

Association of Toll-like receptor 2, Toll-like receptor 4, and MyD88 gene polymorphisms with inflammation in subjects with metabolically healthy obesity

Asociación de polimorfismos de los genes TLR2, TLR4 y MyD88 con inflamación en sujetos con obesidad metabólicamente saludable

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Abstract

Objectives: The aim of this study was to determine the association between the polymorphisms of Toll-like receptors (TLR2), TLR4, and MyD88 with inflammation in individuals with metabolically healthy obesity (MHO). **Methods:** Men and non-pregnant women with obesity were included in a cross-sectional study. Then, subjects diagnosed with MHO were allocated into the groups with and without inflammation. Low-grade inflammation was defined by C-reactive protein concentrations $\geq 3 < 10$ g/dL. **Results:** A total of 64 subjects with MHO were enrolled and allocated into the groups with ($n = 37$) and without ($n = 27$) inflammation. The distribution of alleles and genotypes were not significantly different for the TLR4 rs11536889, TLR2 rs380499, and MyD88 rs7744 polymorphisms between the study groups. However, the genotypes G/A and G/A + A/A of the TLR4 rs10759931 polymorphism were significantly associated with inflammation. **Conclusions:** The TLR4 rs10759931 polymorphism, but not TLR4 rs11536889, TLR2 rs380499, and MyD88 rs7744, is associated with low-grade inflammation in MHO.

Keywords: Obesity. Inflammation. Toll-like receptors. MyD88. Genetics.

Resumen

Objetivo: Determinar la asociación entre los polimorfismos de TLR2, TLR4 y MyD88 con la inflamación de bajo grado en individuos con obesidad metabólicamente saludable (OMS). **Métodos:** Estudio transversal en el que se incluyeron hombres y mujeres no embarazadas con obesidad. Los sujetos diagnosticados con OMS fueron asignados a grupos con y sin inflamación. La inflamación de bajo grado se definió por unas concentraciones de proteína C reactiva ≥ 3 y < 10 g/dl. **Resultados:** Se incluyeron 64 sujetos con OMS en los grupos con ($n = 37$) y sin ($n = 27$) inflamación. La distribución de alelos y genotipos no fue significativamente diferente para los polimorfismos TLR4 rs11536889, TLR2 rs380499 y MyD88 rs7744 entre los grupos de estudio. Sin embargo, los genotipos G/A y G/A + A/A del polimorfismo TLR4 rs10759931 se asociaron significativamente con inflamación. **Conclusiones:** El polimorfismo TLR4 rs10759931, pero no TLR4 rs11536889, TLR2 rs380499 y MyD88 rs7744, se asocia con inflamación de bajo grado en sujetos con OMS.

Palabras clave: Obesidad. Inflamación. TLR. MyD88. Genes.

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Introduction

In the past years, obesity has become an increasing public health problem worldwide, and it is expected to affect around 20% of the global population by 2030.¹ The development of obesity includes increasing body fat and weight gain, which, in turn, leads to chronic inflammation in the adipose tissue, but also in other tissues such as pancreas, liver, and brain. The inflammatory process in obesity induces disturbances in glucose and lipid metabolism, as well as insulin and leptin resistance, resulting in the development of chronic diseases including type II diabetes.²

Derived from several clinical studies, it has been identified a subgroup of subjects who despite excessive body fat accumulation, they do not exhibit cardiometabolic abnormalities commonly associated with obesity, emerging the concept of metabolically healthy obesity (MHO) for this phenotype.³ The biological mechanisms underlying MHO include less visceral and hepatic ectopic fat deposition, higher expandability of subcutaneous adipose tissue, and leg fat deposition. In addition, the close link between metabolism and innate immunity has explained the association between chronic subclinical inflammation and obesity.⁴ The Toll-like receptors (TLRs) are a family of immune system receptors that recognize evolutionarily conserved pathogen-associated molecular patterns and activate the innate immune response.⁵ Furthermore, TLRs may play a central role in obesity-related metabolic disorders by increased expression and activity of TLR2 and TLR4 in adipocytes.⁶

On the other hand, MyD88 is an essential signaling adaptor for most TLRs and the interleukin-1 (IL-1) receptor family.⁷ In this regard, MyD88-dependent pathway induces the synthesis and secretion of pro-inflammatory cytokines through activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B).⁸ It has been reported that the lack of *TLR4* gene or *MyD88* gene is associated with reduced levels of pro-inflammatory cytokines in mice deficient in apolipoprotein E.⁹ Furthermore, the interaction between *TLR4* rs11536889 and *MyD88* rs7744 is related with an increased risk of coronary artery disease, which may be increased by both hyperglycemia and hyperlipidemia.¹⁰ Moreover, *TLR2* rs380499 has been associated with subclinical systemic inflammation by increased C-reactive protein levels in children with gastritis.¹¹ However, to the best of our knowledge,

the polymorphisms of *TLR2*, *TLR4*, and *MyD88* has not been examined in terms of MHO; hence, we investigated whether these polymorphisms are associated with the presence of low-grade inflammation in subjects with MHO.

Methods

This protocol was approved by the National Scientific Research Committee (R-2017-785-078) in accordance with the principles of the Declaration of Helsinki and its later amendments. After obtaining the informed consent signed by the participants, a cross-sectional study was conducted.

Men and non-pregnant women with obesity (body mass index [BMI] ≥ 30 kg/m²) aged 20-55 years were consecutively recruited from the general population of Durango, a city in northern Mexico. Then, subjects with MHO were allocated into the groups with and without low-grade inflammation.

Pregnancy, smoking, alcohol consumption, intense physical activity or sexual intercourse in the previous 72 h, diabetes, high blood pressure, cancer, thyroid disease, acute or chronic infections, renal impairment, and hepatic diseases were exclusion criteria.

Definitions

The presence of MHO was defined by BMI ≥ 30 kg/m² plus one or none of the following cardiovascular risk factors: waist circumference (WC) ≥ 98 cm in men or ≥ 84 cm in women; fasting glucose ≥ 100 mg/dL; triglyceride levels ≥ 150 mg/dL; high-density lipoprotein cholesterol (HDL-C) <40 mg/dL in men or < 50 mg/dL in women; and systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg.¹²

Low-grade inflammation was defined by serum high-sensitivity C-reactive protein (hsCRP) levels $\geq 3 < 10$ g/dL.

Measurements

Anthropometric measurements were performed with the subjects in fasting conditions, standing position, light clothing, and without shoes. Weight and height were measured using a fixed scale with stadiometer (Tanita TBF-215, Tokyo, Japan). The BMI was calculated as weight (kilograms) divided by height (meters) squared. The WC was measured to the

nearest centimeter; the anatomical landmarks used were midway between the lowest portion of the rib cage and the superior border of the iliac crest.

Following the recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, the blood pressure was measured using a sphygmomanometer (Microlife AG, Heerbrugg Switzerland) and stethoscope (3M Littman Classic II, Neuss, Germany).¹³

Assays

The hsCRP was determined by ultra-sensitive competitive immunoassay (Dade Behring, Darmstadt, Germany) with an inter- and intra-assay coefficients of variation of 3.9% and 4.8%, respectively.

Serum glucose was measured using the glucose-oxidase method (intra- and inter-assay coefficients of variation of 1.2% and 1.4%). Triglycerides (inter- and intra-assay coefficients of variation of 1.8% and 2.7%) and total cholesterol (inter- and intra-assay coefficients of variation of 1.9% and 2.9%) were determined by enzymatic methods. The HDL-c fraction was obtained after precipitation by phosphotungstic reagent with and inter- and intra-assay coefficients of variation of 1.4% and 3.1%, respectively. All measurements were performed using an A15 Clinical Analyzer (Biosystems, USA).

DNA extraction and TLR2, TLR4, and MyD88 genotyping

Genomic DNA was extracted from whole blood using a procedure for rapid DNA isolation.¹⁴ The DNA concentration and purity were measured by a Nano-Drop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Germaring, Germany). Genotyping of the SNP rs11536889, rs10759931, rs380499, and rs7744 was performed with StepOne real-time PCR equipment (Applied Biosystems, Carlsbad, CA, USA) using TaqMan assays (SNP ID C__31784034_10, C__2704046_10, C__3007345_10 and C__3094830_10, respectively). The polymerase chain reaction was performed to a final volume of 20 μ L per well containing 10 ng of genomic DNA, 0.625 μ L of Taqman SNP genotyping assay, and 5 μ L of genotyping master mix. Amplification consisted of a first step at 60°C for 30 s and 95°C for 10 min,

followed by 40 cycles of 92°C for 15 s and 60°C for 1 min, and a final step of 60°C for 30 s.

Statistical analysis

Numerical values are reported as mean $\pm$ standard deviation and categorical variables as proportions.

Differences between the study groups were determined by Student's t test (Mann–Whitney U-test for skewed data) for numerical variables and χ^2 test for qualitative variables. Genotype frequencies were tested using the Hardy-Weinberg equilibrium. Odds ratios (ORs) were calculated using the Fisher's exact test to evaluate the association between genotypes and inflammation. Statistical significance was estimated based in 95% confidence interval and/or $p < 0.05$. Data were processed in the Statistical Package for the Social Sciences version 20.0 for Windows.

Results

A total of 647 individuals were screened and 583 were excluded because they did not meet the selection criteria (Fig. 1). Thus, 64 subjects with MHO were enrolled and allocated into the groups with ($n = 37$) and without ($n = 27$) inflammation.

Table 1 shows the characteristics of the study population. Individuals with MHO and inflammation had a higher BMI than those into the group without inflammation; there were no significant differences for other variables between the study groups.

Frequencies of TLR2, TLR4, and MyD88 gene polymorphisms in the study groups are presented in table 2. The distribution of alleles and genotypes were not significantly different for the TLR4 rs11536889, TLR2 rs380499, and MyD88 rs7744 polymorphisms between the study groups. However, the genotypes G/A and G/A + A/A of the TLR4 rs10759931 polymorphism were significantly associated with inflammation.

Discussion

Results of this study show that the TLR4 rs10759931 polymorphism, but not TLR4 rs11536889, TLR2 rs380499, and MyD88 rs7744, is associated with low-grade inflammation in individuals with MHO.

It has been hypothesized that the MHO phenotype is a transient condition that progress with time to unhealthy obesity and that this transition could be

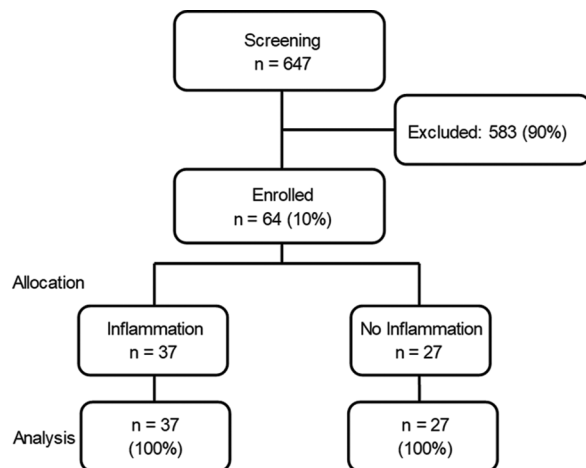


Figure 1. Flow chart for selection of study participants.

Table 1. Characteristics of the study population n = 64

Variables	Inflammation	No inflammation	p
	n = 37	n = 27	
Age, years	38.3 ± 8.8	38.1 ± 10.1	0.93
Women, n (%)	32 (86)	24 (89)	0.21
Waist circumference, cm.	104.5 ± 11.6	101.1 ± 10.2	0.21
Body mass index, kg/m ²	35.4 ± 3.9	33.0 ± 3.3	0.01
Body fat, %	43.6 ± 4.8	41.9 ± 5.0	0.17
Systolic blood pressure, mmHg	113.3 ± 10.4	113.0 ± 8.9	0.88
Diastolic blood pressure, mmHg	73.5 ± 7.2	75.1 ± 5.7	0.33
Fasting glucose, mg/dL	94.2 ± 7.8	91.5 ± 6.0	0.12
HDL-C, mg/dL	51.9 ± 10.8	52.7 ± 9.0	0.73
Triglycerides, mg/dL	122.5 ± 43.2	124.0 ± 54.1	0.90
C-reactive protein, mg/L	6.1 ± 1.8	2.1 ± 0.6	< 0.001

Data are mean ± standard deviation unless otherwise indicated. HDL-C: high-density lipoprotein cholesterol.

related with obesity-related mediators of inflammation.¹⁵⁻¹⁷ In this regard, a recent study reported that CD95+CD86- macrophages might trigger inflammation pathways that could be linked with transition of MHO to unhealthy obesity.¹⁸ Our results, showing that TLR4 *rs10759931* polymorphism is associated with inflammation in individuals with MHO, suggests that signaling pathway of TLR4 could be involved with the transition to unhealthy obesity. To the best of our

knowledge, there are no previous reports on this matter. Given that the polymorphism G/A (OR 6.82) and G/A+A/A (OR 7.36) of TLR4 *rs10759931* were strongly associated with elevated levels of hsCRP in subjects with MHO, our results suggest that allele A is the allele of risk.

With this regard, results about association of the SNP *rs10759931* with inflammation are controversial. Some studies show that TLR4 *rs10759931* polymorphism is associated with the increased expression of TLR4 and chronic inflammation in colon cancer, cardiovascular diseases, and diabetic retinopathy,¹⁹⁻²¹ but others studies show no association.²² It is possible that TLR4 *rs10759931* polymorphism induce the acute secretion of pro-inflammatory cytokines mediated by NF-κB pathway²¹ and or suppression of the Toll-IL-1 receptor domain-containing adapter inducing interferon-β-dependent pathways.²³

About the TLR4 *rs11536889*, it has been observed that the genotype (C/C) increases the TLR4 protein levels²⁴ and that, in Japanese and Chinese population, the C allele is associated with inflammatory diseases.^{25,26} In our population, the frequency of C/C polymorphism was found only in one patient and the G/C and G/C+C/C genotypes were similar between the groups with and without inflammation. Given that frequency of alleles and genotypes varies according the studied population, to elucidate the role TLR4 *rs11536889* in the triggering of inflammation and transition to unhealthy obesity, a large sample size will be required.

It has been reported that the TLR2 *rs3804099* polymorphism is associated with inflammation-related diseases such as severe coronary atherosclerosis and infection by *Helicobacter pylori*, as well as the increased risk of malignancy by *H. pylori* infection.²⁷⁻²⁹ Some studies show that the G/G genotype of the MYD88 *rs7744* polymorphism is associated with increased risk of coronary heart disease.¹⁰ In our study, the C and T alleles of the TLR2 *rs3804099* as well as the A and G alleles of MyD88 *rs7744* polymorphisms were similar between the groups in study. This finding suggests that both polymorphisms do not play an important role in the triggering of inflammation in individuals with MHO. Undoubtedly, further research is needed to verify this hypothesis and clarify the role of TLR signaling pathways in the transition from MHO to unhealthy obesity.

Otherwise, our study has some limitations that should be taken into account. Given that the frequency

Table 2. Frequencies of TLR2, TLR4, and MyD88 gene polymorphisms in the study groups

Polymorphism	Inflammation n (%)	No inflammation n (%)	OR	95% CI	p
TLR4 gene					
rs11536889					
G	65 (16)	49 (91)	Ref		
C	9 (84)	5 (9)	1.35	0.38-5.01	0.77
G/G	29 (78)	22 (81)	Ref		
G/C	7 (19)	5 (19)	3.42	0.69-17.29	1.00
C/C	1 (3)	0 (0)	0	0	1.00
G/C+C/C	8 (22)	5 (19)	3.91	0.82-19.24	1.00
rs10759931					
G	33 (45)	33 (61)	Ref		
A	41 (55)	31 (39)	1.32	0.63-2.73	0.51
G/G	2 (5)	8 (30)	Ref		
G/A	29 (78)	17 (63)	6.82	1.12-31.52	0.01
A/A	6 (16)	2 (7)	12.00	0.92-188.46	0.05
G/A+A/A	35 (94)	19 (70)	7.36	1.23-32.91	0.01
TLR2 gene					
rs380499					
T	54 (73)	38 (70)	Ref		
C	20 (17)	16 (30)	0.87	0.37-2.05	0.90
T/T	18 (49)	14 (7)	Ref		
T/C	18 (49)	10 (26)	1.40	0.43-4.52	0.71
C/C	1 (2)	3 (67)	0.25	0.004-3.76	0.32
T/C+C/C	19 (51)	13 (93)	1.13	0.37 – 3.45	1.00
MyD88 gene					
rs7744					
A	65 (88)	43 (80)	Ref		
G	9 (12)	11 (20)	0.54	0.18-1.55	0.22
A/A	31 (84)	18 (67)	Ref	0.04-1.27	0.07
A/G	3 (8)	7 (26)	0.24		
G/G	3 (8)	2 (7)	0.87	0.10-5.80	1.00
A/G+G/G	6 (16)	9 (33)	0.38	0.10-1.45	0.14

OR: odds ratio; CI: confidence interval.

of the MHO phenotype in the target population was low, the sample size was small; therefore, future studies in large samples are mandatory. Furthermore, the frequencies of some genotypes were low, resulting in limited data to evaluate the association between genotypes and inflammation; thus, our results should be considered preliminary.

Conclusions

The results of the present study revealed that the TLR4 *rs10759931* polymorphism, but not TLR4 *rs11536889*, TLR2 *rs3804099*, and MyD88 *rs7744*, is associated with low-grade inflammation in individuals with MHO.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical considerations

Protection of human subjects and animals. The authors declare to have followed the ethical standards of the relevant experimentation committee, according to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. This study does not involve personal patient data, medical records, or biological samples, and does not require ethical approval. SAGER guidelines do not apply.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial

intelligence was used in the writing or creation of the content of this manuscript.

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