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A functional nonsynonymous toll-like receptor 4 gene polymorphism is associated with metabolic syndrome, surrogates of insulin resistance, and syndromes of lipid accumulation

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Abstract

Toll-like receptor 4 (TLR4) plays a key role in the activation of innate immune responses. Loss-of-function mutations in TLR4 prevent diet-induced obesity and insulin resistance (IR). We conducted a population cross-sectional study to evaluate whether *Asp299Gly* (rs4986790) TLR4 gene polymorphism is associated with metabolic syndrome (MS), surrogates of IR, and syndromes of lipid accumulation (SLAs) in Argentinean healthy male subjects. rs4986790 was genotyped in 621 healthy unrelated male blood donors. National Cholesterol Education Program/Adult Treatment Panel III–MS (NCEP/ATP III-MS); SLAs such as enlarged waist elevated triglyceride syndrome (EWET), hypertriglyceridemic waist (HW), and overweight-lipid syndrome (OLS); and surrogates of IR were assessed. The prevalence of MS, OLS, and EWET was significantly higher among *Asp299Asp* carriers ($P < .05$). These findings were confirmed using 32 000 bootstrap samples. Surrogate markers of IR were also significantly higher in *Asp299Asp* carriers ($P < .05$). Most findings were especially strengthened among individuals with C-reactive protein below the 95th percentile and/or total cholesterol to high-density lipoprotein cholesterol ratio ≥5. This is the first report to find, in Argentinean healthy male blood donors, associations between the *Asp299Asp* genotype of rs4986790 TLR4 gene polymorphism and high risk for NCEP/ATP III-MS, SLAs, and surrogates of IR. These findings are consistent with previous functional and observational studies showing that *Asp299* allele, in comparison with *Gly299*, is associated with increased TLR4 activation, higher levels of inflammatory cytokines, acute-phase reactants and soluble adhesion molecules, and higher risk of atherosclerosis.

1. Introduction

The metabolic syndrome (MS) is a constellation of cardiometabolic risk factors including central obesity, insulin resistance (IR), hypertension, prediabetes/diabetes, hyperinsulinemia, and dyslipidemia [1].

Epidemiologic studies have suggested that nucleotide variations in innate immunity genes could lead to type 2

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diabetes mellitus (T2D) and associated metabolic disorders such as MS [2].

Toll-like receptors (TLRs) are members of the interleukin 1 receptor family, implicated in the activation of innate immune responses in mammals by recognizing conserved pathogen-associated molecular patterns, such as lipopolysaccharides (LPS) [3]. The activation of TLR4 signaling induces up-regulation of inflammatory pathways related to the induction of IR, such as IκB kinase complex (IKKB)/inhibitor of nuclear factor–κB (IκBα)/nuclear factor–κB (NF-κB) and c-Jun NH_2-terminal kinase (JNK) [3]. In C3H/HeJ mice, loss-of-function mutation in TLR4 prevents diet-induced obesity and IR [4]. High TLR4 expression and signaling in muscle from insulin-resistant subjects have been observed [5].

A nonsynonymous single nucleotide polymorphism in the gene region coding for the extracellular domain of TLR4, characterized by a substitution at amino acid position 299 (glycine for aspartate), has been identified (rs4986790). The minor *Gly299* allele affects the inflammatory response to LPS by exhibiting attenuated signaling leading to a dampened response to LPS [6]. *Gly299* carriers have lower levels of inflammatory cytokines, acute-phase reactants, and soluble adhesion molecules, such as interleukin 6 and fibrinogen [7]. We have therefore investigated whether rs4986790 determines susceptibility to MS, IR, and syndromes of lipid accumulation (SLAs) such as enlarged waist elevated triglyceride syndrome (EWET), hypertriglyceridemic waist (HW), and overweight-lipid syndrome (OLS) in healthy men.

2. Subjects and methods

2.1. Subjects

All individuals were unrelated Argentinean men of self-reported European ancestry, especially from southern European countries (Spain and Italy), living in Buenos Aires metropolitan area, recruited between April 2006 and April 2008 at the Department of Haemotherapy of the Hospital "José de San Martín," University of Buenos Aires. All subjects were nondiabetic blood donors with normal findings on medical examination and blood count and were free from any medication. The study was carried out in accordance with the Declaration of Helsinki and was approved by the ethics committee of our hospital. Among 621 individuals (age 37.3 ± 11.0 years; body mass index [BMI] 28.2 ± 4.5 kg/m^2), 172 (27.7%) had National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATP III)–MS, 174 (28.0%) had EWET, 131 (21.1%) had HW, and 276 (44.4%) had OLS (clinical characteristics are summarized in Table 1).

Table 1
Clinical characteristics, surrogates of IR, and SLAs for all participants, stratified by presence or absence of the MS

Clinical characteristics*	All subjects (n = 621)		NCEP/ATP III-MS			
			Yes (n = 172)		No (n = 449)	
	Mean	SD	Mean	SD	Mean	SD
Age (y)	37.34	11.00	42.84	9.57	35.23	10.78
BMI (kg/m^2)	28.19	4.46	32.04	4.26	26.71	3.57
WC (cm)	96.00	12.25	106.38	10.59	92.08	10.38
SBP (mm Hg)	126.44	10.67	132.43	9.68	124.15	10.14
DBP (mm Hg)	79.98	7.14	82.90	7.01	78.86	6.87
TC (mmol/L)	4.85	1.08	5.29	1.06	4.69	1.04
LDL-C (mmol/L)	3.45	0.96	3.84	0.88	3.32	0.96
HDL-C (mmol/L)	1.05	0.28	0.89	0.21	1.12	0.28
TG (mmol/L)	1.62	1.26	2.61	1.78	1.23	0.68
FPG (mmol/L)	5.32	1.54	6.08	2.66	5.04	0.51
CRP (mg/L)[a]	2.41	3.58	3.11	3.44	2.12	3.60
*Surrogates of IR**						
Fasting insulin (μU/mL)	16.52	10.61	23.12	13.44	13.95	7.93
Log(insulin)	1.140	0.265	1.297	0.244	1.079	0.247
40/Insulin (μU/mL)	3.528	2.631	2.366	1.443	3.980	2.842
HOMA-IR	2.136	1.124	3.183	4.421	1.872	0.913
Log(HOMA-IR)	0.274	0.224	0.409	0.200	0.223	0.210
QUICKI	0.325	0.031	0.305	0.024	0.333	0.030
*SLAs**						
EWET (%, n)	28.02 (174)	NA	67.44 (116)	NA	12.92 (58)	NA
HW (%, n)	21.10 (131)	NA	54.07 (93)	NA	8.46 (38)	NA
OLS (%, n)	44.44 (276)	NA	85.47 (147)	NA	28.73 (129)	NA

DBP indicates diastolic blood pressure; SBP, systolic blood pressure; NA, not applicable.

[a] CRP was measured in 545 individuals.

* $P < .0001$ for all parameters, except for CRP ($P = .0027$).

2.2. Clinical measurements

Anthropometric measurements and systolic and diastolic blood pressure were determined by a standardized protocol in every subject. Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were determined in serum by enzymatic methods using commercial kits (TG Triglycerides GPO-PAP, CHOL Cholesterol CHOD-PAP, and Phosphotungstate Precipitant; Roche Diagnostics, Mannheim, Germany).

Fasting plasma glucose (FPG) was determined by a glucose-oxidase method (GLU Glucose GOD-PAP, Roche Diagnostics). Fasting plasma insulin was measured by radioimmunoassay with a commercial kit (Human Insulin Specific RIA kit; Linco Research, St Louis, MO). Cross-reactivity is less than 0.2% to intact human proinsulin. Serum C-reactive protein (CRP) concentration was determined by Tina-quant CRP (Latex) high-sensitive immunoturbidimetric assay (Roche Diagnostics) in a Hitachi 917 autoanalyzer. Within-run and between-day precisions (coefficient of variation) were 0·4% and 3·4%, respectively.

2.3. Calculations

Each subject was assessed for the presence of MS using the NCEP/ATP III criteria [8].

Insulin sensitivity was assessed with (1) fasting insulin, (2) logarithm of fasting insulin, (3) 40/insulin, (4) homeostasis model assessment of insulin resistance (HOMA-IR) using the software HOMA Calculator version 2.2.2 for Windows [9], (5) logarithm of HOMA-IR, and (6) quantitative insulin sensitivity check index (QUICKI) [10].

The following SLAs were assessed: EWET (TG ≥1.45 mmol/L plus waist circumference [WC] ≥95 cm), OLS (TG ≥1.47 mmol/L or TG/HDL-C ≥3, plus BMI ≥25 kg/m^2), and HW (TG ≥1.7 mmol/L plus WC ≥90 cm) [11].

2.4. Genotyping

Genotypes for rs4986790 were scored blindly using polymerase chain reaction–restriction fragment length polymorphism analysis, as previously described [12].

2.5. Statistical analysis

The statistical difference in genotype distribution and allele frequencies among the groups for qualitative variables was assessed by the χ^2 test. Age- and BMI-adjusted analysis of covariance (ANCOVA) was conducted to assess the independent effects of the *Asp299Asp* genotype on surrogates of IR.

Taking into account that TLR4 overexpression can affect insulin sensitivity [5,13], we conducted mediation models, using the test of Sobel, to detect whether a fraction of the influence of rs4986790 on MS and SLAs is mediated by surrogates of IR [14].

An internal cross-validation was conducted by means of bootstrapping (32 000 bootstrap samples) to estimate the most important χ^2-derived odds ratios (ORs), 95% confidence intervals (CIs), and *P* values [15]. A *P* value < .05 was considered statistically significant. Statistical analyses were conducted using the program for Statistical Package for the Social Sciences, version 12.0 (SPSS, Chicago, IL), except for bootstrapping (Simstat for Windows version 2.5.5; Provalis Research, Montreal, Canada).

3. Results

3.1. Asp299Gly and MS

The genotype distribution was *Asp299Asp* 90.3%, *Asp299Gly* 9.5%, and *Gly299Gly*, 0.2%, and was in Hardy-Weinberg equilibrium (*P* > .1). rs4986790 was significantly associated with MS (*P* = .023). *Asp299Asp* carriers had high risk of MS (OR = 2.03; 95% CI, 1.02-

Table 2
rs4986790 and MS: crude and bootstrapped (32 000 samples) χ^2 analyses

	Whole sample		Crude			Bootstrapped (32 000 samples)		
	MS positive (n = 172)	MS negative (n = 449)	OR	95% CI	*P*	OR	95% CI	*P*
Genotypes								
Asp299Gly Gly299Gly[a]	10	50	1.0 (ref)			1.0 (ref)		
Asp299Asp	162	399	2.03	1.02-4.05	.045	2.90	1.37-6.11	.004
χ^2 (*df* 2)					.023			.003
	Individuals with TC/HDL-C ratio ≥5		Crude			Bootstrapped (32 000 samples)		
	MS positive (n = 131)	MS negative (n = 127)	OR	95% CI	*P*	OR	95% CI	*P*
Genotypes								
Asp299Gly Gly299Gly[a]	5	19	1.0 (ref)			1.0 (ref)		
Asp299Asp	126	108	4.43	1.66-11.83	.002	4.43	1.48-13.14	.006
χ^2 (*df* 2)					.002			.007

[a] Because of the small number, *Gly299Gly* (n = 1) and *Asp299Gly* carriers have been combined.

4.05; P = .045). Among individuals with TC/HDL-C ratio ≥5 (n = 258), *Asp299Asp* was strongly associated with high risk of MS (OR = 4.43; 95% CI, 1.66-11.83; P = .002). These analyses were confirmed using 32 000 bootstrap samples (Table 2).

3.2. Asp299Gly and SLAs

rs4986790 was significantly associated with OLS (P = .022). *Asp299Asp* carriers had high risk of OLS (OR = 1.99; 95% CI, 1.12-3.51; P = .018). These findings were more evident among individuals with HDL-C less than 1.0 mmol/L (n = 299, OR = 3.73; 95% CI, 1.60-8.67; P = .002).

rs4986790 was significantly associated with EWET (P = .021). *Asp299Asp* carriers had high risk of EWET (OR = 2.07; 95% CI, 1.04-4.12; P = .039).

rs4986790 was not associated with HW (P =.081).

These analyses were confirmed using 32 000 bootstrap samples (Table 3).

3.3. Asp299Gly and surrogates of IR

According to age- and BMI-adjusted ANCOVA, insulin level in *Asp299Asp* carriers was not different in comparison with that in noncarriers (P = .099). Among individuals with TC/HDL-C ratio of at least 5, *Asp299Asp* carriers had significantly higher insulin levels (19.7 ± 11.7 μU/mL) than noncarriers (14.9 ± 6.8, P = .035).

Asp299Asp carriers had significantly higher log(insulin) levels (1.15 ± 0.26) than noncarriers (1.08 ± 0.28, P = .043) and significantly lower 40/insulin levels (3.45 ± 2.49) than noncarriers (4.22 ± 3.67, P = .029).

The HOMA-IR levels in *Asp299Asp* carriers (2.16 ± 1.14) were not significantly different in comparison with those in noncarriers (1.92 ± 0.94, P = .095). Among individuals with HDL-C less than 1.0 mmol/L, *Asp299Asp* carriers had significantly higher HOMA-IR levels (2.35 ± 1.29) than noncarriers (1.73 ± 0.86, P = .019).

The log(HOMA-IR) levels in *Asp299Asp* carriers (0.28 ± 0.23) were not significantly different in comparison with those in noncarriers (0.23 ± 0.21, P = .13). Among individuals with HDL-C less than 1.0 mmol/L, *Asp299Asp* carriers had significantly higher log (HOMA-IR) levels (0.31 ± 0.24) than noncarriers (0.19 ± 0.23, P = .012).

The QUICKI levels in individuals carrying *Asp299Asp* were significantly lower (0.32 ± 0.03) in comparison with noncarriers (0.33 ± 0.04, P = .021). This difference was more evident among individuals with HDL-C less than 1.0 mmol/L (0.32 ± 0.03 vs 0.35 ± 0.04, P = .0037).

3.4. Mediation analyses

According to the test of Sobel, log(insulin) mediated 34.1% of the effects of *Asp299Asp* genotype on MS (P = .040, ratio of the indirect to the direct effect = 0.52).

The log(insulin) mediated 25.9% of the effects of *Asp299Asp* genotype on OLS (P = .019, ratio of the indirect to the direct effect = 0.35). Although log(insulin) did not mediate the effects of *Asp299Asp* genotype on EWET (P = .059), 33.3% of the effects of *Asp299Asp* on EWET were mediated by QUICKI (P = .030, ratio of the indirect to the direct effect = 0.52). Similar results were found with other surrogates of IR.

Table 3
rs4986790 and SLAs: crude and bootstrapped (32 000 samples) χ^2 analyses

	OLS		Crude			Bootstrapped (32 000 samples)		
	OLS positive (n = 276)	OLS negative (n = 345)	OR	95% CI	P	OR	95% CI	P
Genotypes								
Asp299Gly Gly299Gly[a]	18	42	1.0 (ref)			1.0 (ref)		
Asp299Asp	258	303	1.99	1.12-3.51	.018	1.99	1.13-3.48	.017
χ^2 (df 2)					.022			.020
	EWET		Crude			Bootstrapped (32 000 samples)		
	EWET positive (n = 174)	EWET negative (n = 447)	OR	95% CI	P	OR	95% CI	P
Genotypes								
Asp299Gly Gly299Gly[a]	10	50	1.0 (ref)			1.0 (ref)		
Asp299Asp	164	397	2.07	1.04-4.12	.039	3.00	1.36-6.59	.005
χ^2 (df 2)					.021			.002
	HW		Crude			Bootstrapped (32 000 samples)		
	HW positive (n = 131)	HW negative (n = 490)	OR	95% CI	P	OR	95% CI	P
Genotypes								
Asp299Gly Gly299Gly[a]	10	50	1.0 (ref)			1.0 (ref)		
Asp299Asp	121	440	1.38	0.69-2.76	.38	1.39	0.67-2.85	.38
χ^2 (df 2)					.081			.054

[a] Because of the small number, *Gly299Gly* (n = 1) and *Asp299Gly* carriers have been combined.

3.5. CRP concentration, MS, Asp299Gly, surrogates of IR, and SLAs

The CRP concentration was 2.41 ± SD 3.58 mg/L (n = 545). CRP was significantly higher among individuals with MS (3.11 mg/L) compared with individuals without MS (2.12 mg/dL, P = .0027) in ANCOVA age- and BMI-adjusted analyses. This association was enhanced among individuals with CRP levels below the 95th percentile (7.865 mg/L) (2.37 vs 1.59 mg/L, P < .0001) and with TC/HDL-C ratio ≥5 (2.48 vs 1.41 mg/L, P = .0002), but not above this percentile (P = .30). The CRP level was not different between individuals with (2.70 mg/L) and without high TG (NCEP/ATP III criteria, 2.26 mg/L, P = .19); but among individuals with CRP below the 95th percentile, the criterion high TG was associated with a significantly higher CRP level (2.03 vs 1.71 mg/L, P = .0217). Similar relationships were found for the criteria high FPG (2.18 vs 1.73 mg/L, P = .0091), low HDL-C (2.13 vs 1.54 mg/L, P < .0001), and high WC (2.43 vs 1.58 mg/L, P < .0001), and for TC/HDL-C ratio at least 5 (2.21 vs 1.52 mg/L, P < .0001).

The CRP concentration was not different between individuals carrying *Asp299Asp* (2.43 ± 3.70 mg/L) and noncarriers (2.21 ± 2.27 mg/L, P = .67). Among individuals with high TG and CRP levels below the 75th percentile (2.83 mg/L), *Asp299Asp* carriers showed a significantly higher CRP level (1.34 mg/L) compared with noncarriers (0.81 mg/L, P = .0197). *Asp299Asp* was significantly associated with a higher risk of MS among individuals with CRP levels below the 95th percentile (OR = 2.21; 95% CI, 1.03-4.75; P = .0419), especially among individuals with TC/HDL-C ratio ≥5 (OR = 3.80; 95% CI, 1.38-10.38; P = .0081). *Asp299Asp* was significantly associated with a higher WC (100.65 cm) compared with noncarriers (96.95 cm, P = .0049) among individuals with CRP below the 95th percentile and TC/HDL-C ratio ≥5. Multivariate analyses using logistic regression showed that *Asp299Asp* (OR = 4.19; 95% CI, 1.50-11.67; P = .0062) and CRP levels (OR = 1.17; 95% CI, 1.01-1.36; P = .038) were significantly associated with a high risk of MS among individuals with CRP below the 95th percentile, but not above this percentile (P = .83 and 0.49, respectively). The multivariate association between *Asp299Asp* and MS was significant among individuals with both CRP below the 95th percentile and TC/HDL-C ratio ≥5 (OR = 4.58; 95% CI, 1.31-16.06; P = .0173).

CRP was significantly associated with most surrogates of IR such as insulin (P < .0001), log(insulin) (P < .0001), HOMA-IR (P < .0001), log(HOMA-IR) (P < .0001), and TG/HDL-C ratio (P < .0001), but not 40/insulin (P = .25) and QUICKI (P = .10), in regression age- and BMI-adjusted analyses. These findings were especially significant for most surrogates of IR, including QUICKI and 40/insulin, among individuals with CRP levels below the 95th percentile (or the 90th percentile [5.051 mg/L]), or among individuals with both CRP levels below the 95th percentile (or 90th percentile) and TC/HDL-C ratio ≥5 or high TG (data not shown).

The CRP concentration was higher among individuals with SLAs such as HW (3.09 vs 2.22 mg/L, P = .017), OLS (2.82 vs 2.07 mg/L, P = .013), and EWET (borderline significant, 2.77 vs 2.18 mg/L, P = .059). These relationships were especially significant for all SLAs, including EWET, among individuals with CRP levels below the 95th percentile and/or among individuals with both CRP levels below the 95th percentile and TC/HDL-C ratio ≥5 or high TG (data not shown). The associations between *Asp299Asp* and SLAs were observed among individuals with CRP levels below the 95th percentile (or 90th percentile) or among individuals with both CRP levels below the 95th percentile (or 90th percentile) and TC/HDL-C ratio ≥5 or high TG (data not shown).

4. Discussion

This is the first report that demonstrates significant associations between the rs4986790 TLR4 gene polymorphism and the risk for NCEP/ATP III-MS, IR, and SLAs in white healthy blood donor men from Buenos Aires. *Asp299Asp* carriers had higher prevalence of NCEP/ATP III-MS, lower QUICKI and 40/insulin level indexes, higher log (insulin) values, and higher prevalence of EWET and OLS, especially among individuals with CRP below the 95th percentile and/or TC/HDL-C ratio ≥5.

HW, OLS, and EWET have been associated with risk of cardiovascular disease and T2D [11], [16]. Unfortunately, the SLAs have been overlooked in both cross-sectional and longitudinal genetic and nongenetic studies. It would be interesting to explore whether candidate genes for MS and T2D, such as PPARG, are associated with SLAs, as a proxy for MS and related diseases as T2D. Indeed, it was suggested that exploring the genetic basis of complex diseases might be less difficult if the association studies focus on selected simple pathogenic traits during the predisease period rather than on descriptive parameters after disease diagnosis [17].

Our findings are supported by clinical and experimental evidence. Activation of TLR4 induces up-regulation of inflammatory pathways related to the induction of IR, such as IKKB/IκBα/NF-κB and JNK [3]. Loss-of-function mutation in TLR4 prevented diet-induced obesity and IR in C3H/HeJ mice [4]. Muscle TLR4 overexpression has been reported in individuals with IR [5]. In accordance with our findings, *Asp299* allele has been associated with high risk for T2D, a condition always preceded by inflammation, components of the MS, and IR, in white populations [18,19]. In agreement, TLR4 haplotypes, which included polymorphisms such as rs4986791 (*Thr399Ile*), were associated with high risk for T2D among individuals with high TC/HDL-C ratio levels [20]. In this report, the assay for *Asp299Gly* failed; but it was tagged by *Thr399Ile*, which is in high linkage disequilibrium with *Asp299Gly*.

We cannot exclude the possibility that the associations found in our study are related to the presence of a nearby

gene, such as cyclooxygenase 1 (proinflammatory gene very close to the TLR4 locus, OMIM ∗176805), or a nearby functional polymorphism within the TLR4 gene, such as *Thr399Ile*, in high linkage disequilibrium with *Asp299Gly*. Functional analyses suggested that *Asp299Gly* polymorphism might have a greater functional impact than *Thr399Ile* marker [6]. In silico analyses using the Functional Single Nucleotide Polymorphism database strongly suggest the functional role of rs4986790 (http://compbio.cs.queensu.ca/F-SNP/).

A German study did not found any association between rs4986790 and T2D or components of MS expressed as continuous variables [21]. However, these findings are not comparable with our results because the German study did not explore correlations between this genetic marker and NCEP/ATP III-MS, surrogates of IR, or SLAs and, importantly, included individuals 3 decades older and a smaller sample size of healthy subjects.

Because the *Gly299* allele has been associated with decreased transcriptional activity of TLR4 [22], we hypothesize that detrimental effects of inflammatory stimuli on adipose tissue, especially on visceral fat, were higher in individuals carrying *Asp299* than in noncarriers, thus explaining the significant associations found between *Asp299Asp* and high risk for MS, IR, and SLAs.

Mediation analyses confirmed that surrogates of IR, such as log(insulin), mediated approximately 25% to 35% of the effects of *Asp299Asp* on MS, OLS, and EWET. In agreement, TLR4 overexpression may induce, besides IR, several molecular alterations in the cellular milieu including excess of inflammatory cytokines, acute-phase reactants, hemostatic factors, and soluble adhesion molecules, among others [7,22]. In this sense, we also found a subtle association between *Asp299Asp* and higher CRP levels only among individuals with both high TG and CRP levels below the 75th percentile, which is in part supported by some [18,22,23], but not all, previous reports [21,24,25]. Of note, the association between *Asp299Asp* and MS was observed among individuals with CRP levels below the 95th percentile and TC/HDL-C ratio ≥5 (and, to a lesser extent, high TG), as well as the associations between CRP and MS (and some of its components such as WC). This means that potential interactions among *Asp299Asp*, MS (and its components), and CRP were suggested only excluding the high-extreme CRP phenotype. In accordance, this genetic marker (and TLR4 haplotypes including 9 polymorphisms) was not associated with CRP levels comparing extremely low (<0.2 mg/L) and high (>5 mg/L) CRP levels in white healthy women [25]. Indeed, extremely high CRP levels have been thought to be unreliable because of the likely presence of unrecognized acute inflammation [26]. On the other hand, although CRP is a heterogeneous molecule (ie, pentameric and monomeric forms), there is no reliable commercial assay to well distinguish between the different biological forms of CRP. Ignoring this fact may lead to reductionistic interpretations of artifactual findings or detecting false relationships between CRP levels and variables of interest [26]. In addition, CRP acts not only as an endocrine (systemic) inflammatory marker, but also as a paracrine (local) factor [27], thus increasing complexity even further.

This study has some limitations. The sample size was relatively small. However, this limitation was in part resolved by means of bootstrapping procedures for most χ^2-derived findings.

In conclusion, we have reported novel associations between the functional rs4986790 TLR4 gene polymorphism and the risk for NCEP/ATP III-MS, IR, and SLAs in white healthy blood donor men from Buenos Aires, Argentina, especially among individuals with CRP levels below the 95th percentile and/or TC/HDL-C ratio ≥5. Our findings should be viewed as a preliminary basis for future prospective studies in larger sample sizes and different ethnic groups.

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