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The 9p21 Rs 1333040 polymorphism is associated with coronary microvascular obstruction in ST-segment elevation myocardial infarction treated by primary angioplasty

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Abstract

Background: Microvascular obstruction (MVO) after primary percutaneous coronary intervention (pPCI) leads to higher incidence of both early and late complications. A number of single nucleotide polymorphisms in 9p21 chromosome have been shown to affect angiogenesis in response to ischaemia. In particular, Rs1333040 with its three genotypic vriants C/C, T/C and T/T might influence the occurrence of MVO after pPCI.

Methods: We enrolled ST-elevation myocardial infarction (STEMI) patients undergoing pPCI. The Rs1333040 polymorphism was evaluated by polymerase chain reaction-restriction fragment length polymorphism using restriction endonucleases (Bsml). Two expert operators unaware of the patients' identity performed the angiographic analysis; collaterals were assessed applying Rentrop's classification. Angiographic MVO was defined as a post-pPCI Thrombolysis In Myocardial Infarction (TIMI)<3 or TIMI 3 with myocardial blush grade 0 or 1, whereas electrocardiographic MVO was defined as ST segment resolution <70% one hour after pPCI.

Results: Among our 133 STEMI patients (mean age 63 ± 11 years, men 72%), 35 (26%) and 53 (40%) respectively experienced angiographic or electrocardiographic MVO. Angiographic and electrocardiographic MVO were different among the three variants (p= 0.03 and p=0.02 respectively). In particular, T/T genotype was associated with a higher incidence of both angiographic and electrocardiographic MVO compared with C/C genotype (p=0.04 and p=0.03 respectively). Moreover, Rentrop score <2 detection rate differed among the three genotypes (p=0.03). In particular T/T genotype was associated with a higher incidence of a Rentrop score <2 as compared with C/C genotype (p= 0.02).

Conclusion: Rs1333040 polymorphism genetic variants portend different MVO incidence. In particular, T/T genotype is related to angiographic and electrocardiographic MVO and to worse collaterals towards the culprit artery.

Keywords

ST-segment elevation myocardial infarction, acute coronary syndromes, microvascular obstruction, primary percutaneous coronary intervention, 9p21 polymorphism, Rs 1333040.

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Introduction

In patients with ST-elevation myocardial infarction (STEMI) the primary goal is to reopen an occluded epicardial coronary artery as soon as possible.[1] However, microvascular obstruction (MVO) is an important complication of reperfusion therapy; it occurs in up to 50% of cases despite successful reopening of the occluded epicardial coronary artery, and is associated with a high rate of major adverse cardiac events, including high incidence of adverse left ventricular remodelling, congestive heart failure, and death during hospitalization and at follow-up.[2] The pathogenesis of MVO is complex. Several mechanisms are involved as distal embolization, reperfusion and ischaemic injury; moreover also individual congenital and acquired susceptibility factors play a relevant role.[3] While most of the attention has been focused on ischaemia-reperfusion and distal embolization, little information is available regarding genetic or acquired predisposing mechanisms of MVO.

The chromosomal locus 9p21 is a genetic marker for a variety of cardiovascular and cerebrovascular diseases. Several studies reported a significant association between 9p21 variants and coronary artery disease. In particular, Ye et al. indicated that the sequence variation on chromosome 9p21 influences atherosclerosis development and progression.[4] Furthermore, a previous study reported a correlation between the T/T Rs 1333040 allelic variant, coronary artery disease, early acute coronary syndrome onset and poor prognosis (re-infarction, cardiovascular death).[4] Finally, recent observations[5] suggest that hypoxic neovessel maturation may be impaired in carriers of 9p21 risk alleles, a potential mechanism of MVO mediated by genetic susceptibility to worse reperfusion. Thus, in this study we aim to evaluate the possible correlation between 9p21 Rs 1333040 polymorphism variants and both MVO incidence and other angiographic features, including collateral vessels' development grade, in a consecutive STEMI population treated by primary percutaneous coronary intervention (pPCI).

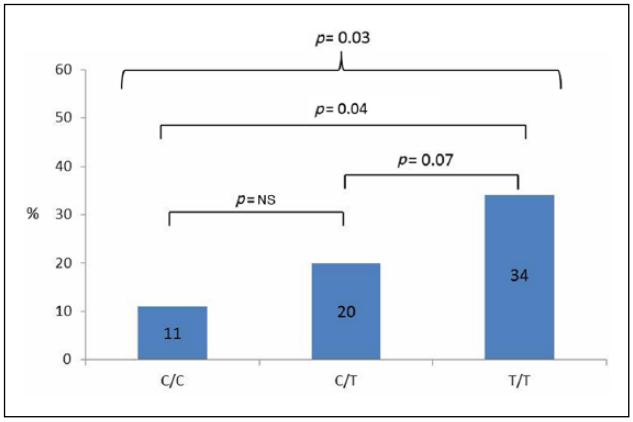

Figure 1. Incidence of angiographic microvascular obstruction according to the Rs 1333040 polymorphism allelic variants.

Methods

We enrolled STEMI patients undergoing pPCI. The Rs 1333040 polymorphism was evaluated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using restriction endonucleases (Bsml). Two expert operators unaware of the patients' identity performed the angiographic analysis. Collaterals were assessed applying Rentrop's classification. Angiographic MVO was defined as a post-pPCI Thrombolysis In Myocardial Infarction (TIMI) <3 or TIMI 3 with myocardial blush grade 0 or 1, whereas electrocardiographic (ECG) MVO was defined as ST segment resolution <70% 1 hour after pPCI. Expanded methods are provided in the Online Supplementary Materials.

Results

Clinical, angiographic, ECG and laboratory data are reported in the Online Supplementary Material tables, stratified according to the three Rs 1333040 polymorphism allelic variants. Our population consisted of 133 patients (mean age 63 ± 11 years, men 72%): 36 (27%) were diabetic, 64 (48%) dyslipidaemic, 69 (52%) hypertensive, 68 (59%) smokers and 46 (35%) with a family history of coronary artery disease (CAD). Only 32 (24%) of them were already taking acetylsalicylic acid at home on their admission, 27 (20%) beta-blockers, 23 (17%) angiotensin-converting enzyme inhibitors, 19 (14%) statins. Left ventricle ejection fraction, as evaluated through echocardiogram on admission, was 48% (40–56%); 41 (31%) patients referred a preinfarction angina. The symptom-onset-to-balloon time was 302 ± 235 min in the whole population. At the time of angiography 75 (56%) patients presented with a multivessel disease and in the half of them (51%) the left anterior descending coronary artery was involved. There was a significant difference in ST-elevation sum at admission ECG among the three study groups (p=0.01); in particular ST-elevation sum was significantly higher in patients with T/T genotype as compared with patients with C/C genotype (7.5 ± 1.5 mm *vs*. 9.0 ± 2.5 mm, p=0.02). A total of 35 (26%) and 53 (40%) patients showed angiographic or ECG evidence of MVO, respectively. A significant difference in angiographic or ECG evidence of MVO was observed among the three allelic variants (p=0.03 and p=0.02 respectively) (Figures 1 and 2). In particular, T/T genotype was associated with a higher incidence of angiographic or ECG MVO compared with the C/C genotype (p=0.04 and p=0.03 respectively). Moreover, a significant difference was observed in the prevalence of Rentrop score < 2 among the three genotypes (p=0.03) (Figure 3). In particular, the T/T genotype was associated with a higher incidence of a Rentrop score < 2 as compared with the C/C genotype (p=0.02). In contrast, no statistically significant differences among the three genotypic variants were observed with regard to demographic, clinical, therapeutic and laboratory data as well as remaining angiographic data.

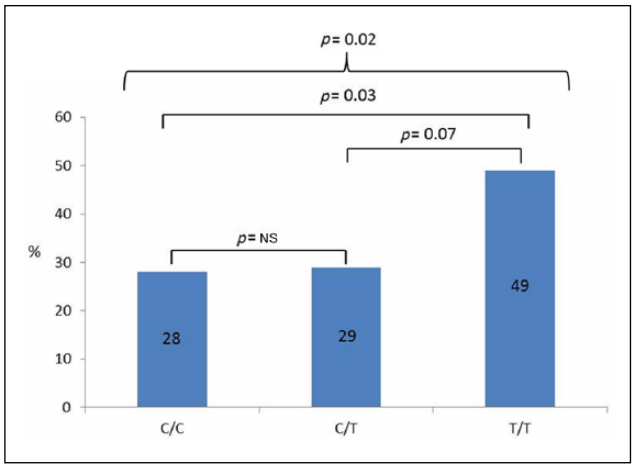

Figure 2. Incidence of electrocardiographic microvascular obstruction according to the Rs 1333040 polymorphism allelic variants.

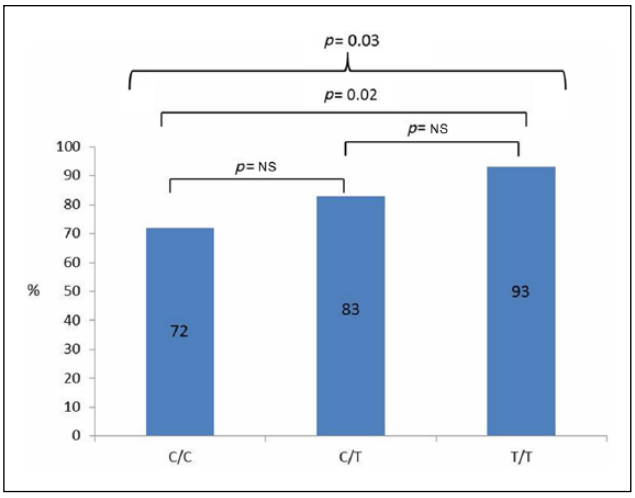

Figure 3. Low Rentrop score (< 2) according to the Rs 1333040 polymorphism allelic variants.

Discussion

This study shows for the first time that the incidence of MVO after pPCI is higher among patients with T/T Rs 1333040 allelic variant of the 9p21 locus than in those with the C/C allelic variant. Conversely, the prevalence of poor collateral development towards the culprit artery as assessed by Rentrop classification is higher in patients with the T/T allelic.

The therapeutic goal in patients presenting with STEMI is to restore the patency of the culprit epicardial coronary artery.[1] Yet, in about one-half of patients treated by pPCI MVO impairs myocardial perfusion. MVO is caused by intravascular plugging due to neutrophil-platelet aggregates and distal embolization, vasoconstriction and extramural compression.[3] Although cardiac MRI is the gold standard for detecting MVO, it is not widely available, thus angiography and ECG are still commonly used to detect MVO and they correlate well with cardiac MRI defined MVO.[6] Moreover, angiographic and ECG indexes of MVO provide independent prognostic information, probably because they interrogate the phenomenon at different time windows.[7] In this study both angiographic and ECG MVO were associated with risk allelic variants of the 9p21 Rs 1333040 locus, suggesting that genetic predisposition may affect quality of reperfusion in STEMI patients treated by pPCI.

Single nucleotide polymorphisms (SNPs) expressed in 9p21 (Rs 1333040, Rs 10757278 and Rs 10757274) have been associated with CAD,[8] stroke, heart failure[9] and vascular malformations.[10] The underlying pathophysiological mechanisms have not, however, been fully elucidated.

Regarding our observations two reasons may explain the association of T/T allelic variant with MVO. The first one is related to the same pathway linking such genotype with atherosclerosis. Indeed, 9p21 SNPs linked to high incidence of stroke and CAD are localized in the proximity of the cyclin-dependent kinase inhibitor (CDKN) gene, with the evidence that the genotypes at high risk for CAD are associated with a reduced expression of CDKN2A and CDKN2B, important regulators of the cellular cycle.[11] Since variations in 9p21 may modify the quantitative expression of CDKN2A and/or CDKN2B, SNPs may modulate the biological life of cells involved in atherosclerosis.[12] Microvascular bed affected by the atherosclerosis process is predisposed to worse reperfusion due to endothelial dysfunction.

A second potential mechanism of the association observed in our study is related to angiogenesis. Of note, CDKN2B loss has been recently associated with defective hypoxic neovessel maturation.[5] Interestingly, in our study, in addition to an association between Rs 1333040 variants and angiographic or ECG evidence of MVO, we also found that T/T genotype carriers had a higher percentage of Rentrop score < 2 (i.e. worse collaterals) compared with C/C genotype carriers and the latter had less severe ischaemia at ECG on admission. Such observations suggest that the amount of microvessels is potentially affected by allelic variants and that the presence of collaterals in coronary circulation may be a pre-existing condition. However, taking into account that the severity of the stenosis underlying an acute coronary occlusion is still an evolving matter of debate,[13] the development of epicardial and microcollaterals may be enhanced in those patients in whom the stenosis, cause of STEMI, is more severe. It cannot be excluded, indeed, that episodes of ischaemia may trigger collateral formation. Collaterals play a central role in patients with STEMI as they may increase the resistance to ischaemia. Myocardial ischaemia and necrosis are powerful stimuli for angiogenesis, a phenomenon mainly occurring in the ischaemic, collateral flow-dependent tissue driven by low oxygen partial pressure.[14] Angiogenesis is a highly regulated process requiring interactions among endothelial cells, extracellular matrix and surrounding cells, mediated

by growth factors, their receptors and intracellular signalling. The most investigated angiogenetic factors in the setting of acute coronary syndromes are vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2). In the coronary system, the predominant role is played by VEGF and Ang-2.[15] Recently, Galaup et al. showed that vascular permeability, haemorrhage, oedema, inflammation, infarct severity and MVO prevalence were increased in Ang-like-4-deficient mice,[16] suggesting that reduced levels of angiogenic factors (i.e. angiopoietin-like 4 protein) are related to poor collaterals and may thus predispose to ischaemia-related injury and MVO. Of note, admission collaterals predicts MVO. Finally, considering that certain genetic variants in the 9p21 genomic region (in which Rs 1333040 is located in linkage disequilibrium with closer genes) affect the expression of CDKN2A, CDKN2B and/or other genes located nearby,[11,17] 9p21 SNPs may be also related to endothelial progenitor cells functions, involved in angiogenesis and collateral development.

Taken together, all the mechanisms described above may explain the association of T/T allelic variant with worse reperfusion and at the same time with poor collateralization of the infarct-related artery.

Our study may have clinical implications for two reasons: 1) patients with the variant associated with higher risk of MVO may benefit from more intensive forms of prevention and treatment, whether confirmed in wider dedicated studies. Indeed, MVO has been shown[18–20] to be evolutive over time and such evolution may be favourably modulated by targeting impaired angiogenesis in predisposed patients; 2) the knowledge of mechanisms of MVO may lead to development of new drugs targeting the CDKN2B pathways.

There are limitations in this study. First, we evaluated MVO by ECG and angiography, usually more available in the clinical practice as compared with cardiovascular magnetic resonance, which is the gold standard for MVO assessment. Nevertheless, both ECG and angiography have been used in previous studies[2,3] as indexes of MVO. Of course, our results need confirmation from wider studies using the gold standard for MVO detection. Second, we used the Rentrop score to assess collateral flow, while the state of the art for its assessment is based on the utilization of pressure-flow wires. Third, the exact mechanisms linking Rs 1333040 variants to MVO has not been assessed in our study, an issue that may be addressed in future basic or translational studies. Last, our sample size is quite small for a genetic association study; however, our work, being the first to evaluate the 9p21 Rs 1333040 polymorphism in MVO predisposition, may open the way to larger studies.

In conclusion, Rs1333040 variants of the 9p21 locus predict the risk of MVO. Future studies should address underlying mechanisms with a special focus on the impact of polymorphisms on microvascular function through modulation of CDKN2B pathways.

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RP and FC equally contributed as last author.

Conflict of interest

The authors have no conflict of interest to declare.

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