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Interleukin-6 gene promoter polymorphisms and cardiovascular risk factors. A family study

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Abstract. Interleukin-6 (IL-6) is a cytokine involved in inflammatory process, as well as in glucose and lipid metabolism. Several studies of the biological relevance of IL-6 gene polymorphisms have indicated a relationship with cardiovascular disease. The aim of this study was to assess whether the –174 G/C and –572 G/C of IL-6 gene polymorphisms are associated with cardiovascular risk factors in Mexican families. Ninety members of 30 Mexican families, in which an index case (proband) had obesity, were included in the study. We evaluated the body composition by bioelectrical impedance. Peripheral blood samples were collected to determine biochemical and hematological parameters. High sensitivity C- reactive protein levels were measurement for nephelometric analysis. Screening for both polymorphisms studied was performed by PCR-RFLP. In the parents, both polymorphisms were in Hardy-Weinberg's equilibrium. The genotypes –174 GC/CC were associated with T2D (OR = 1.23, $IC_{95\%}$ 1.01–1.5) and highest levels of hsCRP ($p = 0.02$), whereas genotype –572 GG was associated with T2D (OR = 1.24, $IC_{95\%}$ 1.04–1.47) with an inflammatory state determined by the increase in the leukocyte count (OR = 1.24, $IC_{95\%}$ 1.02–1.51). The genotypes –174 GC/CC and –572 GG may confer susceptibility for the development of subclinical inflammation and type 2 diabetes in Mexican families.

Keywords: Polymorphisms, Interleukin-6, diabetes, inflammation, family study

1. Introduction

In Mexico, the type 2 diabetes (T2D), cardiac ischemic and cerebrovascular diseases occupy the first places of mortality [1]. Subclinical chronic inflammation is an important mechanism in the pathogenesis of the cardiovascular disease [2] and T2D [3,4]. Increased circulating levels of proinflammatory cytokines have been found in subjects with obesity and/or insulin resistance. Interleukin-6 (IL-6) is a pleiotropic cytokine produced in many types of cells including the adipocytes [5–7]. IL-6 has a key role in stimulating the acute-phase response which elevates the circulating levels of several plasma proteins such as C-reactive protein (CRP) [8]. Furthermore, IL-6 has important effects on glucose [9,10] and lipid metabolism [11,12]. There is evidence that the increase in IL-6 levels is associated not only with T2D but also with impaired glucose tolerance and insulin resistance. The high rate of plasma clearance of IL-6 suggests that the IL-6 concentration is regulated mainly at transcriptional and translational levels [13,14].

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The –174 G/C and –572 G/C IL-6 gene polymorphisms have been proposed as risk factors for type 2 diabetes based on studies of unrelated individuals. These results have been conflicting. Although the genotypes –174 CC and –572 CC polymorphisms have not been associated with T2D in some populations, both have been associated with the increase in the levels of IL-6 [15] and CRP [16], favouring an inflammation state that suggest a relationship between inflammation and diabetes.

Recently, much interest has been focused on the use of family-based association studies to avoid the problem of ethnic confounding [17]. In this study, the analysis of case-parents trios was used to assess whether the –174 G/C and –572 G/C of IL-6 gene polymorphisms are associated with cardiovascular risk factors and subclinical chronic inflammation in Mexican families.

2. Subjects, materials and methods

We recruited 90 members of 30 Mexican families in which a proband had obesity. The families were integrated by case-parents trios. Probands were $>$ 18 years old, with obesity (BMI $>$ 30 kg/m^2). Parents obese or non-obese were included. All families' members were recruited with the presence of one or more of traditional cardiovascular risk factors. The participants were all born in the State of Guerrero, Mexico, with a family history of guerrerenses ancestors, at least back to the third generation. Subjects with evidence of infectious disease or with any treatment that could influence the biochemical and haematological parameters were excluded from the study. Informed written consent was obtained from all subjects before enrollment in the study. Approval for the study was obtained from the Research Ethics Committee of the University of Guerrero.

We evaluated the body composition by bioelectrical impedance (Tanita TBF-300 GS). We defined hypertension by agreement with Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults [18] (BP $\geqslant$ 130/85 mmHg). Type 2 diabetes was defined if fasting serum glucose was $\geqslant$ 126 mg/dL in accord with the American Diabetes Association (ADA) [19].

A fasting blood sample was obtained from each subject and laboratory measurements were available from all subjects. Total serum cholesterol, triglycerides, albumin and glucose levels were obtained using routine biochemical analysis. Hematological parameters were assessed by ADVIA-60 (Bayer Diagnostics). High sensitivity C-reactive protein levels were measured by nephelometric assay (Dade Behring). The intra- and interassay coefficients of variation of CRP were $<$ 4.4% and $<$ 5.7%, respectively, and the analytical sensitivity was 0.175 mg/L.

Analysis of the –174 G/C and –572 G/C of IL-6 gene polymorphisms were performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The –174 G/C polymorphism was detected using the following primers: 5'TGACTTCAGCTTTACTCTTTGT3' (forward) and 5'CTGATTGGAAACCTTATTAAG3' (reverse) under standard conditions using 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.2 μM of each primer, 2.0 U of Taq DNA polymerase (Invitrogen Life Technologies). PCR reaction was initiated by an initial denaturation (5 min at 94 °C), followed by amplification for 35 cycles of denaturation (1 min at 94°C), annealing (1:2 min at 53°C), and extension (1:2 min at 72°C), and a final extension step (5 min at 72°C). The amplified product 198 bp was digested with *SfaNI* according to the manufacturer's instructions (New England Biolabs) and separated on a 3% agarose gel. The CC genotype lacking the *SfaNI* site migrated as a 198 bp fragment, whereas the GG genotype was cleaved and appeared as 140 and 58 bp fragments (Fig. 1).

The –572 G/C polymorphism was detected using the following primers: 5'GGAGACGCCTTGAAGTAACTGC3' (forward) and 5'GAGTTTCCTCTGAC TCCATCGCAG3' (reverse) under standard conditions using 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.2 μM of each primer, 2.0 U of Taq DNA polymerase (Invitrogen Life Technologies). A PCR reaction was initiated by an initial denaturation (5 min at 94°C), followed by amplification for 35 cycles of denaturation (1 min at 94 °C), annealing (1 min at 55°C), and extension (1 min at 72°C), and a final extension step (5 min at 72°C). The amplified product 163 bp was digested with *BsrBI* according to the manufacture's instructions (New England Biolabs) and separated on a 3% agarose gel. The CC genotype lacking the *BsrBI* site migrated as a 163 bp fragment, whereas the GG genotype was cleaved and appeared as 102 and 61 bp (Fig. 1).

The statistical descriptive analysis was performed using chi-square test for categorical variables, mean and standard deviation for symmetrical quantitative variables, median and percentile 5 and 95 for non symmetrical variables. The significance of differences between groups of relatives (fathers, mothers, sons and

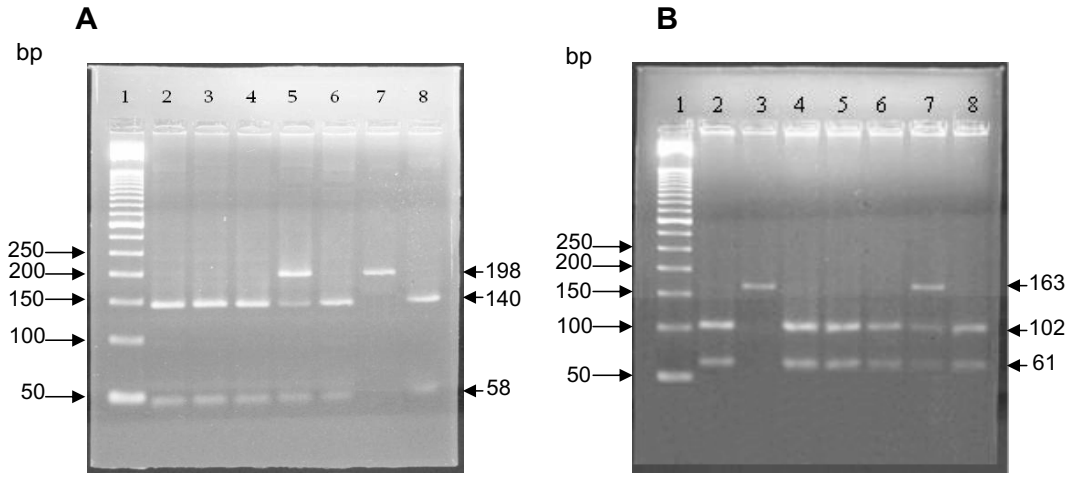

Fig. 1. Panel A. Agarose gel electrophoresis showing pattern restriction of −174 G/C IL-6 polymorphism. Lane 1: 50 bp DNA ladder; lanes 2,3,4,6 and 8: GG genotype; lane 5: GC genotype; and lane 7: CC genotype. Panel B. Agarose gel electrophoresis showing pattern restriction of -572 G/C IL-6 polymorphism. Lane 1: 50 bp DNA ladder; lanes 2,4,5,6 and 8: GG genotype; lane 7: GC genotype; and lane 3: CC genotype.

daughters) was determined using Student's t test (two-tailed) and Mann Whitney. The genotype and allele frequencies for both polymorphisms were determined by direct counting and the significance of the differences between the biochemical and anthropometric parameters for each genotype was determined using ANOVA and by Kruskal-Wallis. Chi-square test was used to evaluate the Hardy-Weinberg equilibrium.

As individuals within a family are not independent, statistical analyses was based on the generalized estimating equation (GEE) technique [20–22], using the STATA v.12 software. Correlations between several variables and hsCRP and other inflammatory markers were assessed with the Pearson's correlation coefficient after logarithmic transformation. Multiple regression generalized analysis was used to determine biological factor variations with –174 G/C and –572 G/C polymorphisms. The models were adjusted for confounding variables: age, fat mass, smoking, exercise, family history of diabetes, obesity and cardiovascular disease. $P < 0.05$ was reported as statistically significant.

3. Results

The 30 Mexican families were integrated by case-parents trios, in which a proband had obesity. The demographic, clinical and biochemical characteristics of all family members are shown in Table 1. The mothers had a significantly higher weight and body fat percentage than the fathers. Between sons and daughters, the sons showed high weight, waist circumference and triglyceride levels, but the daughters had the highest body fat percentage and platelet count. Between mothers and daughters, a significant increase was observed in systolic blood pressure and triglyceride levels in the mothers, whereas the daughters showed elevated platelet count.

The genotype frequencies of –174 G/C IL-6 polymorphism were 76.7% GG, 20% GC, and 3.3% CC. The G and C allele frequencies were 86.7% and 13.3%, respectively. For the –572 G/C IL-6 polymorphism, the frequencies of GG, GC and CC genotypes were 61.1%, 30% and 8.9%, respectively, and the G and C allele frequencies were 76.1% and 23.9%,respectively. In the parents both polymorphisms were in Hardy-Weinberg's equilibrium ($X^2 = 1.09$, $p = 0.30$ for –174 G/C polymorphism, and $X^2 = 0.45$, $p = 0.50$ for –572 G/C polymorphism).

The association between inflammatory markers and some variables studied were assessed with Pearson's correlation coefficient. hsCRP level was logarithmic transformed and was related with BMI ($r = 0.42$, $p < 0.001$), fat mass ($r = 0.40$, $p < 0.001$), body fat percent ($r = 0.36$, $p < 0.001$) and waist circumference ($r = 0.23$, $p = 0.02$). The leukocyte count was associated with BMI ($r = 0.24$, $p = 0.02$), fat mass ($r = 0.27$, $p = 0.007$), waist circumference ($r = 0.23$, $p = 0.02$), diastolic blood pressure ($r = 0.31$, $p = 0.002$), triglyceride level ($r = 0.21$, $p = 0.04$) and hsCRP ($r = 0.23$, $p = 0.02$). The platelet count was only correlated with hsCRP ($r = 0.21$, $p = 0.04$).

Clinical, biochemical and inflammation variables were compared among family members and classi-

Table 1
Demographic, clinical and biochemical characteristics of all family members

Characteristics	Fathers n(30)	Mothers n(30)	$P^{*,a}$ F vs M	Sons n(14)	Daugthers n(16)	$P^{*,a}$ S vs D	$P^{*,a}$ (F *vs* S)	$P^{*,a}$ (M *vs* D)
Age (yr)	53(46–77)	51(40–71)	0.16	25.5(21–51)	26(18–47)	0.41	$< 0.001^{a}$	$< 0.001^{a}$
Weight (kg)	79.4 ± 11.5	80 ± 12.5	0.04*	98.0 ± 11.9	81.8 ± 10.4	0.002*	$< 0.001*$	0.02*
BMI (kg/m^2)	29.7 ± 4.2	30.7 ± 4.5	0.95	33.0 ± 2.8	33.4 ± 2.9	0.99	0.07	0.18
FM (kg)	25.8 ± 8.9	28.3 ± 8.3	0.98	34.4 ± 8.4	34.4 ± 7.1	1.0	0.01*	0.12
BF (%)	31.7 ± 7.0	39.1 ± 5.1	< 0.001*	35.1 ± 5.6	41.8 ± 3.3	0.01*	0.43	0.80
Waist circunference (cm)	100(87–116)	96(83–110)	0.03*	103.5(98–132)	98(90–115)	0.008*	0.06	0.25
Temperature (°C)	35.2(33.7–36.5)	35.5(34–36.6)	0.09	35.9(35.2–36.6)	35.9(35.2–36.7)	0.81	0.005^{a}	0.06
Sistolic BP (mmHg)	128.2 ± 18.9	126.1 ± 22.1	0.96	124.8 ± 9.1	110.8 ± 14.8	0.24	0.96	0.05*
Diastolic BP (mmHg)	80.2 ± 10.9	75.3 ± 11.6	0.64	79.2 ± 9.7	71 ± 14.1	0.33	1.0	0.98
Glucose (mg/dL	100(69–267)	97.5(71–287)	0.68	91.5(69–148)	85(70–193)	0.77	0.10	0.07
Total cholesterol (mg/dL)	217 ± 48.5	208.6 ± 44.6	1.0	222.6 ± 31.7	186.1 ± 32.9	0.12	1.0	0.55
Triglycerides (mg/dL)	200(87–600)	165.5(83–382)	0.11	189(125–444)	133.5(67–307)	0.003^{a}	0.88	0.01^{a}
Albumin (mg/dL)	4.6(2.3–5.5)	4.5(3.0–5.4)	0.91	4.5(2.5–5.7)	4.5(1.8–5.1)	0.44	0.47	0.95
hsCRP (mg/L)	2.87(0.58–17.1)	3.95(1.4–16.4)	0.10	3.68(0.38–17.0)	4.9(0.15–15.3)	0.61	0.57	0.90
Leukocyte count (x10^3/mm^3)	7.20 ± 1.43	6.97 ± 1.72	1.0	8.12 ± 2.16	7.76 ± 1.85	0.96	0.63	0.85
Platelet count (x10^3/mm^3)	268(155–350)	269.5(206–427)	0.36	266(173–410)	336(218–578)	0.03^{a}	0.98	0.03^{a}

For the parametrics variables are shown means $\pm$ SD, median and percentil 5 and 95 are shown for non parametrics variables. FM (Fat Mass), %BF(Body Fat Percentage).The P values between: F vs M = Fathers *vs* Mothers, S *vs* D = Sons *vs* Daughters, F *vs* S = Fathers *vs* Sons, M vs D = Mothers vs Daughters, were obtained with ANOVA* with test Bonferroni and with Mann-Whitney[a] test. $P^{*a} < 0.05$ was statistically significant.

Table 2
Clinical, biochemical and inflammatory variables in all family members classified according to –174 G/C IL-6 polymorphism

Variables	GG $n = 69$	GC+CC (18+3) $n = 21$	$P^{*,a}$ (GG vs GC+CC)
Weight (kg)	79.3 ± 14.2	82.1 ± 15.7	0.44
BMI (kg/m^2)	31.1 ± 4.2	31.7 ± 3.8	0.53
FM (kg)	29.2 ± 9.3	30.5 ± 7.7	0.59
BF (%)	36.3 ± 7.0	37.1 ± 6.4	0.64
Waist circunference (cm)	100(83–115)	100(89–116)	0.46
Temperature (°C)	35.4(33.8–36.6)	35.7(34.8–36.6)	0.20
Sistolic BP (mmHg)	123.6 ± 18.1	124.8 ± 22.3	0.79
Diastolic BP (mmHg)	76.3 ± 11.3	78.4 ± 13.8	0.48
Glucose (mg/dL)	92(70–199)	103(78–303)	0.08
Total cholesterol (mg/dL)	211.2 ± 44.9	205.5 ± 39.2	0.60
Triglycerides (mg/dL)	175(84–444)	177(79–382)	0.66
Albumin (mg/dL)	4.4(2.3–5.5)	4.8(3.4–5.4)	0.28
hsCRP (mg/L)	3.13(0.57–16)	6.39(1.18–16.4)	0.04^{a}
Leukocyte count (x10^3/mm^3)	7.30 ± 1.68	7.58 ± 1.98	0.52
Platelet count (x10^3/mm^3)	272(173–427)	267(222–418)	0.81

For the parametrics variables are shown means $\pm$ SD, median and percentil 5 and 95 are shown for non parametrics variables. FM (Fat Mass), %BF (Body Fat Percentage).The P values were obtained with Student's t test* and Mann Whitney[a]. $P^{*,a} < 0.05$ was statistically significant.

fied according to both IL-6 polymorphisms. For –174 G/C polymorphism, the GC and CC genotypes were grouped. The GC/CC group showed higher hsCRP levels than the GG group (6.39 *vs* 3.13 mg/L, $p = 0.04$) (Table 2). In comparison with the –572 G/C polymorphism, the GG carriers showed an increase in BMI ($p = 0.02$), body fat percent ($p = 0.02$), leukocyte count ($p = 0.01$) and platelet count ($p = 0.02$) (Table 3).

Table 3
Clinical, biochemical and inflammatory variables in all family members classified according to –572 G/C IL-6 polymorphism

Variables	GG $n = 55$	GC $n = 27$	CC $n = 8$	$P^{*,a}$ (GG vs GC vs CC)
Weight (kg)	80.7 ± 15.0	78.9 ± 11.9	77.8 ± 20.3	0.79
BMI (kg/m^2)	31.9 ± 3.8	31.1 ± 4.4	27.6 ± 4.3	0.02*
FM (kg)	30.5 ± 8.6	29.0 ± 9.4	24.1 ± 9.5	0.16
BF (%)	37.5 ± 6.2	36.3 ± 7.6	30.4 ± 5.0	0.02*
Waist circumference (cm)	100(88–116)	100(87–116)	95(81–111)	0.33
Temperature (°C)	35.6(33.9–36.6)	35.3(33.7–36.6)	35.8(33.2–36.2)	0.31
Sistolic BP (mmHg)	121.7 ± 19.8	124.9 ± 16.4	135.1 ± 20.0	0.16
Diastolic BP (mmHg)	76.6 ± 12.9	75.9 ± 9.6	80.6 ± 11.7	0.61
Glucose (mg/dL)	96(71–287)	91(71–173)	96.5(69–257)	0.69
Total cholesterol (mg/dL)	207.7 ± 44.9	207.8 ± 43.1	231.1 ± 31.9	0.35
Triglycerides (mg/dL)	170(80–557)	178(87–440)	186(113–487)	0.73
Albumin (mg/dL)	4.5(1.9–5.4)	4.5(2.3–5.6)	4.0(3.6–5.5)	0.99
hsCRP (mg/L)	3.94(0.66–16.4)	3.02(0.42–17.1)	2.44(1.0–8.1)	0.28
Leukocyte count (x10^3/mm^3)	7.79 ± 1.84	6.64 ± 1.39	6.87 ± 1.40	0.01*
Platelet count (x10^3/mm^3)	290(206–454)	264(155–345)	260(207–316)	0.02[a]

For the parametrics variables are shown means ± SD, median and percentil 5 and 95 are shown for non parametrics variables. FM (Fat Mass), %BF (Body Fat Percentage).The P values were obtained with Student's *t*test* and Mann Whitney[a]. $P^{*,a} < 0.05$ was statistically significant.

To estimate the relative contribution of both polymorphisms to BMI, and metabolic and inflammatory variables, we used multiple linear regression models. After adjustment for age, fat mass, smoking, exercise, and family history for T2D, obesity and cardiovascular disease, it was determined that –174 GC/CC genotypes contributed to a significant increase in glucose levels ($\beta = 36.06$ mg/dL, $p = 0.007$) and hsCRP levels ($\beta =$ 2.5 mg/L, $p = 0.02$) (Table 4). In contrast, the –572 GG genotype contributed to an increase in the leukocyte count ($\beta = 9.57\ 10^3$/mm^3, $p = 0.005$) and platelet count ($\beta = 42.3\ 10^3$/mm^3, $p = 0.003$) (Table 4).

In multiple correlation models, adjusted for the same inconsistencies considered above, the –174 GC/CC genotypes were associated with T2D (OR = 1.23; 95% CI, 1.01 to 1.5; $p = 0.03$), and the –572 GG genotype was associated with T2D (OR = 1.24; 95% CI, 1.04 to 1.47; $p = 0.01$) and leukocyte count 3^{rd} tercil (OR = 1.24; 95% CI, 1.02 to 1.51; $p = 0.02$). However, we didn't find a relationship with overall obesity, abdominal obesity, hypertension, dyslipidemia, hsCRP >3 mg/L, and platelet count 3rd tercil (data not shown).

4. Discussion

This is the first study in Mexican families to investigate the relationship between the IL-6 gene promoter polymorphisms and cardiovascular risk factors. Initially, sex difference in adiposity was observed, women (mothers and daughters) demonstrate a greater body fat percentage. In addition, hipertriglyceridemia was observed in mothers and sons. Similarly, other studies have reported that obesity is associated with increased triglycerides levels. In a Report from the Third National Health and Nutrition Examination Survey of the United States of North America (NHANES), stated that with greater obesity, the risk of dyslipidemia increased by 75% [23].

The increase in the platelet count of women could be related to the increase in IL-6 production by adipose tissue, and consequently IL-6 stimulates the maturation of the megakaryocytes and increases the platelet count. The biologic activity of IL-6 on maturational stages in megakaryocytopoiesis and in platelet production have been demonstrated in animal models [24] and in cell culture *in vitro* [25], suggesting that IL-6 can be an important thrombopoietic factor.

The genotype frequencies of the –174 G/C polymorphism were very similar to those reported for population in western Mexico [26], indicative of an common ancestry. However, a high frequency of 47% [27] and 30% [28] for the –174 CC genotype was observed in Caucasian populations. The genotype and allele frequencies of –572 G/C IL-6 polymorphism are the first reported in Mexico, we identified 76.1% of allele G carriers and 23.9% of C allele. In contrast, low frequencies of 7% and 8% for C allele were reported in UK [29] and Germany [30], respectively. These results clearly show the influence of various races in our genetic background that may explain the allele and genotype differences with Caucasian populations. The Mexican

Table 4
Effect of –174 and –572 polymorphisms on BMI, glucose and inflammatory variables

	Without adjusted		Multiples models[1]	
	§ β (95% CI)	P value	§ β (95% CI)	P value
−174 polymorphism				
Genotypes GC+CC vs GG				
BMI (kg/m^2)	0.56(–1.4–2.5)	0.57	0.15(–0.75–1.06)	0.73
Glucose (mg/dL)	34.1(7.4–60.9)	0.01*	36.06(9.6–62.5)	0.007*
hsCRP (mg/L)	2.31(0.1–4.6)	0.05*	2.5(0.4–4.5)	0.02*
Leukocyte count (x10^3/mm^3)	1.4(–6,68–9,57)	0.72	1.47(–6,7–9,66.)	0.72
Platelet count (x10^3/mm^3)	4.89(–31.8–41.6)	0.79	0.18(–34.0–34.4)	0.99
–572 polymorphism				
Genotypes GG vs CC				
BMI (kg/m^2)	1.50(–0.17–3.19)	0.08	0.59(–0.19–1.38)	0.13
Glucose (mg/dL)	13.6(–10.0–37.3)	0.26	21.9(–1.66–45.5)	0.06
hsCRP (mg/L)	0.85(–1.20–2.91)	0.41	0.41(–1.46–2.3)	0.66
Leukocyte count (x10^3/mm^3)	1.07(4.32–17.2)	0.001*	9.57(2.87–16.2)	0.005*
Platelet count (x10^3/mm^3)	49.8(20.1–79.4)	0.001*	42.3(14.3–70.3)	0.003*

[1] Adjusted by age, fat mass, smoking, exercise, family history of T2D, obesity and CAD.
p* Models generalized regression. Regression coefficient (95%CI).

Mestizo population is result of the mixture of Spanish, pre-Spanish and African genes [31].

We also demonstrated the relationship between inflammatory markers and other variables studied. In particular, the level of hsCRP was related to increased obesity in all measures of body adiposity. Similarly, various studies have documented that the level of hsCRP is strongly related to BMI, waist circumference and adipose body mass [32]. We also showed, in a previous study the relationship of hsCRP with diastolic blood pressure and leukocyte count [33]. In this study, the leukocyte count was correlated with increased adipose tissue, diastolic blood pressure, triglycerides and hsCRP levels. Others investigators have reported that the leukocyte count is associated with components of metabolic syndrome, T2D and dyslipidemias [34]. Moreover, white blood cell count has been correlated with coronary risk factors, and as an independent predictor of Coronary Heart Disease, Ischemic Stroke and mortality by Cardiovascular Disease [35, 36]. The association between white blood cell count and hsCRP levels suggests that the leukocyte count may be of clinical importance as a marker of low-grade systemic inflammation in obese subjects and as predictor of Cardiovascular Disease.

We describe, for first time, in Mexican families that –174 GC/CC genotypes contribute to a significant increase in glucose and hsCRP levels, and an association with T2D. In contrast, some authors have reported a relationship between the –174G allele and insulin resistance [37] and also T2D [38,39]. Other studies reported that –174C allele is associated with increased IL-6 and CRP levels [15,29,30]. The increase in IL-6 and CRP levels have been related with insulin resistance and T2D [38,40]. Interestingly, our results support the hypothesis that type 2 diabetes is a manifestation of an acute-phase response that is characterized by increased concentrations of inflammatory markers such as IL-6 and CRP. Furthermore, we found that the –174 GC/CC genotypes has an effect on glucose levels and can be markers of the susceptibility for T2D in the Mexican population.

On the other hand, the –572 GG genotype was associated with T2D and leukocyte count, which suggest that this genotype can predict the development of T2D and systemic inflammation in the Mexican population. In addition, another study has demonstrated that the –572 GG genotype is associated with high IL-6 levels ($p = 0.02$) [15]. In previous investigations, the –572 GG genotype has been related to an increase in CRP levels [30,41]. In contrast, Ferrari et al, found in healthy postmenopausal women that the GC genotype carriers showed significantly higher CRP levels in comparison with GG genotype carriers ($p = 0.023$) [16]. Huth et al, performed a meta-analysis with eight Caucasian populations. They did not find any association between the –572C allele and T2D (OR $= 1.05$; 95%IC 0.86–1.27) [39]. On the basis of these data, it is possible to speculate that the –572 GG genotype is related with increased IL-6 levels and consequently these cytokine induce the hepatic synthesis of CRP, and the production of leukocytes. In our study, we did not find an association between this genotype and hsCRP levels, but with the leukocyte count.

The association of both polymorphisms with T2D may be explained by previou studies. Most of these

studies have reported that the –174 CC and –572 GG genotypes are associated with increase of IL-6 and CRP levels, and IL-6 may inhibit insulin receptor and insulin receptor substrate type 1 (IRS-1), inducing insulin resistance in hepatocytes, human hepatocarcinoma cell line HepG2 [42,43] and 3T3-L1 Adipocytes [44], therefore may be an mechanism implicated in the development of T2D in carriers of these genotypes.

One limitation of our study is that IL-6 serum levels were not measured; therefore the association of both genotypes with IL-6 levels remains uncertain. Although, serum IL-6 levels may be comparable with both CRP levels and leukocyte number, due to that IL-6 directly induce the production of these inflammatory markers.

In summary, the –174 GC/CC and –572 GG genotypes of IL-6 gene were associated with T2D and inflammatory markers, (hsCRP levels and leukocyte count), suggesting that these genotypes may confer susceptibility for type 2 diabetes and subclinical inflammation in Mexican families.

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