Cytokine 114 (2019) 32–37

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Interleukin 27 polymorphisms, their association with insulin resistance and their contribution to subclinical atherosclerosis. The GEA Mexican study

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ARTICLE INFO

Keywords:
Insulin resistance
Interleukin 27
Hypertension
Hypoadiponectinemia
Polymorphisms
Subclinical atherosclerosis

ABSTRACT

Our previous data suggest that the heterodimeric interleukin-27 (IL-27) could participate in the developing of insulin resistance (IR). Our aim was to assess the participation of *IL-27p28* gene single nucleotide polymorphisms (SNPs) as markers for IR, subclinical atherosclerosis (SA) and cardiovascular risk factors in a Mexican population. Five *IL-27p28* SNPs (rs153109, rs40837, rs17855750, rs26528 and rs181206) were genotyped in 856 individuals with IR and 644 participants without IR. Under inheritance models adjusted for confounding factors, the rs153109*A* (0.78[0.64–0.94] $P_{additive}$ = 0.008, 0.58[0.41–0.82] $P_{recessive}$ = 0.002, 0.57[0.38–0.83] $P_{codominant2}$ = 0.004), rs26528*T* (0.78[0.64–0.94] $P_{additive}$ = 0.008, 0.61[0.43–0.88] $P_{recessive}$ = 0.007, 0.57[0.38–0.84] $P_{codominant2}$ = 0.004) and rs40837*A* (0.76[0.63–0.92] $P_{additive}$ = 0.004; 0.60[0.42–0.86] $P_{recessive}$ = 0.005; 0.54[0.37–0.80] $P_{codominant2}$ = 0.002) alleles were related with a decreased risk of IR. Moreover, *AAATA* haplotype that contains the protector alleles was related with 17% lower risk of presenting IR (0.83 [0.71–0.98], P = 0.023). After adjusting for potential confounding variables, *IL-27p28* SNPs were not associated with SA. However, some SNPs were associated with hypertension (rs26528 and rs40837) and increased total abdominal fat (rs17855750) in non-IR individuals, whereas in IR subjects we observed an association of rs26528 and rs40837 with hypoadiponectinemia. Our evidence suggests that rs40837*A*, rs153109*A*, and rs26528*T* alleles could be envisaged as protective markers for IR. Some polymorphisms showed an association with hypertension, low adiponectin levels, and increased total abdominal fat.

1. Introduction

In 1993, it was shown for the first time that tumor necrosis factor alpha (TNFα) was capable to induce insulin resistance (IR) [1,2]. Since then, epidemiological and clinical studies have confirmed the association between IR and inflammation [3–6]. In fact, IR has been related to cardiovascular risk factors, such as dyslipidemia, obesity and hypertension [7]. On the other hand, it is well recognized that atherosclerosis (AE) and its clinical manifestation –coronary artery disease (CAD)– are characterized as low-grade inflammation states. Inflammation participates in all atherosclerosis phases of the cardiovascular disease: from the fatty streak genesis to the cardiovascular events [8]. It is well known that pro-inflammatory interleukins can produce IR in adipose tissue, liver and skeletal muscle by inhibiting signal transduction of the insulin [2]. Moreover, high concentrations of TNFα, interleukin-8 (IL-8) and interleukin-6 (IL-6) and low concentrations of interleukin-10 (IL-10) have been reported in several IR states [9–13], confirming the association between inflammation and IR. Recently, a new cytokine, interleukin-27 (IL-27), has been involved in the inflammatory process associated with the development of AE [14,15].

Abbreviations: AE, atherosclerosis; BMI, body mass index; CAC, coronary artery calcification; CAD, coronary artery disease; CI, confidence intervals; GEA, Genetics of Atherosclerosis Disease; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-27, interleukin-27; IR, insulin resistance; miRNAs, microRNAs; OR, odds ratio; SA, subclinical atherosclerosis; SNPs, single nucleotide polymorphisms; T2DM, type 2 diabetes mellitus; TNFα, tumor necrosis factor alpha; TAF, total abdominal fat; VAF, visceral abdominal fat

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https://doi.org/10.1016/j.cyto.2018.11.028
Received 29 June 2018; Received in revised form 5 November 2018; Accepted 26 November 2018
Available online 26 December 2018

This heterodimeric cytokine is composed by one β subunit (EB13) and one α subunit (p28) [16]. In humans, the p28 subunit is encoded by the *IL-27p28* gene, which is highly polymorphic and is situated in the 16p11 locus [17]. Previously, our research group reported that *IL-27p28* **single nucleotide** polymorphisms (SNPs) are associated with premature CAD and with some metabolic variables in the Mestizo participants of the Genetics of Atherosclerosis Disease (GEA) Mexican study [18]. Our data also suggested a possible association between these polymorphisms and IR [18]. Thus, the objectives of this study were (a) to further investigate the relation between *IL-27p28* SNPs and IR, (b) to establish their role in the AE early stages by analyzing the relationship of the *IL-27p28* SNPs with subclinical atherosclerosis (SA) –considered as a coronary artery calcification (CAC) score greater than zero–, and (c) to evaluate the relation of *IL-27p28* SNPs to cardiovascular risk factors in subjects with IR and non-insulin resistant (non-IR) participants.

2. Materials and methods

2.1. Ethics statement

The Instituto Nacional de Cardiología Ignacio Chávez (INCICH) Ethics Committee approved the protocol. In accordance with the Declaration of Helsinki, all participants gave written informed consent.

2.2. Subjects

The participants were selected from the population of the GEA Mexican study. This trial was designed to examine the genetic bases of premature CAD and the association of emerging and traditional cardiovascular risk factors in the Mexican population [19]. This is a cross-sectional analysis of the baseline evaluation of the GEA study control group; this group includes 1500 individuals without premature CAD family or personal history, enrolled from social service centers and blood banks. Anthropometric, biochemical, clinical, and demographic parameters, as well as cardiovascular risk factors, were determined in all participants as previously described [19–21]. We followed the methods and criteria definitions reported previously [22–26].

2.3. Quantification of IL-27 plasma concentration

In a carefully selected subsample of 305 non-IR and 139 participants with IR (non-obese and with high-sensitivity C-reactive protein (hsCRP) concentration < 3 mg/L), IL-27 plasma concentrations were quantified using a Bioplex system (Bio-Rad, Contra Costa County, State of California, USA). The data were analyzed using Bio-Plex Manager software. Data were expressed in pg/mL.

2.4. Genetic analysis

We used standard techniques for the isolation of genomic DNA from whole blood containing EDTA and performed the functional prediction of *IL-27p28* SNPs with bioinformatics tools [18]. For the analysis, we selected five *IL-27p28* gene SNPs with minor allele frequencies > 5% and/or possible functional consequences. The information on the rs17855750, rs181206 rs40837 and rs26528 polymorphisms has already been published [18]; rs153109 introduces binding sites for ATF6 and HNF4a transcriptional factors.

Using 5′ exonuclease TaqMan genotyping assays, the rs26528, rs17855750, rs181206 and rs40837 *IL-27* SNPs were genotyped as previously reported [18]. The rs153109 polymorphism was genotyped by restriction fragment length polymorphisms (RFLPs) technique. The primers used to amplify this fragment were Forward 5′- TCAGTCAGT GACCAGGATCG -3′ and Reverse 5′- ACCAAGAAACCCCATCCTCT -3′. Amplification of template DNA (100 ng) was performed in a GeneAmp PCR Systems 9700 (Applied Biosystems, Foster City, CA). To detect a base change, the 224-bp PCR product was incubated at 37 °C overnight with the *XHO* 1 restriction enzyme (Sigma-Aldrich, St. Louis, MO). *XHO*-1 digestion produced two fragments of 179 and 45 bp. The fragment sizes for each genotype were 224 bp for *AA*, 179 bp + 45 bp for *GG*, and 224 bp + 179 bp + 45 bp for *AG*. As positive controls, we included previously sequenced samples of the different SNP genotypes.

2.5. Statistical analysis

Data are expressed as frequencies, median (interquartile range) or mean ± standard deviation, as appropriate. Either Mann–Whitney U or Student's *t*-test were used for continuous variable comparisons, while the chi-squared test was employed for categorical variable comparisons. The frequencies of alleles and genotypes were determined by direct counting. We used the chi-squared test to determine the Hardy-Weinberg's equilibrium. Haplotype analysis and linkage disequilibrium were performed as described previously [18]. IL-27 plasma concentration comparisons were evaluated by the Mann-Whitney *U* test. We used logistic regression analysis [18] (adjusted for confounding variables as appropriate) to test for the relation of studied SNPs to IR, SA, and cardiovascular risk factors. We used SPSS software v15.0 (SPSS Chicago, IL) for all analyses and employed QUANTO software (http://hydra.usc.edu/GxE/) to calculate the statistical power of the association between IR with the studied SNPs. For rs153109, rs26528, and rs40837 (additive, recessive and co-dominant 2 models) the statistical power was > 90%. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Study sample characteristics

Fifteen hundred individuals belonging to the GEA study control group were included in the analyses. According to the homeostasis model assessment of insulin resistance (HOMA-RI) values, 644 individuals were considered non-IR, while 856 participants were insulin resistant. Table 1 shows the tomographic data, study population genotypes, as well as the demographic, lifestyle, clinical and biochemical characteristics. Compared with non-IR individuals, age, percentage of male, waist circumference, body mass index (BMI), diastolic and systolic blood pressure, hypoalphalipoproteinemia, hypertriglyceridemia, hypoadiponectinemia, type 2 diabetes mellitus (T2DM), obesity, hypertension, high visceral (VAF) and total abdominal fat (TAF), and CAC were higher in subjects with IR. On the contrary, physical activity and the rs153109*AA*, rs26528*TT* and rs40837*AA* genotypes were more frequent in non-IR subjects than in insulin resistant ones (Table 1).

3.2. Association of polymorphisms and haplotypes with insulin resistance

All the SNPs were in Hardy-Weinberg equilibrium. Fig. 1 shows the association of *IL-27p28* SNPs with IR. For this analysis, we adjusted for potential confounding variables, such as age, sex, BMI, VAF, diastolic and systolic blood pressure, smoking habit, physical activity, and the concentrations of triglycerides, high density lipoprotein (HDL) and cholesterol. Under additive, recessive and co-dominant 2 models, the rs153109*A* (0.78 [0.64–0.94] $P_{additive} = 0.008$, 0.58 [0.41–0.82] $P_{recessive} = 0.002$, 0.57 [0.38–0.83] $P_{codominant2} = 0.004$), rs26528*T* (0.78 [0.64–0.94] $P_{additive} = 0.008$, 0.61 [0.43–0.88] $P_{recessive} = 0.007$, 0.57 [0.38–0.84] $P_{codominant2} = 0.004$), and rs40837*A* (0.76 [0.63–0.92] $P_{additive} = 0.004$, 0.60 [0.42–0.86] $P_{recessive} = 0.005$, 0.54 [0.37–0.80] $P_{codominant2} = 0.002$) alleles were significantly associated with lower risk of IR.

The five studied SNPs were included in the linkage disequilibrium analysis, and we found that all of them were in linkage disequilibrium ($D' > 0.75$). Seven haplotypes were constructed (*AAATA*, *GACCG*, *GAACG*, *GGACA*, *GAACA* and *GACCA*). The *AAATA* haplotype was related to 17% lower risk of the presence of IR (0.83 [0.71–0.98],

Table 1
Demographic, lifestyle, clinical and biochemical characteristics, tomographic data and genotypes of the studied groups.

	Insulin resistance		P value*
	No (n = 644)	Yes (n = 856)	
Demographic			
Age (years)	52 ± 9	54 ± 9	0.003
Sex (% male)	46.3	52.9	0.012
Biochemical and clinical characteristics			
Body mass index (kg/m^2)	25.9 [23.9–28.2]	29.5 [27.1–32.1]	< 0.001
Waist circumference (cm)	89 ± 10	99 ± 10	< 0.001
Systolic blood pressure (mmHg)	110 [102–121]	118 [108–129]	< 0.001
Diastolic blood pressure (mmHg)	69 [64–75]	73 [67–80]	< 0.001
Insulin (μU/L)	11.9 [9.3–14.1]	22.4 [18.8–28.2]	< 0.001
Interleukin 27 (pg/mL)#	1.00 [0.40–1.71]	0.73 [0.09–1.32]	0.014
Hypoalphalipoproteinemia (%)	38.9	58.5	< 0.001
Hypertriglyceridemia (%)	34.6	59.3	< 0.001
Hypoadiponectinemia (%)	31.0	51.5	< 0.001
Obesity (%)	13.7	43.6	< 0.001
Type 2 Diabetes mellitus (%)	3.1	20.4	< 0.001
Hypertension (%)	14.9	31.1	< 0.001
hsCRP ≥ 3 mg/L (%)	20.1	32.4	< 0.001
Tomography			
Increased TAF (%)	34.4	72.4	< 0.001
Increased VAF (%)	36.6	75.4	< 0.001
Coronary artery calcification (%)	21.6	30.6	< 0.001
Lifestyle			
Current smoking habit (%)	23.1	21.8	0.574
Physical activity	8.0 [7.2–8.9]	7.7 [6.7–8.6]	< 0.001
IL-27p28 genotype (%)			
rs153109 (GG/GA/AA)&	28.9/45.2/26.0	32.8/50.3/16.9	< 0.001
rs26528 (CC/CT/TT)	35.3/46.2/18.5	39.4/47.7/13.0	0.010
rs17855750 (AA/AC/CC)	74.2/23.5/2.3	72.5/25.4/2.1	0.689
rs181206 (AA/AG/GG)	48.4/40.0/11.7	43.2/44.0/12.7	0.141
rs40837 (GG/GA/AA)	34.7/46.7/18.7	39.3/47.8/13.0	0.007

VAF, visceral abdominal fat; TAF, total abdominal fat; hsCRP, high sensitivity C reactive protein. Data are shown as mean ± standard deviation, median [interquartile range] or percentage.

* Mann-Whitney U, chi-squared or Student's *t*-test.

\# Plasma IL-27 concentrations were determined in 455 individuals (144 with IR and 311 without IR).

& rs153109 genotype data was available in n = 1346 subjects (768 with IR and 578 without IR).

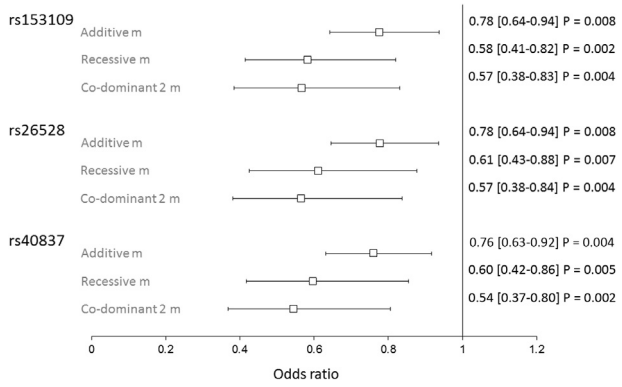

Fig. 1. Association of *IL-27p28* polymorphisms with insulin resistance. All models were adjusted for age, gender, body mass index, smoking habit, physical activity, visceral abdominal fat, systolic and diastolic blood pressure, HDL cholesterol and triglycerides levels. Only the significant associated models are shown. For rs153109, the genotype data were available for 1346 subjects (768 with IR and 578 without IR). The reference genotype was *GG* for rs153109, *CC* for rs26528, and *GG* for rs40837.

Table 2
IL27p28 haplotype frequencies in individuals with and without insulin resistance.

	Haplotypes	Insulin resistance		OR [95% CI]	P value
		Yes	No		
H1	***AAATA***	**0.355**	**0.397**	**0.83 [0.71–0.98]**	**0.023**
H2	***GGACG***	**0.317**	**0.270**	**1.25 [1.06–1.48]**	**0.008**
H3	*GACCG*	0.134	0.120	NS	–
H4	*GAACG*	0.117	0.107	NS	–
H5	*GGACA*	0.029	0.038	NS	–
H6	*GAACA*	0.017	0.024	NS	–
H7	*GACCA*	0.011	0.012	NS	–

OR, odds ratio; CI, confidence interval; NS, not significant. The order of the polymorphism in the haplotype is according to the position in the chromosome (rs40837, rs181206, rs17855750, rs26528, rs153109). Bold numbers indicate significant associations.

P = 0.023); in contrast, the *GGACG* haplotype was related to 25% higher risk of IR (1.25 [1.06–1.48] P = 0.008) (Table 2).

3.3. *IL-27p28* SNPs and their association with SA

Considering that IR has an important participation in the pathogenesis of AE, we explored the association of the five *IL-27p28* SNPs with the presence of SA, defined as the presence of CAC. No association was found between *IL-27p28* SNPs and SA (Supplementary Table 1).

3.4. Cardiovascular risk factors and their relation with *IL-27p28* SNPs

Fig. 2 shows the associations between *IL-27p28* SNPs and cardiovascular risk factors in individuals with and without IR. In non-IR individuals, the rs26528 (OR = 1.45, $P_{additive}$ = 0.029; OR = 2.26, $P_{recessive}$ = 0.004; OR = 2.22, $P_{codominant2}$ = 0.015) and rs40837 (OR = 1.43, $P_{additive}$ = 0.036; OR = 2.32, $P_{recessive}$ = 0.003; OR = 2.16, $P_{codominant2}$ = 0.017) SNPs were related with higher risk of hypertension, whereas rs17855750 (OR = 0.40, $P_{additive}$ = 0.003; OR = 0.36, $P_{recessive}$ = 0.004; OR = 0.41, $P_{codominant2}$ = 0.012) was associated with approximately 60% lower risk of increased TAF (> p75). On the other hand, in the insulin resistant subjects, the rs26528 (OR = 0.56, $P_{recessive}$ = 0.007; OR = 0.61, $P_{codominant2}$ = 0.036) and rs40837 (OR = 0.57, $P_{recessive}$ = 0.009; OR = 0.61, $P_{codominant2}$ = 0.035) SNPs were related with approximately 40% lower risk of the presence of low adiponectin concentrations (Fig. 2).

3.5. Relationship between *IL-27p28* SNPs and IL-27 concentrations

IL-27 plasma concentration of the non-IR subjects was higher than that of the insulin resistant participants (1.00 [0.40–1.71] pg/mL vs 0.73 [0.09–1.32] pg/mL, P = 0.014, respectively). However, when the IL-27 concentrations of each group were analyzed considering the genotypes of the studied *IL-27p28* SNPs, no differences were observed (supplementary Table 2).

4. Discussion

To the best of our knowledge, this work has shown the association of *IL-27p28* SNPs with IR for the first time. Three genetic variants of *IL-27p28* (rs153109*A*, rs26528*T* and rs40837*A*) were related with 20–40% decreased risk of presenting IR. Moreover, the *AAATA* haplotype, containing the three protector alleles (rs153109*A*, rs26528*T*, and rs40837*A*) was associated with 17% lower risk of presenting IR. On the contrary, the *GGACG* haplotype was associated with 25% increased risk of IR. This haplotype includes the rs153109*G*, rs26528*C*, and rs40837*G* alleles with high frequency in individuals with IR (0.580, 0.632 and

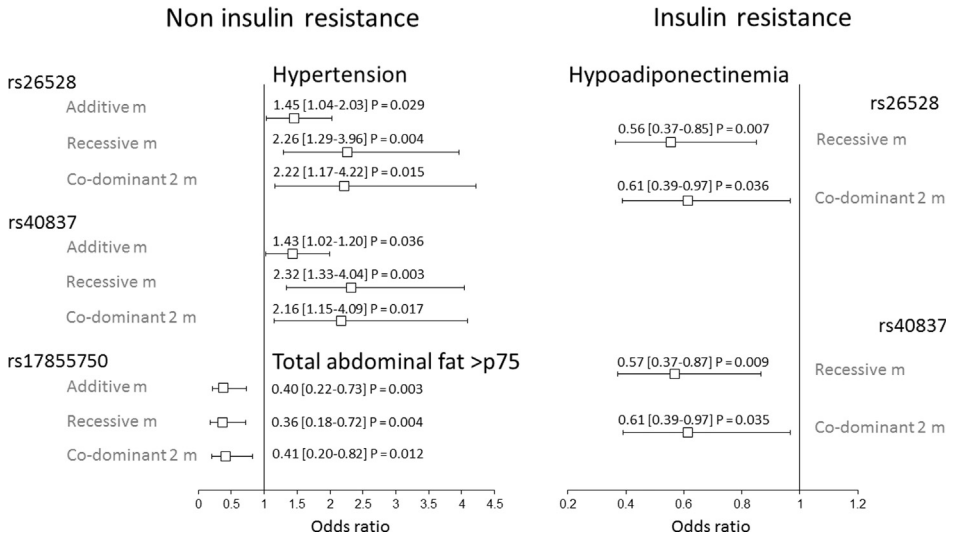

Fig. 2. *IL27p28* gene polymorphism association with cardiovascular risk factors. All models were adjusted for age, gender, BMI, HOMA-IR, visceral abdominal fat, T2DM. The reference genotype was *CC* for rs26528, *GG* for rs40837, and *AA* for rs17855750.

0.632 respectively); however, these alleles were not associated with IR in the independent SNP analysis. It is well known that the construction of the haplotypes of candidate genes across the human genome is convoluted and difficult to analyze and study. This is due to that considerable amount of sequence variation has not been documented yet in some genes. The results obtained in our haplotypes analysis should be take with care owing to the incomplete knowledge of both the genetic variation and the linkage disequilibrium within the IL-27 gene. In addition, we independently evaluated the relation between cardiometabolic variables and the *IL-27p28* gene SNPs in individuals with and without IR. While we found an association with hypertension (rs26528 and rs40837) and increased TAF (rs17855750) in the non-IR subjects, we observed an association with hypoadiponectinemia (rs26528 and rs40837) in the insulin resistant individuals. SA was not associated with *IL-27p28* SNPs. The IL-27 plasma concentration was higher in the non-IR subjects when compared with insulin resistant individuals; however, IL-27 concentrations were not associated with the genotypes of the studied *IL-27p28* SNPs.

Insulin is a pleiotropic hormone; whose target are the liver and adipose tissue. Its functions include the transport of nutrient into cells, enzyme activity modifications, the regulation of energy homeostasis, and gene expression through several intracellular signaling cascades. In liver and adipose tissue, chronic inflammation results in the release of pro-inflammatory cytokines by activated resident macrophages; these cytokines can cause IR by a direct interaction between inflammatory pathways and insulin signaling [27]. After adjusting for confounding variables, *IL-27p28* SNPs were related with IR. The relation between IR and inflammation has been suggested since the 1950s. Additionally, in several IR states, both the chronic production of pro-inflammatory cytokines (such as IL-8, IL-6, and TNFα [9–12]), and reduced serum concentrations of IL-10 have been reported [13]. IL-27 is considered as a pleiotropic cytokine: it modulates the predominant type of immune response and the anti-inflammatory and pro-inflammatory responses [28,29] depending on the disease type and severity. IL-27 receptor is a heterodimer comprising gp130 and WSX-1 subunits [16]; this complex signals via STAT1/STAT3 [30]. In an animal model, Ma et al. showed that STAT3 activation induced by IL-27 attenuates IR injury to rat hearts [31]. This data is consistent with our findings, which suggest a protective role of the *IL-27p28* SNPs against the presence of IR. Moreover, the haplotype *AAATA,* which contain the three protection alleles, showed a 17% risk reduction for IR. Our findings suggest that rs40837*A*, rs153109*A* and rs26528*T* alleles could be envisaged as potential markers for IR in our population.

Adiponectin is a potent adipocytokine produced by adipose tissue [32]. This cytokine reduces IR and stimulates adipocyte differentiation. Obesity, in particular central adiposity, and IR have been associated with hypoadiponectinemia [33,34]. It has been reported that when adipocytes overexpress adiponectin, adipocyte differentiation is increased and IR is reduced [33]. Moreover, it has been reported that hypoadiponectinemia may result in IR and diabetes mellitus [35]. In this study, the rs26528*TT* and the rs40837*AA* genotypes were significantly related to a greater than 40% reduced risk for hypoadiponectinemia. The rs26528 polymorphism is positioned in the intron 1, whereas rs40837 is in the 3′-UTR region of the *IL-27p28* gene. Introns are noncoding regions that can modulate the expression of a gene by cleaving RNA transcripts or repressing translation [36]. On the other hand, 3′-UTR controls gene expression at post-transcriptional level [37,38]. The bioinformatics analysis revealed that the rs40837 polymorphism introduces binding site for several miRNAs. No evidence of protein interactions was shown for rs26528. However, these two SNPs (rs26528 and rs40837) are in high linkage disequilibrium ($D' > 0.96$). Previous research has established that miRNAs participate in adipogenesis, insulin biosynthesis, lipid metabolism regulation, glucose homeostasis, among other processes [39,40]. Some of these miRNAs could modulate IL-27 transcription and modify the inflammatory signaling and response in the protector genotype carriers. Consequently, miRNAs may have an impact on insulin sensitivity and thus in the production of adiponectin by the adipose tissue. This may explain the protective association observed in our study between the rs26828 and rs40837 SNPs and the presence of low adiponectin concentrations.

In humans and animal models, the evidence shows that there is macrophage accumulation in the adipose tissue [41,42]. Moreover, adiposity and the size of the adipocyte is directly related with the macrophage percentage in a given adipose tissue depot [42]. In mice, adipose tissue macrophages with the pro-inflammatory phenotype have been associated with the development of IR [41]. Interestingly, rs17855750 SNP (associated with 60% lower risk of having high TAF accumulation) creates a binding site for the proteins SF/ASF, which participate in alternative splicing [43]. This SNP is in linkage disequilibrium ($D' > 0.80$) with the three SNPs (rs153109, rs26528 and rs40837) associated with lower risk for IR. This polymorphic site may modulate possible IL-27 isoforms with relevance to the susceptibility of abdominal fat accumulation.

For many years, it has been shown that IR is associated with hypertension and has been defined as a risk factor for this condition [44,45]. Recently, the immune system has been considered as a

relevant participant in the physio-pathogenesis of hypertension [46]. High IL-6 levels have been reported in hypertensive conditions. Moreover, JAK/STAT3 activation by IL-6 has an important participation in the hypertension induced by angiotensin II [47]. We found an association of rs26528 and rs40837 SNPs with hypertension. As mentioned above, IL-27 receptor signals via STAT1/STAT3 [30] and is present in several types of cells. The complexity of its receptor may explain the wide-ranging immunomodulatory function of this cytokine, including the relation of the *IL-27p28* SNPs to the presence of hypertension.

Although the studied SNPs were not associated with IL-27 plasma concentrations, individuals without IR had higher IL-27 concentrations compared with insulin resistant participants. Epigenetic modifications and changes at DNA level are part of the complex mechanisms that modulate the production of several molecules, including IL-27; therefore, the lack of association between the *IL-27p28* SNPs and IL-27 plasma concentrations could be expected. Furthermore, it must also be considered that we measured IL-27 concentration only in a subsample of the population with specific characteristics.

A key strength of our work is that the GEA Mexican Study participants constitute a large cohort of Mexican individuals with biochemical, clinical and tomographic data and thorough phenotyping. This data enabled us to adjust the statistical analyses for an extensive number of potential confounders. However, the limitations of the present work need to be recognized. Firstly, the cross-sectional design of the GEA Mexican Study basal phase does not allow to establish causality. Secondly, the findings may not be applicable to the general population, considering that individuals were not randomly selected. Thirdly, IR was not determined by the gold standard: the euglycemic clamp; nevertheless, insulin sensitivity can be accurately estimated by the HOMA-IR index that has proven to be a reliable measure [48]. Fourthly, our data may not apply to other ethnicities, considering that the GEA participants are exclusively Mexican-Mestizo subjects: a population with genetic characteristics that are particular and different from other ethnic groups [49–52]. The *IL-27p28* polymorphism associations detected in our study should be investigated in other populations to establish if they are shared with other ethnic groups or are specific for the Mexican population.

In summary, our findings show for the first time that *IL-27p28* SNPs were significantly related with IR, hypertension, increased total abdominal fat and low adiponectin levels in the Mexican population. Despite all the evidence on the role of IL-27 in AE, the studied polymorphisms were not associated with SA. Our findings suggest that rs40837*A*, rs153109*A* and rs26528*T* alleles could be envisaged as potential protective markers for IR in our population.

Acknowledgments

The authors are grateful to A. Rene López-Uribe and M del Carmen González-Salazar for their technical assistance. This project was supported by Consejo Nacional de Ciencia y Tecnología, Mexico City, Mexico (Grant number: Fronteras de las Ciencia 2016-01-1958).

Conflicts of interest

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2018.11.028.

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