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A single nucleotide polymorphism in the *p27^{Kip1}* gene is associated with primary patency of lower extremity vein bypass grafts

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Abstract

Objective—Factors responsible for the variability in outcomes after lower extremity vein bypass grafting (LEVBG) are poorly understood. Recent evidence has suggested that a single nucleotide polymorphism (SNP) in the promoter region of the *p27^{Kip1}* gene, a cell-cycle regulator, is associated with coronary in-stent restenosis. We hypothesized an association with vein graft patency.

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Methods—This was a retrospective genetic association study nested within a prospective cohort of 204 patients from three referral centers undergoing LEVBG for claudication or critical ischemia. The main outcome measure was primary vein graft patency.

Results—All patients were followed up for a minimum of 1 year with duplex graft surveillance (median follow-up, 893 days; interquartile range, 539-1315). Genomic DNA was isolated and SNP analysis for the *p27*^{Kip1}-838C>A variants was performed. Allele frequencies were correlated with graft outcome using survival analysis and Cox proportional hazards modeling. The *p27*^{Kip1}-838C>A allele frequencies observed were CA, 53%; CC, 30%; and AA, 17%, satisfying Hardy-Weinberg equilibrium. Race (P = .025) and history of coronary artery disease (P = .027) were different across the genotypes; all other baseline variables were similar. Primary graft patency was greater among patients with the −838AA genotype (75% AA vs 55% CA/CC at 3 years; P = .029). In a Cox proportional hazards model including age, sex, race, diabetes, critical limb ischemia, redo (vs primary) bypass, vein type, and baseline C-reactive protein level, the *p27*^{Kip1}-838AA genotype was significantly associated with higher graft patency (hazard ratio for failure, 0.4; 95% confidence interval, 0.17-0.93). Genotype was also associated with early (0-1 month) changes in graft lumen diameter by ultrasound imaging.

Conclusions—These data suggest that the *p27*^{Kip1}-838C>A SNP is associated with LEVBG patency and, together with previous reports, underscore a central role for *p27*^{Kip1} in the generic response to vascular injury.

First described more than 60 years ago,[1] autogenous vein bypass grafting remains a key therapeutic option for patients with extensive peripheral artery disease as well as coronary artery disease. In the United States Medicare population, more than 100,000 lower extremity and 200,000 coronary bypass graft procedures are performed each year for relief of ischemia.[2,3] Although vein grafts in the lower extremity are durable in many cases, the development of de novo stenosis within the graft occurs in 30% to 50% of patients within the first several years, often necessitating repeat intervention.[4-8] Despite attention to vein harvesting trauma, improved surgical techniques, modification of conventional atherosclerosis risk factors (eg, smoking cessation, lipid-lowering drugs), and antithrombotic therapies, the incidence of vein graft disease has not changed perceptibly for 3 decades. Furthermore, there is limited understanding, beyond technical factors, of the variable nature of vein graft remodeling and clinical outcomes among individual patients.[9]

The prototypic response of blood vessels to mechanical trauma, namely, the development of neointimal thickening, may become clinically manifest as lumenal renarrowing after angioplasty, stent placement, and bypass grafting. The acute injury triggers a proliferative responsive in resident vascular smooth muscle cells (VSMCs) and adventitial cells via cell-cycle activation. Normally quiescent in the uninjured vessel, VSMCs rapidly respond to local cytokine and growth factor signals and are released from growth inhibition by coordinated activity of cell cycle proteins.[10] The cyclin-dependent kinase (CDK) inhibitor p27^{Kip1} is a critical gatekeeper of the G1-S checkpoint, blocking cell-cycle entry by inhibiting CDK-cyclin interactions, specifically that between cyclin E-CDK2 and cyclin D-CDK4.[11] Numerous lines of evidence suggest that p27^{Kip1} plays an important role in the response to vascular injury and in atherosclerosis.[12-14]

Recent studies have demonstrated the potential role of genetic variability as a determinant of clinical outcomes in patients with cardiovascular disease and after clinical interventions. Of interest, a single nucleotide polymorphism (SNP) in the *p27*^{Kip1} gene (−838C>A; rs36228499) was recently identified as a potential risk factor for myocardial infarction.[15] In a retrospective association study in two Dutch cohorts of patients who had undergone percutaneous placement of bare-metal stents (BMS) in coronary arteries, this single nucleotide polymorphism (SNP) was identified as a strong predictor of in-stent restenosis.[16]

We hypothesized that genetic factors related to neointimal disease in venous bypass grafts would be similar to those in injured arteries and that variability in the *p27*^{Kip1} gene would be associated with vein graft disease. Our findings support a potentially central role for p27^{Kip1} as a global determinant of cardiovascular intervention outcomes.

METHODS

Study design and cohorts

This was a retrospective study designed to test the specific hypothesis that the *p27*^{Kip1}-838*C*>*A* SNP (rs36228499) is associated with primary patency of lower extremity vein bypass grafts (LEVBGs).

The primary cohort of 204 patients was derived from a prospective study examining the relationship between systemic inflammation and clinical outcomes after LEVBG at three Boston hospitals (Brigham and Women's Hospital, Beth Israel Deaconess Medical Center, Boston VA Medical Center). This study was sponsored by the National Heart, Lung and Blood Institute (HL 75771). The inclusion and exclusion criteria of this cohort have been described elsewhere.[17,18] Briefly, patients were eligible for enrollment if they were undergoing primary or redo lower extremity bypass surgery with autogenous vein for lifestyle-limiting claudication or critical limb ischemia. Importantly, patients were excluded if they had a recent pre-existing condition likely to influence systemic inflammation, including myocardial infarction, stroke, major illness, or major operation ≤30 days of the bypass surgery, evidence of foot infection, or current use of immunosuppressant medications. Patients were also excluded if any portion of the bypass was constructed with nonautogenous material.

All patients provided written informed consent, and the study protocol was approved by the respective Institutional Review Boards (IRBs) at the participating sites. Patients were enrolled between 2004 and 2007 and were followed up for a minimum of 1 year (median follow-up, 32 months). A preoperative blood sample was drawn on all study participants, and aliquots of anticoagulated whole blood were frozen at −80° C. Of the 225 individuals enrolled in this study, DNA samples were available for genotyping from 204, which are the subject of the analysis.

A second cohort of 51 patients examined in this study was derived from two Seattle hospitals (University of Washington Medical Center and the VA Puget Sound Health Care System). These individuals were enrolled in a series of prospective, observational pilot studies examining the associations between graft stenosis, platelet/monocyte activity, and growth patterns of cells obtained from vein grafts (Supplementary Table I).[19,20] The studies were approved by the IRBs at both institutions, and all patients gave informed consent. Patients were excluded if they were unable to give informed consent or to return for follow-up examinations. Participants in the Seattle cohort were recruited between 2004 and 2009. Patients were followed up for a minimum of 12 months. Anticoagulated whole blood was obtained at baseline and frozen at −80°C.

Clinical assessments and end point definitions

All patients in the Boston cohort were followed up by their vascular surgeons for clinical and graft-related events at 1, 3, 6, 9, and 12 months, and every 6 months thereafter until termination from the study. Study personnel recorded clinical or graft-related events during the postoperative visits, including rehospitalizations, major adverse cardiovascular events, amputations, graft revisions, or graft occlusions. Under the study protocol, patients underwent duplex ultrasound surveillance of their bypass grafts at each visit at 1, 3, 6, 9, and 12 months, and thereafter at 6-month intervals. For the Seattle cohort, the follow-up

assessment schedule included clinical and duplex ultrasound graft examinations at 6 weeks and at 3, 6, and 12 months after surgery. Primary and secondary graft patency were defined in accordance with accepted guidelines for reporting of lower extremity revascularization.[21]

Genomic DNA and SNP analysis

Genomic DNA was isolated from whole blood using a purification kit and the manufacturer's suggested protocol (Wizard; Promega Corp, Madison, Wisc). The *p27*^{Kip1}-838C>A SNP was genotyped by polymerase chain reaction using the following primers: forward: TCCAGGTCCCGGCTTCCCGGt, reverse: CCTGCTCTGGCTGGCCTCGGAG. A mismatch creating a Taq1 site when −838C is present is shown in lower case. Reactions were performed using a programmable thermocycler (MJ Research, St. Bruno, Quebec, Canada), and the reaction product was digested with Taq1 (10 hours at 65°C) and resolved on a 3.5% agarose gel with ethidium bromide staining.

Ultrasound imaging substudy

We prospectively enrolled patients from one of the study sites (Brigham and Women's Hospital in Boston) in an IRB-approved imaging substudy designed to examine remodeling patterns in vein bypass conduits using high-resolution ultrasound imaging. The methods for this substudy have been reported previously.[18] In brief, after informed consent, participants underwent serial ultrasound assessment of a defined, registered 5-cm region of their bypass graft using B-mode, M-mode, and Doppler modalities. The designation of the region of interest of the conduit (index segment) was made in the operating room and was specifically selected as a straight, valveless vein segment ≥5 cm away from the proximal anastomosis and in a superficial location. Surgical clips were placed as a reference, and the distance from the proximal anastomosis was recorded for subsequent identification. The initial set of images was acquired in the operating room after completion of the bypass graft and before wound closure. Five high-resolution M-mode cross-sectional images of the vein were recorded at each 1-cm interval along the index segment using an ATL HDI 3000 ultrasound machine (Advanced Technology Laboratories, Bothell, Wash) with a 10-MHz transducer and cardiac gating. Lumen diameter was calculated as the mean of these 25 measurements. At postoperative visits, in addition to standard Duplex graft surveillance, these patients had detailed imaging acquisition of the index segment of the conduit using the same protocol.

Statistical methods

Hardy-Weinberg equilibrium was first evaluated among the full Boston cohort and then in the subset who self-reported as white. There were no significant deviations from Hardy-Weinberg equilibrium ($P > .20$). The primary analyses were performed using the larger Boston cohort, with the Seattle cohort analyzed separately as a confirmatory population. Graft patency rates were estimated by life-table analysis. Univariate associations between genotype and graft outcomes were performed by log-rank test. A Cox proportional hazards model was used incorporating demographic (age, race, sex) variables and other variables relevant to LEVBG outcomes, including diabetes, critical limb ischemia as the indication, redo bypass, and baseline high-sensitivity C-reactive protein level, in addition to the *p27* genotype. A value of $P < .05$ was considered statistically significant for all tests.

RESULTS

Characterization of the study population by *p27* genotype

Characteristics of subjects in the Boston cohort by *p27*^{Kip1}-838C>A genotype are summarized in Table I (see Supplementary Table II, online only, for the Seattle cohort).

Mean age was 70 years, 82% were white, and 45% were women. Race ($P = .025$) and history of coronary artery disease ($P = .027$) were different across the genotypes; all other baseline variables including use of anti-platelet and statin medications were similar between the groups. There were no failures of genotyping for this SNP.

The *p27*^{Kip1}-838C>A allele frequencies observed were CA, 53%; CC, 30%; and AA, 17% in the Boston cohort and CA, 65%, CC 23%, and AA 12% in the Seattle cohort. Age, race, and sex distributions for the Seattle cohort were similar to that of the Boston cohort.

Clinical outcomes

For the Boston cohort, 78 patients lost primary patency during follow-up. By life-table analysis, the overall primary patency rate was 69% ± 3% at 1 year and 60% ± 4% at 3 years. Secondary patency was 85% ± 3% and 82% ± 3% at 1 and 3 years respectively.

Early (30-day) event rates were not different by genotype (Table II). Primary graft patency tended to be associated with *p27*^{Kip1}-838 genotype, with the patients having the AA genotype demonstrating improved patency ($P = .066$ by log-rank test; Table II; Fig 1). Because the observed pattern was consistent with a recessive model, we combined the CA and CC groups for subsequent analysis. In this analysis, AA genotype was significantly associated with primary graft patency ($P = .029$ by log-rank test; Fig 2). In the Seattle cohort, a similar trend was observed in primary patency by *p27*^{Kip1}-838C>A genotype (83% AA vs 60% CA/CC at 1 year; $P = .27$ by χ^2; Supplementary Table III, online only).

Multivariable model for primary graft patency

A Cox proportional hazards model using the Boston cohort data revealed that the *p27*^{Kip1}-838C>A genotype was significantly associated with primary graft patency (hazard ratio for AA, 0.41; 95% confidence interval, 0.18-0.97; $P = .039$), adjusting for age, race, diabetes, redo bypass, indication (critical ischemia vs claudication), and baseline high-sensitivity C-reactive protein (Table III). Analyses restricted to the individuals who self-reported as white race had very similar point estimates (hazard ratio for AA, 0.39; 95% confidence interval, 0.16-0.99; $P = .048$) including adjustment for the same set of covariates.

Vein remodeling

We analyzed data from the imaging substudy to look for associations between patterns of vein remodeling after arterialization and the *p27*^{Kip1}-838 genotype. There were 55 patients who participated in the ultrasound substudy, had imaging data available from the intraoperative and 1-month scans, and had been genotyped for the *p27* SNP of interest. We found that individuals with the homozygous CC genotype had significantly less early dilation (0-1 month) of the venous conduit ($P = .045$ by analysis of variance; Fig 3). This association was unchanged when restricted to the 45 white patients. Owing to the modest size of the substudy cohort, we were unable to define significant associations between genotypes and later remodeling changes or to conduct further multivariable analysis across genotypes.

Comparison with other cohorts

Table IV summarizes the genotype frequencies, prevalence of stenosis (artery or graft), and point estimate effect size for the Boston and Seattle vein graft cohorts compared with the coronary BMS outcomes in the Dutch cohort reported by van Tiel et al.[16] Reflective of a largely white population in the current study populations, likely enriched for European descent, the genotype frequencies seen are broadly similar across the North American and Dutch cohorts. The protective AA genotype was present in 12% to 21% of these populations. The strikingly similar estimates of a protective association between the AA

genotype and target vessel stenosis across the peripheral bypass and coronary BMS studies suggest a fundamental association between *p27*^{Kip1}-838C>A genotype and the vascular injury response in disparate vessels and circulatory beds.

DISCUSSION

To our knowledge, this report identifies the first potential genetic marker for LEVBG outcomes, a common SNP in the promoter region (position −838) of the gene for the cell-cycle inhibitor, *p27*^{Kip1}. Patients homozygous for the minor variant A allele, roughly one of six individuals in the study population, experienced a 2.5-fold reduction in subsequent vein graft failure. This association was independent of demographic and clinical risk factors even in a modestsized population, which suggests it is likely robust. Moreover, the magnitude of the effect was strikingly similar across two independent vein graft cohorts, as well as a Dutch coronary BMS population. Although these findings require further prospective validation, they suggest a potentially important global marker of genetic variability in the vascular injury response.

The cell-cycle inhibitor p27^{Kip1} is known to play a critical role in the regulation of vascular cell proliferation, with complementary evidence from both animal models of disease and human vascular lesions.[10,13,22] After arterial injury, increased p27^{Kip1} expression coincides with decreased cellular proliferation by 5-bromo-2-deoxyuridine staining.[12] Genetic studies in mice demonstrate a prominent inhibitory role of p27^{Kip1} on atherogenesis and injury-induced neointimal hyperplasia.[23] In a rabbit vein graft model, local treatment with rapamycin resulted in elevated levels of p27^{Kip1} that correlated directly with reduced proliferation and less early intimal thickening.[24]

Current understanding of the adaptive process of vein arterialization and its relationship to subsequent bypass graft disease in humans remains quite incomplete. After implantation in the arterial circulation, veins must undergo structural remodeling in response to acutely elevated shear and tensile forces, leading to some requisite wall thickening. An integrated biomechanical/biochemical approach is needed to explain the observed variability in this response, both along the course of a given conduit and among individual patients.[25] Lumen caliber in the vein graft is determined by wall thickness and remodeling. We have previously reported the time course and variability of vein graft remodeling in the lower extremity, highlighting the importance of early outward remodeling on subsequent clinical outcomes.[18,26] In our imaging substudy cohort, individuals with the homozygous *p27*^{Kip1}-838CC genotype had notably inferior remodeling during the first postoperative month. These data, although preliminary, suggest that genetic variability in *p27*^{Kip1} may influence the early adaptive dilation response in the arterialized vein. Because ultrasound imaging is limited to lumen dimensions, we are not able to discriminate changes in wall thickness or composition associated with these findings. Graft failure is a complex phenotype consisting of several factors, including remodeling, wall thickness, and thrombosis. We postulate that the apparent discrepancy between a dominant vs a recessive influence of the A allele on early remodeling vs clinical patency may reflect these different components.

Interest in blocking cell cycle activation as a means of reducing neointimal thickening in bypass grafts stems from a large body of animal and in vitro studies. Of note, the PRoject of Ex-Vivo vein graft ENgineering via Transfection (PREVENT) clinical trials tested a molecular strategy of cell-cycle inhibition via a transcription factor “decoy” (antagonist of E2F) in two large phase 3 trials (coronary and peripheral), both of which were negative.[27,28] The reasons for failure of the test agent in these studies remain unclear; however, variability in clinical outcomes across a range of patient-level factors, including race and sex, was

observed in these large multicenter studies. There are few prior reports describing genetic association studies in vein bypass outcomes,[29-31] and none involving peripheral grafting. Importantly, our study was not a broad exploratory investigation of genetic associations but rather a hypothesis-based testing of a single suspected genetic marker based on the recent coronary studies.

One of the salient findings from this study is the striking concordance in both prevalence and beneficial association of the *p27*^{Kip1}-838C>A SNP across our two geographic centers and one in Europe, and two distinct types of vascular injury (stent in the coronary artery and vein bypass in the leg). Although clearly requiring further larger-scale validation studies and expanding to larger numbers of nonwhite individuals, these findings suggest that this marker may be of unique clinical and biologic significance. The initial report from Gonzalez et al[16] linking the *p27*^{Kip1}-838C>A SNP to an increased risk of myocardial infarction is also important to consider because it appears contradictory in clinical terms. We consider the hypothesis proposed by van Tiel et al,[16] that is the differential role of VSMC proliferation in maintaining fibrous cap integrity of native atherosclerosis lesions vs promoting restenosis, as a logical explanation that requires further study.

This investigation has a number of important limitations, among which the modest size and limited diversity of the study population is paramount. Nevertheless, this limitation is counterbalanced by the considerable power of long-term imaging surveillance of the bypass grafts in these individuals, allowing for accurate assessment of the timing and progression of vein graft lesions that is not generally obtainable in coronary studies.

Second, we do not have evidence to directly link the *p27*^{Kip1}-838C>A SNP to expression of p27^{Kip1} within tissue from the patients. Therefore we cannot discriminate between association and causation in our findings. Of note, van Tiel et al[16] examined the potential functional significance of the *p27*^{Kip1}-838C>A SNP using a recombinant promoter-luciferase construct in human embryonic kidney 293 cells, demonstrating a large increase in promoter activity with the −838A construct compared with the −838C construct. In preliminary studies using these identical constructs (courtesy Carlie J.M. deVries), we have confirmed these relative findings in both human embryonic kidney 293 cells and in primary cultured adventitial fibroblasts from human saphenous vein (Supplementary Fig, online only), However, more direct evidence linking tissue expression to *p27*^{Kip1} genotype is needed to support the hypothesis for causation.

CONCLUSIONS

Failure rates of clinical interventions for the treatment of peripheral arterial disease remain high, and the prediction and prevention of such failures is a significant unmet clinical need. Our studies support the need for broader efforts to identify the role of genetic variability in treatment outcomes, which may lead to improvements in patient selection, surveillance, and postinterventional therapies to reduce the burden of restenosis. These findings highlight that such efforts should consider common phenotypic patterns between different types of interventions and vascular beds as well as other related forms of mechanical and surgical trauma. As one example, the *p27*^{Kip1}-838C>A SNP is a commonly encountered genetic variant that may help to explain some of the unpredictable failure risk associated with therapeutic cardiovascular interventions.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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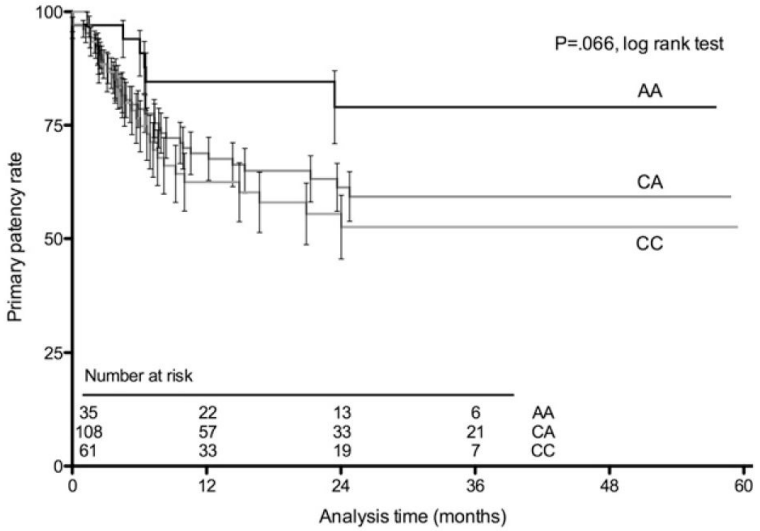

Fig 1.

Life-table plot shows primary lower extremity vein bypass graft patency by *p27*^{Kip1}-838 genotype for the 204 individuals in the Boston cohort. Data are shown with the standard error of the mean brackets.

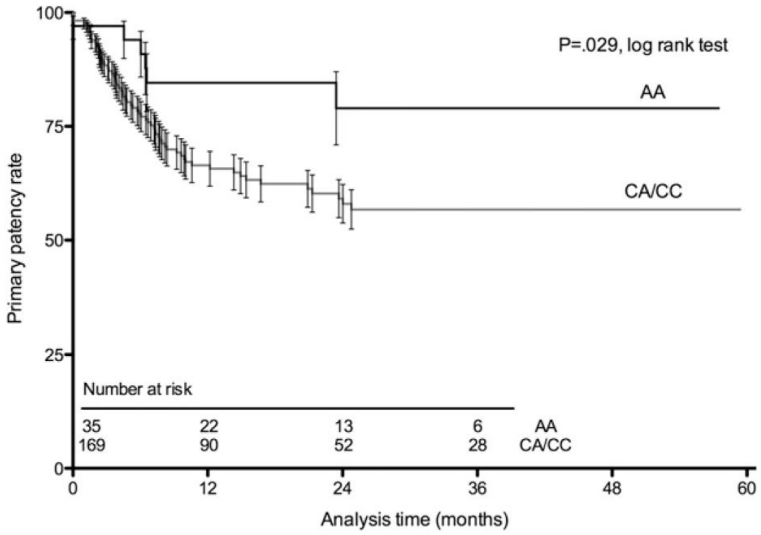

Fig 2.

Life-table plot shows primary lower extremity vein bypass graft patency by *p27^{Kip}-838* genotype, using a recessive model (AA vs CA + CC). Data are shown with the standard error of the mean.

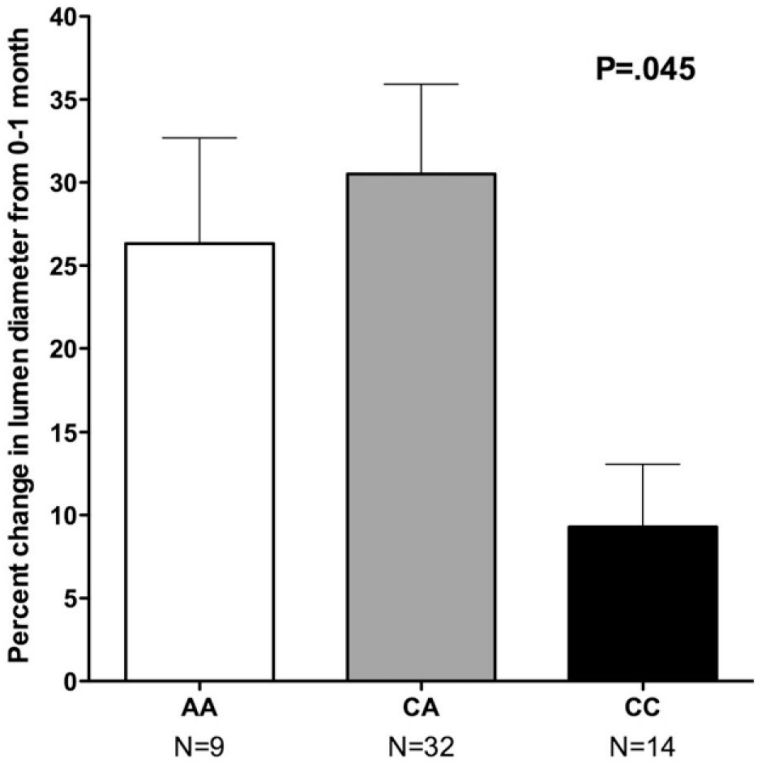

Fig 3.

Percentage change (mean ± standard deviation) is shown in vein graft lumen diameter from baseline (intraoperative after implantation) to 1 month, in a subset of 55 patients who took part in a detailed ultrasound imaging substudy,[18] by $p27^{Kip1}$-838 genotype ($P = .045$ by analysis of variance).

Table I

Characteristics of Boston lower extremity vein bypass (LEVB) graft cohort by the *p27*^{Kip1}-838 genotype

Variable [a]	−838AA	−838CA	−838CC	P[a]
Patients	35 (17.2)	108 (52.9)	61 (29.9)	
Age, years	67.3 ± 12.1	66.8 ± 67.5	67.8 ± 9.95	.891
Male sex	26 (74.3)	81 (75.0)	40 (65.6)	.402
Race				
Caucasian	31 (88.6)	99 (91.7)	47 (77.1)	.025
African	0	8 (7.4)	8 (13.1)	.069
American Hispanic	3 (8.6)	1 (0.93)	6 (9.8)	.020
hsCRP >5 mg/L	14 (40.0)	36 (33.3)	24 (39.3)	.650
CLI	20 (57.1)	62 (57.4)	37 (60.7)	.908
Diabetes mellitus	19 (54.3)	53 (49.1)	35 (57.4)	.567
CAD	14 (40.0)	56 (51.9)	41 (67.2)	.027
Current tobacco use	10 (28.6)	46 (42.6)	22 (36.1)	.305
BMI, kg/m^2	27.1 ± 5.03	29.1 ± 7.4	28.7 ± 7.36	.325
Tissue loss	9 (25.7)	33 (30.6)	21 (34.4)	.670
Redo LEVB	3 (8.6)	11 (10.2)	6 (9.8)	.962
SSGSV conduit	32 (91.4)	91 (84.3)	46 (75.4)	.114
Nonreversed SSGSV	23 (65.7)	66 (61.1)	35 (57.4)	.719
Infrapopliteal target	17 (48.6)	47(43.5)	36 (59.0)	.153
Statin use	30 (85.7)	89 (82.4)	47 (77.1)	.532
Antiplatelet Rx	29 (82.9)	86 (79.6)	49 (80.3)	.916

BMI, Body mass index; *CAD*, coronary artery disease; *CLI*, critical limb ischemia; *hsCRP*, high-sensitivity C-reactive protein; *SSGSV*, single-segment great saphenous vein.

[a] Continuous data re shown as mean ± standard deviation and categoric data as number (%).

[b] χ^2 test.

Table II

Summary of clinical outcomes in Boston cohort by *p27*^{Kip1}-838 genotype

Variable[a]	AA No. (%)	CA No. (%)	CC No. (%)	P[b]
30-day				
Graft failure	3 (8.6)	6 (5.6)	0 (0)	.921
MACE	3 (8.6)	7 (6.5)	2 (3.3)	.908
	% (SEM)	*% (SEM)*	*% (SEM)*	
Primary patency				
1 year	81.0 (7.0)	67.5 (4.7)	62.1 (6.4)	.066
3 years	76.5 (7.9)	58.2 (5.4)	51.2 (7.4)	.066
Secondary patency				
1 year	90.6 (5.1)	80.4 (4.3)	86.1 (8.6)	.163
3 years	90.6 (5.1)	76.8 (4.6)	83.8 (5.0)	.163
3-year outcome				
Limb loss	91.0 (4.9)	95.0 (2.1)	96.5 (2.4)	.721
Survival	74.9 (8.5)	74.9 (8.5)	83.5 (5.1)	.903

MACE, Major adverse cardiovascular event, including death, myocardial infarction, stroke; *SEM*, standard error of the mean.

[a] All rates >30 days are by life-table analysis, shown as % (SEM); 30-day event rates are raw proportion.

[b] Univariate *P*-values by log-rank or logistic regression analysis.

Table III

Cox proportional hazards model for primary graft patency

Variable	*HR (95% CI)*	P[a]
Age	1.01 (0.99-1.03)	.387
Non-white race	2.0 (1.12-3.60)	.019
Diabetes	1.58 (0.95-2.62)	.075
Critical limb ischemia	1.52 (0.86-2.69)	.151
Re-do bypass	1.99 (1.03-3.84)	.041
Baseline hs-CRP >5 mg/L	1.23 (0.77-2.16)	.335
p27^{Kip1}-838AA	0.41 (0.18-0.97)	.039

CI, Confidence interval; *HR*, hazard ratio; *hs-CRP*, high-sensitivity C-reactive protein.

[a]There is no corresponding footnote for “a” in Table III.

Table IV

Relative effects size of the AA genotype in three different cohorts

Cohort	No.	Overall prevalence of stenosis (%)	Frequency of −838AA genotype (%)	Point estimate of effect size
Boston	202	34.8	17.2%	.41 (HR)
Seattle	51	37.3	11.8%	.30 (OR)
Netherlands[16]	598	18	21.2%	.29 (HR)

HR, Hazard ratio, *OR*, odds ratio.