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Report

Role of the CDKN1A/p21, CDKN1C/p57, and CDKN2A/p16 Genes in the Risk of Atherosclerosis and Myocardial Infarction

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ABSTRACT

Atherosclerosis is characterized by excessive proliferation of neointimal leukocytes and vascular smooth muscle cells (VSMCs). In mice, the manipulation of cell cycle inhibitors such as *CDKN1B* (p27) and *CDKN1A* (p21) modifies the risk of developing atherosclerosis. In humans, *CDKN1A*, *CDKN1B* and *CDKN1C* (p57) are differentially expressed in normal versus atherosclerotic vessels. A DNA-polymorphism within the *CDKN1B* promoter has been associated with myocardial infarction (MI). In the present study, we analyzed the effect of *CDKN1A*, *CDKN1C* and *CDKN2A* (p16) polymorphisms on MI-risk.

A total of 316 patients (all male, < 55 years) and 434 controls were genotyped, and the allele and genotype frequencies were compared between the two groups. Two *CDKN1C* polymorphisms, a promoter GT-repeat and a variable number of repeats of the amino acid PAPA-motif, were associated with MI. The presence of two alleles ≤ 11-repeats (9/11, 10/11 and 11/11 genotypes) was significantly less frequent among patients ($p < 0.001$). This difference was also significant when analyzing the subpopulation of smokers ($p = 0.004$), suggesting a protective role for these low-repeat genotypes (OR = 0.49, 95%CI = 0.32–0.73). The PAPA-BB homozygotes were significantly less frequent in patients, but this could be attributed to a linkage disequilibrium between the 11-repeats and B alleles. No significantly different frequencies between patients and controls for the four *CDKN1A* (-1026A/G, -754G/C, -369G/C and Ser31Arg) and the three *CDKN2A* (-523 G/A, +22 G/A and Ala148Thr) polymorphisms was found. In conclusion, we provide here genetic evidence for the association between DNA-variants in the *CDKN1C*/p57 gene and the risk of atherosclerosis and MI.

INTRODUCTION

Abnormal proliferation of leukocytes and vascular smooth muscle cells (VSMCs) within the artery wall is a hallmark of the atherosclerotic process.[1] This pathological response is part of a complex inflammatory reaction caused by endothelial dysfunction triggered by chemical and mechanical injury to the vessel wall. Cell proliferation depends on the sequential activation of several holoenzymes composed by a catalytic and a regulatory subunit dubbed cyclin-dependent kinase (CDK) and cyclin, respectively. In resting cells, cyclin-CDK complexes are inhibited by the reversible association with CDK inhibitory proteins (CKIs) of the Ink4 ($p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$ and $p19^{Ink4d}$) and Cip/Kip ($p21^{cip1}$, $p27^{kip1}$ and $p57^{kip2}$) families.[2]

In recent years, accumulating evidence has implicated various CKIs as important endogenous regulators of leukocyte and VSMCs proliferation in various pathophysiological situations.[3-5] The regulation of CKI expression/activity influences the response of VSMCs to mechanical stimuli (such as blood flow) and to molecules (such as nitric oxide, interleukines, angiotensin II and growth factors), and is mediated through the regulation of CKI expression/activity.[6-10] For example, reduced expression of p27 has been reported in primary atherosclerotic and restenotic tissue versus nondiseased arterial tissue.[11-13] Fat-fed mice deficient in both apolipoprotein E (apoE) and p27 display increased neointimal VSMC and macrophage proliferation and accelerated atherogenesis, compared to apoE-null mice with an intact p27 gene.[14] Remarkably, selective inactivation of p27 in hematopoietic cells, which leads to augmented neointimal macrophages proliferation, is sufficient to accelerate atherosclerosis in apoE-null mice.[15] In rabbits, adenovirus-mediated overexpression of p57 suppressed VSMCs proliferation.[16] In cultured rat thoracic aorta VSMCs, mitogen-dependent G_1-to-S transition coincided with downregulation of p57 and, conversely, was inhibited by supplemental p57 overexpression.[17] Surprisingly, p21 ablation in apoE-null mice has a protective effect against atherosclerosis.[18] In the absence

of p21, atherosclerotic lesions exhibited increased apoptosis and a reduced expression of inflammatory mediators. Moreover, transplantation of p21-null bone marrow cells into irradiated apoE-null mice reproduced most of the protective effect of whole-body p21 inactivation. These findings suggest that the absence of p21 expression in neointimal macrophages is important to the beneficial effect against atherosclerosis, possibly due to the prevention of p21-dependent induction of inflammatory responses in macrophages.[18] Compared with p27 and p21, p16 was a weak inhibitor of intimal VSMC proliferation in an in vivo model of arterial injury.[19] Moreover, p16 expression was not detected in normal or injured arteries, suggesting that this CKI plays a limited role in atherosclerosis.[11]

Both acquired/environmental (e.g., smoking, hypertension and hypercholesterolaemia) and inherited/genetic factors contribute to the development of atherosclerotic lesions and their clinical manifestation, such as coronary artery disease (CAD) and associated ischaemic events. Among others, polymorphisms in the components of the renin-angiotensin system, nitric oxide synthases, apolipoproteins, and several cytokines/chemokines and growth factors could modulate the risk of suffering CAD.[20] These polymorphic genes fulfil the main criteria to be considered candidate modifiers of CAD-risk, since they encode proteins involved in vascular physiology or in atheroma development. In view of the role of CKIs in the atherosclerotic process, we hypothesised that DNA-variation at the CKI-genes could modify the risk for CAD. Indeed, we have previously reported an increased frequency of the p27/*CDKN1B* -838 A allele among MI patients.[21] In addition, this allele was associated with significantly lower *CDKN1B* gene promoter activity in vitro, compared with the -838 C variant, suggesting that reduced p27 expression in -838 A carriers may facilitate the proliferative response associated to atherosclerosis and thus increase MI risk.[21] The present study was designed to assess whether *CDKN1A*/p21, *CDKN1C*/p57 and *CDKN2A*/p16 gene polymorphisms are associated with the risk of developing atherosclerotic disease and MI.

METHODS

Subjects. All participating subjects were from the region of Asturias (Northern Spain, total population 1 million), and gave their written informed consent to participate in the study, which was approved by the Ethical Committee of Clinical Investigation of Asturias. A total of 340 male patients < 55 years attended our Cardiology Department in the period 2000–2005, and fulfilled the WHO MONICA criteria for MI (chest pain, electrochardiographic changes and elevated enzymatic blood-levels).[22] These patients underwent coronary angiography for diagnostic purposes, and 316 (93%) had at least one atherosclerotic-diseased coronary vessel (luminal narrowing of at least 70%). All 316 patients agreed to participate in a research on the genetic factors involved in MI, and none refused to participate.[23,24] Patients were defined as hypertensives if they had a documented history of hypertension, with a systolic blood pressure >140 mmHg in more than one determination. Patients with a history of hypercholesterolaemia or showing total cholesterol concentrations >250 mg/dl were defined as hypercholesterolaemics. Patients with a history of diabetes or having a basal glucaemia > 120 mg/dl were considered diabetics. A smoking history of patients and controls was obtained through a structured questionnaire.

The control group consisted of 434 male healthy individuals < 65 years, recruited through the blood bank and the Cardiology Department at our Institution. Although these controls were apparently healthy and did not have suffered episodes of cardiovascular disease, they were not angiographically evaluated to exclude the presence of diseased coronary vessels. Table 1 shows the main clinical, biochemical and anthropometric characteristics of patients and controls.

Table 1 **Clinical, biochemical and anthropometric characteristics of patients and controls**

	Patients (n = 316)	Controls (n = 434)
Mean age ± SD (years)[1]	45 ± 7	48 ± 7
Male	316 (100%)	434 (100%)
Smokers[2]	294 (93%)	156 (36%)
Hypertensives[3]	114 (36%)	65 (15%)
Total cholesterol (mg/dl)[4]	222 ± 58	203 ± 38
Hypercholesterolaemics (total cholesterol >250 mg/dl)	57 (18%)	65 (15%)
HDL-cholesterol (mg/dl)[5]	33 ± 9	51± 12
Triglycerides (mg/dl)6	161 (95)	109 (81)

[1]$p < 0.05$, mean age in patients vs. controls; [2]$p < 0.0001$; OR = 21.68, 95%CI = 13-35, patients vs. controls; [3]sistolic blood pressure > 140 mmHg; $p < 0.0001$; OR = 2.96, 95%CI = 2.19-4.01.; [4]$p < 0.001$; [5]$p < 0.001$; [6]median (interquartile range); $p < 0.001$.

Polymerase chain reaction genotyping. Blood (10 ml) was collected from patients and controls, and the genomic DNA was extracted from the leukocytes following a salting-out method.[25] Polymerase chain reaction (PCR) was used to amplify the specific fragments containing the polymorphic sites. Table 2 summarizes the primer sequences and the conditions to genotype each polymorphism. PCR primers were derived from the UCSC genome sequences for *CDKN1A* (NM-078467), *CDKN1C* (NM-000076), and *CDKN2A* (NM-000077) (www.genome.ucsc.edu). Each PCR consisted of 100 ng of DNA, 10 pmol of each primer pair, 2 mM $MgCl_2$, 1X Taq-polymerase buffer, and 0.5 U of Taq-DNA polymerase, in a final vol of 20 µl. After an initial 3 min denaturation at 95°C, fragments were amplified through 32 cycles of 95°C-30 s, the annealing temperature for 60 s, and 72°C-60 s, followed by a final extension of 72°C-5 min. The amplified fragments were used for genotyping through digestion with a restriction enzyme (PCR-RFLP) followed by electrophoresis on agarose gels, automated capillary electrophoresis, or single strand conformation analysis (SSCA). In order to confirm the accuracy of these genotyping methods, we sequenced samples representative of each genotype through direct sequencing of PCR-fragments, using BigDye chemistry and an ABI PRISM 310 system (Applied Biosystems). In addition, each set of PCR and genotyping assays included samples from the possible genotypes as positive quality-controls.

***CDKN1C*/p57 polymorphisms.** The GT dinucleotide repeat polymorphism is at the 5' upstream (promoter) region of the *CDKN1C* gene (position -1120 from the transcription initiation site). The forward primer was 5'-end labelled with the dye FAM. Two µl of the PCR were mixed with 20 µl of formamide, denatured at 95°C, and size-fractioned through electrophoresis on an automated ABI310 system (Applied Biosystems). The TAMRA-500 size marker and the GENESCAN software were used to define the size of the alleles and the genotype of patients and controls.

Genotypes for the polymorphic PAPA repeat in exon 2 of *CDKN1C* were determined through SSCA. Two µl of the PCR-product were mixed with 20 µl of formamide and denatured, and 5 µl were

Table 2 **Primers used to amplify the *CDKN1A*, *CDKN1C* and *CDKN2A* fragments, size of the PCR-fragments and genotyping method used for each polymorphism**

Polymorphism*	Primers **	Size #	Genotyping	Allele-sizes &
CDKN1C				
-1120 (GT)n	F-CCTGGCTGGGGTGAAGCA	146 bp	Capillary electrophoresis	142 (9-repeats) to
(rs34738237)	R-CCGAGGTCATAGGGGAAGGG			150 (13-repeats)
PAPA repeat	F-GAGGCGCCGGAGCAGCT	300 bp	SSCA	SSCA
	R-CTGGTTCGCGCCCTGCTC			
CDKN1A				
-1026 A/G	F-CATTTCTTTGCTGCATGATCTGAGTT	186 bp	RFLP	A:186 bp
(rs2395655)	R-CCCTACACTCACCTGAACAGAAGG		(*Hinf I*)	G:166+20 bp
-754 G/C	F-GTTCAGTGGACCTCAATTTCCTC	272 bp	RFLP	G: 272 bp
(rs730506)	R-ACTCTGGCAGGCAAGGATTTA		(*DdeI*)	A: 152+120 bp
-369 G/C	F-GATTTGTGGCTCACTTCGTGGGG	153 bp	RFLP	G: 153 bp
(rs4135239)	R-GCTCCTGGCTGCCCAGCGT		(*Taq I*)	C: 134 +19 bp
S31R	F-CATGCGGCAGCAAGGCCTG	240 bp	SSCA	SSCA
(rs1801270)	R-CTCCTCCCAACTCATCCCGG			
CDKN2A				
-523 G/A	F-CATCTTTTCAGAGTCTGCTC	346 bp	SSCA	SSCA
(rs 3731238)	R-CCTAACTGCCAAATTGAATC			
+22 G/A	F-CCCTATGACACCAAACAC	366 bp	SSCA	SSCA
(rs3814960)	R-GGTATCTTTCCAGGCAAG			
A148T	F-CTGGACGTGCGCGATGCCTG	307 bp	RFLP	A: 126+148 bp
(rs3731249)	R-GCAGGGCGATAGGGAGACTC		(*BstUI*)	G:126+133+38 bp

For those polymorphisms genotyped through RFLP, the restriction enzyme and the size of the alleles are also indicated. *The reference number for each polymorphism was obtained from the ENSEMBL database (www. Ensembl.org). The PAPA-repeat has no reference number in this database. ** F: forward primer; R: reverse primer. # Size of the PCR fragment in base pairs (bp). & Alleles in the SSCA were identified according to the electrophoretic patters.

electrophoresed on 8% polyacrylamide gels. After silver staining, PAPA-repeat alleles were visualised as bands of 300, 288, 276, and 282 bp, corresponding to the PAPA alleles A (Normal sequence), B (12 bp deletion, APVA), C (24 bp deletion, APVA + PAPA), and D (6 bp deletion, VA), respectively.[26]

***CDKN2A*/p16 polymorphisms.** For genotyping the Ala148Thr polymorphism in exon 2 of the *CDKN2A* gene, a 307 bp fragment was PCR amplified and each reaction was digested with the restriction enzyme *Bst*UI, followed by electrophoresis on 4% agarose gels and ethidium-bromide staining. Alleles were visualised as fragments of 181 bp (148 A) or 133 + 38 bp (148 G).

Two single nucleotide polymorphisms (SNPs) in the CDKN2A promoter (-523 G/A), and the 5'-nontranslated region (+22G/A) were genotyped through SSCA. Three μl of each PCR were mixed with 8 μl of formamide and denatured at 95°C for 2 min, and 2 μl of the mixture were electrophoresed on 12% polyacrilamide gels (29:1 acrilamide:bis-acrilamide; 30 cm long gels), at 8 W for 22 h. After silver-staining, the patterns corresponding to each sample were visualized to determine the -523 and +22 genotypes in patients and controls.

***CDKN1A*/p21 polymorphisms.** We analyzed three SNPs in the 5'-promoter sequence of the *CDKN1A*/p21 gene: -1026 A/G, -754 G/C, and -369 G/C. For genotyping -1026 A/G the DNA was PCR-amplified, and digested with *Hinf*I. After electrophoresis on 4% agarose gels, alleles were visualized as bands of 186 bp (G) and 166 + 20 bp (A). For genotyping the -754 G/C, PCR-products were digested with *Dde*I, and alleles visualized as bands of 272 bp (-754 G) and 152 + 120 bp (-754 C) after electrophoresis on 3% agarose gels. The -369 G/C was genotyped through amplification followed by digestion with the restriction enzyme *Taq*I. Alleles were visualised as bands of 153 bp (G) and 134 + 19 bp (C) after electrophoresis on 4% agarose gels.

To genotype the p21 exon 2, codon 31 Ser/Arg (AGC/AGA) polymorphism, a fragment of 240 bp was PCR-amplified and patients and controls were genotyped through SSCA, as described above.

Statistical analysis. Frequencies of genotypes and alleles in patients and controls were compared through a Yate´s corrected Chi-squared test. The Chi^2 was also used to analyse the deviation from the Hardy-Weinberg equilibrium of the genotype frequencies. Odds ratio (OR) and the 95% confidence intervals (CI) were also obtained to calculate the relative risk of MI conferred by each genotype. ANOVA was used to evaluate differences in mean values for onset-age, cholesterol, and triglycerides between the genotypes. The SPSS package (v. 11.0) was used for all the statistical analysis.

RESULTS

Clinical and biochemical data. Table 1 summarizes the main characteristics of patients and controls. Early MI was strongly associated with smoking in our population ($p < 0.0001$; OR = 21.68, 95%CI = 13.5–35.1). In addition, we found a higher frequency of hypertensives among patients ($p < 0.001$). Compared to controls, patients displayed significantly higher mean total cholesterol and triglyceride values, and lower HDL-cholesterol ($p < 0.001$ for all comparisons). Mean age was higher in controls compared to patients (48 vs. 45 years; $p < 0.05$).

***CDKN1C* GT and PAPA-repeat polymorphisms.** The GT-repeat of the *CDKN1C* gene is localized at position -1120 from the transcription initiation site. We found in our population individuals carrying alleles containing between 9 and 13 repeats. Alleles with 9 and 10 repeats were rare (allele frequency < 1.5%; Table 3). We genotyped the 316 MI-patients and 434 controls for this polymorphism, and found a significantly higher frequency of the 12 and 13-repeat alleles compared to the 9, 10, and 11 alleles among the patients ($p < 0.001$). Carriers of two alleles ≤ 11-repeats (9/11, 10/11, 11/11 genotypes) were significantly less frequent among patients ($p<0.001$). When we analyzed the subpopulation of smokers, the difference between patients and controls remained significant ($p = 0.004$), suggesting a protective role for the low-repeat genotypes (OR = 0.49, 95%CI = 0.32-0.73) (Table 3). Patients with two alleles ≤ 11-repeats had a non significantly lower onset-age for MI compared to the other genotypes (44 ± 3 vs. 45 ± 6 years, respectively).

We found four alleles for the *CDKN1C* PAPA-repeat polymorphism. Table 4 shows the distribution of the PAPA-genotypes in patients and controls. Individuals homozygous for the B allele were significantly more frequent among the controls ($p = 0.04$). When we analysed the subpopulation of smokers, the difference remained significant ($p = 0.037$; OR = 0.39, 95%CI = 0.17-0.91). Thus, the PAPA-BB genotype may have a protective effect against MI. Mean onset ages for MI did not differ between PAPA-BB and the other genotypes (45 ± 4 vs. 44 ± 7 years, respectively). We also investigated the distribution of the GT-polymorphism according to the PAPA-genotypes. Among the BB-controls (n = 35), 87% were 11/11-homozygotes, while all the BB-patients (n = 13) were 11/11. Therefore, the observed protective effect of the BB genotype may be a consequence of its transmission with the 11/11 genotype.

***CDKN1A* and *CDKN2A* polymorphisms.** We did not find significantly different allele and genotype frequencies between patients and controls for the four *CDKN1A*/p21 polymorphisms analysed (Table 5). Likewise, allele and genotype frequencies of three polymorphisms in the *CDKN2A*/p16 gene did not differ between patients and controls (Table 6). This lack of association was maintained when classical cardiovascular risk factors were introduced in the statistical analysis. Onset ages for MI did not differ between the different *CDKN1A* and *CDKN2A* genotypes (data not shown).

Table 3 **Frequency of the GT-repeat genotypes and alleles in the *CDKN1C* promoter in patients and controls (%)**

(GT)n Genotypes	Patients (n = 316)	Controls (n = 434)	Patients smokers (n = 294)	Controls smokers (n = 156)
9/11*	4 (1)	9 (2)	4 (1)	6 (4)
9/12	2 (<1)	2 (<1)	2 (<1)	2 (1)
9/13	1 (<1)	0	1 (<1)	0
10/11*	1(<1)	6 (1)	0	2 (1)
10/12	2 (<1)	0 (<1)	2 (<1)	0
11/11*	101 (32)	194 (45)	95 (32)	70 (45)
11/12	112 (34)	145 (33)	108 (38)	47 (30)
11/13	51 (16)	37 (9)	44 (14)	14 (9)
12/12	23 (7)	27 (6)	23 (8)	8 (5)
12/13	16 (5)	14 (3)	13 (4)	7 (5)
13/13	3 (<1)	0	2 (<1)	0
(GT)n alleles	N = 632	N = 868	N = 588	N = 312
9	7 (1)	11 (1)	7 (1)	8 (3)
10	3 (<1)	6 (<1)	2 (<1)	2 (<1)
11	370 (59)	585 (67)	346 (59)	209 (67)
12	178 (28)	215 (25)	171 (29)	72 (23)
13	74 (12)	51 (6)	62 (11)	21 (7)

* carriers of two alleles ≤ 11-repeats (11/11+10/11+9/11); $p < 0.0001$; OR = 0.54, total patients vs. total controls; P = 0.0004; OR = 0.49, 95%CI = 0.32-0.73; smokers, patients vs. controls

Table 4 **Frequencies of the PAPA-repeat genotypes and alleles in the *CDKN1C* gene in patients and controls (%)**

PAPA Genotypes#	Patients (n = 316)	Controls (n = 434)	Patients Smokers (n = 294)	Controls Smokers (n = 156)
AA	153 (48)	208 (47)	139 (47)	66 (42)
AB	140 (44)	178 (41)	132 (45)	69 (44)
BB*	13 (4)	35 (8)	11 (4)	14 (9)
AC	5 (1)	9 (2)	5 82)	4 (3)
AD	1 (<1)	1 (<1)	1 (<1)	1 (<1)
BC	3 (1)	1 (<1)	1 (<1)	1 (<1)
CD	1 (<1)	0	1 (<1)	0
BD	0	1 (<1)	0	0
CC	0	1 (<1)	0	1 (<1)
PAPA Alleles	**N = 632**	**N = 868**	**N = 588**	**N = 312**
A	452 (72)	604 (70)	422 (72)	206 (66)
B	169 (27)	250 (29)	157 (27)	98 (31)
C	9 (1)	12 (81)	7 (1)	7 (82)
D	2 (<1)	2 (<1)	2 (<1)	1 (<1)

* p = 0.04 (BB vs. the other genotypes), total patients vs. total controls; p = 0.037 (OR = 0.39, 95%CI = 0.17-0.91), patients smokers vs. control smokers; # A: Normal (complete) sequence; B: 12 bp deletion (APVA sequence); C: 24 bp deletion (APVA + PAPA sequences); D: 6 bp deletion (VA sequence).

DISCUSSION

VSMC and macrophage proliferation within the artery wall is a major event in the development of the atherosclerotic lesion, a pathological hallmark of CAD. Several recent studies have suggested an important role for various CKIs, particularly for those of the Cip/Kip family, as regulators of arterial cell proliferation and atheroma development.[3] We had previously reported the association between a functional promoter polymorphism in the p27 gene and MI.[21] In this report, we analyzed the effect of several polymorphisms in the *CDKN1A*/p21, *CDKN1C*/p57 and *CDKN2A*/p16 genes on the risk of suffering MI in a group of patients with atherosclerotic-diseased vessels. The polymorphisms included in our study were in functionally relevant regions of these genes, such as the promoter and the coding sequence, and some of them could have a functional effect because have been previously related with the risk of developing cancer.

We found a significantly reduced frequency of carriers of two low-repeat (9, 10, and 11) alleles in the *CDKN1C* promoter among

Table 5 **Genotype frequencies for *CDKN1A*/p21 polymorphisms in patients and controls (%)**

Genotypes	Patients (n = 316)	Controls (n = 434)
-1026 AA	76 (24)	126 (29)
AG	163 (52)	221 (51)
GG	77 (24)	87 (20)
-754 GG	186 (59)	282 (65)
GC	113 (36)	139 (32)
CC	17 (5)	13 (3)
-369 GG	224 (71)	330 (76)
GC	82 (26)	100 (23)
CC	10 (3)	4 (1)
Codon 31 ser/ser	290 (92)	386 (94)
ser/arg	26 (8)	47 (6)
arg/arg	1 (<1)	1 (<1)

Table 6 **Genotype frequencies for *CDKN2A*/p16 polymorphisms in patients and controls (%)**

Genotypes	Patients (n = 316)	Controls (n = 434)
-523 GG	300 (95)	407 (94)
GA	16 (5)	26 (6)
AA	0	1 (<1)
+22 GG	54 (17)	74 (17)
GA	152 (48)	217 (50)
AA	110 (35)	143 (33)
148 ala/ala	297 (94)	394 (91)
ala/leu	19 (6)	39 (9)
leu/leu	0	1 (<1)

the patients. This association was maintained when patients and controls were matched for classical cardiovascular risk factors (smoking, hypercholesterolaemia, hypertension). Previous studies have suggested that the antiproliferative effect of p57 may protect against neointimal lesion development. Nakano et al found that p57 downregulation was involved in the progression through the cell cycle in the VSMCs from embryonic rat thoracic aorta.[17] After mitogenic stimulation of quiescent cells, the level of p57 rapidly decreased coinciding with the activation of G_1 cyclin/CDKs. Moreover, forced overexpression of p57 inhibited the activation of G_1 cyclin/CDKs, prevented the subsequent hyperphosphorylation of retinoblastoma proteins, and blocked the G_1/S transition of the cell cycle.[16] Likewise, adenovirus-mediated overexpression of p57 into cultured proliferating VSMCs suppressed DNA synthesis and caused cell cycle arrest in G_1.[16] Consistent with this findings, intraluminal delivery of p57 overexpressing adenovirus significantly suppressed neointimal lesion formation in balloon-injured rabbit carotid artery.

The *CDKN1C*-microsatellite polymorphism is located in the promoter region, involved in the regulation of gene expression. GT-dinucleotide repeats in the promoters of several genes have been associated with the risk of suffering breast cancer, schizophrenia, melanoma, and atherosclerosis, among other diseases.[27-31] Microsatellite polymorphisms in the promoter of the heme oxygenase-1 and matrix metalloproteinase-9 genes have been associated with angiographic restenosis after coronary stenting and the progression of intima-media thickening and constrictive remodeling of carotid atherosclerotic plaques, respectively.[30,31] (GT)n repeat is one of the most frequent variation in the human genome and, when located in a promoter region, may affect transcriptional activity.[32] Longer GT repeats have been linked to decreased in vitro transcription. Increased *CDKN1C* gene expression linked to the low-repeat alleles may confer protection against atherosclerosis and MI.

We found a protective effect for the PAPA-BB genotype. The PAPA-repeat is in the proline-rich domain of p57, and is not conserved between humans and mice.[33,34] This suggests a lack of functional effect of this gene variation, and could explain the lack of association with some diseases, such as breast cancer.[34] Because the B-allele is in linkage disequilibrium with the 11-repeats allele, the protective effect observed in the present study could be due to the existence of a common B-11 haplotype. We also analyzed four *CDKN1A* and three *CDKN2A* polymorphisms which covered the promoter and transcribed regions. Our results revealed no association between the risk of suffering MI and none of these polymorphisms. Although this suggests a lack of effect of these *CDKN1A* and *CDKN2A* gene variants in MI-risk, we can not exclude the possibility that other rare variations in these genes may be associated with the disease in some cases.

Our study has some limitations. First, we analyzed a limited number of patients. However, the total number of patients and controls was sufficient to reach a statistical power > 80% for all the genotype comparisons. Second, the possibility of false-positive results due to population stratification must be considered in association studies, but this is unlikely to occur in our study because we analysed individuals from a homogeneous Caucasian population. In addition, genotype frequencies for the *CDKN1A*, *CDKN1C* and *CDKN2A* polymorphisms analysed were in the Hardy-Weinberg equilibrium in both groups, suggesting that the observed frequencies are representative of cases and controls. Third, the controls in our study were not angiographically evaluated. Coronographic analysis is potentially dangerous, and was only performed in the patients for diagnostic purposes. Thus, the role of these polymorphisms in modulating the origin of atherosclerotic plaque could not be established, and our study could underestimate the risk conferred by the *CDKN1C* GT-repeat polymorphism if it was associated with the risk of developing atherosclerosis.

In conclusion, we have found a significant association between MI in patients with atherosclerotic vessels and microsatellite variants within the *CDKN1C* promoter. If confirmed in larger cohorts, genotyping of the *CDNK1C* GT-repeat could be a valuable tool to quantify the individual risk of suffering MI. Also, this polymorphisms could be analysed as a pharmacogenetic marker in patients treated with drugs directed to reduce atherosclerosis and the risk of MI.

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