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The rs1805193, rs5361, and rs5355 single nucleotide polymorphisms in the *E-selectin* gene (*SEL-E*) are associated with subclinical atherosclerosis: The Genetics of Atherosclerotic Disease (GEA) Mexican study

Gilberto Vargas-Alarcon[a], Oscar Perez-Mendez[a], Gabriel Herrera-Maya[a], Carlos Posadas-Romero[b], Rosalinda Posadas-Sanchez[b], Julian Ramirez-Bello[c], Galileo Escobedo[d], Jose Manuel Fragoso[a,*]

[a] Department of Molecular Biology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico
[b] Department of Endocrinology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico
[c] Research Unit on Endocrine and Metabolic Diseases, Hospital Juarez de México, Mexico City, Mexico
[d] Unit of the Experimental Medicine, Hospital General de Mexico, Dr. Eduardo Liceaga, Mexico City, Mexico

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ABSTRACT

The aim of this study was to evaluate the association of rs1805193, rs5361, and rs5355 *E-selectin* gene single nucleotide polymorphisms (SNPs) with the risk of developing subclinical atherosclerosis (SA) in a group of Mexicans individuals. SNPs were determined by *TaqMan* genotyping assays in a group of 287 individuals with SA and 688 healthy controls. Under different models, the *T* allele of the 5′*UTR G98 T* (rs1805193) (OR = 1.71, 95%CI: 1.00–2.93, $pC_{Co\text{-}dominant}$ = 0.0006, OR = 2.02, 95%CI: 1.21–3.38, $pC_{Dominant}$ = 0.004, and OR = 2.14, 95%CI: 1.34–3.44, $pC_{Additive}$ = 0.0015) and the *C* allele of the Ser128Arg *A561C* (rs5361) (OR = 1.60, 95%CI: 0.92–2.79, $pC_{Co\text{-}dominant}$ = 0.012, OR = 1.78, 95%CI: 1.04–3.06, $pC_{Dominant}$ = 0.038, and OR = 1.87, 95%CI: 1.13–3.11, $pC_{Additive}$ = 0.016) polymorphisms were associated with an increased risk of development of SA. In the same way, under co-dominant model, the *CT* genotype of the *Leu575Phe C1880T* (rs5355) polymorphism was associated with an increased risk of SA as compared to *CC* genotype (OR = 2.34, 95%CI: 1.33–4.11, pC = 0.0035). All models were adjusted by traditional cardiovascular risk factors. In summary, this study demonstrates that the 5′*UTR G98 T,* Ser128Arg *A561C, and* Leu575Phe *C1880T* polymorphisms are associated with an increased risk of developing SA.

1. Introduction

The atherosclerosis is the main cause of vascular disease and different methods are used to detect it in subclinical stages. Recently, the coronary artery calcification (CAC, usually expressed as the Agatston's score) has been established as a marker of subclinical atherosclerosis (SA, CAC > 0). CAC provides a distinct approach to measure the extent atherosclerotic lesion, and is an established predictor for adverse cardiovascular events (Budoff et al., 2006; Osawa et al., 2016; Kianoush et al., 2017). Endothelial dysfunction, and inflammation localized within the blood vessel wall are the first stages of the atherosclerotic process. In this context, inflammation is modulated and regulated by adhesion molecules including selectins, integrins, immunoglobulins and chemokines (Gonzales and Selwyn, 2003; Mallika et al., 2007; Fernandez-Borja et al., 2010). The selectins constitute a class of cell adhesion molecules that is involved in chronic and acute inflammation processes, generally expressed on endothelial cells after stimulation by inflammatory cytokines (Galkina and Ley, 2007; Fernandez-Borja et al., 2010). E-selectin is a member of the family of selectins, which mediates lymphocytes and monocyte recruitment, rolling, and diapedesis to the areas of inflammation (Galkina and Ley, 2007; Fernandez-Borja et al., 2010). The E-selectin has been considered as a key endothelial product in the chain of events leading to plaque formation and atherosclerosis; SEL-E directly promotes adhesive interaction between monocyte and activated endothelial cell and thereby initiates a cascade leading to migration of monocytes into subendothelial space (Zhao et al., 2012;

Abbreviations: SNP, single nucleotide polymorphism; SEL-E, E-selectin; SA, subclinical atherosclerosis; UTR, untranslated region

* Corresponding author at: Department of Molecular Biology, Instituto Nacional de Cardiología "Ignacio Chávez", Juan Badiano No. 1, Tlalpan, 14080, Mexico CDMX, Mexico.
E-mail address: mfragoso1275@yahoo.com.mx (J.M. Fragoso).

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Issac et al., 2014).

The *SEL-E* gene located on the chromosome 1q12 encodes for a carbohydrate-binding protein of 110-kDa, usually expressed on activated endothelial cells and platelets (Zhao et al., 2012). Recent studies have associated three single nucleotide polymorphisms (SNPs) in the *SEL-E* gene, one in the UTR'5 region [position *G98T* (rs1805193)], and two located in the exons 4, and 10 [*Ser128Arg A561C* (rs5361), and *Leu575Phe C1880T* (rs5355), respectively] with the risk of developing coronary artery disease (CAD), coronary heart disease, myocardial infarction, acute coronary syndrome, ischemic stroke, Kawasaki disease, and hypertension, in different populations (Yoshida et al., 2003; Zak et al., 2008; Mallik and Majumder, 2011; Shirakawa et al., 2012; Wang et al., 2012; Zhao et al., 2012; Sandoval-Pinto et al., 2014).

Considering the prominent contribution of the E-selectin to the chain of events leading to plaque formation and atherosclerosis, we assumed that *SEL-E* gene polymorphisms have a measurable influence on the development of SA that has not been demonstrated yet. Therefore, in this study, we analyzed the UTR'5 *G98 T* (rs1805193), Ser128Arg *A561C* (rs5361), and Leu575Phe *C1880T* (rs5355) polymorphisms in a sample of Mexican individuals to establish whether they are associated with developing to SA (CAC > 0).

2. Materials and methods

2.1. Study population

The study included 975 apparently healthy individuals belonging to the Genetics of Atherosclerotic disease (GEA) study. The primary aim of the GEA study is to investigate genetic factors associated with premature CAD and atherosclerosis in the Mexican population. We determined the CAC score in every participant by computed tomography; tomography of the chest and abdomen was performed using a 64-channel multidetector helical computed tomography system (Somatom Cardiac Sensation, 64, Forchheim, Germany) and interpreted by experienced radiologists. Scans were read to assess and quantify the following parameters: (a) Tissue abdominal fat (TAF), subcutaneous abdominal fat (SAF), and visceral abdominal fat (VAF) areas, as described by Mongraw-Chaffin et al., 2015; (b) liver to spleen attenuation ratio (L:SAR) as described by Machann et al., 2006; and (c) CAC score using the Agatston method (Ahmed et al., 2015). After the computed tomography, 287 individuals were classified in the SA group (those individuals with CAC score > 0) and 688 in the control group (individuals with CAC score = 0). Exclusion criteria for controls and individuals with SA were congestive heart failure, liver, renal, thyroid or oncological disease and premature CAD. Demographic, clinical, anthropometric, biochemical parameter and cardiovascular risk factors were evaluated in both patients and controls as previously described (Posadas-Sanchez et al., 2017). All subjects included in this study were ethnically matched, and considered Mexican Mestizo only those individuals whose ascendance had been born in Mexico for three generations, including their own. A Mexican Mestizo is defined as someone born in Mexico who is a descendant of the original autochthonous inhabitants of the region and of individuals, mainly Spaniards of Caucasian and/or Black origin, who came to America during the sixteenth century. The study complies with the declaration of Helsinki and was approved by the Ethics Committee of the Instituto Nacional de Cardiologia Ignacio Chavez (INCICH). All participants provided their written informed consent.

After a 12-h overnight fasting, EDTA blood samples were drawn, centrifuged within 15 min after collection, plasma was separated into aliquots, and immediately analyzed or frozen at −80 °C until analysis. Cholesterol and triglycerides plasma concentrations were determined by enzymatic/colorimetric assays (Randox Laboratories, UK). The dyslipidemia was defined as a cholesterol > 200 mg/dl, LDL-C > 130 mg/dl, HDL-C < 40 mg/dl, and triglycerides > 150 mg/dl, according with the National Cholesterol Education Project (NCEP) Adult Treatment Panel (ATP III) (http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3_rpt.htm).

2.2. Genetic analysis

DNA extraction was performed from blood peripheral in agreement with the proposed method by Lahiri and Nurnberger (Lahiri and Nurnberger, 1991). The 5′*UTR G98 T* (rs1805193), *Ser128Arg A561C* (rs5361), and *Leu575Phe C1880T* (rs5355) single nucleotide polymorphisms were genotyped using 5′ exonuclease TaqMan genotyping assays on a 7900 H T Fast Real-Time PCR system according to manufacturer's instructions (Applied Biosystems, foster City, USA). Samples previously sequenced for the different genotypes of the studied polymorphisms were included as positive controls.

2.3. Statistical analysis

The Mann Whitney U test was used for comparison of continuous variables between control and SA groups. For categorical variables, Chi^2 or Fisher's exact tests were carried out. We performed the analysis of association of the polymorphisms (5′*UTR G98T*, Ser128Arg *A561C*) with SA by logistic regression analysis. To this purpose, we used the following models: co-dominant (major allele homozygotes versus over-dominant, and major allele homozygotes versus minor allele homozygotes), dominant (major allele homozygotes versus over-dominant + minor allele homozygotes), over-dominant (homozygote for the minor allele + homozygote for the major allele versus over-dominant) and additive (major allele homozygotes versus over-dominant versus minor allele homozygotes). Nonetheless, the analysis of the Leu575Phe *C1880T* SNP was carried out under the co-dominant model because the *TT* genotype was not observed in neither cases nor controls. In addition, this analysis could not be performed under the other models. Models were constructed in order to identify the variables that better explain the risk of developing SA. Furthermore, models were built which incorporated one variable at a time, whereas final models included variables with biological relevance and statistical significance. When a principal effect model was reached, the effect modification was also tested and interaction terms were constructed between the polymorphisms and various variables; the terms were included in the model when the significance of the p-value was higher or equal to 0.05. All p-values were corrected (pC) by the Bonferroni test. pC values less than 0.05 were considered statistically significant, and all odds ratios (OR) are presented with 95% confidence intervals. The occurrence of SA in our population was based in the OR values: OR = 1 does not affect odds of developing SA, OR > 1 is associated with higher odds of developing SA, and OR < 1 is associated with lower odds of developing SA. The linkage disequilibrium analysis (LD, D") of the analyzed polymorphisms as well as the haplotypes construction were performed with Haploview version 4.1 (Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA). The analysis of data was performed with SPSS version 18.0 (SPSS, Chicago, Il) statistical package. The statistical power to detect an association with SA was 0.80, and was estimated with the QUANTO software [http://biostats.usc.edu/software].

2.4. Functional prediction analysis

Two *in silico* programs [FastSNP (http://fastsnp.ibms.sinica.edu.tw) and SNP Function Prediction (http://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/snpfunc.cgi)] were used to predict the potential effect of *SEL-E* gene polymorphisms. Both programs (FastSNP and SNPinfo) analyze the location of the SNP (eg. 5′-upstream, 3′-untranslated regions, intronic) and its possible functional effects such as amino acid changes in protein structure, transcription factor binding sites in promoter or intronic enhancer regions, and alternative splicing regulation by disrupting exonic splicing enhancers (*ESE*) or silencers (Yuan et al., 2006;

Table 1
Demographic characteristics and biochemical parameters of the studied individuals.

		SA (n = 287) Median	(percentile 25-75)	Healthy controls (n = 688) Median	(percentile 25-75)	*P* value
Age (years)		55	(50-61)	51.2	(47-56)	< 0.001
BMI (kg/m^2)		28.1	(26-31)	28	(25.3-30.5)	0.26
Blood pressure (mmHg)	Systolic	120	(112-129)	115	(106-123)	< 0.001
	Diastolic	77	(69-82)	72	(66-77.5)	< 0.001
Glucose (mg/dl)		94	(87-104)	89	(84-97)	< 0.001
Total cholesterol (mg/dl)		198	(170-218)	190	(167-208)	< 0.001
HDL-C (mg/dl)		43	(36-50)	47	(35-46)	0.001
LDL-C (mg/dl)		123	(102-144)	115	(96-132)	0.002
Triglycerides (mg/dl)		156	(118-202)	141	(151-191)	< 0.001
Gender n (%)	Male	206	(72)	463	(67)	0.173
	Female	81	(28)	225	(33)	
Smoking n (%)	Yes	85	(29)	579	(64)	< 0.001
Alcohol n (%)	Yes	224	(78)	500	(55)	< 0.001

Data are expressed as median and percentiles (25th-75th). P values were estimated using Mann-Whitney *U* test continuous variables and Chi-square test for categorical values.

Xu and Taylor, 2009).

3. Results

3.1. Characteristics of the study population

Demographic characteristics and biochemical parameters of the SA individuals and healthy controls included in the study are presented on Table 1.

3.2. Association of polymorphisms with SA

Allele and genotype frequencies of the *SEL-E* polymorphism in SA and healthy controls are shown in Table 2. Frequencies were in Hardy-Weinberg equilibrium. In our study, the polymorphisms [5′*UTR G98 T* (rs1805193), Ser128Arg *A561C* (rs5361), and Leu575Phe *C1880T* (rs5355)] were associated with increased risk of developing SA. Under co-dominant, dominant, and additive models, the *T* allele of the 5′*UTR G98 T* SNP was associated with an increased risk of SA (OR = 1.71, 95%CI: 1.00–2.93, $pC_{Co\text{-}dominant}$ = 0.0006, OR = 2.02, 95%CI: 1.21–3.38, $pC_{Dominant}$ = 0.004, and OR = 2.14, 95%CI: 1.34–3.44, $pC_{Additive}$ = 0.0015, respectively). Also, under co-dominant, dominant, and additive models, the *C* allele of the Ser128Arg *A561C* SNP was associated with increased risk of developing SA (OR = 1.60, 95%CI: 0.92–2.79, $pC_{Co\text{-}dominant}$ = 0.012, OR = 1.78, 95%CI: 1.04–3.06, $pC_{Dominant}$ = 0.038, and OR = 1.87, 95%CI: 1.13–3.1, $pC_{Additive}$ = 0.016, respectively). On the other hand, under co-dominant model, the *CT* genotype of the Leu575Phe *C1880 T* was associated with increased risk of SA as compared to *CC* genotype (OR = 2.34, 95%CI: 1.33–4.11, pC = 0.0035). All models were adjusted by common cardiovascular risk factors: gender, age, blood pressure, BMI, glucose, total cholesterol, HDL-C, LDL-C, triglycerides, smoking and alcohol consumption.

3.3. Linkage disequilibrium analysis

The analysis of haplotypes was performed using the Haploview version 4.1 program. In this analysis, two SNPs [5′*UTR G98T* and Ser128Arg *A561C*] out of three SNPs were in linkage disequilibrium (D' = 0.90) and were used to construct two haplotypes "*AG*" and "*CT*". Haplotype *CT* was associated with the increased risk of developing SA as compared to controls (OR = 1.80, 95% CI: 1.12–2.87, P = 0.0001). On the other hand, the haplotype *AG* showed a decreased frequency in individuals with SA when compared to healthy controls (OR = 0.46, 95% CI: 0.31-0.71, P = 0.009) (Table 3). Haplotype *CT*, present with heightened frequency in SA individuals was considered as a risk

Table 2
Distribution of *SEL-E* polymorphisms in patients with SA and healthy controls.

		Genotype frequency		MAF	Model	OR (95%CI)	pC
SEL-E UTR'5 G98T (rs1805193)							
Control	GG	GT	TT				
(n = 688)	634 (0.921)	54 (0.079)	0 (0.0)	0.040	Co-dominant	1.71 (1.00–2.93)	0.0006
					Dominant	2.02 (1.21–3.38)	0.004
SA	250 (0.871)	32 (0.111)	5 (0.017)	0.070	Over-dominant	1.67 (0.98–2.85)	0.064
(n = 287)					Log-additive	2.14 (1.34–3.44)	0.0015
SEL-E A561C (rs5361)							
Control	AA	AC	CC				
(n = 688)	638 (0.927)	50 (0.073)	0 (0.0)	0.040	Co-dominant	1.60 (0.92–2.79)	0.012
					Dominant	1.78 (1.04–3.06)	0.038
SA	255 (0.888)	29 (0.101)	3 (0.010)	0.060	Over-dominant	1.58 (0.90–2.75)	0.11
(n = 287)					Log-additive	1.87 (1.13–3.11)	0.016
SEL-E C1880T (rs5355)							
Control	CC	CT	TT				
(n = 688)	646 (0.939)	42 (0.061)	0 (0.0)	0.030	Co-dominant	2.34 (1.33–4.11)	0.0035
SA	254 (0.885)	33 (0.0115)	0 (0.0)	0.060			
(n = 287)							

SA, Subclinical Atherosclerosis; MAF, Minor allele frequency; OR, odds ratio; CI, confidence interval; pC, *P*-value. The p-values were calculated from logistic regression analysis, and ORs were adjusted for gender, age, blood pressure, BMI, glucose, total cholesterol, HDL-C, LDL-C, triglycerides, smoking and alcohol consumption. Bold numbers indicate significant associations.

Table 3
Frequencies of *SEL-E* haplotypes in SA and healthy controls individuals.

	SA (n = 287)	Controls (n = 688)	OR	95%CI	*p*-value
Haplotype	Hf	Hf			
AG	0.916	0.959	0.46	0.31-0.71	0.0001
CT	0.061	0.035	1.80	1.12-2.87	0.009

Abbreviations: Hf = Haplotype frequency, p = *p*-value, OR = odds ratio, 95%CI = confidential interval. The order of the polymorphisms in the haplotypes is according to the positions in the chromosome (rs5361 and rs1805193). Bold numbers indicate significant associations.

haplotype, whereas haplotype *AG*, present with reduced frequency, was regarded as a protective haplotype.

3.4. Functional prediction

The functional prediction analysis showed that the presence of the *T* allele of the *SEL-E 5′UTR G98 T* (rs1805193) polymorphism potentially produces a binding motif in the OCT3/4 transcription factor. The analysis also revealed that the *C* allele of the *SEL-E A561C* (rs5361) polymorphism may generate binding motifs for SF2/ASF, and Srp55 proteins. In contrast, the analysis of the *SEL-E C1880T* (rs5355) polymorphism did not exhibit evidence of potential functional motifs.

4. Discussion

In this study, we examined the relationship between three [5′*UTR G98 T* (rs1805193), Ser128Arg *A561C* (rs5361), and Leu575Phe *C1880T* (rs5355)] *E-selectin* gene SNPs and SA risk. The local inflammation and endothelial dysfunction are the first stages of the atherosclerotic processes. In this context, the E-selectin is considered as an important endothelial product in the chain of events leading to plaque formation and atherosclerosis; E-selectin plays an important role in recruitment, rolling, and diapedesis of lymphocytes and monocytes in the blood vessel wall (Gonzales and Selwyn, 2003; Galkina and Ley, 2007; Mallika et al., 2007; Fernandez-Borja et al., 2010**).** As far as we know, this is the first study that describes the association of the *SEL-E* polymorphisms with risk of developing SA. Interestingly, we found that the *C* allele of the *SEL-E A561C* SNP was associated with increased risk of developing SA in our population. In agreement with our data, Zhao et al., studied the same SNPs and reported the association of the *C* allele of the Ser128Arg *A561C* polymorphism with increased risk of developing ischemic stroke (OR = 2.80) in Han Chinese population (Zhao et al., 2012). Similarly, Wang et al., reported that carriers of *CC* genotype of this polymorphism had a heightened risk for development of essential hypertension (OR = 3.81) (Wang et al., 2012). In line with these data, Liao et al. reported in a meta-analysis the association of the *CC* genotype (OR = 1.91) of the *Ser128Arg A561C* polymorphism, as well as of the *TT* genotype (OR = 2.82) of the 5′*UTR G98 T* polymorphism with an increased risk for CAD in Asian population (Liao et al., 2016). In the same vein, Wu et al. reported in a meta-analysis that the *C* allele of the Ser128Arg *A561C* polymorphism increased risk for CAD (OR = 2.07) in an Asian population, but not among Caucasians (Wu et al., 2015). We also found the association of the *CT* genotype of the Leu575Phe *C1880 T* polymorphism with increased risk of development of SA. However, in contrast with our results, Issac et al. studied the Leu575Phe *C1880T* (rs5355) polymorphism and reported that this SNP not is associated with the risk of development of carotid atherosclerosis in end-stage renal disease in Egyptian population (Issac et al., 2014).

The haplotype analysis showed that the *CT* haplotype conformed by Ser128Arg *A561C* and 5′*UTR G98 T* SNPs increased risk of developing SA, whereas that the *AG* haplotype was associated with a decreased risk. Evidently, in the *CT* haplotype the presence of the *C* and *T* alleles of the Ser128Arg *A561C* and 5′*UTR G98 T* polymorphisms represents the risk haplotype. In addition, it is important to note that in the independent analysis of the SNPs, the *C* and *T* alleles were associated with risk of developing SA, indicating that both alleles have an important role in development of SA. In addition, even if Leu575Phe *C1880T* polymorphism was not in linkage disequilibrium with the SNPs Ser128Arg *A561C* and *G98T*, it is likely that the former may have an important role as a single SNP in the development of SA. Nevertheless, we consider that other studies are needed to investigate the true role of these SNPs in risk of developing SA and other cardiovascular diseases in populations with different ethnic origins.

To our knowledge, there are not functional studies concerning the 5′*UTR G98T*, and Leu575Phe *C1880 T* polymorphisms. However, we determined the potential effect of the polymorphisms associated with the SA development using bioinformatics tools. The analysis of the Leu575Phe *C1880T* polymorphism did not reveal any evidence of functional effects of this polymorphism. Moreover, the analysis of the 5′*UTR G98 T* polymorphism showed that presence of the *T* allele produces a binding site for the *OCT3/4* transcription factor. Nonetheless, the potential functional role of the *A561C* (Ser128Arg*)* polymorphism is still controversial; for example, Yoshida et al., reported that the change of the *Arg (C) > Ser (A)* in the *A561C (*Ser128Arg*)* polymorphism modified the binding specificity of E-selectin, and enhanced the adhesion of leukocytes (Yoshida et al., 2003). In addition, the *Arg (C)* allele was associated with greater levels of phosphorylation of extracellular signal regulated kinase 1 and 2 and p38 mitogen-activated protein kinase, suggesting an altered endothelial signaling pathway (Yoshida et al., 2003). In contrast, previous reports indicated that the *C* allele of the *A561C (*Ser128Arg*)* polymorphism had no effect on E-selectin plasma levels (Saldoval-Pinto et al., 2014; Miller et al., 2005). Additional to this information, we determined by bioinformatics tools that the *C* allele of the Ser128Arg *A561C* polymorphism generates an exonic splicing enhancer binding sites for SF2/ASF and SRp55 proteins that regulate alternative splicing. These proteins may result inappropriate splicing of the mRNA transcript resulting in abnormal protein product. In our opinion, the functional consequence of these polymorphisms deserves to be specifically addressed in future studies.

We recognize the relatively small sample of number of individuals as a main limitation of this study. In spite of this limitation, our study contributes with a new argument in which the 5′*UTR G98 T,* Ser128Arg *A561C,* and Leu575Phe *C1880 T* polymorphisms may have a role in the development of SA. Therefore, these results with Mexican population justify the design of additional studies with a larger number of individuals to further confirm the role of these polymorphisms as markers of risk of or protection against developing SA and other cardiovascular diseases.

In summary, this study demonstrates that the 5′*UTR G98 T,* Ser128Arg *A561C,* and Leu575Phe *C1880 T* polymorphisms are associated with an increased risk of developing SA. In addition, we distinguished one haplotype (*CT*) associated with an increased risk of develop SA.

Conflicts of interest

There are no competing financial interests in this study.

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