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Original article

The *PLAU* P141L Single Nucleotide Polymorphism Is Associated With Collateral Circulation in Patients With Coronary Artery Disease

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ABSTRACT

Introduction and objectives: Urokinase-type plasminogen activator, which is encoded by the *PLAU* gene, plays a prominent role during collateral arterial growth. We investigated whether the *PLAU* P141L (C > T) polymorphism, which causes a mutation in the kringle domain of the protein, is associated with coronary collateral circulation in a cohort of 676 patients with coronary artery disease.
Methods: The polymorphism was genotyped in blood samples using a TaqMan-based genotyping assay, and collateral circulation was assessed by the Rentrop method. Multivariate logistic regression models adjusted by clinically relevant variables to estimate odds ratios were used to examine associations of *PLAU* P141L allelic variants and genotypes with collateral circulation.
Results: Patients with poor collateral circulation (Rentrop 0-1; n = 547) showed a higher frequency of the TT genotype than those with good collateral circulation (Rentrop 2-3; n = 129; *P* = .020). The T allele variant was also more common in patients with poor collateral circulation (*P* = .006). The odds ratio of having poorly developed collaterals in patients bearing the T allele (adjusted for clinically relevant variables) was statistically significant under the dominant model (odds ratio = 1.83 [95% confidence interval, 1.16-2.90]; *P* = .010) and the additive model (odds ratio = 1.73 [95% confidence interval, 1.14-2.62]; *P* = .009).
Conclusions: An association was found between coronary collateral circulation and the *PLAU* P141L polymorphism. Patients with the 141L variant are at greater risk of developing poor coronary collateral circulation.

El polimorfismo de un solo nucleótido *PLAU* P141L se asocia con el grado de circulación colateral en pacientes con enfermedad arterial coronaria

Palabras clave:
Activador del plasminógeno
Polimorfismo rs2227564
Circulación colateral
Estudio de asociación

RESUMEN

Introducción y objetivos: El gen *PLAU*, que codifica para el activador del plasminógeno tipo urocinasa, desempeña un papel destacado en el crecimiento colateral. Se ha investigado si el polimorfismo *PLAU* P141L (C > T), que causa una mutación en el dominio *kringle* de la proteína, se asocia con la circulación colateral coronaria en una cohorte de 676 pacientes con enfermedad arterial coronaria.
Métodos: Se genotipificó el polimorfismo de muestras de sangre mediante prueba basada en TaqMan, y la circulación colateral se evaluó por el método Rentrop. Las asociaciones de las variantes alélicas y los genotipos con la circulación colateral se examinaron mediante modelos de regresión logística multivariable ajustados por las variables clínicamente relevantes.
Resultados: Los pacientes con circulación colateral deficiente (Rentrop 0-1; n = 547) presentaron mayor frecuencia del genotipo TT que aquellos con buena circulación colateral (Rentrop 2-3; n = 129; p = 0,020). Por otra parte, el alelo T fue más frecuente en los pacientes con circulación deficiente (p = 0,006). La *odds ratio* de los portadores del alelo T de presentar una circulación colateral deficiente (ajustada por variables clínicamente relevantes) fue estadísticamente significativa en el modelo dominante (*odds ratio* = 1,83 [intervalo de confianza del 95%, 1,16-2,90]; p = 0,010) o el aditivo (*odds ratio* = 1,73 [intervalo de confianza del 95%, 1,14-2,62]; p = 0,009).

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Conclusiones: Se demuestra una asociación entre la circulación colateral coronaria y el polimorfismo *PLAU* P141L. Los pacientes con la variante 141L tienen mayor riesgo de sufrir una circulación colateral deficiente.

Abbreviations

CAD: coronary artery disease
CCC: coronary collateral circulation
DM: diabetes mellitus
SNP: single nucleotide polymorphism
u-PA: urokinase-type plasminogen activator

INTRODUCTION

Coronary artery disease (CAD) is the leading cause of death in industrialized countries.[1–4]. Collateral coronary artery growth (coronary arteriogenesis) is a recognized alternative source of blood supply to a myocardial area affected by ischemia in CAD.[1,3] Patients with high collateralization have a 36% lower risk of death than those with low collateralization.[5] Chronic ischemia and the extent of atherosclerotic burden are important triggers in the etiology of collateral artery formation. Nonetheless, the ability to induce coronary collateral growth is widely heterogeneous in CAD patients, even in those with completely occluded arteries,[6] and it may well be a genetically determined process.[7,8]

Fulton[9] demonstrated that the human heart contains an extensive collateral network that is present even before the appearance of obstructive atherosclerotic disease (reviewed by Van Royen et al[10]). In arteriogenesis, these collateral arterioles interconnect lateral branches proximal and distal to the occluded artery (anastomosis).[10,11] Following arterial occlusion, the blood flow is redirected through the preexisting arteriole bridges, which triggers an increase in mechanical forces on the arterial wall, such as shear stress and circumferential stress, resulting from the pressure gradient formed between the high-pressure region proximal to the occlusion, and the low-pressure region located distally.[11–13] These forces induce collateral coronary artery growth. This process implies complete structural remodeling of the arterial wall, which involves proliferation of endothelial and smooth muscle cells and ultimately, reorganization of the extracellular component and the internal elastic lamina. Completion of these phases reduces artery wall shear stress to baseline physiologic values.[14]

Endothelial cells perceive shear stress and transform the signal into changes in gene expression.[11–15] The endothelium becomes activated and promotes attraction and adhesion of monocytes, which secrete growth factors and cytokines to the growing arteries.[11] In the early stages, collateral arterial growth is associated with an increase in fibroblast growth factor receptor-1 expression at the site of vessel remodeling, while monocytes supply ligands (fibroblast growth factors) via paracrine signaling to promote growth.[15] Fibroblast growth factor receptor-1 activation by fibroblast growth factors leads to activation of the Ras/Raf, MEK1/2, ERK1/2 pathway and finally, increased expression of early growth response 1 factor, which is involved in inducing an invasive, migratory phenotype in endothelial and smooth muscle cells. Early growth response 1 factor induces expression of urokinase-type plasminogen activator (u-PA).[14]

The u-PA is crucial for regulation of cell adhesion, migration, and proliferation,[16] for remodeling the artery wall affected by mechanical injury, and for arteriogenesis.[16–19] The uPA converts plasminogen into plasmin, which, in turn, activates growth factors, latent forms of cytokines, and matrix metalloproteinases 2 and 9. These are involved in a strictly localized degradation of the extracellular matrix, a critical step to enable smooth muscle cell migration and neointima formation[16,17] during collateral vessel growth.

PLAU, the gene that codes for u-PA, is located on chromosome 10q22.2, between 2 regions that have shown a link with Alzheimer disease.[20] The single nucleotide polymorphism (SNP) rs2227564, a C/T polymorphism of the second base of codon 141 of the *PLAU* gene, causes a missense mutation (from proline to leucine) in the kringle domain of u-PA, which has a functional effect on the binding of u-PA to the zymogen of fibrin.[21]. Although this SNP has been linked to several diseases,[22–29] there have been no reports of its association with coronary arteriogenesis. The aim of this study was to evaluate the association between the *PLAU* P141L polymorphism and collateral coronary artery growth, focusing on the hypothesis that the *PLAU* L141 variant is associated with a decreased arteriogenic response in patients with CAD.

METHODS

Study Design and Patient Selection

This study was performed in accordance with the Declaration of Helsinki, and the protocol was approved by the Bioethics Committee of *Centre Cardiovascular Sant Jordi* and *Hospital Universitari de la Vall d'Hebron* for the period of 2008 to 2012. All patients gave informed consent and written authorization for participation.

Patients undergoing diagnostic coronary artery catheterization were consecutively selected and enrolled in the study. The cohort comprised of 677 patients with CAD and severe ($\geq$ 70%) stenosis. Patients were excluded if they had a recent ($<$ 1 month) acute myocardial infarction, anemia, recent angioplasty, a previous percutaneous coronary revascularization procedure, coronary artery bypass surgery, infection, inflammation, or chronic renal failure. Each patient's clinical history was studied in detail and the demographic, clinical, and laboratory data were recorded. Hypertension, diabetes mellitus (DM), DM type, hypercholesterolemia, hypertriglyceridemia, smoking history, family history of heart disease, history of angina, type of angina, and acute myocardial infarction were recorded as categorical variables (presence or absence).

Coronary Angiography and Evaluation of the Coronary Collateral Circulation

Coronary angiography was performed in multiple orthogonal projections using the Judkins technique. Angiographic evaluation of coronary collateral circulation (CCC) was carried out according to the method of Rentrop et al,[30] with the following scale to grade collateral artery filling: 0, no visible filling of collaterals; 1, collateral filling of the side branches of the vessel to be dilated, with no contrast reaching the epicardial segment of the vessel; 2, partial filling of the epicardial segment by collateral vessels, and 3, complete filling of the epicardial segment by collateral vessels.