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Original article

Association Between Coronary Artery Disease Genetic Variants and Subclinical Atherosclerosis: An Association Study and Meta-analysis

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ABSTRACT

Introduction and objectives: Recent studies have identified several genetic variants associated with coronary artery disease. Some of these genetic variants are not associated with classical cardiovascular risk factors and the mechanism of such associations is unclear. The aim of the study was to determine whether these genetic variants are related to subclinical atherosclerosis measured by carotid intima media thickness, carotid stiffness, and ankle brachial index.

Methods: A cross-sectional study nested in the follow-up of the REGICOR cohort was undertaken. The study included 2667 individuals. Subclinical atherosclerosis measurements were performed with standardized methods. Nine genetic variants were genotyped to assess associations with subclinical atherosclerosis, individually and in a weighted genetic risk score. A systematic review and meta-analysis of previous studies that analyzed these associations was undertaken.

Results: Neither the selected genetic variants nor the genetic risk score were significantly associated with subclinical atherosclerosis. In the meta-analysis, the rs1746048 (*CXCL12*; n = 10581) risk allele was directly associated with carotid intima-media thickness (β = 0.008; 95% confidence interval, 0.001-0.015), whereas the rs6725887 (*WDR12*; n = 7801) risk allele was inversely associated with this thickness (β = −0.013; 95% confidence interval, −0.024 to −0.003).

Conclusions: The analyzed genetic variants seem to mediate their association with coronary artery disease through different mechanisms. Our results generate the hypothesis that the *CXCL12* variant appears to influence coronary artery disease risk through arterial remodeling and thickening, whereas the *WDR12* risk variant could be related to higher plaque vulnerability.

Asociación entre variantes genéticas de enfermedad coronaria y aterosclerosis subclínica: estudio de asociación y metanálisis

Palabras clave:
Variantes genéticas
Aterosclerosis
Grosor intimomedial carotídeo
Rigidez carotídea
Índice tobillo-brazo
Metanálisis
Subclínica

RESUMEN

Introducción y objetivos: En estudios recientes se han identificado varias variantes genéticas asociadas a la enfermedad coronaria. Algunas de estas variantes genéticas no se asocian a factores de riesgo cardiovascular clásicos y no están claros los mecanismos por los que se producen tales asociaciones. El objetivo de este estudio es determinar si estas variantes genéticas están relacionadas con la aterosclerosis subclínica medida con el grosor intimomedial carotídeo, la rigidez carotídea y el índice tobillo-brazo.

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Métodos: Se llevó a cabo un estudio transversal anidado en el seguimiento de la cohorte REGICOR. El estudio se llevó a cabo en 2.667 individuos. Se realizaron mediciones de la aterosclerosis subclínica con métodos estandarizados. Se determinaron los genotipos relativos a nueve variantes genéticas para evaluar las asociaciones con la aterosclerosis subclínica, individualmente y con una puntuación de riesgo genético ponderada. Se llevó a cabo una revisión sistemática y metanálisis de los estudios previos que analizaron esas asociaciones.

Resultados: Ninguna de las variantes genéticas estudiadas ni la puntuación de riesgo genético mostraron una asociación significativa con la aterosclerosis subclínica. En el metanálisis, el alelo de riesgo del rs1746048 *(CXCL12)* (n = 10.581) mostró asociación directa con el grosor intimomedial carotídeo (β = 0,008; intervalo de confianza del 95%, 0,001-0,015), mientras que el alelo de riesgo rs6725887 *(WDR12)* (n = 7.801) mostró asociación inversa (β = –0,013; intervalo de confianza del 95%, –0,024 a –0,003).

Conclusiones: Las variantes genéticas analizadas parecen intervenir en la asociación con la enfermedad coronaria por diferentes mecanismos. Estos resultados generan la hipótesis de que la variante *CXCL12* parece influir en el riesgo de enfermedad coronaria a través del remodelado y el engrosamiento de las arterias, mientras que la variante de riesgo *WDR12* podría estar relacionada con una mayor vulnerabilidad de la placa.

Abbreviations

ABI: ankle-brachial index
CAD: coronary artery disease
cCVRFs: classical cardiovascular risk factors
IMT: intima-media thickness
SNPs: single nucleotide polymorphisms

INTRODUCTION

Coronary artery disease (CAD), the leading cause of death[1] and disability worldwide,[2] has a significant genetic component. Recent genome-wide association studies have identified several single nucleotide polymorphisms (SNPs) associated with myocardial infarction and CAD.[3–11] Some of these variants are also associated with classical cardiovascular risk factors (cCVRFs), but others are not, and the biologic mechanisms underlying their association with CAD remain unclear.

Atherosclerosis is the main etiopathogenic mechanism related to CAD. It begins with a subclinical phase, starting early in life,[12] and the clinical manifestations represent the end stage of this chronic process. Therefore, the measurement of early and intermediate stages of subclinical atherosclerosis may be used to identify the progression of this process and to predict the future risk of cardiovascular or coronary events. Intima-media thickness (IMT), stiffness of the carotid artery, and ankle-brachial index (ABI) are accurate, quantifiable, reproducible, and noninvasive markers of atherosclerosis and well-established predictors of future cardiovascular events such as myocardial infarction and stroke.[13–15]

The study of the association between the presence of subclinical atherosclerosis and of SNPs associated with CAD independently of cCVRFs could shed some light on the biological mechanisms underlying this relationship. This study had two aims: *a)* to determine whether genetic variants associated with CAD but not with cCVRFs are related to subclinical atherosclerosis as assessed by carotid IMT, carotid stiffness, and ABI in a population-based survey, and *b)* to perform an updated systematic review and meta-analysis of previous studies that analyzed these associations.

METHODS

Design

A cross-sectional study nested in the Girona Heart Registry (REGICOR) cohort study.[16,17] We used data from the follow-up of 2 population-based cohorts originally enrolled in 1995 and 2000 (with response rates of 72.4% and 70.0%, respectively) from towns that represent the geographic diversity of Girona province.[16,17] During 2007-2010, the surviving, noninstitutionalized participants residing in these towns were invited to participate in a follow-up visit; the response rate was 78.4%. We selected participants aged 35 to 74 years at the time of the basal exams, who were free of cardiovascular disease at that time, and for whom DNA was available. This study was approved by the local ethics committee, and participants gave written informed consent.

Genetic Variants Selection, Genotyping, and Multilocus Risk Score Generation

We selected 9 genetic variants associated with CAD but not with cCVRFs: blood pressure, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, diabetes, and smoking. A multilocus genetic risk score was generated as previously described.[17] The first 7 variants selected were associated with CAD in genome-wide association studies: rs17465637 in *MIA3*, rs6725887 in *WDR12*, rs9818870 in *MRAS*, rs12526453 in *PHACTR1*, rs1333049 near *CDKN2A/2B*, rs1746048 near *CXCL12* and rs9982601 near *SCL5A3*. We also included the rs10455872 variant in *LPA*[18] and the haplotype B (rs10507391, rs17216473, rs9315050, rs17222842) in *ALOX5AP*,[19] which have been associated with CAD risk independently of cCVRFs.

The DNA samples were genotyped by Spain's National Center for Oncology Research in Madrid using the Cardio inCode chip (Ferrer inCode; Barcelona, Spain), which is based on Veracode (Illumina; San Diego, United States) and KASPar (KBioscience; Hoddesdon, United Kingdom) technologies. Quality control criteria were applied both to individual samples and to selected SNPs, including Hardy-Weinberg equilibrium analyses. The overall percentage of agreement of the chip with reference technology was 99.9% and the analytical sensitivity and specificity were greater than 98.6%.