.
32. **Hamann A, Mantzoros C, Vidal-Puig A, Flier JS.** Genetic variability in the TNF-alpha promoter is not associated with type II diabetes mellitus (NIDDM). *Biochem Biophys Res Commun*. 1995;211:833–9.
33. **Hayakawa T, Nagai Y, Taniguchi M, et al.** Tumor necrosis factor-beta gene NcoI polymorphism decreases insulin resistance in Japanese men. *Metabolism*. 2000;49:1506–9.
34. **Heijmans BT, Westendorp RG, Droog S, et al.** Association of the tumour necrosis factor alpha −308G/A polymorphism with the risk of diabetes in an elderly population-based cohort. *Genes Immun*. 2002;3:225–8.
35. **Herrmann SM, Ricard S, Nicaud V, et al.** Polymorphisms of the tumour necrosis factor-alpha gene, coronary heart disease and obesity. *Eur J Clin Invest*. 1998;28:59–66.
36. **Ishii T, Hirose H, Saito I, et al.** Tumor necrosis factor alpha gene *G*-308*A* polymorphism, insulin resistance, and fasting plasma glucose in young, older, and diabetic Japanese men. *Metabolism*. 2000;49:1616–8.
37. **Kankova K, Marova I, Jansen EH, et al.** Polymorphism NcoI in tumor necrosis factor B is associated with fasting glycemia and lipid parameters in healthy non-obese Caucasian subjects. *Diabetes Metab*. 2002;28:231–7.
38. **Ko GT, Lee SC, Pu YB, et al.** Tumour necrosis factor-alpha promoter gene polymorphism at −308 (genotype AA) in Chinese subjects with Type 2 diabetes. *Diabet Med*. 2003;20: 167–8.
39. **Lee SC, Pu YB, Thomas GN, et al.** Tumor necrosis factor alpha gene *G*-308*A* polymorphism in the metabolic syndrome. *Metabolism*. 2000;49:1021–4.
40. **Li H, Groop L, Nilsson A, Weng J, Tuomi T.** A combination of human leukocyte antigen DQB1*02 and the tumor necrosis factor alpha promoter G308A polymorphism predisposes to an insulin-deficient phenotype in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2003;88:2767–74.
41. **Morris AM, Heilbronn LK, Noakes M, Kind KL, Clifton PM.** −308 Nco I polymorphism of tumour necrosis factor alpha in overweight Caucasians. *Diabetes Res Clin Pract*. 2003;62:197–201.
42. **Padovani JC, Pazin-Filho A, Simoes MV, et al.** Gene polymorphisms in the TNF locus and the risk of myocardial infarction. *Thromb Res*. 2000;100:263–9.
43. **Pihlajamaki J, Ylinen M, Karhapaa P, Vauhkonen I, Laakso M.** The effect of the −308A allele of the TNF-alpha gene on insulin action is dependent on obesity. *Obes Res*. 2003;11:912–7.
44. **Romeo S, Sentinelli F, Capici F, et al.** The *G*-308*A* variant of the tumor necrosis factor-alpha (TNF-alpha) gene is not associated with obesity, insulin resistance and body fat distribution. *BMC Med Genet*. 2001;2:10.
45. **Sheu WH, Lee WJ, Lin LY, Chang RL, Chen YT.** Tumor necrosis factor alpha −238 and −308 polymorphisms do not associate with insulin resistance in hypertensive subjects. *Metabolism*. 2001;50:1447–51.
46. **Shiau MY, Wu CY, Huang CN, et al.** TNF-alpha polymorphisms and type 2 diabetes mellitus in Taiwanese patients. *Tissue Antigens*. 2003;61:393–7.
47. **Vendrell J, Fernandez-Real JM, Gutierrez C, et al.** A polymorphism in the promoter of the tumor necrosis factor-alpha gene (−308) is associated with coronary heart disease in type 2 diabetic patients. *Atherosclerosis*. 2003;167:257–64.
48. **Wybranska I, Malczewska-Malec M, Niedbal S, Naskalski JW, Dembinska-Kiec A.** The TNF-alpha gene NcoI polymorphism at position −308 of the promoter influences insulin resistance, and increases serum triglycerides after postprandial lipidemia in familiar obesity. *Clin Chem Lab Med*. 2003;41: 501–10.
49. **Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K.** A comprehensive review of genetic association studies. *Genet Med*. 2002;4:45–61.
50. **Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN.** Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet*. 2003;33:177–82.
51. **Neaton JD, Kuller L, Stamler J, Wentworth DN.** Impact of systolic and diastolic blood pressure on cardiovascular mortality. In: Laragh JH, Brenner BM, eds. *Hypertension: Pathophysiology, Diagnosis, and Management*. New York: Raven Press; 1995, pp. 127–144.
52. **Patel JN, Jager A, Schalkwijk C, et al.** Effects of tumour necrosis factor-alpha in the human forearm: blood flow and endothelin-1 release. *Clin Sci (Lond)*. 2002;103:409–15.

53. **Klemm P, Warner TD, Hohlfeld T, Corder R, Vane JR.** Endothelin 1 mediates ex vivo coronary vasoconstriction caused by exogenous and endogenous cytokines. *Proc Natl Acad Sci USA*. 1995;92:2691–5.
54. **Sardo MA, Castaldo M, Cinquegrani M, et al.** Effects of AT1 receptor antagonist losartan on sICAM-1 and TNF-alpha levels in uncomplicated hypertensive patients. *Angiology*. 2004;55:195–203.
55. **Hotamisligil GS, Peraldi P, Budavari A, et al.** IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science*. 1996;271:665–8.
56. **Kras KM, Hausman DB, Martin RJ.** Tumor necrosis factor-alpha stimulates cell proliferation in adipose tissue-derived stromal-vascular cell culture: promotion of adipose tissue expansion by paracrine growth factors. *Obes Res*. 2000;8:186–93.
57. **Wiederer O, Loffler G.** Hormonal regulation of the differentiation of rat adipocyte precursor cells in primary culture. *J Lipid Res*. 1987;28:649–58.
58. **Schulz S, Schagdarsurengin U, Suss T, et al.** Relation between the tumor necrosis factor-alpha (TNF-alpha) gene and protein expression, and clinical, biochemical, and genetic markers: age, body mass index and uric acid are independent predictors for an elevated TNF-alpha plasma level in a complex risk model. *Eur Cytokine Netw*. 2004; 15:105–11.
59. **Perneger TV.** What's wrong with Bonferroni adjustments? *BMJ*. 1998;316:1236.
60. **Ioannidis JPA, Ntzani EE, Trikalinos TA.** "Racial" differences in genetic effects for complex diseases. *Nat Genet*. 2004;36:1312–8.
61. **Bouma G, Crusius JB, Oudkerk PM, et al.** Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles: relevance for inflammatory bowel disease. *Scand J Immunol*. 1996;43:456–63.
62. **Blumenthal T.** Gene clusters and polycistronic transcription in eukaryotes. *Bioessays*. 1998;20:480–7.