# No Association of Chromosome 9p21.3 Variation With Clinical and Angiographic Outcomes After Placement of Drug-Eluting Stents

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**Objectives** After novel findings from genomewide association studies that sequence variation on chromosome 9p21.3 is a genetic factor for coronary heart disease, we investigated whether this locus influenced the clinical and angiographic outcomes after implantation of drug-eluting stents in coronary arteries.

**Background** Recently, genomewide association studies have identified a locus on chromosome 9 (approximately 100 kb in band p21.3) as the strongest genetic factor for coronary heart disease.

**Methods** We studied the rs7865618, rs1537378, rs1333040, and rs1333049 polymorphisms located on chromosome 9p21.3 in a cohort of 2,028 patients who were treated with percutaneous coronary intervention and implantation of sirolimus- or paclitaxel-eluting stents. Records of 3-year adverse clinical outcomes were obtained from all stented patients. Follow-up angiography at 6 to 8 months after stenting was performed in 1,683 patients (83%).

**Results** The polymorphisms were not significantly related with clinical outcomes at 3 years, including death ($p \geq 0.18$), myocardial infarction ($p \geq 0.19$), repeat revascularization ($p \geq 0.08$), and the composite end point of adverse events (death, myocardial infarction, repeat revascularization) ($p \geq 0.34$). No association of the polymorphisms was found with angiographic measures at follow-up, including minimal lumen diameter ($p \geq 0.51$), diameter stenosis ($p \geq 0.31$), late lumen loss ($p \geq 0.05$), and binary restenosis ($p \geq 0.31$).

**Conclusions** Specific polymorphisms in the chromosome 9p21.3 region that were shown to be associated with coronary heart disease in genomewide analyses were not related to the clinical and angiographic outcomes after the placement of drug-eluting stents in coronary arteries.   (J Am Coll Cardiol Intv 2009;2:1149–55) © 2009 by the American College of Cardiology Foundation

Drug-eluting stents (DES) have become the treatment of choice for patients with symptomatic coronary artery disease (CAD) undergoing percutaneous coronary revascularization. These stents combine the mechanical scaffolding properties of metallic stents with the site-specific delivery of an antiproliferative agent designed to inhibit vascular responses to arterial injury, thereby reducing restenosis (1–5). The 4- and 5-year data from randomized trials comparing DES and bare-metal stents have shown rates of target vessel revascularization to be reduced to one-half to two-thirds with DES, such that the need for repeat intervention was ~10% to 15% for DES cohorts at long-term follow-up (6,7).

However, restenosis is far from being completely defeated by current technology, and the question is whether genetic elements are related to unfavorable clinical outcomes after implantation of DES. Genomewide analyses of single nucleotide polymorphisms (SNPs) have recently identified a locus on chromosome 9 (~100 kb in band p21.3) as the as yet strongest genetic factor for CAD and myocardial infarction (MI) (8–11). Among the sequence variations within this region, the SNPs rs7865618, rs1537378, rs1333040, and rs1333049 were found to strongly influence disease risk (8–16). For example, the strength of the association between rs1333049 and CAD was p = $6.04 \times 10^{-10}$ (odds ratio: 1.24 per 1 risk allele [95% confidence interval: 1.20 to 1.29]), according to a result of a meta-analysis that included 12,004 cases and 28,949 controls (14). As progression of coronary atherosclerosis and critical outcomes after stenting share common mechanisms, including inflammatory processes, we asked whether the same 4 SNPs were related to adverse clinical events and angiographic restenosis after placement of DES in coronary arteries of patients with CAD.

**Abbreviations and Acronyms**

**CAD** = coronary artery disease

**DES** = drug-eluting stent(s)

**MI** = myocardial infarction

**SNP** = single nucleotide polymorphism

**TLR** = target lesion revascularization

## Methods

**Study population.** The study included 2,028 patients with ischemic symptoms or evidence of myocardial ischemia in the presence of ≥50% de novo stenosis located in native coronary vessels. Patients were treated with percutaneous coronary intervention and sirolimus- (Cypher, Cordis, Johnson & Johnson Company, Warren, New Jersey) or paclitaxel-eluting (Taxus, Boston Scientific, Natick, Massachusetts) stent implantation. An oral loading dose of 600 mg of clopidogrel was administered to all patients at least 2 h before the intervention. During the procedure, patients were given intravenous aspirin, heparin, or bivaluridin; glycoprotein IIb/IIIa inhibitor usage was at the discretion of the operators. After the intervention, all patients received 200 mg/day aspirin indefinitely, 150 mg clopidogrel for the first 3 days (or until discharge) followed by 75 mg/day for at least 6 months and other cardiac medications (including beta-blockers, angiotensin-converting enzyme inhibitors, statins) according to the judgment of the patient's physician. After stenting, patients remained in the hospital for at least 48 h. Re-hospitalization for repeat angiography was scheduled between 6 and 8 months or earlier if noninvasive evaluation or clinical presentation suggested the presence of ischemia. Clinical follow-up by office visit or direct telephone call to the patient was scheduled at 36 months. The study protocol was approved by the institutional ethics committee responsible for both participating centers, Deutsches Herzzentrum München and 1. Medizinische Klinik, Klinikum rechts der Isar, Technische Universität München, Munich, Germany. All subjects gave their written, informed consent for participation in the study. The reported investigations were in accordance with the principles of the Declaration of Helsinki.

**Data management, end points, and definitions.** Relevant data were collected and entered into a computer database by specialized personnel of the participating clinics joint data management center. Qualitative morphological lesion conditions were characterized by means of standard criteria

**Table 1.** Genotype Distributions and Minor Allele Frequencies in the Study Cohort (n = 2,028)

| SNP* | Position† | Alleles | Genotype Distribution | | | Minor Allele Frequency |
| | | | Maj | Het | Min | |
|---|---|---|---|---|---|---|
| rs7865618 | 22021005 | A>G | 768 (37.9) | 988 (48.7) | 272 (13.4) | 0.38 |
| rs1537378 | 22051614 | C>T | 832 (41.0) | 967 (47.7) | 229 (11.3) | 0.35 |
| rs1333040 | 22073404 | T>C | 736 (36.3) | 951 (46.9) | 341 (16.8) | 0.40 |
| rs1333049 | 22115503 | C>G | 526 (25.9) | 1018 (50.2) | 484 (23.9) | 0.49 |

Genotype values are n (%). *Single nucleotide polymorphism (SNP) identification number according to the National Center for Biotechnology Information SNP database; †position of SNP on chromosome 9 according to genome build 36.3.

Het = frequency of heterozygous patients; Maj = frequency of patients homozygous for the major allele; Min = frequency of patients homozygous for the minor allele.

(17). Baseline, post-procedural, and follow-up coronary angiograms were digitally recorded and assessed off-line in the quantitative angiographic core laboratory (ISAR Center) with an automated edge-detection system (CMS version 7.1; MEDIS Medical Imaging Systems, Leiden, the Netherlands) by 2 independent experienced operators. All measurements were performed on cineangiograms recorded after the intracoronary administration of nitroglycerin using the same single worst-view projection at all times. The contrast-filled nontapered catheter tip was used for calibration. Quantitative analysis was performed on both the 'in-stent' and 'in-segment' area (including the stented segment, as well as both 5-mm margins proximal and distal to the stent). The primary end point of the study was the composite of all-cause death, MI, or need for target lesion revascularization (TLR) over a period of 3 years. Diagnosis of MI required the presence of new Q waves on the electrocardiogram and/or elevation of creatinine kinase or its MB isoform $\geq 3\times$ the upper limit of normal in no fewer than 2 blood samples. TLR was defined as any revascularization procedure involving the target lesion due to luminal renarrowing in the presence of symptoms or objective signs of ischemia. Secondary end point was 'in-segment' binary angiographic restenosis, defined as diameter stenosis $\geq 50\%$ in the 'in-segment' area at follow-up angiography, regardless of clinical symptoms. Stent thrombosis was classified as definite stent thrombosis according to recently agreed Academic Research Consortium criteria (18).

**Genetic analysis.** TaqMan allelic discrimination assays (19,20) were designed and used for genotyping of rs7865618, rs1333040, and rs1333049 (pairwise $r^2 \leq 0.46$), which were defined as lead SNPs in genomewide association studies (8–10), and rs1537378, the SNP with the strongest association with CAD in a fine mapping analysis (14). The sequences of oligonucleotide primers and TaqMan probes are shown in Online Table 1. About 100 different polymerase chain reaction products obtained with each TaqMan system were sequenced to test whether 1 or more additional polymorphisms were present in the probe-binding section of the amplicons, because they may interfere with TaqMan reactions and result in wrong genotype assignments. The selected SNPs were identified as the only sequence variabilities in the probe-binding regions, which implicated that the probability of genotyping errors because of possible further sequence variations was relatively low. In addition, the genotype results obtained with sequence analyses and the corresponding TaqMan reactions were in full agreement. With each SNP, retyping of 20% of the DNA samples was done to control for correct sample handling and data acquisition. Clinicians responsible for diagnosis and treatment were not aware of the genetic data. All genetic analyses were blinded with regard to patient characteristics and study outcomes.

**Table 2.** Baseline, Lesion, and Procedural Characteristics in the Groups of Patients With and Without Adverse Clinical Events (Death, MI, TLR) Within 3 Years After Stenting (n = 2,028)

| | With Event (n = 444) | Without Event (n = 1,584) | p Value |
|---|---|---|---|
| Age, yrs | 68.5 ± 10.5 | 65.6 ± 10.4 | <0.0001 |
| Women | 99 (22.3) | 344 (21.7) | 0.79 |
| Arterial hypertension | 250 (56.3) | 929 (58.6) | 0.38 |
| Hypercholesterolemia | 304 (68.5) | 1,126 (71.1) | 0.29 |
| Current smoking | 58 (13.1) | 240 (15.2) | 0.27 |
| Diabetes mellitus | 145 (32.7) | 411 (25.9) | 0.005 |
| Angina class | | | 0.003 |
|   CCS I | 123 (27.7) | 498 (31.4) | |
|   CCS II | 120 (27.0) | 516 (32.6) | |
|   CCS III | 99 (22.3) | 305 (19.3) | |
|   CCS IV | 102 (23.0) | 265 (16.7) | |
| Previous MI | 186 (41.9) | 547 (34.5) | 0.004 |
| Previous bypass surgery | 72 (16.2) | 146 (9.2) | <0.0001 |
| Multivessel disease | 384 (86.5) | 1,280 (80.8) | 0.006 |
| Left ventricular ejection fraction, % | 52.2 ± 14.2 | 55.4 ± 12.4 | <0.0001 |
| Target coronary vessel | | | 0.37 |
|   LMCA | 25 (5.6) | 105 (6.6) | |
|   LAD | 209 (47.1) | 689 (43.5) | |
|   LCx | 116 (26.1) | 403 (25.4) | |
|   RCA | 94 (21.2) | 387 (24.4) | |
| Lesion data | | | |
|   Lesion length, mm | 13.4 ± 7.8 | 13.6 ± 7.4 | 0.59 |
|   Complex lesion | 342 (77.0) | 1,191 (75.2) | 0.43 |
|   Restenotic lesion | 137 (30.9) | 396 (25.0) | 0.01 |
|   Chronic occlusion | 41 (9.2) | 121 (7.6) | 0.27 |
| Before stenting | | | |
|   Reference diameter, mm | 2.70 ± 0.57 | 2.75 ± 0.55 | 0.06 |
|   Minimal luminal diameter, mm | 1.06 ± 0.52 | 1.08 ± 0.48 | 0.40 |
|   Diameter stenosis, % | 61.1 ± 15.9 | 60.9 ± 14.7 | 0.88 |
| Procedural data | | | |
|   Number of treated lesions | 1.24 ± 0.52 | 1.16 ± 0.42 | 0.001 |
|   Measured balloon diameter, mm | 3.09 ± 0.57 | 3.13 ± 0.55 | 0.15 |
|   Maximal balloon pressure, atm | 14.7 ± 3.0 | 14.5 ± 2.9 | 0.06 |
|   Balloon-to-vessel ratio | 1.16 ± 0.12 | 1.15 ± 0.12 | 0.14 |
|   Stented segment length per lesion, mm | 23.5 ± 9.7 | 23.1 ± 8.9 | 0.38 |
|   Number of stents per lesion | 1.12 ± 0.37 | 1.10 ± 0.31 | 0.10 |
|   Drug-eluting stent type | | | 0.04 |
|     Cypher stent | 247 (55.6) | 969 (61.2) | |
|     Taxus stent | 197 (44.4) | 615 (38.8) | |
|   Periprocedural abciximab therapy | 62 (14.0) | 223 (14.1) | 0.95 |
| Immediately after stenting* | | | |
|   Minimal lumen diameter, mm | 2.19 ± 0.62 | 2.24 ± 0.59 | 0.09 |
|   Diameter stenosis, % | 23.4 ± 12.5 | 22.8 ± 12.1 | 0.37 |

Values are mean ± SD or n (%). *Minimal lumen diameter and diameter stenosis were determined in the 'in-segment' area. Angina classes were defined according to the Canadian Cardiovascular Society (CCS) grading system. Complex lesions were defined as lesion types B2 and C according to the American College of Cardiology/American Heart Association grading system.

LMCA = left main coronary artery; LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; MI = myocardial infarction; RCA = right coronary artery; TLR = target lesion revascularization.
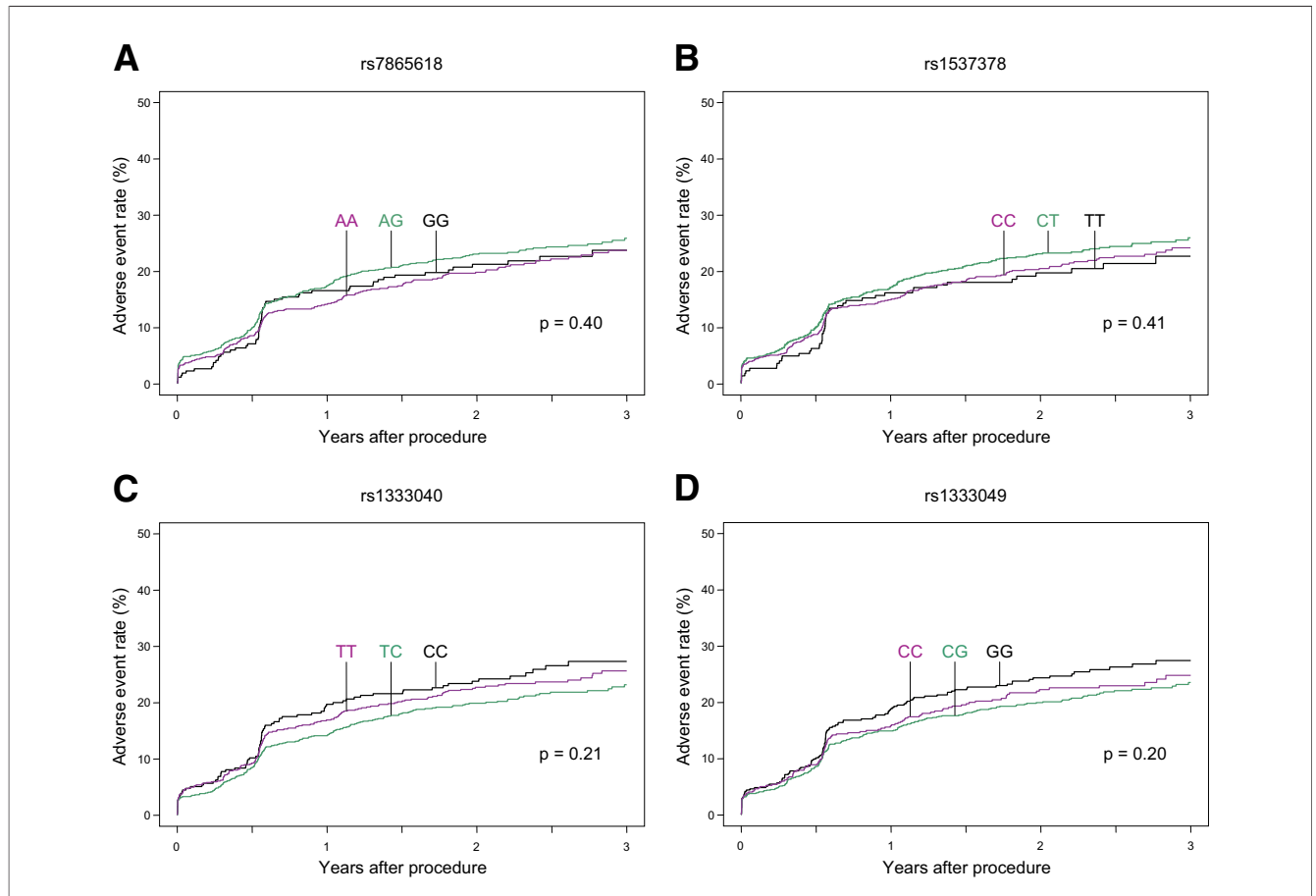
**Figure 1. Kaplan-Meier Estimates for 3-Year Adverse Event Rates**

Kaplan-Meier curves are shown for combined adverse event rates related to **(A)** rs7865618, **(B)** rs1537378, **(C)** rs1333040, and **(D)** rs1333049 during a 3-year period. Curves in **purple** are for the patients homozygous for the major alleles, curves in **green** are for the heterozygous patients, and curves in **black** are for the patients homozygous for the minor alleles of the respective single nucleotide polymorphism.

**Statistical analysis.** The analysis consisted of a comparison of baseline, lesion, procedural, and post-procedural characteristics between the group of patients who experienced the occurrence of adverse clinical events (primary study end point) and the group without events. Patient characteristics were also compared between the group of patients with binary angiographic restenosis (secondary study end point) and the group without restenosis. Discrete variables were assessed with the use of the chi-square test. Continuous variables are expressed as mean ± SD and were compared by means of the unpaired 2-sided *t* test. In the end point analyses, additive genetic models were used, and the p values are reported from trend tests based on the number of variant alleles in each SNP. A Cox proportional hazards model for the composite end point of adverse clinical events and a multivariable logistic regression model of angiographic restenosis were applied to adjust for potentially confounding effects of baseline and lesion-related characteristics. All statistical analyses were performed using S-Plus software (TIBCO Software Inc., Palo Alto, California).

## Results

**Genotype distributions and minor allele frequencies.** The genotypes related to rs7865618, rs1537378, rs1333040, and rs1333049 were determined in the patients (n = 2,028), and their distributions and the minor allele frequencies are shown in Table 1. Similar genotype distributions and minor allele frequencies were observed in the cohort with symptomatic CAD assessed here and patient groups with CAD examined in prior studies with participants of European ancestry from Germany, Iceland, the United Kingdom, and the U.S. (Online Table 2).

**Clinical outcome.** Three-year follow-up was complete for the study cohort. The composite of all-cause death, MI, or need for TLR, the primary end point of the study, was observed in 444 patients (21.9%). Baseline, lesion-related, and procedural parameters at the time of intervention in the groups with and without adverse clinical events are shown in Table 2. The incidences of stent thrombosis (n = 24; 1.2%), death (n = 163; 8.0%), MI (n = 91; 4.5%), TLR (n = 242;

11.9%), and the combined incidence of death, MI, and TLR were not associated with the SNPs in additive genetic models (Online Table 3). Kaplan-Meier curves illustrate the lack of relationship between each of the SNPs and combined adverse event rates (Fig. 1). After adjustments were made for all baseline and lesion-related characteristics shown in Table 2, no SNP was found to exert a significant independent influence on the composite end point of adverse events in a Cox proportional hazards model (p ≥ 0.07).

**Angiographic results.** Follow-up coronary angiography at 6 to 8 months was performed in 1,683 patients (83%). Angiographic restenosis (diameter stenosis ≥50% in the 'in-segment' area), the secondary end point, was observed in 227 patients (13.5%). Table 3 shows baseline, lesion-related, and procedural parameters at the time of intervention in the group of patients with restenosis and the group without restenosis. Minimal lumen diameter ('in segment'), diameter stenosis ('in segment'), late lumen loss ('in stent'), and incidence of binary restenosis at follow-up were not significantly associated with the SNPs in additive genetic models (Online Table 4). After adjusting for all baseline and lesion-related variables displayed in Table 3, no association of the SNPs with angiographic binary restenosis was observed in a multivariable logistic regression model (p ≥ 0.08).

## Discussion

Association with CAD and MI was shown for multiple SNPs across a region of approximately 100 kb on chromosome 9 (band p21.3), including rs7865618, rs1537378, rs1333040, and rs1333049 (8–16). Significantly increased disease risks were attributed to rs7865618-A, rs1537378-C, rs1333040-T, and rs1333049-C, the major alleles of these SNPs (8–16). We assessed the association of the same 4 CAD- and MI-related SNPs with clinical and angiographic outcomes after the placement of DES in coronary arteries of patients with symptomatic CAD. In essence, the results of the study provided no evidence for a relationship of the SNPs with adverse clinical events or angiographic measures of restenosis. The sample size provided the analysis with 80% power to detect increases of 20% of composite adverse events and 33% of angiographic restenosis in the carriers of the putative risk alleles (2-sided alpha-level of 0.01).

Using 4 SNPs of the chromosome 9p21.3 region that were different from those employed here, association with clinical outcomes after interventions in patients with angiographically documented CAD was examined previously (21). These SNPs, rs2383206, rs2383207, rs10757274, and rs10757278, are all in relatively tight pairwise linkage disequilibrium among each other ($0.84 \leq r^2 \leq 0.96$) (21), as compared with the much wider genetic variation covered by

**Table 3.** Baseline, Lesion, and Procedural Characteristics in Patients With and Without Angiographic Restenosis (n = 1,683)

| | With Restenosis (n = 227) | Without Restenosis (n = 1,456) | p Value |
|---|---|---|---|
| Age, yrs | 67.0 ± 10.7 | 65.5 ± 10.4 | 0.05 |
| Women | 58 (25.6) | 293 (20.1) | 0.06 |
| Arterial hypertension | 143 (63.0) | 832 (57.1) | 0.10 |
| Hypercholesterolemia | 166 (73.1) | 1,047 (71.9) | 0.70 |
| Current smoking | 31 (13.7) | 213 (14.6) | 0.70 |
| Diabetes mellitus | 62 (27.3) | 370 (25.4) | 0.54 |
| Angina class | | | 0.94 |
| CCS I | 69 (30.4) | 466 (32.0) | |
| CCS II | 72 (31.7) | 453 (31.1) | |
| CCS III | 48 (21.1) | 288 (19.8) | |
| CCS IV | 38 (16.7) | 249 (17.1) | |
| Previous myocardial infarction | 91 (40.1) | 516 (35.4) | 0.17 |
| Previous bypass surgery | 40 (17.6) | 144 (9.9) | 0.0005 |
| Multivessel disease | 193 (85.0) | 1,180 (81.0) | 0.15 |
| Left ventricular ejection fraction, % | 53.3 ± 13.7 | 55.3 ± 12.3 | 0.02 |
| Target coronary vessel | | | 0.38 |
| LMCA | 9 (4.0) | 99 (6.8) | |
| LAD | 99 (43.6) | 632 (43.4) | |
| LCx | 59 (26.0) | 381 (26.2) | |
| RCA | 60 (26.4) | 344 (23.6) | |
| Lesion data | | | |
| Lesion length, mm | 14.3 ± 8.6 | 13.2 ± 7.2 | 0.04 |
| Complex lesion | 188 (82.8) | 1,078 (74.0) | 0.004 |
| Restenotic lesion | 79 (34.8) | 380 (26.1) | 0.006 |
| Chronic occlusion | 31 (13.7) | 102 (7.0) | 0.0006 |
| Before stenting | | | |
| Reference diameter, mm | 2.61 ± 0.47 | 2.76 ± 0.57 | 0.0001 |
| Minimal lumen diameter, mm | 0.96 ± 0.47 | 1.09 ± 0.49 | 0.0003 |
| Diameter stenosis, % | 63.3 ± 16.1 | 60.7 ± 14.8 | 0.02 |
| Procedural data | | | |
| Number of treated lesions | 1.33 ± 0.64 | 1.16 ± 0.40 | <0.0001 |
| Measured balloon diameter, mm | 3.01 ± 0.49 | 3.14 ± 0.57 | 0.002 |
| Maximal balloon pressure, atm | 15.1 ± 3.20 | 14.4 ± 2.81 | 0.002 |
| Balloon-to-vessel ratio | 1.16 ± 0.11 | 1.15 ± 0.12 | 0.04 |
| Stented segment length per lesion, mm | 24.9 ± 9.8 | 22.8 ± 8.9 | 0.001 |
| Number of stents per lesion | 1.15 ± 0.39 | 1.09 ± 0.30 | 0.008 |
| Drug-eluting stent type | | | 0.03 |
| Cypher stent | 122 (53.7) | 894 (61.4) | |
| Taxus stent | 105 (46.3) | 562 (38.6) | |
| Periprocedural abciximab therapy | 25 (11.0) | 209 (14.4) | 0.18 |
| Immediately after stenting* | | | |
| Minimal lumen diameter, mm | 2.05 ± 0.58 | 2.26 ± 0.59 | <0.0001 |
| Diameter stenosis, % | 26.0 ± 13.6 | 22.5 ± 11.8 | <0.0001 |

Values are mean ± SD or n (%). *Minimal lumen diameter and diameter stenosis were determined in the 'in-segment' area. Angina classes were defined according to the CCS grading system. Complex lesions were defined as lesion types B2 and C according to the American College of Cardiology/American Heart Association grading system.
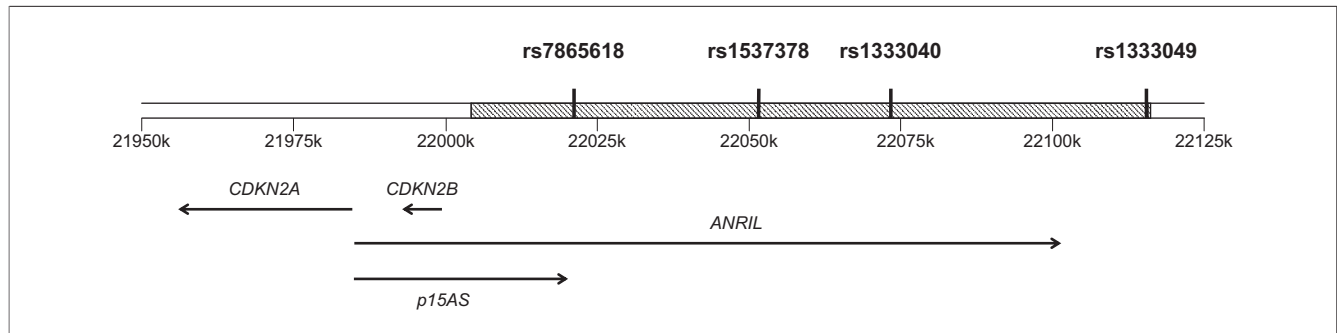Abbreviations as in Table 2.

**rs7865618    rs1537378    rs1333040    rs1333049**

21950k    21975k    22000k    22025k    22050k    22075k    22100k    22125k

*CDKN2A*    *CDKN2B*

*ANRIL*

*p15AS*

**Figure 2. Genes Close to or Overlapping With a Region on Chromosome 9p21.3 Associated With Coronary Heart Disease**

A ~100 kb region on chromosome 9p21.3 containing multiple single nucleotide polymorphisms strongly associated with coronary heart disease (8–16) is marked with **stripes**. The positions of the polymorphisms, rs7865618, rs1537378, rs1333040, and rs1333049, addressed in this report are indicated. Loci and orientations (directions of transcription) of genes near and within this sequence and potentially involved in disease mechanisms are shown as **arrows**. *CDKN2A*, gene encoding the cyclin-dependent kinase inhibitors p16$^{INK4a}$ and p14$^{ARF}$; *CDKN2B*, gene encoding the cyclin-dependent kinase inhibitor p15$^{INK4b}$; *ANRIL*, gene for antisense noncoding RNA in the *INK4* locus; *p15AS*, antisense RNA directed against the (sense) RNA for p15$^{INK4b}$.

the SNPs used in the current study ($0.21 \leq r^2 \leq 0.81$), and are also tightly linked to rs1333049 ($0.81 \leq r^2 \leq 0.97$). Corresponding to the present results, the SNPs, which showed strong associations with CAD and MI in genome-wide approaches (8–11), were not associated with incident MI or death during longitudinal follow-up in the prior study (21). The results of the present study are also in accord with recent data showing no association between rs2383206, rs2383207, rs10757278, and rs1333049 and the severity and progression of coronary atherosclerosis in patients with CAD (22).

The polymer-regulated, site-specific delivery of agents, such as sirolimus and paclitaxel, from DES were shown to inhibit tissue growth after coronary stent implantation compared with bare-metal stents (2,3). Likely due to delayed and incomplete endothelialization (23,24), the incidence of stent thrombosis, especially after the first year of implantation, is increased with these DES compared with their bare-metal counterparts (7,25). It was not possible to address a potential association of the SNPs with stent thrombosis in the present cohort, because the study was underpowered for this relatively uncommon complication.

Genotype distributions and allele frequencies in the cohort were typical of samples with CAD, as demonstrated by a comparison with data from prior studies (Online Table 2). The present findings suggest that the CAD-associated SNPs are not related with adverse processes that may ensue from stenting in coronary arteries, most prominently thrombosis culminating in MI and neointima formation leading to restenosis. Associations of SNPs on chromosome 9p21.3 with cardiovascular disease, as reported from genomewide studies and subsequent replications using identified SNPs or their neighbor SNPs (8–16), may reflect an influence on the development and progression of coronary atherosclerosis in native arteries. Higher prevalences of the risk alleles in case groups with MI than in control groups (10–12,14,16) may

be related to ongoing atherosclerosis in these patients rather than critical complications of the disease resulting in MI, such as rupture of vulnerable plaques and thrombosis. Supportive evidence for this possibility came from observations involving rs1333049 or rs2891168, a SNP in strong linkage disequilibrium with rs1333049, showing that associated risks in CAD patients with MI were not higher but rather somewhat lower than in CAD patients without MI (12,14).

The pathomechanism underlying the association of chromosome 9p21.3 SNPs with CAD is not known. Two genes, *CDKN2A*, encoding p16$^{INK4a}$ and p14$^{ARF}$, and *CDKN2B*, encoding p15$^{INK4b}$ (26,27), are located at some distance from the SNPs most closely related with CAD (Fig. 2). p16$^{INK4a}$, p14$^{ARF}$, and p15$^{INK4b}$ are critical inhibitors of pathways involved in the regulation of cell proliferation, aging, senescence, and apoptosis (28,29), which are important features of atherosclerosis. Many of the SNPs showing the strongest association with CAD map within a gene, termed *ANRIL* (Antisense Non-coding RNA in the *INK4* Locus), spanning a region of 126.3 kb and specifying noncoding RNA products of unknown function (Fig. 2) (12,30). *ANRIL* is expressed in vascular endothelial cells, monocyte-derived macrophages, coronary smooth muscle cells, and atheromatous human vessels, and evidence for a coordinated transcriptional regulation of *ANRIL*, *CDKN2A*, and *CDKN2B* has been obtained (12,30). The 5' portion of *ANRIL* gives rise to a natural antisense RNA of 34.8 kb, named *p15AS* RNA, that was shown to specifically interfere with transcription of *p15$^{INK4b}$* (sense) RNA from *CDKN2B* in tumor cells (Fig. 2) (31). It remains to be determined whether gene silencing or some other mechanism involving *CDKN2A*, *CDKN2B*, and antisense RNA can explain the well-documented association between SNPs in the chromosome 9p21.3 region and CAD (12,30–32).

## Conclusions

No support was obtained for associations of specific CAD-related SNPs on chromosome 9p21.3 with adverse clinical events and angiographic results after placement of DES in coronary arteries.

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**Key Words:** atherosclerosis ■ coronary artery disease ■ drug-eluting stents ■ restenosis ■ genetics ■ single nucleotide polymorphism.

▷ **APPENDIX**

**For supplementary materials, please see the online version of this article.**