UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

FACULTAD DE MEDICINA

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EVALUACIÓN DE POLIMORFISMOS EN LOS GENES ENOS, RANTES, SEL-P Y SEL-E EN LA SUSCEPTIBILIDAD GENÉTICA AL DESARROLLO DE ATEROSCLEROSIS

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

M. EN C. HERRERA MAYA GABRIEL

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CIUDAD UNIVERSITARIA, CD. MX. , MAYO, 2021

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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS
FACULTAD DE MEDICINA
OFICIO CPCB/360/2021
ASUNTO: Oficio de Jurado

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted qué en la reunión ordinaria del Subcomité de Ecología y Manejo Integral de Ecosistemas y Biología Evolutiva y Sistemática del Posgrado en Ciencias Biológicas, celebrada el día **22 de marzo de 2021** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **HERRERA MAYA GABRIEL** con número de cuenta **305127745** con la tesis titulada **"EVALUACIÓN DE POLIMORFISMOS DE LOS GENES eNOS, RANTES, SEL-P, SEL-E EN LA SUSCEPTIBILIDAD GENÉTICA AL DESARROLLO DE ATEROSCLEROSIS",** realizada bajo la dirección del **DR. JOSÉ MANUEL FRAGOSO LONA,** quedando integrado de la siguiente manera:

Presidente:	DR. JESÚS KAZUO YAMAMOTO FURUSHO
Vocal:	DR. EUSTACIO GALILEO ESCOBEDO GONZÁLEZ
Vocal:	DR. JULIAN RAMÍREZ BELLO
Vocal:	DR. JULIO GRANADOS ARRIOLA
Secretario:	DRA. MARTHA ESTHELA PÉREZ RODRÍGUEZ

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Cd. Universitaria, Cd. Mx., a 27 de abril de 2021

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

AL POSGRADO EN CIENCIAS BIOLÓGICAS DE LA UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO. POR PERMITIR TENER UN ESPACIO DE DEDICACIÓN AL CONOCIMIENTO Y LA INFRAESTRUCTURA PARA PODER DESARROLLARME PROFESIONALMENTE DURANTE MI EDUCCIÓN PROFESIONAL HASTA EL GRADO DE DOCTOR EN CIENCIAS.

AGRADEZCO AL CONSEJO NACIONAL DE CIENCIA Y TECNOLOGÍA (CONACYT) POR LA BECA OTORGADA CON NÚMERO DE CVU 545322 DURANTE EL DESARROLLO DEL PRESENTE TRABAJO PARA LA OBTENCIÓN DE GRADO DOCTOR EN CIENCIAS. ASI MISMO SE GARADECE AL CONACYT POR EL FINANCIAMIENTO DE ESTE PROYECTO.

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Índice

Resumen

El objetivo del estudio fue evaluar si los polimorfismos de un solo nucleótido (SNPs) de los genes *SEL-E, eNOS, CCL-5 y SEL-P* se asocian con el desarrollo de la placa aterosclerótica que conlleva al síndrome isquémico coronario agudo (SICA) y la aterosclerosis. La determinación de polimorfismos presentes en los genes *SEL-E, eNOS, CCL-5* y *SEL-P [SEL-E 98 G/T (rs1805193), SEL-E 602 A/C (rs5361), SEL-E 1040 A/G (rs5364), SEL-E 1559 C/T (rs5368) and SEL-E 1839 C/T (rs5355), eNOS -786 T/C (rs2070744), eNOS 894 G/T (rs1799983), CCL-5 -28 G/C (rs2280788), CCL-5 -109 C/T (rs1800825), CCL-5 -403 G/A (rs2107538), SEL-P -1969 A/G (rs1800805), SEL-P 1087 G/A (rs6131), SEL-P 2013 G/T (rs6133)* y *SEL-P 2361 A/C (rs6136)]* se realizó utilizando ensayos TaqMan 5' exonucleasa (Applied Biosystems), en un grupo de 287 individuos con aterosclerosis subclínica, 625 pacientes con SICA y 700 individuos control. Así mismo, se realizó la correlación de los polimorfismos asociados con los niveles plasmáticos de las moléculas propuestas en este estudio. De acuerdo con los resultados obtenidos en este trabajo, la distribución alélica y genotípica de los polimorfismos *eNOS -786 T/C* y *eNOS 894 G/T eNOS* fue similar en pacientes con SICA y controles sanos. No obstante, el análisis de haplotipos demostró que el haplotipo "*TT*" conformado por los polimorfismos del gen *eNOS -786 T/C* y *eNOS 894 G/T* se asoció con una disminución de riesgo al desarrollo de SICA en nuestra población. Por otra parte, bajo los modelos de herencia co-dominate, dominante y aditivo el alelo *T* del SNP *SEL-E* 98 *G/T*, así como el alelo *C* del polimorfismo *SEL-E* 561 *A/C* se asociaron con mayor riesgo al desarrollo de aterosclerosis. Además, bajo el modelo co-dominante, el genotipo *CT* del polimorfismo *SEL-E* 1880 *C/T* se asoció con mayor riesgo al desarrollo de aterosclerosis en comparación con el genotipo *CC*. Por otro lado, el alelo *G* del polimorfismo del gen *CCL-5 -109 C/T* se asoció con mayor riesgo al desarrollo de SICA, bajo los modelos co-dominante, dominante y aditivo. Así mismo, bajo los modelos co-dominante y recesivo, el alelo *A* del polimorfismo *CCL-5* -403 *G/A* fue asociado con mayor riesgo al desarrollo de SICA. Por otro lado, el análisis los polimorfismos del gen *SEL-P 1087 G/A*, *SEL-P* 2013 *G/T y SEL-P* 2361 *A/C* mostro que bajo los modelos de herencia (co-dominate, dominante, heterocigoto y aditivo) el alelo *A* del polimorfismo *SEL-P 1087 G/A* y el alelo *C* del polimorfismo *SEL-P* 2361 *A/C* reducen el riesgo de desarrollar SICA. Respecto al análisis del efecto funcional de los polimorfismos asociados, los resultados mostraron que los portadores de los siguientes genotipos (*GG* del polimorfismo *CCL-5 -109 C/T*, *AA* del polimorfismo *CCL-5* -403 *G/A*, *AA* del polimorfismo *SEL-P 1087 G/A y CC* del polimorfismo *SEL-P* 2361 *A/C*) disminuye la concentración de RANTES y selectina-P, respectivamente. En resumen, en este estudio se demuestra que los polimorfismos *SEL-E* 98 *G/T*, *SEL-E* 561 *A/C* y *SEL-E* 1880 *C/T* del gen *SEL-E* están asociados con un mayor riesgo de desarrollar aterosclerosis. Por otra parte, se demostró que los polimorfismos *CCL-5* -109 *G/A* y *CCL-5* -403 *G/A* se asocian con el riesgo de desarrollar SICA, así como con baja concentración de RANTES en plasma. Finalmente, los polimorfismos *SEL-P* 1087 *G/A* y *SEL-P 2361 A/C* del gen *SEL-P* se asociaron con bajo riesgo de desarrollar SICA y bajos niveles de selectina-P en plasma.

Abstract

The aim of the present study was to evaluate whether single nucleotide polymorphisms (SNPs) of *SEL-E, eNOS, CCL-5* and *SEL-P* genes are associated with the development of atherosclerotic plaque that leads to acute coronary ischemic syndrome (ACS) and atherosclerosis. The determination of polymorphisms present in *SEL-E, eNOS, CCL-5* and *SEL-P* [*SEL-E* 98 *G/T* (rs1805193), *SEL-E* 561 *A/C* (rs5361), *SEL-E* 1040 *A/G* (rs5364), *SEL-E* 1559 *C/T* (rs5368) and *SEL-E* 1839 *C/T* (rs5355), *eNOS* -786 *T/C* (rs2070744), *eNOS* 894 *G/T* (rs1799983), *CCL-5* -28 *G/C* (rs2280788), *CCL-5* -109 *C/T* (rs1800825), *CCL-5* -403 *G/A* (rs2107538), *SEL-P* -1969 *A/G* (rs1800805), *SEL-P* 1087 *G/A* (rs6131), *SEL-P* 2013 *G/T* (rs6133) and *SEL-P* 2361 *A/C* (rs6136)] was performed using TaqMan5'exonuclease assays (Applied Biosystems), in a group of 287 individuals with subclinical atherosclerosis, 625 patients with SICA and 700 control individuals. Likewise, the correlation of polymorphisms *eNOS* -786 *T/C*, and *eNOS* 894 *G/T* was similar in patients with SICA and healthy controls. However, the haplotype analysis showed that the "TT" haplotype conformed by the *eNOS* -786 *T/C* and *eNOS* 894 *G/T* gene was associated with decrease risk of developing SICA in our population. On the other hand, under co-dominant, dominant and additive inheritance models, the T allele of the *SEL-E* 98 *G/TSEL-E* SNP, as well as the C allele of the *SEL-E* 561 *A/C* polymorphism were associated with higher risk of developing subclinical atherosclerosis. In addition, under co-dominant model, the *CT* genotype of the *SEL-E* 1880 *C/T* polymorphism was associated with a higher risk of developing atherosclerosis compared to the *CC* genotype. Besides, the *G* allele of the *CCL-5* -109 *G/A* were associated with higher risk of developing SICA, under co-dominant, dominant and additive models. Likewise, under co-dominant and recessive models, the A allele of the *CCL-5* -403 *G/A* polymorphism were associated with higher risk of developing SICA. Finally, the analysis of the polymorphisms *SEL-P 1087 G/A* and *SEL- P*2361 *A/C* showed that under inheritance models (co-dominate, dominant, heterozygous and additive) the A allele (290Asn) and the C allele (715Pro) reduce risk of developing SICA. Regarding the analysis of the functional effect associated with polymorphisms, the results showed that carriers of the following genotypes (*CCL-5* -109 GG, *CCL-5* -403 AA, *SEL-P* 290 Asn / Ans, and *SEL-P* 715 Pro / Pro) decrease the concentration of RANTES and P-selectin, respectively. In summary, this study shows that *SEL-E* 98 *G/T, SEL-E* 561 *A/C* and *SEL-E* 1880 *C/T* polymorphisms of the *SEL-E* gene are associated with increased risk of developing sub-clinical atherosclerosis. Besides, it was shown that the polymorphisms *CCL-5*-109 *G/A* and *CCL-5* -403 *G/A* are associated with risk of developing SICA, as well as low concentration of RANTES in plasma. Finally, the *SEL-P 1087 G/A* and *SEL-P 2361 A/C* polymorphisms of the *SEL-P* gene were associated with a low risk of developing SICA and low levels of P-selectin in plasma.

Capítulo I
Introducción general

Introducción

Aterosclerosis

La aterosclerosis es una enfermedad progresiva y multifactorial condicionada por factores genéticos y ambientales caracterizada por la acumulación de lípidos en las capas íntima y media arterial, ahora considerada como enfermedad con inflación crónica (1, 2). La aterosclerosis se desarrolla silenciosamente a lo largo de los años y suele estar muy avanzada cuando aparecen los síntomas (3-5). En los últimos años cambió el paradigma que explicó por mucho tiempo la arteriosclerosis como resultado de una compleja interacción de factores no accesibles a intervención médica. En ese paradigma las alteraciones del metabolismo de los lípidos fueron el concepto clave asociado a la aterosclerosis como enfermedad crónico-degenerativa (6). En los últimos 20 años, se ha demostrado que la disfunción endotelial, así como la inflamación tienen crucial importancia en la patogenia de la aterosclerosis y de sus complicaciones; actualmente la aterosclerosis se ha considerado una enfermedad inflamatoria causa de la disfunción endotelial (7-10). Las consecuencias clínicas de la aterosclerosis, siendo estas el primer paso para el desarrollo de diversas patologías, incluyen los síndromes isquémicos coronarios agudos (SICA) (11).

Síndrome isquémico coronario agudo

Los SICA se definen como cualquier síntoma clínico que se asemejé a la isquemia cardiaca, lo que incluye a la angina inestable, infarto agudo de miocardio con y sin elevación del segmento ST. La identificación y la clasificación del infarto agudo de miocardio con elevación del segmento ST y el infarto agudo de miocardio sin elevación del segmento ST se obtiene mediante las características clínicas, los cambios electrocardiográficos y los marcadores bioquímicos de necrosis cardiaca (isoezimas de la fosfocinasa o troponina I que se deben de encontrar por encima del límite superior normal). Se sugiere que el diagnostico siga las definiciones de la Sociedad Europea de Cardiología (ESC, por sus siglas en ingles) y el colegio americano de cardiología (ACC). El diagnostico que no se asoció a infarto agudo de miocardio con elevación del segmento ST o infarto agudo de miocardio sin elevación del segmento ST fue asociado a angina inestable. El diagnostico de angina inestable se determina mediante los síntomas de malestar en reposo con cambios del segmento ST en el electrocardiograma que indicaban isquemia [depresión del segmento ST o elevación transitoria (≥1 mm) en al menos dos derivaciones contiguas y/o inversión de la onda T prominente] y un biomarcador positivo que indica necrosis miocárdica. (11-15).

El infarto agudo de miocardio es consecuencia de la interrupción abrupta del flujo sanguíneo arterial, asociada a los eventos trombóticos agudos con lesiones coronarias relacionadas a placas ateroscleróticas. En la perdida de irrigación se observan alteraciones inicialmente a nivel de los miocitos lo cual modifica la anatomía regional y parietal cardiaca con irregularidades hemodinámicas y compensación neurohormonal (16, 17). En el caso de la angina inestable no se interrumpe el flujo sanguíneo, lo cual podemos aportar a un trombo no oclusivo, considerado la causa de la disminución aguda del flujo sanguíneo (8, 17).

La rotura de la placa genera hemorragia intralesional a través de la superficie rota, se desarrolla la trombosis con progresión rápida, generando la lesión coronaria (18). Posteriormente a la proliferación de células de musculo liso y aumento de la matriz extracelular, inflamación superficial, adherencia de células de inmunidad innata y factores de crecimiento sanguíneos contribuyen a la progresión del evento cardiovascular (19, 20).

De esta manera se puede concluir que los SICA son consecuencia del desequilibrio entre el aporte y la demanda de oxígeno, considerando la reducción aguda o subaguda de dicho aporte, excluyendo trastornos como la hipertensión arterial, hipertensión pulmonar arteritis, trombocitosis u anomalías congénitas, que también son capaces de provocar un evento isquémico (21-23).

Epidemiologia

En los últimos diez años el síndrome isquémico coronario agudo ha constituido la causa de hasta el 23% de los fallecimientos en los países industrializados (12, 24). En México, los SICA son el principal grupo de causas de muerte desde 1990, la mayor mortalidad se ha observado en los hombres (25). Una de las razones por la cual se ha mantenido tantos años como principal causa de muerte se debe a que 60 % de la población adulta en México presenta al menos algún factor de riesgo relacionado al desarrollo de SICA (tabaquismo, obesidad y sobrepeso, hipertensión, diabetes o dislipidemia) (26). Otro de los factores involucrados al desarrollo de SICA es el proceso de envejecimiento de la población general lo que conlleva al incremento de enfermedades asociadas a la edad (como son los SICA) (27, 28). Las proyecciones dejan claro que la mortalidad por SICA tiene tendencia al aumento Fig. 1 (28, 29). Predicciones epidemiológicas de los Estados Unidos de América (30) y países Europeos (31), así como de la Secretaría de Salud de la República Mexicana (32) prevén que esta enfermedad permanecerá en un lugar predominante entre los problemas de salud para las siguientes dos décadas.

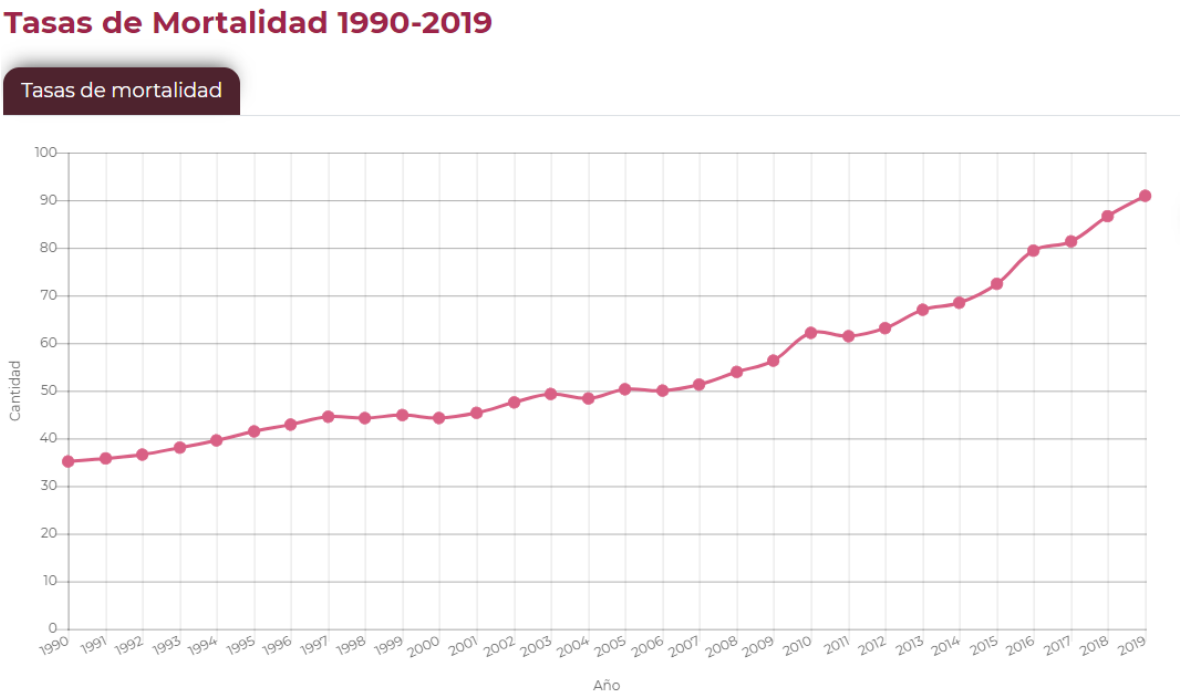

Figura 1. Tasa de mortalidad por cada 100,000 habitantes a nivel nacional (Sistema de Información de la Secretaría de Salud).

Fisiopatología síndrome isquémico coronario agudo

Disfunción endotelial

El endotelio es una monocapa de células que recubre la pared luminar de los vasos sanguíneos, regula la interacción de las células y las proteínas circulantes con las células residentes en la pared vascular, ejerciendo un papel central como sensor y transmisor de señales. Protege a la pared frente al desarrollo de lesiones, contribuyendo a la homeostasis vascular. El endotelio tiene funciones antitrombóticas (inhibe la adhesión plaquetaria, la coagulación, y regula el sistema fibrinolítico), controla la actividad de las células musculares lisas de la capa media, así como la adhesión de leucocitos (monocitos/macrófagos, linfocitos T) a la pared arterial. El endotelio también modula el tránsito de macromoléculas, como las lipoproteínas hacia el espacio subendotelial. En este contexto, la disfunción endotelial puede definirse como un desequilibrio en la biodisponibilidad de sustancias activas de origen endotelial que predispone a la inflamación, a la vasoconstricción y al aumento de la permeabilidad vascular, facilitando el desarrollo de aterosclerosis, agregación plaquetaria y trombosis (33-36). Aunado a esto, existen otros factores conocidos que participan en el desarrollo de la aterosclerosis, entre estos tenemos a las lipoproteínas de baja densidad (LDL) oxidadas, el tabaquismo, la diabetes, la hipertensión, la obesidad, el género, la edad, el alcoholismo los radicales libres de oxígeno, la homocisteína, el déficit estrogénico y las infecciones, que facilitan el desarrollo de la aterosclerosis (35, 36).

La pérdida paulatina de la capacidad del endotelio para controlar el tráfico de macromoléculas hacia el interior de la pared permite un mayor depósito de moléculas

circulantes, como las LDL, iniciando así el fenómeno inflamatorio (37); las LDL circulantes quedan atrapadas en la matriz extracelular del espacio subendotelial y se oxidan, convirtiéndose en factores quimiotácticos para monocitos circulantes. Al penetrar al espacio subendotelial, los monocitos se diferencian a macrófagos, exacerbando la respuesta inflamatoria al fagocitar a las lipoproteínas oxidadas e incrementan la expresión de moléculas de adhesión, la producción excesiva de elementos de la matriz extracelular, quimiocinas y citocinas que van provocando un aumento en el volumen del ateroma y la consecuente disminución de la luz arterial llegando incluso a ocluirla por completo lo que generará isquemia del tejido irrigado por tal vaso. Sin lugar a duda la disfunción endotelial y el fenómeno inflamatorio desempeñan un importante papel en el desarrollo de la ateroesclerosis coronaria (9, 10, 38, 39)). Los datos acumulados demuestran que una baja o elevada expresión de las moléculas en estudio conllevan a una disfunción endotelial, y por consecuencia al fenómeno inflamatorio en individuos asintomáticos, así como en pacientes con enfermedad arterial coronaria, hipertensión y aterosclerosis (40-44). Entre los factores que participan en la disfunción endotelial y en el fenómeno inflamatorio destacan el óxido nítrico sintasa-endotelial (*eNOS*), la proteína quimioatrayente "RANTES", las moléculas de adhesión P y E selectinas (*SEL-P* y *SEL-E*). Moléculas que tienen un papel fundamental en la formación de la placa ateromatosa, ya que están involucradas en la regulación del endotelio, así como en la adhesión, rodamiento y migración de los monocitos/macrófagos al espacio subendotelial.

Migración de lipoproteínas de baja densidad

Estudios en animales y humanos han demostrado que la hipercolesterolemia compuesta en su mayoría por LDL, causa activación focal en el endotelio y espacio subendotelial de las arterias coronarias. Esta provoca la infiltración y retención de LDLs en la íntima arterial que inicia una respuesta inflamatoria en la pared arterial. (45-47). Así mismo, se sabe que las LDL y su concentración tienen íntima relación en el desarrollo de la placa ateromatosa. Las concentraciones que hasta ahora se sabe que afectan y contribuyen al desarrollo de la placa aterosclerótica son aquellos casos donde el paciente tiene valores mayores de 200 mg/dl de LDL, que se asociaron al desarrollo de eventos coronarios agudos. Por otro lado los pacientes con colesterol total mayor a 300 mg/dl no presentan evidencia de enfermedad coronaria, sino, hasta edades avanzadas. También se ha visto que en pacientes con igual grado de concentración de hipercolesterolemia y valores de LDL, presentan diferente evolución clínica, convirtiéndose la hipercolesterolemia en el punto clave en un evento coronario agudo. La internalización y acumulación extravascular del colesterol, LDLs y esteres de colesterol depende probablemente de dos mecanismos, uno activo dependiente de receptores específicos en el endotelio y otro pasivo de receptores independientes, presumiblemente cuando el daño endotelial es más avanzado (5, 48).

Una vez dentro de la capa íntima y media arterial la LDL se oxida, sufriendo cambios principalmente en la subunidad apo-B. Posteriormente se oxidan otras subunidades de la LDL siendo estos pasos necesarios para que las LDL oxidadas (LDLox) puedan ser reconocidas por los receptores del monocito/macrófago. Los diferentes grados de oxidación de LDL estimulan la producción de factor tisular, siendo esta considerada citotóxica. Por lo tanto, a medida que aumenta el grado de oxidación de LDL, disminuye su capacidad para inducir mediadores de inflamación y aumenta su capacidad toxica sobre la célula (49, 50).

La LDLox altera la actividad de la óxido nítrico sintetiza (NOS), provocando la disminución de la producción de óxido nítrico (ON), un importante vasodilatador que mantiene el tono vascular, disminuyendo la expresión de la molécula de adhesión celular vascular 1 (VCAM-1), molécula de adhesión intracelular 1(ICAM-1) y la proteína quimioatrayente de monocitos 1 (MCP-1), entre otras moléculas. Por otro lado, el anión superóxido (o2-), aumentado en la hipercolesterolemia, produce inactivación oxidativa del ON produciendo peroxinitrito (ONOO-), que a bajas concentraciones puede tener igual efecto que el ON ; pero en elevadas concentraciones es tóxico para las células endoteliales, provocando disfunción endotelial (51, 52). Por otro lado, la LDLox puede activar también al factor NFKB que estimula la producción de MCP-1 y el factor estimulador de colonias de macrófagos (MCSF). Otro juego de moléculas que intervienen en la adhesión, rodamiento y migración del endotelio, lo que contribuye a la disfunción endotelial, son las lisofosfatídil colinas presentes en las LDL que provocan la expresión de moléculas como las Selectinas E, P, VCAM-1 e ICAM-1, con lo cual se logra el ingreso de los monocitos al espacio subendotelial (53, 54).

Matriz extracelular y proceso oxidativo

El fenómeno de infiltración de LDL al espacio subendotelial considera la retención y acumulación de lipoproteínas en la matriz extracelular en conjunto con la alta concentración de lipoproteína en plasma. La matriz extracelular está compuesta por colágeno tipo I, elastina, proteoglicanos y proteínas como la fibronectina, laminina y tenascina. La matriz extracelular se extiende de la membrana basal de las células del endotelio a la íntima media interna del vaso, formando un continuo contacto con la región pericelular de las células de musculo liso (55-57).

Dentro de la matriz extracelular los proteoglicanos se distribuyen en dos formas: los proteoglicanos que se encuentran en el ambiente pericelular, anclados a la membrana plasmática, y los secretados por células que forman parte del espacio extracelular que usualmente no se conecta físicamente con otras células de la matriz. Los proteoglicanos son macromoléculas compuestas por un grupo complejo de proteínas lineares y de largas cadenas de carbohidratos, llamados glicosaminoglicanos (GAGs). Estos son distintos tipos: el condroitin sulfato (CS), dermatan sulfato (DS), heparan sulfato (HS), keratan sulfato y la hialurona. Varias clases de CS y DS son fundamentales en la arteria e incluyen también al versican y decorin, dos pequeños proteoglicanos ricos en leucina contenidos en DS y CS respectivamente (58-63).

Las células que regulan la producción de los proteoglicanos dentro de la arteria son las células del musculo liso, endoteliales y macrófagos. Estas son responsables de la síntesis de proteoglicanos en la pared arterial (64-67). S Jimi, y colaboradores (68) exponen como participantes principales en la unión de los mucopolisacáridos de las arterias a las betalipoproteínas, que en conjunto con las moléculas de fibrinógeno forman uniones iónicas de grupos aminos cargados negativamente que se unen a las moléculas de apoB cargados positivamente, siendo estos los responsables de la interacción entre mucopolisacáridos y betalipoproteínas la subunidad apoB100. Por otro lado, se sabe por los trabajos de Flood y colaboradores (69) que la interacción también se da por la subunidad apoB48 en los cuales

la participación de los proteoglicanos es más afín. Esta evidencia en conjunto con la retención de LDL por el colágeno tipo I y III se propone que la retención y acumulación de LDL conllevan al desarrollo de la placa ateromatosa con el consecuente SICA (69-75).

El siguiente proceso a la retención de moléculas de LDL es la oxidación de las mismas. Un evento importante para que se inicie la formación de la placa ateromatosa es el transporte de la LDLox a través del endotelio hacia la pared arterial (76) donde encontramos células endoteliales, células de musculo liso y macrófagos, entre otras, que son fuentes importantes de especies reactivas de oxígeno (ROS) como el superoxido ($-O_2$) (77, 78). La LDLox desencadena inflamación aguda en la cual hay activación de moléculas pro- y antiinflamatorias que conllevan a eventos isquémicos coronarios agudos (79). El oxígeno es una molécula abundante en los organismos biológicos, que a pesar de ser un radical, reacciona con moderación por los dos electrones desapareados que presenta. El oxígeno molecular dentro del organismo sufre reducción univalente a forma (O_2) por medio de enzimas como la NADPH, NADH y Xantin oxidasa. No enzimáticamente, el oxígeno también puede convertirse a superóxido por la reacción con compuestos de actividad redox semejantes a la semiubiquinona en la cadena de transporte de electrones en la mitocondria (80-82). Por otro lado, la superóxido dismutasa (SOD) a partir del anión O_2 produce peróxido de hidrógeno (H_2O_2), que puede reaccionar con otros radicales como la transición del metal FE^{2+} a FE^{3+} que produce grupos hidroxilo(OH^-) altamente reactivos; esto es conocido como la reacción de Fenton (83).

Además, durante el proceso de fagocitosis es secretada una hemoproteina que amplifica el potencial oxidativo del H_2O_2, ya que interacciona con el Cl en condiciones fisiológicas formando hipoclorito (HOCL), que interacciona con la molécula de O2 produciendo radicales (OH^-) 76-79F. Así mismo, el complejo NADPH oxidasa, tal vez la mayor fuente de O2, después del transporte de electrones en la mitocondria, se relaciona íntimamente con la vasculatura de las arterias (84).

El complejo NADPH oxidasa está compuesto por subunidades que se encuentran en la membrana de las células endoteliales, fibroblastos y células de musculo liso vascular. Está compuesto por enzimas de la familia Nox (1,2 y 4), p47phox, p67phox y proteínas G rac1 y rac2 (85). Este complejo aumenta la concentración de O2 durante la formación de la placa ateromatosa, el complejo NADPH oxidasa y la producción de O2 son incrementados en células vasculares y endoteliales por un numero de agonistas (angiotensina II, trombina, PDGF y el factor de necrosis tumoral alfa) asociados con la patogenia. En humanos Azzumi *et al*. describen producción de ROS y LDLox asociados a la subunidad p22phox sugiriendo que las ROS catalizan la formación de LDLox, que son reconocidas por macrófagos en el espacio subendotelial dando como resultado la activación y formación de células espumosas (86). Otro sistema, no menos importante, es la activación dela xantin oxidasa, que genera O2 por la catalizacion de hipoxantina y xantina a ácido úrico. Bajo condiciones fisiopatológicas se perpetúa esta fuente de estrés oxidativo en células endoteliales y de musculo liso. El desacoplamiento de (NOS), en especial la NOS endotelial también son fuente importante de O2 en la patología. (87)

Proceso inflamatorio

La inflamación es pieza central en el desarrollo de los SICA. En los últimos años, un número creciente de observaciones ha demostrado que la inflamación es crucial en la patogenia de la aterosclerosis y de sus complicaciones, por lo cual ahora es considerada como una enfermedad inflamatoria (3, 4, 8).

El fenómeno inflamatorio se inicia cuando las lipoproteínas circulantes quedan atrapadas en la matriz extracelular subendotelial y se oxidan, adquiriendo así propiedades pro-inflamatorias que dan lugar a eventos que van desde el depósito de monocitos circulantes que exacerban la respuesta inflamatoria al fagocitar los lípidos, la producción excesiva de elementos de la matriz extracelular y reclutamiento de nuevas células. Todos estos eventos provocan aumento en el volumen del ateroma o placa ateromatosa y la consecuente disminución de la luz arterial llegando incluso a ocluir por completo, lo que genera isquemia del tejido irrigado por tal vaso. Sin embargo, otro evento frecuente es que ocurra la ruptura de la placa, con los que se exponen componentes subendoteliales altamente trombogénicos que generan trombosis arterial y por lo tanto isquemia, de menor o mayor lesión al tejido, dependiendo del sitio de la lesión, en especial cardiaco (3, 4, 9, 88).

Sin lugar a dudas el fenómeno inflamatorio desempeña un importante papel en el desarrollo de la aterosclerosis coronaria y probablemente constituye el factor de transformación de un síndrome coronario estable e inestable (10, 38, 39). La respuesta inflamatoria no solo promueve el inicio de un proceso aterosclerótico, sino que también contribuye al posterior crecimiento del ateroma y a la precipitación de sucesos trombóticos agudos. (3, 4, 7).

Los datos acumulados demuestran que la concentración elevada de marcadores circulantes de inflamación predice la respuesta cardiovascular desfavorable en individuos asintomáticos, en pacientes can cardiopatía isquémica estable y en pacientes con síndromes coronarios agudos (89, 90). Varios estudios “in vitro” y en animales experimentales son apoyados por hallazgos clínicos de incremento de marcadores inflamatorios en pacientes con angina estable crónica, angina inestable e infarto agudo de miocardio (91, 92).

De las moléculas inflamatorias que participan en esta serie de eventos son enfocamos en las moléculas relacionadas a la inmunidad innata como los son las selectinas, quimiocinas y productoras de óxido nítrico (4). Sus principales efectos en el sistema cardiovascular incluyen el incremento en la expresión de moléculas de adhesión, la producción de citocinas endoteliales y óxido nítrico lo que conlleva a la permeabilidad vascular, reducción de la actividad de lipoproteína lipasa, incremento de la síntesis de ácidos grasos hepáticos y efecto protrombotico (93).

Óxido nítrico sintasa endotelial

El óxido nítrico es una molécula que tiene diversas funciones que van desde la neurotransmisión, inhibición de la síntesis de ADN, regulación de la expresión genética, traducción del ARN mensajero, modificaciones postraduccionales hasta la regulación del tono vascular. En modelos murinos se ha descubierto que es molécula clave en el desarrollo y morfogénesis de pulmón. Esta molécula puede ser desactivada al reaccionar con especies reactivas de oxígeno, debido a que tiene dominios reductasa y dominios oxygenasa que funcionan transportando electrones. El sustrato comprende una molécula de NADPH que

terminara en la generación de ON y L- citrulina. Al encontrarse desacoplada puede interrumpir la trasportación del electrón que reaccionara con los componentes del citosol para formar especies reactivas de oxígeno (94). La *eNOS* tiene un papel crucial en la regulación y función del endotelio vascular; Es expresada mayormente en células endoteliales y plaquetas (94) debido a que participa en la vasodilatación; inhibe potentemente la agregación plaquetaria, así como la proliferación y migración de células de músculo liso (94, 95). Está involucrado tan fuertemente con la vasodilatación que la pérdida del gen codificante de esta enzima, en modelos murinos, provoca elevación de la presión arterial y defectos en la neovascularización. Además, evita la adhesión de leucocitos al endotelio mediante la inhibición de la expresión o la interferencia con las moléculas de adhesión CD11/CD18 en la membrana de las células endoteliales; es capaz de inhibir la apoptosis por la liberación de citocinas proinflamatorias y la reducción de factores proaterogenicos ya que reduce la presencia de especies reactivas de oxígeno (94).

La expresión de *eNOS* es estimulada el incrementar las concentraciones de Ca2+ a través de la inducción de la unión de calmodulina a *eNOS*. También proteínas como hsp90 se han encontrado asociadas en humano, mismas que tiene función como modulador alostérico de la enzima *eNOS* en las arterias (94). Estudios previos han demostrado que la baja expresión del gen de la *eNOS* y su baja actividad enzimática, incrementan la contracción vascular por vasoconstrictores como la endotelina-1, tromboxanos y serotonina, intensificando la permeabilidad vascular, la formación de trombos, exacerbando la proliferación y migración de células músculo liso vascular en eventos ateroscleróticos (96-99). Así mismo, estudios in vitro con células endoteliales de arteria coronaria humana han demostrado que hay una significativa disminución de la expresión del gen *eNOS* en pacientes con aterosclerosis (52, 100). Por otro lado, estudios experimentales y clínicos han demostrado que las LDLox, el TNF-alfa (101-106), especies reactivas de oxígeno (94) y otros estímulos aterogénicos suprimen la expresión del gen de la *eNOS* (101-106). Además, estos estudios han puesto en evidencia que la *eNOS* no sólo se asocia con la disfunción endotelial, sino también tiene un papel muy importante en la progresión de la aterosclerosis(102-111).

Los mecanismos moleculares de la disfunción endotelial involucran la potencialización del estrés oxidativo. Lo que convierte a *eNOS* en una molécula generadora de aniones superóxido. El mal acoplamiento de *eNOS* provoca que el sitio de asociación de la molécula BH4 pierda afinidad y se ha asociado a modelos de enfermedad cardiovascular (94). Por otra parte, los aniones súperoxido dan origen a otras especies reactivas de oxígeno como: peróxido de hidrogeno, radicales hidroxilo y moléculas sínglate de oxígeno. Experimentalmente se asocia a la capacidad de despolimerizar ácido hialurónico, degradar colágena, oxidar lípidos, lesionar DNA y atacar membranas. Dentro de células fagociticas, estos radicales libres de oxígeno alteran la integridad de la membrana fagosomal escapando primero al citoplasma y de aquí al exterior de las células (112).

La ET-1 bajo condiciones fisiológicas normales se produce en pequeñas cantidades principalmente por células endoteliales y cardiomiocitos (113, 114), tiene un papel muy importante en la regulación del endotelio vascular, ya que participan en la regulación del tono vascular, remodelación vascular, inflamación, trombosis y homeostasis (99). La ET-1 disminuye la producción de óxido nítrico (ON) por que regula a la baja al gen de la *eNOS* en células endoteliales durante la isquemia miocárdica (115, 116) (46, 47). También se ha

observado que la ET-1 incrementa la producción de especies reactivas de oxígeno; induce la expresión de gp91phox del complejo NADPH oxidasa, aumentando la producción de superóxido que inactiva al ON por la formación de peroxinitrito que incrementa el daño al endotelio vascular (117-121). Por otro lado, la ET-1 a concentraciones elevadas induce la liberación de mediadores pro-inflamatorios y quimiotácticos, incluyendo a el TNF-alfa, IL-1, IL-6, y moléculas de adhesión (122-124), favoreciendo el proceso aterosclerótico (125, 126).

Ligando 5 de quimiocina con motivos C-C

La quimiocina *CCL-5* (también llamada RANTES) es un potente quimioatrayente para monocitos, linfocitos, eosinófilos y basófilos, que participa en la regulación endotelial, así como en la remodelación vascular (127, 128). Se ha observado que en placas ateroscleroSas de humano la expresión de RNAm de RANTES se encuentra elevada (127-130). Estudios in vitro han demostrado que elevadas concentraciones de LDLox o LDL nativas son un potente inductor de RANTES, sugiriendo que las LDL intensifican la expresión de RANTES en estados tempranos de la aterosclerosis (131, 132). También se ha detectado una elevada concentración de RANTES en plasma de pacientes con enfermedad arterial coronaria (133). De esta manera RANTES tiene un papel fundamental en el fenómeno inflamatorio, debido a que promueve la migración de los monocitos/macrófagos hacia el espacio subendotelial, así como la liberación de mediadores pro-inflamatorios, aumento de la expresión de moléculas de adhesión, la producción de especies reactivas de oxígeno, el aumento de la permeabilidad vascular, la disfunción endotelial, así como perpetuación de la enfermedad aterosclerótica (127-132, 134).

Selectina E y P

Las moléculas de adhesión *SEL-E* y *SEL-P*, tienen un papel importante en estadios tempranos de la placa ateroscleroticа (135). Ambas se expresan en endotelio, macrófagos, leucocitos y la P-selectina se expresa además en plaquetas. Las selectinas P y E son parcialmente responsables de la adhesión, el rodamiento y migración de los monocitos hacia la pared vascular (54, 136-140). Se ha observado que bajas concentraciones de ON o la deficiente función de la *eNOS* incrementan la expresión de las selectinas E y P en individuos asintomáticos (141, 142). Estudios experimentales han demostrado que moléculas como las LDLox, lipoproteína (a) y citocinas, promueven la expresión de estas selectinas en las placas ateroscleróticas (143, 144). Además, varios estudios in vitro han demostrado que una elevada expresión de estas moléculas aumenta la adhesión, el rodamiento de los monocitos hacia la pared vascular, provocando un acumulo de lipoproteínas de baja densidad oxidadas, promoviendo la formación temprana de la placa aterosclerótica (145-149).

La fisiopatología que comienza con la acumulación de LDLs en torrente sanguíneo hasta la formación de la placa arteriosclerosa la podemos observar a manera de esquema en la figura 1.

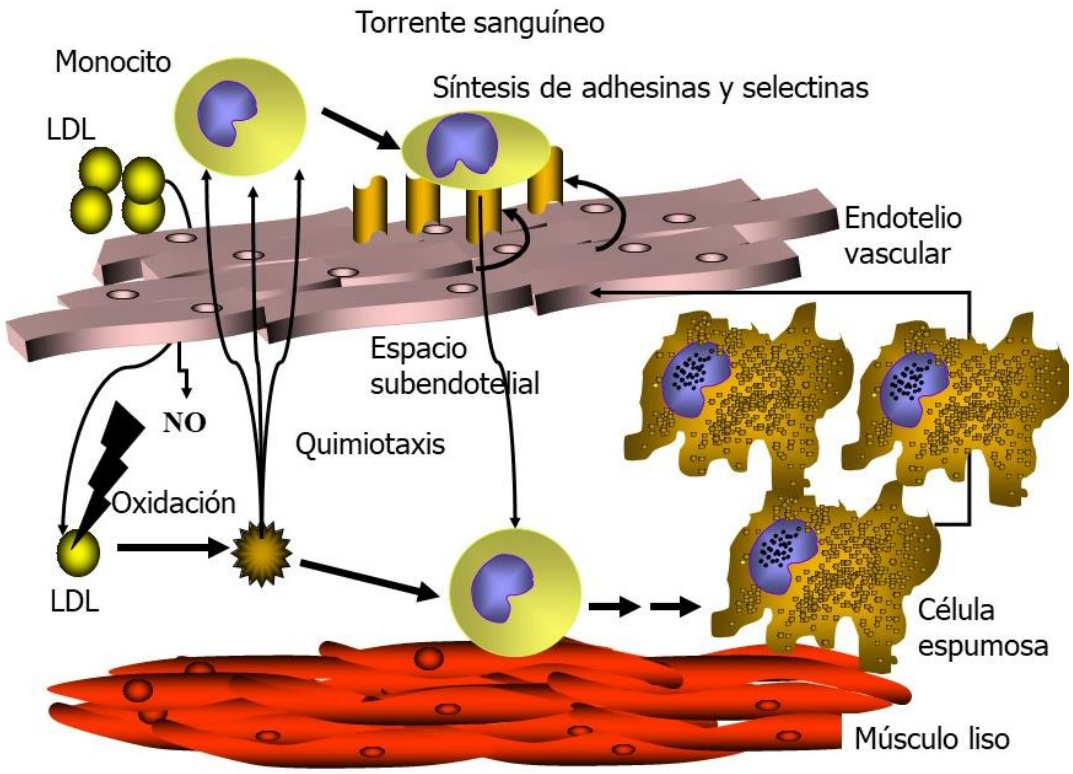

Figura1. El desarrollo de aterosclerosis comienza con la acumulación de LDL en torrente sanguíneo que desestabiliza al endotelio vascular generando disfunción endotelial, estas LDL pasan por el endotelio vascular al espacio subendotelial donde sufren oxidación. Las LDL oxidadas generan la liberación moléculas quimiotácticas que reclutan monocitos. Los monocitos se adhieren a adhesinas y selectinas en el endotelio vascular y migran al espacio subendotelial transformándose en macrófagos que fagocitan LDL oxidadas. Posteriormente los macrófagos con demasiadas vesículas, llenas de LDL oxidadas, se denominan células espumosas. Las células espumosas perpetúan el ciclo de estimulación y producción de citocinas inflamatorias, síntesis de selectinas y quimiotaxis. La síntesis de selectinas y moléculas quimioatrayentes de monocitos perpetua la generación de disfunción endotelial generando un ciclo de inflamación alrededor de la placa aterosclerótica.

Trombosis, rotura y erosión de la placa ateromatosa

La trombosis es inducida por la ruptura o erosión de la placa. Puede dar lugar a cambios rápidos en la severidad de la estenosis y dar lugar a oclusión vascular total. El conjunto de lípidos, que es expuesto tras la ruptura es altamente trombogénico y tiene una alta concentración de factor tisular (150-153). Se ha encontrado que la actividad pro coagulante sistémica de los monocitos esta elevada en pacientes con angina inestable (154).

Este proceso implica factores relacionados con la hipercoagulabilidad sistémica, la hipercolesterolemia, el fibrinógeno y la infección en la génesis del trombo. EL trombo que se observa en el síndrome isquémico coronario agudo es rico en plaquetas. La obstrucción inicial del flujo coronario se debe a agregación plaquetaria pero la fibrina es importante para la posterior estabilización del trombo. La respuesta trombotica de la placa es dinámica, es decir, se producen de manera simultánea los fenómenos tromboticos y los trombolíticos,

dando lugar a interrupción intermitente del flujo coronario que explican episodios transitorios de oclusión y sub-oclusión que se asocia con cambios en el electrocardiograma y en la clínica de los pacientes (155, 156).

Las placas vulnerables (con tendencia a la rotura) tienen una gran acumulación de lípidos, baja densidad de células de musculo liso, alta densidad de macrófagos, una delgada capa fibrosa con colágeno desorganizado y una alta concentración de factor tisular y constituyen el sustrato de entre dos tercios y tres cuartos de los trombos coronarios (46, 47, 153). El núcleo lipídico de las placas con tendencia a la ruptura tiene un gran contenido de ésteres de colesterol con gran proporción relativa de los diferentes ácidos grasos contribuye a la agregación y activación plaquetaria local, así como a la formación del trombo (157-159).

La ruptura de la placa aterosclerótica se produce como consecuencia de la conjunción de varios mecanismos:
Mecanismo activo: Se debe a la secreción de enzimas proteolíticas por los macrófagos, que pueden debilitar la capa fibrosa.
Mecanismo pasivo: Se relaciona con fuerzas físicas aplicadas sobre la zona más débil de la capa fibrosa, que corresponde con la zona más delgada de esta, a nivel de la unión con la pared arterial normal.
La vulnerabilidad de la placa puede relacionarse con localización, tamaño y composición del núcleo lipídico y también con el impacto del flujo en la superficie luminar de la placa (160, 161). Junto a la rotura de la placa, los fenómenos erosivos de la misma también se han descrito como mecanismos subyacentes en el síndrome isquémico coronario agudo (162-166). Parece ser más frecuente en hombres, diabéticos e hipertensos; así mismo existen datos que indican que se producirá con mayor frecuencia en individuos que desarrollen estenosis de alto grado y en estenosis localizada en la arteria coronaria (164-167). El proceso de trombosis que conlleva a los SICA lo podemos observar esquemáticamente en la figura 2.

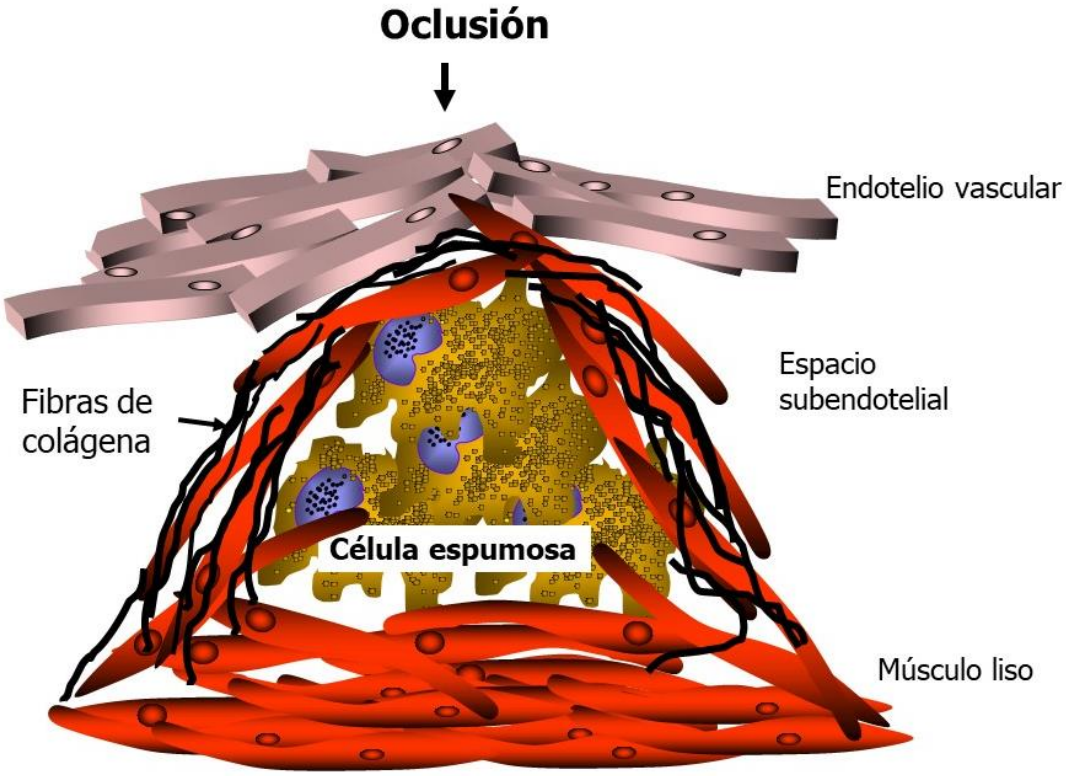

Figura 2. La acumulación de células espumosas en el espacio subendotelial conlleva a la migración de células de musculo liso al espacio subendotelial. Posteriormente fibras de colágeno se depositan en la parte externa de la capa de células de musculo liso para contener a las células espumosas. Cuando la cantidad de células espumosas es superior a la capacidad de las células de musculo liso y las fibras de colágena de contenerlo se comienza a desestabilizar el endotelio vascular. La desestabilidad del endotelio vascular produce liberación de factores coagulantes que generan el trombo y posteriormente la oclusión. También existe el escenario en el cual la oclusión es provocada por el gran tamaño de la placa aterosclerótica.

Genética de las moléculas activadoras de la respuesta inmune innata

De acuerdo con los antecedentes anteriores se sugiere que la alteración en la cantidad o calidad de la producción de estas moléculas pueden ser un factor importante en la perpetuación del daño arterial y aparición de la placa aterosclerótica y/o aterosclerosis. Estudios previos han demostrado que sitios polimórficos presentes en los genes *eNOS*, RANTES, *SEL-P* y *SEL-E* se asocian con aterosclerosis, enfermedad arterial coronaria, hipertensión e infarto agudo de miocardio en diferentes poblaciones (41-44, 95, 168-180). Dentro de los que destacan, tres sitios polimórficos del gen *eNOS*, dos en la región promotora -786 T/C y -1474 T/A y uno en la posición 894G/T (Glu298Asp) del exón 7. Del gen RANTES en la región promotora los sitios -28 G/C, -109 C/T y -403 *G/A*. Del gen de la *SEL-P* en la región promotora el sitio -1969 A/G y de la región codificante las posiciones 1087 *G/A* (Ser290Asp), 2013 G/T (Val599Leu) y 2361 A/C (Thr715Pro) de los exones 7,12 y 13, respectivamente. Del gen de la *SEL-E* el sitio polimórfico 98 G/T ubicado en la región 5'UTR y de la región codificante las posiciones 602 A/C (Ser128Arg), 1040 A/G (Lys295Glu), 1559 C/T (Tyr468His) y 1839 C/T (Leu554Phe) ubicadas en los exones 4, 6, 9 y 11, respectivamente. El objetivo de nuestro trabajo es evaluar la participación de los

polimorfismos de los genes que codifican para estas moléculas en la susceptibilidad genética al desarrollo de la aterosclerosis y los SICA. Además de lo anterior pretendemos determinar para Selectina P y RANTES si los polimorfismos están relacionados con variaciones en los niveles de las moléculas en plasma.

Justificación

La aterosclerosis es una de las principales causas de muerte a nivel mundial. Según datos de la Secretaria de Salud de la República Mexicana, en México se registran 50 mil muertes al año por algún evento cardiovascular causa de la aterosclerosis. Este padecimiento es de origen multifactorial, es decir en su desencadenamiento participan tanto factores genéticos como ambientales. El estudio de los polimorfismos presentes en los genes *eNOS, RANTES, SEL-P y SEL-E,* que codifican para moléculas relevantes en la aterosclerosis, así como, el estudio funcional y de expresión génica, serán de mucha importancia para definir si estos pueden ser marcadores de susceptibilidad y/o resistencia en el desarrollo de aterosclerosis y que en un futuro permitirán mejores y más dirigidos tratamientos. Algunos de los polimorfismos que se pretenden estudiar en este proyecto ya se han reportado en la literatura de manera aislada y los hallazgos reportados hasta el momento han sido controversiales entre las diferentes poblaciones de estudio, así mismo, no en todos estos estudios estos hallazgos se han demostrado con mediciones en suero y/o cultivo celular. En el presente proyecto estudiaremos algunos polimorfismos previamente reportados y otros que no han sido estudiados en este tipo de pacientes, con la finalidad de identificar si uno o más sitios polimórficos presentes en los diferentes genes propuestos en este proyecto participan en la susceptibilidad a desarrollar aterosclerosis en la población mexicana. Por otro lado, el identificar uno o más sitios polimórficos en un mismo gen permitirá construir haplotipos e identificar si estos haplotipos tienen un efecto en la susceptibilidad a desarrollar aterosclerosis en la población de estudio. No obstante, con la finalidad de comprobar los hallazgos obtenidos se hizo la medición de las moléculas en suero y en cultivo celular con la finalidad de comprobar si los resultados genéticos tienen una consecuencia funcional. Cabe destacar que hasta el momento en la literatura no hay ningún trabajo publicado en el cual se analicen en conjunto al menos 3 genes de los 5 propuestos en este trabajo y por consiguiente tampoco se han definido haplotipos y/o trabajos en los que se haga medición en suero y cultivo celular en un mismo estudio.

Hipótesis

Si las moléculas codificadas por los genes *eNOS, RANTES, SEL-P* y *SEL-E* regulan la función endotelial y el proceso inflamatorio que conllevan al desarrollo de la aterosclerosis, y sus genes presentan sitios polimórficos que tiene un efecto en su producción y/o función, algunos de sus alelos podrían diferenciar a los pacientes de los individuos control y ser por tanto marcadores de susceptibilidad y/o resistencia para este padecimiento en la población mexicana.

Objetivos

General

Determinar el papel de los polimorfismos presentes en los genes *eNOS, RANTES, SEL-P y SEL-E* en la susceptibilidad y/o resistencia al desarrollo de la aterosclerosis y SICA en la población mexicana y para aquellos polimorfismos que tengan asociación de susceptibilidad y/o resistencia, establecer su relación utilizando los niveles séricos o expresión en membrana de estas moléculas en los individuos de estudio.

Específicos

- Determinar la frecuencia de los sitios polimórficos de los genes que codifican para *SEL-E* en el grupo de pacientes mexicanos con aterosclerosis y *eNOS, RANTES y SEL-P* en el grupo de pacientes mexicanos con SICA. Determinar estas mismas variantes en el grupo control.
- Una vez definidas estas frecuencias compararlas entre los dos grupos de estudio y establecer si existe algún polimorfismo de los genes estudiados que confiera susceptibilidad o resistencia para el desarrollo de la aterosclerosis y/o SICA. Por medio de los modelos de herencia (co-dominante, dominante, recesivo, heterocigoto y aditivo).
- Determinar el desequilibrio de ligamiento entre los diferentes polimorfismos de cada uno de los genes estudiados y construir los distintos haplotipos.
- Hacer el análisis de correlación entre los polimorfismos, y los factores de riesgo cardiovascular, con el fin de definir si alguna variable en conjunto con estos pudiera estar confiriendo susceptibilidad y/o resistencia para el desarrollo de estos padecimientos en la población mexicana.
- Establecer si los niveles séricos de las moléculas *SEL-E*, *eNOS*, RANTES y *SEL-P* se asocian al genotipo.

Capítulo II
Artículo requisito

The Ser290Asn and Thr715Pro Polymorphisms of the SELP Gene Are Associated with A Lower Risk of Developing Acute Coronary Syndrome and Low Soluble P-Selectin Levels in A Mexican Population

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MDPI

Article

The Ser290Asn and Thr715Pro Polymorphisms of the *SELP* Gene Are Associated with A Lower Risk of Developing Acute Coronary Syndrome and Low Soluble P-Selectin Levels in A Mexican Population ‡

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‡ Running Head: *SELP* gene polymorphisms in acute coronary syndrome.

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Abstract: Recent studies have shown that P-selectin promotes the early formation of atherosclerotic plaque. The aim of the present study was to evaluate whether the *SELP* gene single nucleotide polymorphisms (SNPs) are associated with presence of acute coronary syndrome (ACS) and with plasma P-selectin levels in a case-control association study. The sample size was estimated for a statistical power of 80%. We genotyped three *SELP* (*SELP* Ser290Asn, *SELP* Leu599Val, and *SELP* Thr715Pro) SNPs using 5′ exonuclease TaqMan assays in 625 patients with ACS and 700 healthy controls. The associations were evaluated with logistic regressions under the co-dominant, dominant, recessive, over-dominant and additive inheritance models. The genotype contribution to the plasma P-selectin levels was evaluated by a Student's t-test. Under different models, the *SELP* Ser290Asn (OR = 0.59, $pC_{\text{Co-Dominant}}$ = 0.047; OR = 0.59, pC_{Dominant} = 0.014; OR = 0.58, $pC_{\text{Over-Dominant}}$ = 0.061, and OR = 0.62, pC_{Additive} = 0.015) and *SELP* Thr715Pro (OR = 0.61, pC_{Dominant} = 0.028; OR = 0.63, $pC_{\text{Over-Dominant}}$ = 0.044, and OR = 0.62, pC_{Additive} = 0.023) SNPs were associated with a lower risk of ACS. In addition, these SNPs were associated with low plasma P-selectin levels. In summary, this study established that the *SELP* Ser290Asn and *SELP* Thr715Pro SNPs are associated with a lower risk of developing ACS and with decreased P-selectin levels in plasma in a Mexican population.

Keywords: acute coronary syndrome; P-selectin; genetics; polymorphisms; susceptibility

1. Introduction

Acute coronary syndrome (ACS) comprises a spectrum of obstructive coronary artery diseases that most commonly arise from plaque rupture and/or erosion, leaving the vulnerable lipid-rich core exposed to the circulation. As a result, platelets and the coagulation cascade are activated, leading to acute thrombotic occlusion [1,2]. This syndrome is a consequence of atherosclerosis associated with a strong inflammatory component, which is immune mediated by chemokines. These molecules have an important role in the development of atherosclerotic plaque [3–5]. P-selectin is a chemokine, which mediates lymphocyte and monocyte recruitment, rolling, and diapedesis to the areas of inflammation [4–6]. Experimental studies have shown that higher expression of SELP increases adhesion, monocytes rolling to the vascular wall, accumulation of oxidized low-density lipoproteins, and the early formation of atherosclerotic plaque and other inflammatory diseases [4–7].

P-selectin contains 17 exons and is encoded by the *SELP* gene located on chromosome 1q21-q24 spanning <50 kb [8]. Recently, three single nucleotide polymorphism (SNPs) in the *SELP* gene in the exons 7, 12, and 13 [positions *G1057A* Ser290Asn (rs6131), *G1980T* Leu599Val (rs6133), and *A2331C* Thr715Pro (rs6136)] have been associated with myocardial infarction, hypertension, coronary heart disease, lupus erythematosus, type 2 diabetes mellitus (T2DM), and atherosclerosis [8–13]. Nonetheless, the association between these SNPs and other inflammatory diseases, such as diabetic retinopathy and multiple sclerosis is controversial, with negative results [14,15].

Considering the prominent role of P-selectin as a key in the chain of events leading to atherosclerotic plaque formation, the aim of this study was to investigate the association of three *SELP* SNPs (Ser290Asn, Leu599Val and Thr715Pro) with the risk of developing ACS. Furthermore, we evaluated whether these SNPs were associated with plasma P-selectin levels in a Mexican population sample.

2. Subjects and Methods

2.1. Study Population

This case-control study was carried out at the Instituto Nacional de Cardiologia Ignacio Chavez. The sample size was calculated for unmatched cases and controls with OpenEpi software (http://www.openepi.com/SampleSize/SSCC.html) with a statistical power of 80% and an alpha error of 0.05. Using this criterion, we included 625 patients with ACS (82% men and 18% women with a mean age of 57.97 ± 10.5 years) who were diagnosed based on clinical characteristics, electrocardiographic changes and biochemical markers of cardiac necrosis, according to guidelines from the European Society of Cardiology (ESC) and American College of Cardiology (ACC) [16,17]. The exclusion criteria were (1) patients with clear inflammatory pathologies on admission, such as infection established by clinical, laboratory, or image investigations, and (2) patients with an autoimmune disease or cancer previously diagnosed or documented during their hospitalization. Moreover, we included 700 healthy controls (66% men and 34% women with a mean age of 54.37 ± 7.65 years) coming from the Genetics of Atherosclerosis Disease (GEA) Mexican study previously described by Rosalinda-Posadas et al [18]. All healthy controls were asymptomatic and apparently healthy individuals without a family history of CAD and with a negative calcium score, indicative of the absence of subclinical atherosclerosis [18]. The exclusion criteria included not only the use anti-dyslipidemic, anti-hypertensive, and anti-diabetic drugs at the time of the study, but also congestive heart failure, as well as liver, renal, thyroid or oncological disease. All GEA participants were unrelated and of self-reported Mexican ancestry (3 generations). A Mexican mestizo was defined as a person who (1) was born in Mexico and (2) is a descendant of the original autochthonous inhabitants and of individuals (Caucasian and/or African, mainly Spaniards) who migrated to America in or after the XVI century. This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics and Research committee

of our institution (registration number: 17CI09012010). Written informed consent was obtained from all individuals enrolled in the study.

2.2. Laboratory Analyses

After a 12-h overnight fast, EDTA blood samples were drawn and centrifuged within 15 min after collection; the plasma was separated into aliquots and immediately analyzed or frozen at −80 °C until analysis. Cholesterol and triglyceride plasma concentrations were determined by enzymatic/colorimetric assays (Randox Laboratories, UK). The phosphotungstic acid-Mg^{2+} method was used to determine HDL-C concentrations. LDL-C was estimated in samples with a triglyceride level lower than 400 mg/dl, using the modified Friedewald formula [19]. Plasma lipid concentrations were determined within 24 h after blood sample collection. We followed the National Cholesterol Education Project (NCEP) Adult Treatment Panel (ATP III) guidelines and thus defined dyslipidemia with the following levels: cholesterol > 200 mg/dl, LDL-C > 130 mg/dl, HDL-C < 40 mg/dl, and triglyceride > 150 mg/dl (http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3_rpt.htm). Type 2 diabetes mellitus (T2DM) was defined with a fasting glucose ≥ 126 mg/dL and was also considered when participants reported glucose-lowering treatment or a physician diagnosis of T2DM. Hypertension was defined by a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or the use of oral antihypertensive therapy [18].

2.3. Genetic Analysis

DNA extraction was performed from peripheral blood in agreement with the method of Lahiri and Nurnberger [20]. The *SELP G1057A* Ser290Asn, *SELP G1980T* Leu599Val, and *SELP A2331C* Thr715Pro SNPs were genotyped using 5′ exonuclease TaqMan assays on a 7900HT Fast Real-Time PCR system according to manufacturer's instructions (Applied Biosystems, foster City, CA, USA). In order to avoid genotyping errors, ten percent of the samples were determined twice; the results were concordant for all cases.

2.4. Determination of P-Selectin Levels

Samples were aliquoted and stored at −70 °C for further use. Plasma P-selectin levels were measured using a quantitative sandwich enzyme immunoassay technique (ELISA) kit in accordance with the manufacturer's instructions (Human P-Selectin/CD62P Quantikine ELISA Kit, R&D systems). The detection range was 0.8–50.00 ng/mL and the sensitivity was equal to the minimal detectable dose of this kit (≥ 0.121 ng/mL).

2.5. Functional Prediction Analysis

Two in silico programs, the ESEfinder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home) and SNP Function Prediction (http://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/snpfunc.cgi) were used to predict the possible functional effect of the *SELP* SNPs. Both programs (ESEfinder2.0 and SNPinfo) analyzed the localization of the SNPs (e.g., 5′-upstream, 3′-untranslated regions, intronic) and their 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 [21,22].

2.6. Statistical Analysis

All statistical analysis in this study was performed using SPSS version 18.0 (SPSS, Chicago, Il). Data of continuous variables were expressed as median and percentiles (25^{th}–75^{th}), while data of discrete variables [e.g., frequency (n, %)] were analyzed using Chi-squared or Fisher's exact tests. We used logistic regression tests to associate the SNPs with ACS under five inheritance models [16].

The correction of the p-values (pC) was performed with the Bonferroni test. Using the HAPLOVIEW version 4.1 software (Cambridge, MA, USA), we performed the haplotypes construction and linkage disequilibrium analysis (LD, D''). We tested whether our study population was in Hardy–Weinberg equilibrium (HWE) with a Chi-square test. Furthermore, we used the QUANTO software [http://biostats.usc.edu/software] to calculate the statistical power of our study and found it was 0.80. Using the Student's t-test, we analyzed the contribution of the genotypes on the P-selectin plasma levels. The values were expressed as means ± SD. The level of significance was set at $p < 0.05$.

3. Results

3.1. Characteristics of the Study Population

Clinical and biochemical characteristics of the ACS patients and healthy controls are shown in Table 1. There were significant differences between the ACS patients and healthy controls. Compared to healthy controls, the ACS patients had a higher frequency of T2DM, hypertension, dyslipidemia, and smoking habit. Conversely, the total cholesterol, triglycerides, and LDL-C levels in ACS patients were lower than those in the control group; this effect may be due to their treatment with statins.

Table 1. Clinical characteristics and biochemical parameters of the study individuals.

		ACS (n = 625)	Healthy Controls (n = 700)	p-value
		Median (percentile 25–75)	Median (percentile 25–75)	
Age (years)		57.72 (51–65)	54.39 (49–59)	<0.001
BMI (kg/m²)		27.3 (25–29)	28.3 (26–31)	0.001
Blood pressure (mmHg)	Systolic	130.61 (114–144)	117.32 (106–126)	<0.001
	Diastolic	80.1 (70–90)	72.47 (66–77)	<0.001
Glucose (mg/dl)		158.51 (102–188)	98.73 (84–99)	<0.001
Total cholesterol (mg/dl)		164.22 (128–198)	190.4 (164–210)	<0.001
HDL-C (mg/dl)		38.32 (32–44)	44.6 (35–53)	<0.001
LDL-C (mg/dl)		106.4 (76–133)	115.8 (94–134)	<0.001
Triglycerides (mg/dl)		169.2 (109–201)	175.1 (112–208)	0.218
Gender n (%)	Male	510 (82)	463 (66)	<0.001
	Female	115 (18)	237 (34)	
Smoking n (%)	Yes	225 (35)	155 (22)	<0.001
Hypertension	Yes	355 (57)	206 (29)	<0.001
Diabetes mellitus	Yes	218 (35)	68 (10)	<0.001
Dyslipidemia n (%)	Yes	534 (85)	501 (71)	<0.001

Data are expressed as median and percentiles (25th–75th). p-values were estimated using Mann–Whitney U test for continuous variables and chi-square test for categorical values. ACS: acute coronary syndrome patients.

3.2. Allele and Genotype Frequencies

Genotype frequencies of the SNPs were in HWE. The frequencies of the *SELP Leu599Val* SNP was similar in ACS patients and healthy controls. Nonetheless, the SNPs [*SELP* Ser290Asn, and *SELP* Thr715Pro] were associated with a lower risk of ACS (Table 2). Under co-dominant, dominant, over-dominant, and additive models, the *A* (290Asn) allele of the *SELP* Ser290Asn SNP was associated with a lower risk of ACS (OR = 0.59, $pC_{Co\text{-}Dom} = 0.047$; OR = 0.59, $pC_{Dom} = 0.014$; OR = 0.58, $pC_{Over\text{-}Dom} = 0.061$, and OR = 0.62, $pC_{Add} = 0.015$, respectively). In the same way, under dominant, over-dominant, and additive models, the *C* (715Pro) allele of the *SELP* Thr715Pro SNP was associated with a lower risk of ACS (OR = 0.61, $pC_{Dom} = 0.028$; OR = 0.63, $pC_{Over\text{-}Dom} = 0.044$, and OR = 0.62, $pC_{Add} = 0.023$, respectively). All models were adjusted for gender, age, blood pressure, BMI, glucose, total cholesterol, HDL-C, LDL-C, triglycerides, and smoking habit.

Considering that the prevalence of T2DM (35%) and hypertension (57%) are highest in ACS patients versus healthy controls (10% and 29%, respectively), we performed a sub-analysis of the polymorphisms associated with a low risk of ACS (*SELP* Ser290Asn and *SELP* Thr715Pro). This analysis was made comparing individuals with and without T2DM and the other hand, individuals with and without hypertension. The results show that both polymorphisms were not associated with T2DM or with hypertension (Supplementary Tables S1 and S2). Therefore, this analysis corroborates that the genetic variation of these polymorphisms of the *SELP* gene are associated to the ACS, and not comes of the T2DM or hypertension.

Table 2. Distribution of *SEL-P* polymorphisms in ACS patients and healthy controls.

				MAF	Model	OR (95%CI)	*pC*
SELP G1057A Ser290Asn (rs6131)							
	GG	GA	AA				
Control (*n* = 691)	569 (0.823)	115 (0.166)	7 (0.010)	0.09	Co-dominant	0.59 (0.38–0.92)	0.047
					Dominant	0.59 (0.39–0.90)	0.014
					Recessive	0.58 (0.12–2.83)	0.49
ACS (*n* = 617)	541 (0.877)	73 (0.118)	3 (0.005)	0.06	Over-dominant	0.61 (0.39–0.92)	0.019
					Additive	0.62 (0.42–0.92)	0.015
SELP G1980T Leu599Val (rs6133)							
	GG	GT	TT				
Control (*n* = 682)	563 (0.825)	114 (0.167)	5 (0.007)	0.09	Co-dominant	0.28 (0.02–3.32)	0.46
					Dominant	1.07 (0.73–1.58)	0.73
					Recessive	0.27 (0.02–3.26)	0.26
ACS (*n* = 611)	505 (0.827)	105 (0.172)	1 (0.002)	0.09	Over-dominant	1.12 (0.75–1.66)	0.57
					Additive	1.02 (0.71–1.49)	0.90
SELP A2331C Thr715Pro (rs6136)							
	AA	AC	CC				
Control (*n* = 685)	580 (0.847)	97 (0.141)	8 (0.012)	0.08	Co-dominant	0.63 (0.40–0.99)	0.075
					Dominant	0.61 (0.39–0.95)	0.028
					Recessive	0.36 (0.04–2.95)	0.32
ACS (*n* = 607)	537 (0.884)	67 (0.110)	3 (0.005)	0.06	Over-dominant	0.63 (0.40–0.99)	0.044
					Additive	0.62 (0.41–0.94)	0.023

ACS, acute coronary syndrome, MAF, minor allele frequency, OR, odds ratio, CI, confidence interval, *pC*, *p*-value corrected. The p-values were calculated by the logistic regression analysis, and the ORs were adjusted for gender, age, blood pressure, BMI, glucose, total cholesterol, HDL-C, LDL-C, triglycerides, and smoking habit.

3.3. Linkage Disequilibrium Analysis

We used the Haploview version 4.1 program for the analysis of the linkage disequilibrium and construction of haplotypes. In this analysis, the *SELP* Thr715Pro and *SELP* Leu599Val SNPs showed a strong linkage disequilibrium (D′ = 0.95). In addition, Haploview revealed strong evidence of recombination of the polymorphisms *SELP* Thr715Pro versus *SELP* Ser290Asn and *SELP Leu599Val* versus *SELP* Ser290Asn (D '= 0.17 and D' = 0.28, respectively; data not shown). This analysis marked three haplotypes with different distributions in ACS patients and healthy controls (Table 3). The "Thr-Leu-Ser" haplotype was associated with a higher risk of developing ACS (OR = 1.28, 95% CI: 1.05–1.54, pC = 0.006), while the "Pro-Leu-Ser" and "Thr-Leu-Asn" haplotypes were associated with a lower risk of developing ACS (OR = 0.72, 95% CI: 0.52-0.99, pC = 0.022, and OR = 0.71, 95% CI: 0.51–1.00, pC = 0.027, respectively).

Table 3. Haplotype frequencies (Hf) of *SEL-P* haplotypes in ACS patients and healthy controls.

Haplotypes	Thr715Pro	Leu599Val	Ser290Asn	ACS (n = 605)	Controls (n = 676)	OR	95%CI	P
				Hf	Hf			
H1	Thr	Leu	Ser	0.804	0.763	1.28	1.05–1.54	0.006
H2	Thr	Val	Ser	0.073	0.067	1.08	0.80–1.47	0.32
H3	Pro	Leu	Ser	0.057	0.077	0.72	0.52–0.99	0.022

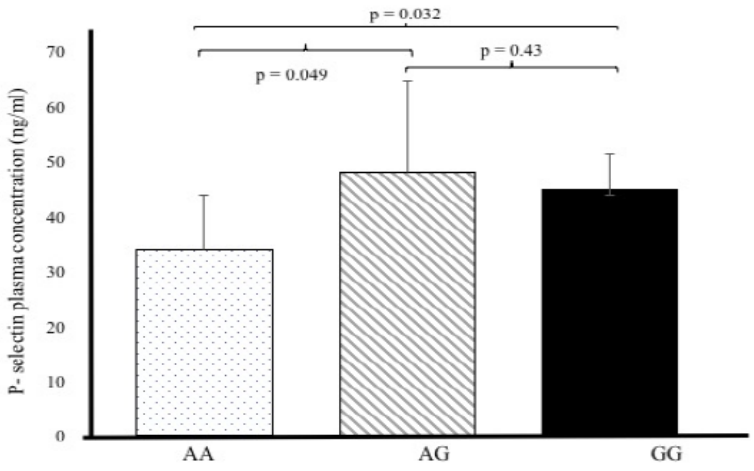
H4	Thr	Leu	Asn	0.049	0.066	0.71	0.51–1.00	0.027
H5	Pro	Val	Asn	0.014	0.022	0.62	0.33–1.13	0.063

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 (*SELP A2331C Thr715Pro* (rs6136), *SELP G1980T* Leu599Val (rs6133), and *SELP G1057A* Ser290Asn (rs6131). Bold numbers indicate significant associations.

3.4. Association of Polymorphisms with Plasma P-Selectin Levels

In order to define the functional effect of the *SELP* Ser290Asn and *SELP* Thr715Pro SNPs associated with a lower risk of ACS, we determined the plasma levels of P-selectin in individuals with different genotypes of these two polymorphisms. For this analysis, we included a subgroup of 30 healthy controls for *SELP* Ser290Asn (7 *AA*, 11 *GA* and 12 *GG*) and a subgroup of 30 healthy controls for the *SELP* Thr715Pro SNP (8 *CC*, 11 *AC* and 11 *AA*). In this study, we did not include the analysis of plasma P-selectin levels in patients with ACS, due to the fact that in the setting of the coronary syndrome, the comorbidities, such as insulin resistance/T2DM, hypertension, and inflammatory processes, as well as the use of the anti-dyslipidemic and/or anti-hypertensive drugs, may have altered the inflammatory markers levels, such as inflammatory cytokines, adhesion molecules, and C-reactive protein, masking the real impact of *SELP* polymorphisms on plasma P-selectin levels [23–25]. In this context, subjects carrying the *AA* (Ans/Ans) genotype of the *SELP* Ser290Asn SNP had a lower P-selectin plasma concentration (33.93 ± 9.79 ng/mL) than carriers of the *GG* (Ser/Ser) (44.76 ± 6.54 ng/mL, p =0.032) or GA (Ser/Ans) genotypes (48.04 ± 16.57 ng/mL, p = 0.049) (Figure 1A). On the other hand, the analysis of the *SELP* Thr715Pro polymorphism showed that individuals with the *CC* (Pro/Pro) genotype had a lower concentration of P-selectin (26.44 ± 10.77 ng/mL) than *AA* (Thr/Thr) carriers (55.35 ± 14.05 ng/dl, p = 0.001). In addition, the individuals with the *AC* (Thr/Pro) genotype had lower P-selectin levels than *AA* (Thr/Thr) carriers (34.91 ± 14.46 ng/dl, p = 0.005) (Figure 1B).

Fig. 1A *SELP* G1057A *Ser290Asn* (rs6131)



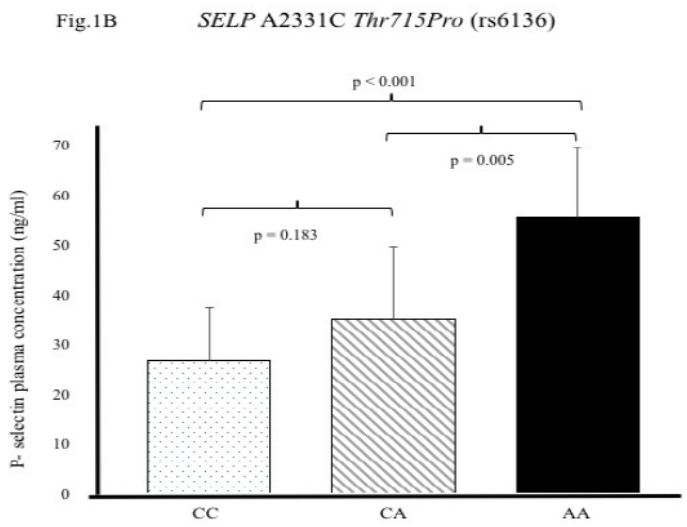


Figure 1. Genetic contribution of the *SELP G1057A* and *SELP A2331C* polymorphisms on P-selectin levels. (**A**) P-selectin plasma levels in individuals with different genotypes of the *SELP G1057A* polymorphism. (**B**) P-selectin plasma levels in individuals with different genotypes of the *SELP A2331C* polymorphism.

3.5. Functional Prediction

The functional prediction analysis showed that the presence of the *A* (Asn) allele of the *SELP* Ser290Asn polymorphism potentially produces a binding motif for Srp40 protein. In contrast, no evidence of potentially functional motifs was found for the *SELP* Thr715Pro polymorphism.

4. Discussion

In this study, we analyzed three relevant polymorphisms (Ser290Asn, Leu599Val, and Thr715Pro, respectively) of the *SELP* gene. The association of these SNPs with several inflammatory diseases in different populations is controversial, with positive and negative results [8–15]. In our study, the distribution of the *SELP* Leu599Val SNP was similar in both ACS patients and healthy controls. Nonetheless, the presence of the 290*Asn* and 715*Pro* alleles (*SELP* Ser290Asn and *SELP* Thr715Pro polymorphisms, respectively) was associated with a lower risk of developing ACS. In the same way, Reiner et al. reported in the CARDIA study that the *SELP* Ser290Asn and *SELP* Thr715Pro SNPs are associated with carotid intima-media thickness in young adults; however, these associations are different in European-American and African-American individuals [9]. In line with these data, Nasibullin et al. reported that the 290*Ans* allele of the *SELP* Ser290Asn SNP is associated with a lower risk of MI in a Russian population [13]. Similarly, the study of the risk of atherosclerosis in communities (ARIC), as well as the study of the Framingham heart (FHS) have shown that the genotype Pro715Pro is associated with a decreased risk of atherosclerosis in American and European populations [26,27]. In contrast with these data, in the ARIC study, Volcik et al. reported that the 290*Ans* and 715*Pro* alleles (*SELP* Ser290Asn and *SELP* Thr715Pro SNPs, respectively) were associated with the development of coronary heart disease in white but not in African Americans [11]. Similarly, Timasheva et al. reported that the *290*Ans allele of the *SELP* Ser290Asn SNP is associated with the development of hypertension in ethnic Tatars originating from the Republic of Bashkortostan (Russian Federation) [12]. By the same token, Kou et al. reported that *Thr715Pro* or *Pro715Pro* genotypes of the *SELP* Thr715Pro polymorphism increased the risk of developing cardiovascular diseases (CVD) in a Chinese Han population [28]. Additionally, we found that the H3 (Pro-Leu-Ser) and H4 (Thr-Leu-Asn) haplotypes were associated with a lower risk of developing ACS, whereas H1 (Thr-Leu-Ser) was associated with a

higher risk. As can be seen, the haplotypic combinations between *SELP* Thr715Pro and *SELP* Ser290Asn polymorphisms were not in linkage disequilibrium. Nonetheless, the protection haplotypes carry *715Pro* and 290*Ans* alleles, and both of them were associated independently with a lower risk of cardiovascular diseases and other inflammatory diseases. This finding corroborated the role of these two alleles with the presence of ACS, whether they were analyzed independently or as haplotypes.

It is important to note that ACS patients and the healthy donors have much greater variation in blood glucose (102-188 versus 84-99) and diabetes mellitus (35% versus 10%). Considering these data, it is important to establish whether the polymorphisms are associated with T2DM or hypertension. In a sub-analysis, we showed that both polymorphisms were not associated with T2DM or with hypertension.

As can be seen, the associations of the *SELP* Ser290Asn and *SELP* Thr715Pro polymorphisms with ACS are contradictory in different study populations. We suggest that these discrepancies could be due to the classical cardiovascular risk factors and the environmental factors, such as diet, exercise, and lifestyle, which have an important role in the development of inflammatory diseases [29,30]. Another reason may be the fact that the allelic distribution of these polymorphisms varies according to the ethnic origin of the study populations. According to data obtained from the National Center for Biotechnology Information, populations from European, Asian, and African ancestry in Southwest US present a higher frequency of the *A* allele of the *SELP G1057A* Ser290Asn (rs6131) polymorphism (21.7%, 20.2% and 32.9%, respectively) when compared to Mexican mestizos and white American populations with a lower frequency of the *A* allele (9% and 14%, respectively). Concerning the *SELP A2331C* Thr715Pro (rs6136) SNP, Mexican mestizos, Europeans, and white Americans present a higher frequency of the *C* allele (8%, 8.8%, and 8.2%, respectively) than populations with Asian and African ancestry (0.2% and 2.5%, respectively) (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/), (https://www.ensembl.org/index.html).

We further determined the effect of the *SELP* gene polymorphisms on plasma P-selectin levels using genotype groups. We found that the *AA* (290 Asn/Asn) and *CC* (715 Pro/Pro) genotypes were associated with low P-selectin levels. As far as we know, this is the first study that showed the association of the *SELP* Ser290Asn and *SELP* Thr715Pro polymorphisms in P-selectin levels in individuals without the use of the anti-dyslipidemic or anti-hypertensive drugs. These drugs may modify the levels of the inflammatory markers, such as pro-inflammatory cytokines, adhesion molecules and C-reactive protein, masking the real impact of *SELP* gene polymorphisms on plasma P-selectin [23–25]. Nonetheless, the results concerning the association between P-selectin plasma levels and heart diseases are still contradictory. For example, Reiner et al. reported in the CARDIA study that the *A* (290Asn) and *C* (715Pro) alleles are associated with decreased plasma P-selectin levels and with the risk of developing atherosclerosis [9]. By the same token, Volcik et al. documented that the 715Pro allele is associated with lower P-selectin levels in the Atherosclerosis Risk in Communities (ARIC) study [27]. Similarly, Lee et al. determined that the lower serum levels of P-selectin decreased the risk of atherosclerosis [26]. At the same time, other reports have shown that the 715Pro *(C)* allele increased the expression of SELP mRNA, as well as the concentration of P-selectin levels in other inflammatory diseases, such as rheumatoid arthritis and T2DM [8,31]. As far as we know, the precise mechanism by which low and/or high P-selectin levels are associated with ACS remains to be elucidated. Nonetheless, recent data provide evidence that P-selectin upregulation on the endothelial cell surface mediates the effects of angiotensin II (Ang II), which has an important role in the development atherosclerosis [32]. In addition, Ang II stimulates not only the production of several molecules (adhesion molecules, chemokines, and cytokines) but also the oxidation and uptake of LDL, which promotes endothelial dysfunction [6,32]. On the other hand, Ang II triggers the synthesis of matrix metalloproteinases, the plasminogen activator inhibitor-1, and the proliferation of vascular smooth cells; this effect leads to the destabilization of atherosclerotic plaques [6]. Furthermore, using bioinformatics tools, we determined the potential effect of the *SELP* gene polymorphisms associated with ACS. The analysis of the *SELP* Thr715Pro

polymorphism did not provide evidence of potential functional motifs. Nonetheless, the analysis of the *SELP* Ser290Asn polymorphism showed that the 290 Asn (*A)* allele generates a binding site for the Srp40 proteins. These proteins have multiple functions in the pre-mRNA splicing process, as well as in the regulation of alternative splicing, which leads to the production of protein isoforms [33,34]. In this context, we think that future investigations are warranted to understand the effect of these polymorphisms on P-selectin levels.

Some limitations should be considered. The P-selectin levels were only measured in a small sample of control individuals and experiments on RNA transcription or protein stability were not made. Considering these limitations, the effect of the SNPs on P-selectin plasma levels should be taken with care and studies in a large number of individuals are necessary to corroborate this association. In the same way, in our study it was not possible to determine the expression levels of P-selectin on the leukocyte's surface to confirm the data obtained in plasma.

In summary, this study demonstrated that the *SELP* Ser290Asn and *SELP* Thr715Pro polymorphisms are associated with a lower risk of developing ACS in a Mexican population. It was possible to distinguish two haplotypes (Pro-Leu-Ser and Thr-Leu-Asn) associated with a lower risk of developing ACS. On the other hand, both polymorphisms were associated with lower P-selectin levels in plasma. Lastly, due to the specific genetic characteristics of the Mexican population, we consider that additional studies will need to be undertaken in a larger number of individuals and in populations with different ethnic origins; these studies could help define the true role of these polymorphisms as markers of risk or protection from developing ACS and other cardiovascular events.

Supplementary Materials: **Table S1**. Distribution of Ser290Asn and Thr715Pro SEL-P polymorphisms in individuals with and without T2DM. **Table S2**. Distribution of Ser290Asn and Thr715Pro SEL-P polymorphisms in individuals with and without hypertension.

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Abbreviations

HDL-C: High-density lipoprotein–cholesterol;

LDL: Low-density lipoprotein–cholesterol;

SELP: P-selectin gene;

T2DM: Type 2 diabetes mellitus;

SNP: Single nucleotide polymorphism;

ACS: Acute coronary syndrome.

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Capítulo III
Artículo científico

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.

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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

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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

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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 HT 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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Capítulo IV
Artículo científico

Two genetic variants in the promoter region of the *CCL-5* gene are associated with the risk of acute coronary syndrome and with a lower plasma *CCL-5* concentration.

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Two genetic variants in the promoter region of the *CCL5* gene are associated with the risk of acute coronary syndrome and with a lower plasma CCL5 concentration

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ABSTRACT

Acute coronary syndrome (ACS) is a multi-factorial condition with a strong inflammatory component, which is immune-mediated by chemokines. The CCL5 is a chemokine that has been suggested to be an important participant in the development of the atherosclerotic plaque. Therefore, in this work, we evaluated whether three polymorphisms located in the promoter region of the *CCL5* gene [*CCL5* -28 *G/C* (rs2280788), *CCL5−109 G/A* (rs1800825), and *CCL5−403 G/A* (rs2107538)] are significantly associated with the acute coronary syndrome (ACS), and plasma CCL5 levels. The determination of the gene polymorphisms was performed by 5′exonuclease TaqMan assays in 625 patients with ACS and 700 control individuals. Plasma CCL5 levels were evaluated by ELISA. Under co-dominant, dominant, and additive models, the *G* allele of the *-109 G/A* polymorphism was associated with a higher risk of ACS (OR = 1.27, $pC_{\text{Co-dom}} = 0.041$, OR = 1.33, $pC_{\text{Dom}} = 0.03$, and OR = 1.33, $pC_{\text{Add}} = 0.015$, respectively). In the same way, under co-dominant and recessive models, the *A* allele of the *-403 G/A* polymorphism was associated with an increased risk of ACS (OR = 1.62, $pC_{\text{Co-dom}} = 0.042$, and OR = 1.63, $pC_{\text{Res}} = 0.012$, respectively). The *CCL5−109 G* allele carriers had a lower concentration of the CCL5 than subjects with the *A* allele. Also, carriers of *CCL5−403 A* allele showed a lower concentration of the CCL5 than individuals with the *G* allele. Our data suggest the association of the *CCL5−109 G/A* and *CCL5−403 G/A* polymorphisms with the risk of developing ACS and with a lower concentration of CCL5 in our population.

1. Introduction

At present, acute coronary syndrome (ACS) constitutes a worldwide public health problem. It is a multi-factorial condition determined by both environmental and genetic factors [1,2]. This syndrome is a consequence of atherosclerosis associated with a strong inflammatory component, which is immune-mediated in part by chemokines [3–5]. The chemokine (C-C motif) ligand 5 (CCL5), also known as RANTES has been proposed to play an important role in the development of the atheroma. CCL5 is a potent chemoattractant for monocytes, lymphocytes, eosinophils, and basophils into inflammatory sites [3–5]. Recent studies have shown that this chemokine promotes the transendothelial migration of leukocytes and macrophages, contributing to the pathogenic process of atherosclerotic plaque [6–9]. Data in the literature have suggested that CCL5 levels are associated with atherosclerotic plaque development [10–13]. This chemokine is encoded by the *CCL5* gene

Abbreviations: CCL5, chemokine (C-C motif) ligand 5; RANTES, regulated on activation, normal T cell expressed and secreted; HDL-C, High-density lipoprotein-cholesterol; LDL-C, Low-density lipoprotein – cholesterol; T2DM, Type 2 diabetes mellitus; SNP, Single nucleotide polymorphism; ACS, Acute coronary syndrome.

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located in a region of 8.8 kb on chromosome 17. Interestingly, some polymorphisms of this gene have been associated with cardiovascular diseases [10,14,15].

In the last decade, three single nucleotide polymorphisms (SNPs) in the promoter region of *CCL5* gene [*CCL5* -28 *G/C* (rs2280788), *CCL5−109 G/A* (rs1800825), and *CCL5−403 G/A* (rs2107538)] have been associated, in different ethnic populations, not only with the expression of CCL5, but also with susceptibility to develop coronary artery disease [9,10,14,15].

Considering the important role of the CCL5 chemokine in the inflammatory process associated with the atherosclerotic plaque formation, the present study aimed to establish the role of the *CCL5* −28 G/C, *CCL5−109 G/A*, and *CCL5−403 G/A* SNPs in the susceptibility to develop ACS. Furthermore, we evaluated whether these SNPs are associated with CCL5 plasma concentrations in a Mexican population sample.

2. Material and methods

2.1. Study population

The sample size was calculated for unmatched cases and controls with OpenEpi software (http://www.openepi.com/SampleSize/SSCC.html) with a statistical power of 80 %, and an alpha error of 0.05. The study included 1325 Mexican mestizos, 625 consecutive patients with ACS, and 700 healthy controls. From July 2007 to July 2017, 625 patients with ACS (82 % men and 18 % women, with a mean age of 57.72 ± 10.4) were referred to the Instituto Nacional de Cardiología Ignacio Chavez. From these patient population, 501 were diagnosed with myocardial infarction and 124 with unstable angina. The myocardial infarction was classified in ST-elevation myocardial infarction (STEMI) and non-ST-elevation ACS (NSTE-ACS) based on clinical characteristics, electrocardiographic changes, and biochemical markers of cardiac necrosis (creatinine kinase isoenzymes, creatinine phosphokinase, or troponin I above the upper limit of normal); the European Society of Cardiology (ESC) and American College of Cardiology (ACC) definitions were followed [16,17]. The diagnosis of NSTE-ACS included non-STEMI and unstable angina. The diagnosis of non-STEMI was angina or discomfort at rest with ST-segment changes on ECG indicating ischemia [ST-segment depression or transient elevation (≥1 mm) in at least two contiguous leads and/or prominent T-wave inversion], with positive biomarker indicating myocardial necrosis. Patients with clinical features and/or electrocardiographic expression of non-STEMI (albeit with normal cardiac biomarker levels) were diagnosed with unstable angina [16,17]. The exclusion criteria were (1) patients with clear inflammatory pathologies on admission, such as infection established by clinical, laboratory, or image investigations, and (2) patients with an autoimmune disease or cancer previously diagnosed or documented during their hospitalization. Moreover, we included 700 controls (66 % men and 34 % women, with a mean age of 54.39 ± 7.65) recruited from the Genetics of Atherosclerosis Disease (GEA) Mexican study database. All subjects were asymptomatic and healthy individuals without a family history of coronary artery disease (CAD) or atherosclerosis recruited from June 2009 to June 2015 from blood bank donors and with the assistance of brochures posted in social service centers. The exclusion criteria included the use of anti-dyslipidemic or anti-inflammatory drugs at the time of the study, congestive heart failure, and liver, renal, thyroid, or oncological disease [18]. Also, the control subjects had a coronary calcium score of zero determined by computed tomography, indicating the absence of subclinical atherosclerosis. All the included subjects were ethnically matched and considered Mexican mestizos only if they were at least third-generation Mexicans and had been born in the country. This study was conducted according to the principals of the Declaration of Helsinki and was approved by the Ethics and Research commission of Instituto Nacional de Cardiologia Ignacio Chavez - registration number: 17CI09012010. Written informed consent was obtained from all individuals enrolled in the study.

2.2. Laboratory analyses

Cholesterol and triglycerides plasma concentrations were determined by enzymatic/colorimetric assays (Randox Laboratories, UK). HDL-cholesterol concentrations were determined after precipitation of the apo B-containing lipoproteins by the method of the phosphotungstic acid-Mg^{2+}. The LDL-C concentration was determined in samples with a triglyceride level lower than 400 mg/dl with the Friedewald formula [19]. Dyslipidemia was defined as one or more of the following characteristics: cholesterol > 200 mg/dl, LDL-C > 130 mg/dl, HDL-C < 40 mg/dl, or triglycerides > 150 mg/dl, according to the guidelines of the National Cholesterol Education Project (NCEP) Adult Treatment Panel (ATP III) (http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3_rpt.htm). Type 2 diabetes mellitus (T2DM) was defined with a fasting glucose ≥ 126 mg/dl and was also considered when participants reported glucose-lowering treatment or a physician diagnosis of T2DM. Hypertension was defined by a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or the use of oral antihypertensive therapy [18].

2.3. Genetic analysis

DNA extraction was performed from peripheral blood in agreement with the method described by Lahiri and Nurnberger [20]. The *CCL5* -28 *G/C* (rs2280788), *CCL5−109 G/A* (rs1800825), and *CCL5−403 G/A* (rs2107538) SNPs 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).

2.4. Determination of CCL5 levels

To assess the contributions of the - *109 G/A*, and - *403 G/A* genotypes on the CCL5 levels, we included an additional group of healthy individuals (also belonging to the GEA Mexican Study database) who fulfilled the above-mentioned inclusion criteria for control subjects. We select a subgroup of 45 healthy controls for the *-109 G/A* (9 *GG*, 18 *GA*, and 18 *GG*) SNP, and 45 for the *-403 G/A* (15 *GG*, 15 *GA*, and 15 *AA*) SNP. We did not include the analysis of CCL5 plasma levels in patients with ACS because, in the setting of the coronary syndrome, these levels may be altered by the use of the anti-dyslipidemic or anti-hypertensive drugs [21–24]. Besides, comorbidities such as insulin resistance/T2DM, hypertension, and inflammatory processes also alter the cytokine plasma levels and mask the real impact of *CCL5* SNPs on plasma CCL5 levels. Plasma CCL5 levels were measured using a quantitative enzyme-linked immunosorbent assay (ELISA) kit by the manufacturer's instructions (RANTES Human Instant ELISA Kit, ThermoFisher Scientific, USA). The detection range was 31.3 – 2000 pg/ml and the sensitivity was equal to the minimal detectable dose of this kit (≥ 4.2 pg/mL).

2.5. Statistical analysis

All statistical analysis in this study was performed using SPSS version 18.0 (SPSS, Chicago, Il). The Mann Whitney *U* test was used to compare continuous variables, such as age, body mass index (BMI), blood pressure, glucose, total cholesterol, HDL-C, LDL-C, and triglycerides between control and ACS groups. For categorical variables as gender, hypertension, T2DM, dyslipidemia, and smoking habit, Chi^2 or Fisher's exact tests were performed. Using co-dominant, dominant, recessive, overdominant, and additive models, we analyzed the association of the SNPs with ACS by logistic regression, adjusting by cardiovascular risk factors. All p values were corrected (pC) by the Bonferroni test. The values of pC < 0.05 were considered statistically significant, and all odds ratios (OR) are presented with 95 % confidence intervals. The occurrence of the ACS in our population was based in the OR values: OR = 1

does not affect the odds of developing ACS, OR > 1 is associated with higher odds of developing ACS, and OR < 1 is associated with lower odds of developing ACS. The linkage disequilibrium analysis (LD, D") of the SNPs, as well as the haplotypes construction, was performed with Haploview version 4.1 (Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA, USA). Hardy-Weinberg equilibrium (HWE) was evaluated by the Chi^2 test. The CCL5 levels were expressed as means ± SD and its association with the CCL5 genotypes was evaluated by the Student's *t*-test and p values < 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of the study population

Demographic, anthropometric, biochemical parameters and cardiovascular risk factors of the ACS patients and healthy controls are presented in Table 1. As expected, ACS patients presented a higher frequency of T2DM, hypertension, dyslipidemia, and smoking habit (35 %, 57 %, 85 %, and 35 %, respectively) when compared to healthy controls (10 %, 29 %, 71 %, and 22 %, respectively). ACS patients had a higher systolic and diastolic blood pressures [systolic = 130.61 (114−144) mmHg), and diastolic = 80.1 (70−90) mmHg] when compared to healthy controls [systolic =117.32 mmHg (106−126)), and diastolic =72.47 mmHg (66−77)]. Also, we observed statistically significant differences in the glucose plasma levels, 158.51 (102−188) mg/dl in patients vs. 98.73(84−99) mg/dl in controls. Nonetheless, the ACS patients showed lower levels of total cholesterol, HDL-C, and LDL-C [164.22 mg/dl (128−198), 38.32 mg/dl (32−44), and 106.4 mg/dl (76−133), respectively] than healthy controls [190.4 mg/dl (164–210), 44.6 mg/dl (35−53), and 115.8 mg/dl (94−134), respectively], whereas triglyceride levels were similar in both groups. As an effect of the statins, total cholesterol and LDL-C were lower in patients than in controls.

3.2. Allele and genotype frequencies

Genotype frequencies in the polymorphic sites were in HWE. The allele and genotype frequencies of the *CCL5* SNPs in ACS patients and healthy controls are shown in Table 2. Distribution of genotypes was significant different in the 3 study SNPs (p = 0.033 for -28 *G/C*, p = 0.013 for -109 *G/A*, and p = 0.042 for -403 *G/A*), whereas allele distribution only was different in the -109 *G/A* (p = 0.006) SNP.

Table 1
Demographic, anthropometric, biochemical parameters, and cardiovascular risk factors of the study individuals.

Characteristic		ACS patients (n = 625) Median (percentile 25−75)	Healthy controls (n = 700) Median (percentile 25−75)	p-value
Age (years)		57.7 [51−65]	54.4 [49−59]	<0.001
Gender n (%)	Male	510 (82)	463 (66)	<0.001
	Female	115 (18)	237 (34)	
BMI (kg/m2)		27.3 [25–29]	28.3 [26–31]	0.001
Blood pressure (mmHg)	Systolic	130.6 [114−144]	117.3 [106−126]	<0.001
	Diastolic	80.1 [70−90]	72.5 [66−77]	<0.001
Glucose (mg/dl)		158.5 [102−188]	98.7 [84−99]	<0.001
Total cholesterol (mg/dl)		164.2 [128−198]	190.4 [165−210]	<0.001
HDL-C (mg/dl)		38.3 [32−44]	44.6 [35−53]	<0.007
LDL-C (mg/dl)		106.4 [76−133]	115.8 [94−134]	<0.001
Triglycerides (mg/dl)		169.2 [109−201]	175.1 [112−208]	0.218
Hypertension n (%)	Yes	355 (57)	206 (29)	<0.001
Type II diabetes mellitus n (%)	Yes	218 (35)	68 (10)	<0.001
Dyslipidemia n (%)	Yes	534 (85)	501 (71)	<0.001
Smoking n (%)	Yes	222 (36)	147 (22)	<0.001

Data are expressed as median and percentiles (25th-75th). ACS: Acute coronary syndrome.

3.3. Association of CCL5 SNPs with ACS

Under co-dominant, dominant and additive models, the *G* allele of the *-109 G/A* SNP was associated with a higher risk of ACS (OR = 1.27, 95 % CI = 0.98–1.65, $pC_{Co\text{-}dom}$ = 0.041, OR = 1.33, 95 % CI = 1.03−1.71, pC_{Dom} = 0.03, and OR = 1.33, 95 %CI = 1.06−1.67, pC_{Add} = 0.015, respectively). In the same way, under co-dominant and recessive models, the *A* allele of the *-403 G/A* SNP was associated with increased risk of ACS (OR = 1.62, 95 % CI = 1.08−2.41, $pC_{Co\text{-}dom}$ = 0.042, and OR = 1.63, 95 %CI = 1.11−2.41, pC_{Res} = 0.012, respectively) (Table 3). All models were adjusted for gender, age, blood pressure, BMI, glucose, total cholesterol, HDL-C, LDL-C, triglycerides, and smoking habit.

3.4. Linkage disequilibrium analysis

We analyzed the haplotypes using the Haploview version 4.1 program. The analysis of the linkage disequilibrium showed a moderated linkage disequilibrium (D' < 0.60) between -28 *G/C*, -109 *G/A*, and -403*G/A* SNPs. Also, two of five allelic combinations (*GAG* and *GGA)* presented different frequencies in patients and healthy controls (Table 4). In this context, the "*GGA*" haplotype was associated with risk of developing ACS (OR = 3.12, 95 % CI: 1.45−6.72, *pC* = 0.003), whereas the "*GAG*" haplotype was associated with a lower risk of developing ACS (OR = 0.81, 95 % CI: 0.69−0.95, *pC* = 0.010).

3.5. Association of the CCL5 SNPs with CCL5 plasma levels

To define the possible functional effect of the *-109 G/A* and *-403 G/A* SNPs associated with the presence of ACS, we determined the plasma levels of CCL5 in individuals with different genotypes of these two SNPs. Subjects carrying *-109 GG* genotype had a lower CCL5 plasma

Table 2
Allele and genotype distribution of *CCL5* SNPs in the study sample.

SNP		ACS n = 625	Controls n = 693	*p
CCL5 −28 G/C (rs2280788)	Allele			
	G	1221 (0.976)	1366 (0.985)	NS
	C	29 (0.023)	20 (0.014)	
	Genotype			
	GG	596 (0.953)	674 (0.972)	
	GC	29 (0.046)	18 (0.026)	0.033
	CC	0 (0.0)	1 (0.001)	
CCL5−109 G/A (rs1800825)	Allele			
	A	1068 (0.855)	1231 (0.888)	
	G	180 (0.144)	155 (0.112)	0.006
	Genotype			
	AA	460 (0.737)	547 (0.789)	
	AG	148 (0.237)	137 (0.197)	0.013
	GG	16 (0.025)	9 (0.013)	
CCl5−403 G/A (rs2107538)	*Allele*			
	G	866 (0.692)	998 (0.720)	
	A	384 (0.307)	388 (0.279)	NS
	Genotype			
	GG	309 (0.494)	353 (0.509)	
	GA	248 (0.396)	292 (0.421)	0.042
	AA	68 (0.109)	48 (0.069)	

Data are shown as n and frequency. *chi-square test.
NS: No significant.

Table 3
Association of *CCL5* SNPs with ACS.

		n (Genotype frequency)		MAF	Model	OR (95 %CI)	pC
CCL5 −28 G/C (rs2280788)							
Control	GG	GC	CC	C			
(n = 693)	674 (0.972)	18 (0.026)	1 (0.001)	0.013	Co-dominant	1.80 (0.99–3.29)	0.07
					Dominant	1.71 (0.95–3.08)	0.07
ACS	596 (0.953)	29 (0.046)	0 (0.0)	0.023	Over-dominant	1.81 (0.99–3.30)	0.05
(n = 625)					Additive	1.59 (0.89–2.81)	0.12
CCL5−109 G/A (rs1800825)							
Control	AA	AG	GG	G			
(n = 693)	547 (0.789)	137 (0.197)	9 (0.013)	0.112	Co-dominant	1.27 (1.02–1.65)	**0.041**
					Dominant	1.33 (1.03–1.71)	**0.03**
ACS	460 (0.737)	148 (0.237)	16 (0.025)	0.144	Recessive	2.10 (0.92–4.82)	0.07
(n = 624)					Over-dominant	1.25 (0.96–1.62)	0.10
					Additive	1.33 (1.06–1.67)	**0.015**
CCL5−403 G/A (rs2107538)							
Control	GG	GA	AA	A			
(n = 693)	353 (0.509)	292 (0.421)	48 (0.069)	0.279	Co-dominant	1.62 (1.08–2.41)	**0.042**
					Dominant	1.07 (0.86–1.32)	0.57
ACS	309 (0.494)	248 (0.396)	68 (0.109)	0.307	Recessive	1.63 (1.11–2.41)	**0.012**
(n = 625)					Over-dominant	0.91 (0.73–1.13)	0.39
					Additive	1.14 (0.96–1.35)	0.12

ACS, Acute coronary syndrome; MAF, Minor allele frequency; OR, odds ratio; CI, confidence interval; pC, *p*-value. The p-values were calculated with the logistic regression analysis, and ORs were adjusted for gender, age, blood pressure, BMI, glucose, total cholesterol, HDL-C, LDL-C, triglycerides, and smoking habit.

Table 4
Haplotype frequencies (Hf) of *CCL5* haplotypes in ACS and healthy controls.

Haplotype	ACS (n = 624) Hf	Controls (n = 693) Hf	OR	95 %CI	pC
H1 (GAG)	0.555	0.605	0.81	0.69–0.95	**0.010**
H2 (GAA)	0.279	0.269	1.05	0.88–1.24	0.60
H3 (GGG)	0.122	0.105	1.18	0.93–1.51	0.18
H4 (GGA)	0.020	0.007	3.12	1.45–6.72	**0.003**
H5 (CAG)	0.016	0.010	1.59	0.80–317	024

Abbreviations: Hf, Haplotype frequency; *pC*, *p*-value corrected; OR, odds ratio; 95 %CI, confidential interval. The order of the polymorphisms in the haplotypes is according to the positions in the chromosome (rs2280788, rs1800825, and rs2107538). Bold numbers indicate significant associations.

concentration (369 ± 293.93 pg/mL) than carriers of both *AA* (944.81 ± 312.04 pg/mL, p = 0.001) and *AG* genotypes (787.05 ± 352.71 pg/mL, p < 0.0001) (Fig. 1A). In addition, this difference remained significant under the dominant model (*AA vs AG + GG*, p = 0.002) (Fig. 1B). On the other hand, individuals with *AA* genotype of *-403* SNP showed a lower concentration of CCL5 (478 ± 184.86 pg/mL) than individuals with either *AG* (821.85 ± 275.76 pg/dl, p = 0.013) or *GG* genotypes (1032.56 ± 301.39 pg/dl, p = 0.001) (Fig.1C). The difference remained significant under a recessive model (*AG + GG vs AA*, p < 0.001) (Fig. 1D).

4. Discussion

ACS is a multifactorial and polygenic disorder that results from an excessive inflammatory response to various forms of harmful stimuli to the arterial wall. The inflammatory process is a component of several pathophysiological conditions such as endothelial dysfunction, obesity, dyslipidemia, hypertension, and diabetes [1,2]. In this study, we focused on the chemokine (C-C motif) ligand 5 (CCL5), a molecule related to the inflammatory process involved in the genesis of the atheroma. We studied three SNPs (*−28 G/C, -109 G/A*, and *-403 G/A)* located in the promoter region of the *CCL5* gene in ACS patients and healthy controls. We aimed to establish the role of these SNPs in the genetic susceptibility to this syndrome and their effect in the plasma CCL5 levels. In this study, the *-109 G/A* and *-403 G/A* SNPs were associated with the risk of developing ACS, as well as with lower plasma CCL5 concentrations. These SNPs have been studied in cardiovascular diseases with contradictory results. For example, in agreement with our data, Simeoni et al. reported that the - *403 A* allele may increase the risk of developing ACS (OR = 1.36) and CAD (OR = 1.30) in a Caucasian population [10]. Ting et al. demonstrated that the - *403 AA* genotype was associated with the risk of CAD (OR = 3.06) in an Asian population [15]. In the same way, Vogiatzi et al. found an association between - *403 A* allele and the presence and severity of CAD in a Caucasian population [9]. Lipkova et al. reported, also in a Caucasian, population that the - *403 A* allele was associated with a higher risk of acute heart failure in patients with myocardial infarction [25]. Similarly, Wang et al. revealed in a meta-analysis that the - *403 A* allele was associated with CAD in a Caucasian population [26]. In contrast, Herder et al. in conjunction with MONICA/KORA Augsburg studies and CARDIoGRAM Consortia Investigators recently established that the *-403 G/A* SNP was not associated with CAD in a Caucasian population [13]. By the same token, Zhang et al. proved in a meta-analysis that this SNP was not associated with the risk of T2DM in a Chinese population [27]. Contrary to our results, in a Saudi Arabian population, Jabir et al. documented that *-109 G/A* SNP was associated with neither risk of developing CAD nor with plasma CCL5 levels [28]. The same result was found by Vogiatzi et al. in a Caucasian population [9]. Also, we found that the *GGA* haplotype was associated with a higher risk of developing ACS, whereas the *GAG* was associated with a lower risk. Although there was not a strong linkage disequilibrium between SNPs studied in our work, these haplotypes have different alleles in positions *-109* and *-403*. The risk haplotype has the G and A alleles in these positions, and both alleles were associated independently with the risk of developing ACS. This finding corroborated the role of these two alleles in the genetic susceptibility to ACS whether they were analyzed independently or as haplotypes. Recent studies have demonstrated that a local Th1 inflammatory response may contribute to plaque instability, increasing the risk of clinical manifestations of atherothrombosis [29]. In this context, CCL5 is secreted by the compromised endothelial cells around the atheroma constituting one of the main chemoattractant factors for $CD4^+$ T cells and favoring such Th1 inflammatory process. Therefore, it is likely that CCL5 (RANTES) gene SNPs modulate the secretion of CCL5 chemokine, and in consequence, the amount of $CD4^+$ T cells infiltrated in the atheroma, thus explaining the association of such SNPs with the incidence of ACS found in the present study. Furthermore, we analyzed the impact of the CCL5 SNPs on CCL5 plasma concentration as a possible mechanism that explains the relationship between these SNPs and the higher risk of ACS [9–11,13,14,25,28,30]. In our study, the association of the -109 *G/A* and -403 *G/A* SNPs with CCL5 levels was conducted in healthy controls. This analysis showed that the -109 *GG* and -403 *AA* genotypes are associated with low

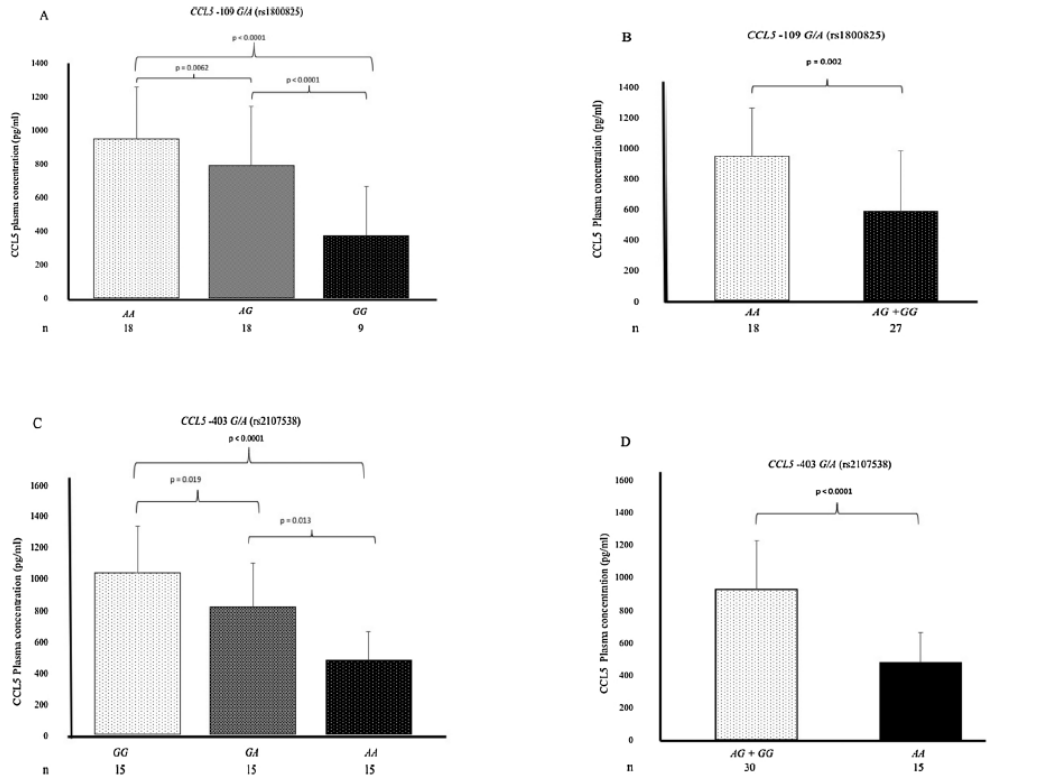

Fig. 1. Genetic contribution of the *CCL5 -109 G/A* and *CCL5 - 403 G/A* SNPs on CCL5 levels. A) Comparison of the genotype of the *CCL5 - 109 G/A* SNP with CCL5 levels in plasma. B) Comparison of CCL5 levels in plasma using the dominant model. C) Comparison of the genotype of the *CCL5 - 403 G/A SNPs* with CCL5 levels in plasma. D) Comparison of CCL5 levels in plasma with *CCL5 - 403 G/A* genotypes using the recessive model.

CCL5 levels. As far as we know, this is the first study that showed the association of the -109 G/A and -403 G/A SNPs with CCL5 levels in individuals without the use of the anti-dyslipidemic or anti-hypertensive drugs. It is well-known that these drugs may modify the levels of inflammatory molecules such as pro-inflammatory cytokines, adhesion molecules, and C-reactive protein, masking the real impact of CCL5 gene SNPs on plasma CCL5 levels [21–24]. Nonetheless, the results concerning the relationship between CCL5 plasma levels and heart diseases are still controversial with positive and negative results. For example, Lipkova et al. described that serum CCL5 levels were lower in patients with myocardial infarction [25]. By the same token, Cavausoglu et al. documented that low levels of plasma CCL5 are associated with cardiac mortality after myocardial infarction [11]. Similarly, Rothenbacher et al. determined that the lower serum levels of CCL5 were associated with a progression and a risk of coronary heart disease [31]. In our study, the results showed that the -109 *GG* and - *403 AA* genotypes were associated with low CCL5 levels. In contrast, Parissis et al. reported increased serum levels of CCL5 in patients with myocardial infarction; however, the authors did not analyze the CCL5 SNPs [7].

As far as we know, the precise mechanism by which low and/or high CCL5 levels are associated with ACS remains to be elucidated. Nonetheless, recent studies in the murine model have shown that the Y-box binding protein-1 (YB-1) is a key regulator of the expression of the CCL5 chemokine, as well as the mRNA processing. YB-1 has a binding site named Y-box or CCAAT inverted box, which has a high affinity for the position -204/ -173 in the promoter region of the *CCL5* gene [32]. In this context, the experimental evidence showed that the overexpression of the YB-1 increased the transcriptional activity in reporter assays, mRNA, and the protein expression in atherosclerosis-prone mice. In the same way, Hanssen et al., reported that low YB-1 expression decreased the CCL5 levels in sera from sepsis patients, as well as in the murine model [33]. In this context, we suggest that low levels of CCL5 could be due to a decrease in the transcriptional activity by the low affinity of the YB-1 to the promoter region of the *CCL5* gene. However, we think that future investigations are warranted to understand the contribution of these SNPs to CCL5 levels.

Finally, our data, showed that the CCL5−403 *G/A* and CCL5−109 *G/A* SNPs were associated with the risk of developing ACS, data controversial with reports in other populations. The observed differences when are study different populations could be due to the effect of the cardiovascular risk factors that play an important role in the development of coronary heart diseases [13], as well as, to the different distribution of the SNPs in the study populations. Data obtained from the National Center for Biotechnology Information revealed that Mexican mestizos, Colombians, and individuals with Mexican ancestry from Los Angeles had a high frequency of the -109 *G* allele (11, 5, and 6%, respectively) compared with Caucasians (2%). In an Asian population, the frequency of the *G* allele is null since the *-109 G/A* SNP is monomorphic. On the other hand, individuals from Los Angeles with Mexican ancestry, Mexican mestizos, Peruvians, as well as an Asian population had a high frequency of the -403 A allele (30, 28, 24, and 32 %, respectively), whereas in Caucasian population this allele had a low frequency (16 %)

(https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). Of note, the Mexican population has a characteristic genetic background and important ethnic differences with other populations [34–36]. Therefore, we consider that studies with a greater sample in populations with different ethnic origins may explain the true role of *CCL5* SNPs in the risk of developing ACS.

In summary, this study demonstrated that the *CCL5−109 G/A* and *CCL5−403 G/A SNPs* of the *CCL5* gene were associated with an increased risk of developing ACS in a Mexican population. Also, it was possible to distinguish one haplotype (*GGA*) associated with an increased risk of developing ACS. There was a statistically significant association of both *CCL5−109 G/A* and *CCL5−403 G/A* SNPs with lower CCL5 levels in plasma. According to our data, we suggest that the *CCL5* gene SNPs could be a factor more in the etiology of ACS. Besides, we strongly believe that these results could be contributing to a future comprehensive panel of genetic background to assess the risk of ACS.

Author contributions

Gabriel Herrera-Maya and Gilberto Vargas-Alarcon contributed equally to this study.

Gabriel Herrera-Maya, Gilberto Vargas-Alarcon, and Jose Manuel Fragoso were responsible for the conception and design of the study.

Oscar Perez-Mendez, Gabriel Herrera-Maya, Rebeca Lopez-Marure, Rosalinda Posadas-Sanchez, Julio Granados, and Betzabe Nieto-Lima, participated in the generation and collection of the samples.

Oscar Perez-Mendez, Gabriel Herrera-Maya, Rosalinda Posadas-Sanchez, Julian Ramirez-Bello, and Betzabe Nieto-Lima, contributed in the analysis, and interpretation of data.

Drafting or revision of the manuscript was handled by Gilberto Vargas-Alarcon, and Jose Manuel Fragoso.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Capítulo V

Discusión

En un primer estudio se llevó a cabo la determinación de cuatro polimorfismos presentes en el gen *SEL-E* (*SEL-E* 98 *G/T*, *SEL-E* 561 *A/C*, *SEL-E* 1880 *C/T* y *SEL-E* 1040 *G/A*) en pacientes con aterosclerosis e individuos control, como originalmente se tenía pensada en este estudio. En el cual se observó la asociación de los polimorfismos *SEL-E* 98 *G/T*, *SEL-E* 561 *A/C*, y *SEL-E* 1880 *C/T* fue con aterosclerosis subclínica y no con aterosclerosis *per se (*Véase Capitulo III*)*. Una vez obtenidos estos datos nosotros observamos que la tendencia de los pacientes con aterosclerosis culminaba en el desarrollo final de SICA, lo que en principio nos permitió replantear la hipótesis y continuar el estudio en la población de pacientes que desarrollaron SICA e individuos control. En este contexto, los polimorfismos que se estudiaron en los pacientes con SICA e individuos control fueron los siguientes: del gen *eNOS* los polimorfismos *eNOS* -786 *T/C*, *eNOS* 894 *G/T*; del gen *CCL-5* los polimorfismos *CCL-5* -28 *G/C*, *CCL-5* -109 *C/T*, *CCL-5* -403 *G/A* y del gen *SEL-P* los sitios polimórficos *SEL-P* -1969 *A/G*, *SEL-P* 1087 *G/A*, *SEL-P* 2013 *G/T* y *SEL-P* 2361 *A/C*. Cabe destacar que por causas ajenas a nosotros los polimorfismos (*SEL-E* 1040 *A/G*, *SEL-E* 1559 *C/T* y *SEL-P* -1969 *A/G*) no se realizaron por el mal diseño de la casa comercial Applied Biosystems (Thermo Fisher Scientific).

La reciente evidencia ha sugerido que la molécula de selectina-E se involucra de manera importante en la disfunción endotelial y la inflamación localizada dentro de la pared de los vasos sanguíneos (4). Además, las selectinas P y E se considera un producto endotelial clave en el desarrollo de aterosclerosis y SICA (181, 182). En nuestro estudio, encontramos la asociación de tres polimorfismos del gen *SEL-E*. El alelo *T* del polimorfismo *SEL-E* 5'UTR *G*98*T*, el alelo *C* del polimorfismo *SEL-E* 561 *A/C* y el genotipo *CT* del polimorfismo *SEL-E* 1880 *C/T* se asociaron con riesgo al desarrollo de aterosclerosis. Concordantemente el polimorfismo *SEL-E* 561 *A/C* en el alelo *C* ha sido asociado a riesgo al desarrollo de accidente cerebrovascular isquémico, hipertensión arterial e infarto de miocardio, en estudios previos en población china, sudanesa y metaanálisis (182-185). Dentro de estas investigaciones también se encontró que el genotipo *TT* del polimorfismo *SEL-E* 5'UTR *G*98*T* se asocia a riesgo al desarrollo de aterosclerosis en población asiática y caucásica, similar a los resultados obtenidos en nuestro estudio (183). En nuestro estudio también se encontró asociación de riesgo para el genotipo CT del polimorfismo *SEL-E* 1880 *C/T*, aunque este último no se describe en población asiática como polimorfismo de riesgo (181). Adicionalmente, nuestros datos demostraron que el haplotipo *AT* compuesto por los polimorfismos *SEL-E* 561 *A/C* y *SEL-E* 98 *G/T* se asocia con un mayor riesgo de desarrollar aterosclerosis, mientras que el haplotipo *AG* se asoció con un menor riesgo de desarrollar aterosclerosis. Como se puede ver, estos haplotipos se diferencian por la presencia de los alelos *A* y *T* de los polimorfismos *SEL-E* 561 *A/C* y *SEL-E* 98 *G/T*, que de manera independiente o en haplotipo como se demuestra en este estudio, se asocian con riesgo a desarrollar algún evento cardiovascular. Gracias a estos datos fue posible publicar un primer artículo en aterosclerosis subclínica (Véase Capitulo III).

El análisis de los polimorfismos del gen *eNOS* (*eNOS* -786 *T/C* y *eNOS* 894 *G/T)* no mostraron asociación al desarrollo de SICA en nuestra población. Debido a que la distribución alélica y genotípica fue similar entre los grupos de estudio. No obstante,

interesantemente, el análisis de haplotipos demostró que el haplotipo "TT" conformado por los polimorfismos del gen *eNOS* -786 *T/C* y *eNOS* 894 *G/T* se asocian a protección al desarrollo de SICA en nuestra población. Controversial con nuestros resultados, datos en la literatura han demostrado que, el haplotipo *"TT"*, se encuentra asociado a variabilidad diastólica y sistólica de la presión arterial en Europea. E incluso se ha asociado a la falta de respuesta al tratamiento antihipertensivo (186, 187). El polimorfismo del gen *eNOS* -786 *T/C* se asocia con hipertensión esencial (OR= 1.84) en población de Sudán (188). Similar con lo reportado por Kong y colaboradores quien determina en meta-análisis que el alelo *T* se asocia con protección al desarrollo de infarto de miocardio en poblaciones asiática caucásica (189). Por otra parte, el polimorfismo del gen *eNOS* 894 *G/T* ha demostrado que confiere susceptibilidad en modelos de herencia dominante, codominante y recesivo (OR= 1.2) a desarrollar accidentes cerebrovasculares isquémicos, desde la perspectiva de meta-análisis en población China Han y del norte de la India (190). Hasta donde sabemos no hay reportes en la literatura que muestren la presencia del haplotipo *"TT"* como protector al desarrollo de eventos cardiovasculares. En este contexto, nosotros consideramos no solo continuar con la confirmación de nuestro hallazgo, sino creemos que futuros estudios en una cohorte mayor de pacientes con SICA en diferentes poblaciones podrían mostrar el verdadero papel de estos sitios polimórficos.

Por otro lado, el análisis de los polimorfismos genéticos *CCL-5* -109 *C/T* y *CCL-5* -403 *G/A* del gen *CCL-5* que codifica para la proteína RANTES mostraron que los alelos *G* del polimorfismo *CCL-5* -109 *C/T* y el alelo *A* del polimorfismo *CCL-5* -403 *G/A* se asociaron con el riesgo al desarrollo de SICA. Datos que nos permitieron se publicara un segundo manuscrito (Véase Capitulo IV). En línea con nuestros datos. Simeoni y colaboradores reportan que el alelo *A* del polimorfismo del gen *CCL-5* -403 *G/A* aumenta el riesgo al desarrollo de SICA (134), similar con lo reportado en otros estudios en diferentes trabajos en poblaciones caucásicas y asiáticas (191-194) . Al hacer el análisis de haplotipos dos de cinco posibles combinaciones (GAG y GGA) de los polimorfismos *CCL-5* -28 *G/C*, *CCL-5* -109 *C/T* y *CCL-5* -403 *G/A* resultaron en asociación a riesgo (OR = 3.12, 95% IC 1.45 – 6.72, pC = 0.003) y protección (OR = 0.81, 95% IC 0.69 – 0.95, pC = 0.010) al desarrollo de SICA, respectivamente.

Finalmente, se analizaron los polimorfismos *SEL-P* 1087 *G/A*, *SEL-P* 2013 *G/T*, *SEL-P* 2361 *A/C* del gen *SEL-P*. Los resultados mostraron los alelos *A* del polimorfismo *SEL-P* 1087 *G/A* y *C* del polimorfismo *SEL-P* 2361 *A/C*, respectivamente, se asocian con una disminución en el riesgo de desarrollar SICA. Datos en la literatura tiene bien reportado que los SNPs *SEL-P* 1087 *G/A* y *SEL-P* 2361 *A/C* se asocian a enfermedades cardiovasculares, siendo las poblaciones estudiadas de origen Europeo/asiático que de origen Africano y/o mestizo (178, 195-198). Adicionalmente, a este análisis nosotros determinamos que los haplotipos "*A T C"* y "*A T A"* compuestos por de los polimorfismos *SEL-P* 1087 *G/A*, *SEL-P* 2013 *G/T*, *SEL-P* 2361 *A/C* del gen *SEL-P* se asocia con menor riesgo al desarrollo de SICA (Véase Capitulo II).

Una vez determinada la asociación de los polimorfismos con la presencia de SICA. Se analizó el posible papel funcional de los polimorfismos asociados con la concentración de las moléculas. En este contexto, las concentraciones de *SEL-E* en membrana no se analizaron. Este análisis contemplaba el uso de células del endotelio del cordón umbilical,

material que no nos permitió analizar los genotipos de los polimorfismos involucrados, por la frecuencia a la que se distribuye el alelo variante. Por esta razón no se incluyen en las publicaciones asociadas. De la misma manera, en el caso de *eNOS* no encontramos asociación de riesgo de los polimorfismos por lo cual no se procedió al análisis de nitritos orgánicos por prueba de Griess.

Sin embargo, cuando hacemos la correlación de los alelos asociados del gen *SEL-P* con la concentración de selectina-P en suero, el alelo *A* del polimorfismo *SEL-P* 1087 *G/A*, así como el alelo *C* del polimorfismo *SEL-P* 2361 *A/C*, se asociaron con baja concentración de selectina-P. En línea con nuestros datos, tres grandes estudios como son: el estudio CARDIA, el estudio del riesgo de aterosclerosis en las comunidades (ARIC), así como el estudio del corazón de Framingham (FHS) reportan que estos alelos se asocian con bajo riesgo de desarrollar aterosclerosis, e IAM. Así como, con bajos niveles de selectina-P (178, 195-197).

Similar a nuestros resultados investigaciones como CARDIA, concuerdan con los datos obtenidos en esta investigación, en los cuales se determinan niveles bajos de selectina-P en suero. Controversialmente estos estudios se determinan asociación a riesgo del alelo *A* del polimorfismo *SEL-P* 1087 *G/A*, así como el alelo *C* del polimorfismo *SEL-P* 2361 *A/C* con niveles bajos de selectina-P en suero al desarrollo de aterosclerosis, pero no al desarrollo de SICA (178, 196, 197). Nuestros datos demuestran que en población mexicana los polimorfismos *SEL-P* 2013 *G/T, SEL-P* 1087 *G/A* están asociados a protección. Similar efecto mostraron los alelos G y *A* de los polimorfismos *CCL-5* -109 *C/T* y *CCL-5* -403 *G/A*, que se asociaron a una baja concentración de RANTES en suero. Nuestros datos correlacionan con los resultados obtenidos en varias poblaciones. El grupo de trabajo de Simeoni, reporta al alelo *A* del polimorfismo *CCL-5* -403 *G/A* como potencial alelo de riesgo al desarrollo de SICA en población caucásica (134). Por otro lado, Ting y su grupo de trabajo demuestran que el genotipo *AA* del polimorfismo *CCL-5* -403 *G/A* se asocia a riesgo en enfermedades cardiovasculares en población de Asia (194). Controversial a nuestros resultados, el grupo de trabajo de Jabir reporta que no hay asociación de riesgo o protección del polimorfismo *CCL-5* -109 *C/T* en población de Arabia Saudita (199). Hipótesis que también es apoyada por el grupo de trabajo de Vogiatzi en población caucásica (193). Nuestros resultados resaltan por la medición de concentraciones de RANTES en suero haciendo separación por genotipos de los polimorfismos *CCL-5* -109 *C/T* y *CCL-5* -403 *G/A* (191, 200, 201).

Adicionalmente, se determinó por medio de herramientas *in silico*, el posible papel funcional que pudiesen tener los polimorfismos asociados a la aterosclerosis y al SICA. En este análisis se determinó que el alelo *T* del polimorfismo *SEL-E* 98 *G/T* genera un motivo de unión para el factor de transcripción OCT3/4 (202), así como el alelo A de polimorfismo *SEL-E A561C* produce un sitio de unión para las proteínas SF2/ASF y Srp55, que tiene un papel importante en el splicing alternativo (203). Por otra parte, el alelo A del polimorfismo *SEL-P* 1087 *G/A* produce un sitio de unión a proteínas de splicing alternativo como la Srp40 (203). Sin embargo cuando analizamos los polimorfismos los genes *eNOS* y *CCL-5* in silico no mostraron efecto funcional alguno.

En este contexto, nosotros proponemos esquemáticamente (Figura. 3), que la información obtenida en esta investigación conjunta los fenómenos biológicos involucrados en el desarrollo de aterosclerosis y SICA intrínsecamente relacionados. Debido a que nuestra investigación se centra en el fenómeno de disfunción endotelial como una de las principales causales de la aterosclerosis y el SICA. En la disfunción endotelial la selectina-E y P en concentraciones elevadas perpetua el fenómeno de disfunción endotelial, producción de especies reactivas de oxígeno y migración de células inmunes a la capa íntima y media de las aterías. La disfunción endotelial también promueve el paso de LDL a la misma capa íntima y media de las arterias. Esto genera que el proceso inflamatorio que se perpetúa a partir de la disfunción endotelial continúe no solamente por el estilo de vida y hábitos alimenticios del individuo sino por el gradual aumento de los procesos involucrado en la aparición de placas ateroscleróticas que conllevan al SICA (7-10).

De acuerdo con la premisa anterior, la asociación obtenida de los SNPs, los datos obtenidos *in silico* y funcionales, nos llevaron a sugerir una seria de vías metabólicas que podría estar involucradas en el desarrollo de la aterosclerosis y el SICA (Figura 3). De acuerdo con nuestros datos los polimorfismos A561C y G98T del gen de la *SEL-E* se asociaron al riesgo al desarrollo de aterosclerosis. Así mismo, bajo estudios *in silico* se observó que el alelo *C* del polimorfismo A561C del gen *SEL-E*, genera mayor afinidad de la proteína por su ligando promoviendo una mayor atracción de monocitos al endotelio vascular. Por otro lado, el alelo T del polimorfismo 5'UTR G98T (rs1805193), simula la región promotora del factor transcripcional de motivo de octamero 3/4 (OCT3/4) (212). Esta pseudoregion promotora generada por el polimorfismo, promueve la activación del óxido nítrico sintetasa inducible (iNOS) a través de OCT1 (212). Posteriormente iNOS recluta a la DNA Polimerasa III que es la encargada de la transcripción del RNA ribosomal y de transferencia (cita original). Patológicamente la expresión de iNOS por el factor transcripcional OCT1 promueve la transcripción de iNOS, que promueve la sobre producción de ROS (204). Estos ROS generan el ambiente que promueve angiogénesis (inflamación y vasoconstricción), perpetuando el desarrollo de la placa aterosclerótica.

Los mecanismos que generan disfunción endotelial son los primeros eventos previos a el desarrollo de SICA, por esta razón se busca relacionar polimorfismos entre la población de aterosclerosis y la población de SICA. Siendo unos de los principales involucrados en le generación de especies reactivas de oxígeno y por la facilidad con que es desestabilizada, la *eNOS* estuvo dentro de nuestras consideraciones desde el inicio del proyecto. Los resultados obtenidos de los polimorfismos de *eNOS* no arrojaron asociación a riesgo para el desarrollo de SICA. Sin embargo, la *eNOS* es uno de los componentes que aumenta la concentración de ROS, debido a que la evidencia muestra que concentraciones elevadas de ROS promueven la inestabilidad de la *eNOS*, inestabilidad que provoca la donación de los electrones que serían destinados a la síntesis de óxido nítrico sean tomados por oxígeno, produciendo iones súper óxido y peroxinitritos (94). Interesantemente, la *eNOS* también es regulada por la ruta de PI3K-AKT, en este caso le *eNOS* puede ser fosforilada en el residuo Ser1177 por la cinasa AKT produciendo mayor flujo de electrones por el dominio reductasa generando incremento en la concentración de óxido nítrico (205).

La ruta PI3K-AKT también se relaciona con la regulación del miRNA-200b, en la cual la expresión es inversamente proporcional a la expresión de *CCL-5* (206, 207). En

nuestro estudio los niveles de RANTES fueron bajos de acuerdo a los genotipos de los polimorfismos asociados a riesgo. Nosotros sugerimos que estos niveles bajos se podrían explicar bajo las siguientes premisas: i) la primera radica en el supuesto de que a bajas concentraciones de RANTES, se sobre expresa el miRNA-200b que promueve la señalización de la ruta PI3k-AKT, NFKB y VEGF incrementado la angiogénesis, inflamación y vaso constricción (208). ii) la segunda radica en la proteína de unión a caja Y-1 (siglas en inglés: YB-1); la cual tiene un papel importante en la regulación de la expresión de RANTES, así como en el procesamiento del RNA mensajero (41). Debido a que esta tiene un sitio de unión 5'-*CCAAT-'3* que tiene una alta afinidad por la posiciones -204/-173 de la región promotora en el gen de *CCL-5*, incrementando la expresión de RANTES [35]. En este contexto, la baja expresión de YB-1 disminuye podría estar involucrada en la baja expresión de RANTES, por la baja afinidad al sitio 204/-173 de la región promotora.

Otra de las moléculas relacionada a la ruta de PI3K-AKT es la angiotensina II, la cual es regulada por selectina-P (209). En relación a nuestros resultados los polimorfismos del gen *SEL-P*, se asociaron a bajo riesgo de desarrollar SICA y a una baja concentración de selectina-P en suero. Debido a que se ha comprobado que una sobre expresión de selectina-P en la superficie de las células endoteliales medía los efectos de la angiotensina II (Ang II), que tiene un papel importante en el desarrollo de la aterosclerosis (210, 211). Estimulando la producción de moléculas de adhesión, quimiocinas y citocinas, así como la oxidación de LDLs, promoviendo la disfunción endotelial (210). Adicionalmente, una posible explicación basada en estudio in silico, radica en el sitio de unión que produce alelo A del polimorfismo *SEL-P* 1057 *G/A* para la proteína Srp40 involucrada en el proceso de splicing alternativo. Lo que sugiere que podrían presentarse isoformas de la selectina-P con o sin funcionalidad.

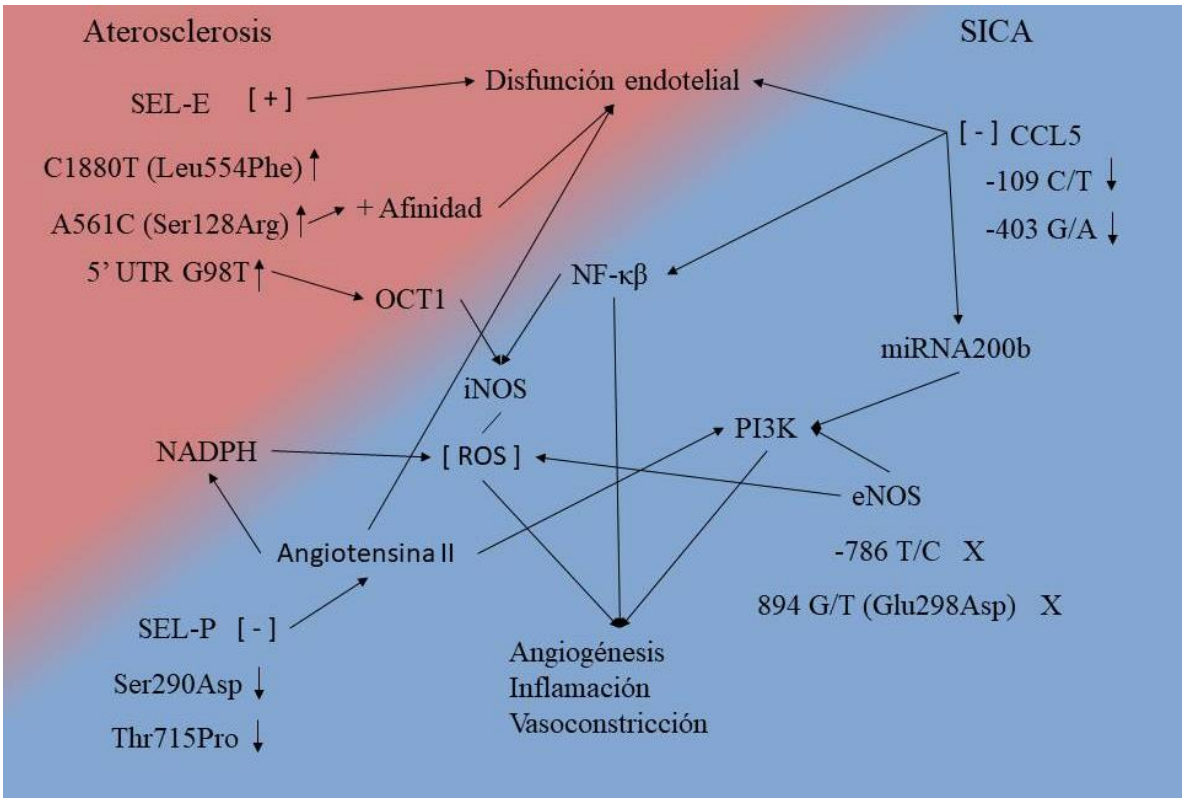

Figura 3. Representación gráfica de la interacción entre los polimorfismos, el papel funcional de los polimorfismos y las enfermedades trabajadas en esta tesis. En rojo podemos observar las interacciones sugeridas en aterosclerosis y como interaccionan con el desarrollo de SICA (azul). Entre corchetes podemos observar la representación de la concentración en plasma de las proteínas analizadas ([-] siendo menor concentración y [+] siendo mayor concentración) de cada proteína. Las flechas a los costados de los polimorfismos expresan la asociación a riesgo (flecha apuntando hacia arriba) o protección (flecha apuntando hacia abajo).

Finalmente, en nuestro estudio los siguientes SNPs (*SEL-E* 98 *G/T* , *SEL-E* 561 *A/C* y *SEL-E* 1880 *C/T* del gen *SEL-E*; *eNOS* -786 *T/C* y *eNOS* 894 *G/T* del gen *eNOS*; *CCL-5* -28 *G/C*, *CCL-5* -109 *C/T* y *CCL-5* -403 *G/A* del gen *CCL5* y *SEL-P* 1087 *G/A* y *SEL-P* 2361 *A/C* del gen *SEL-P*) se asociaron con la presencia de aterosclerosis y el SICA. No obstante, la asociasen de estos polimorfismos es controversial con datos positivos y negativos en diferentes poblaciones. En este contexto, nosotros sugerimos que la asociación de estos SNPs puede deberse a los factores de riesgo cardiovascular que juegan un papel importante en el desarrollo de las enfermedades coronarias (212-215), así como al origen étnico de cada población. De acuerdo a los datos obtenidos del Centro Nacional de Información Biotecnológica, se reveló que una muestra de mestizos mexicanos, colombianos y personas de Los Ángeles con ascendencia mexicana tenía una alta frecuencia del alelo G del polimorfismo *CCL-5* -109 *C/T* (11, 5 y 6%, respectivamente) en comparación con caucásicos (2%). Polimorfismo que se encontró asociado a riesgo en nuestra población (216-218). Hasta la fecha este es el primer estudio que muestra la asociación de los polimorfismos *CCL-5* -109 *C/T* y *CCL-5* -403 *G/A*, sin el uso de fármacos

antidislipidémicos o antihipertensivos que pueden modificar los niveles de marcadores inflamatorios como las citocinas proinflamatorias, las moléculas de adhesión y la proteína C reactiva, enmascarando el impacto real de los polimorfismos del gen *CCL-5* en la expresión plasmática de RANTES (88, 219-221). En el caso del gen *SEL-P* la frecuencia del alelo *A* del polimorfismo *SEL-P* 1087 *G/A* para poblaciones Europeas, Asia y África (21.7%, 20.2% y32.9%) es menor a la descrita en la población de mexicanos mestizos y caucásicos americanos (9% y 14%). En el caso del alelo *C* del polimorfismo *SEL-P* 2361 *A/C* las poblaciones de mexicanos mestizos, europeos y caucásicos americanos (8%, 8.8% y 8.2%) es mayor a las poblaciones de Asia y África (0.2% y 2.5%)

Conclusión

En resumen, este trabajo se determinó que los polimorfismos *SEL-E* 98 *G/T*, *SEL-E* 561 *A/C* y *SEL-E* 1880 *C/T* del gen *SEL-E* tienen participación importante en el riesgo a desarrollar aterosclerosis. Así como, los polimorfismos *CCL-5* -109 *C/T* y *CCL-5* -403 *G/A* del gen *CCL-5* se asocian con el riesgo de desarrollar SICA en nuestra población. Por otra parte, los polimorfismos *SEL-P* 1087 *G/A* y *SEL-P* 2361 *A/C* del gen *SEL-P* se asocian con bajo riesgo de desarrollar SICA en nuestra población. Adicionalmente en este estudio, se logró identificar que los haplotipos "*AT*" conformados por los polimorfismos *SEL-E* 98 *G/T* y *SEL-E* 561 *A/C*, así como el haplotipo "*GGA*" conformado por polimorfismos *CCL-5* -28 *G/C*, *CCL-5* -109 *C/T* y *CCL-5* -403 *G/A* del gen *CCL5* incrementan el riesgo de desarrollar SICA en nuestra población. Mientras que los haplotipos "*A T C"* y "*A T A"* compuestos por de los polimorfismos *SEL-P* 1087 *G/A*, *SEL-P* 2013 *G/T*, *SEL-P* 2361 *A/C* del gen *SEL-P* disminuyen el riesgo de desarrollar SICA. Similar al haplotipo "*TT*" conformado por los polimorfismos *eNOS* -786 *T/C* y *eNOS* 894 *G/T* del gen *eNOS*.

Cabe destacar que el número de individuos incluidos en nuestro estudio y las características genéticas específicas de la población mexicana nos hace considerar que los estudios adicionales en un mayor número de individuos y en otras poblaciones podrían ayudar a definir la relación de estos polimorfismos como marcador para deducir el pronóstico de aterosclerosis y SICA, respectivamente, así como en otros eventos cardiovasculares.

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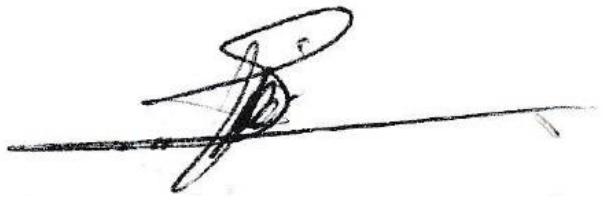

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