# Genetic Variants Associated with Vein Graft Stenosis after Coronary Artery Bypass Grafting

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## ABSTRACT

**Background:** Vein graft stenosis after coronary artery bypass grafting (CABG) is common. Identifying genes associated with vein graft stenosis after CABG could reveal novel mechanisms of disease and discriminate patients at risk for graft failure. We hypothesized that genome-wide association would identify these genes.

**Methods:** We performed a genome-wide association study on a subset of patients presenting for cardiac catheterization for concern of ischemic heart disease, who also underwent CABG and subsequent coronary angiography after CABG for clinical indications (n = 521). Cases were defined as individuals with ≥50% stenosis in any vein graft on any cardiac catheterization, and controls were defined as those who did not have vein graft stenosis on any subsequent cardiac catheterization. Multivariable logistic regression was used to assess the association between single nucleotide polymorphisms (SNPs) and vein graft stenosis.

**Results:** Sixty-nine percent of patients had vein graft failure after CABG. Seven SNPs were significantly associated with vein graft stenosis, including intronic SNPs in the genes *PALLD* (Rs6854137, $P = 3.77 \times 10^{-6}$), *ARID1B* (Rs184074, $P = 5.97 \times 10^{-6}$), and *TMEM123* (Rs11225247, $P = 8.25 \times 10^{-6}$); and intergenic SNPs near the genes *ABCA13* (Rs10232860, $P = 4.54 \times 10^{-6}$), RMI2 (Rs9921338, $P = 6.15 \times 10^{-6}$), *PRM2* (Rs7198849, $P = 7.27 \times 10^{-6}$), and *TNFSF4* (Rs17346536, $P = 9.33 \times 10^{-6}$).

**Conclusions:** We have identified novel genetic variants that may predispose to risk of vein graft failure after CABG, many within biologically plausible pathways. These polymorphisms merit further investigation, as they could assist in stratifying patients with multi-vessel coronary artery disease, which could lead to alterations in management and revascularization strategy.

## INTRODUCTION

Coronary artery bypass grafting (CABG) remains the

standard revascularization strategy for patients with three-vessel coronary artery disease. Despite the improved patency of arterial grafts, saphenous vein grafts are the most frequently used vascular conduits for these procedures. Unfortunately, stenosis of these grafts is common. Up to 14% of vein grafts are completely occluded 1 year after CABG [Desai 2004], and by 10 years after surgery only 50% of grafts remain patent [Grondin 1989]. This graft failure predisposes to angina, myocardial infarction, need for repeat revascularization, and premature mortality.

Although the pathophysiology of vein graft stenosis is well described, therapies to prevent vein graft failure have been generally unsuccessful [Alexander 2005]. Additionally, determining which patients are at highest risk for vein graft failure after CABG is difficult. Identifying these patients preoperatively could result in changes in patient management including use of total arterial grafting or more aggressive postoperative antiplatelet therapy.

Genome-wide association studies (GWAS) have been used to elucidate the genes involved in numerous complex disease processes, with the goal of improving the detection, treatment, and prevention of these diseases [Manolio 2010]. Identification of the genes involved in vein graft stenosis may allow clinicians to: (1) identify patients at highest risk for vein graft failure after CABG, and (2) identify novel targets for potential therapies to prevent vein graft failure. Therefore, the objective of this study was to identify genes associated with vein graft stenosis after CABG. We hypothesized that genome-wide association would identify these genes.

## MATERIALS AND METHODS

### Patients

The study population consisted of a subset of patients from the CATHGEN biorepository, which contains clinical and follow-up data as well as blood samples from >9000 patients presenting for cardiac catheterization at Duke University Medical Center (DUMC). Details of the biorepository have been previously published [Shah 2010]. Briefly, fasting blood samples are collected in EDTA tubes via an arterial sheath at the time of cardiac catheterization, prior to the administration of supplemental heparin (if given). Blood tubes are then chilled to 4°C, centrifuged within 30 minutes of collection, separated into aliquots, and frozen at -80°C. Genotypic data was available on 3255 of these patients. In this study, we included patients who had undergone CABG either before or after enrollment and

Table 1. Patient Characteristics

|  | Vein Graft Failure (n = 361) | No Vein Graft Failure (n = 160) |
|---|---|---|
| Mean age, y | 63.9 ± 10.9 | 65.6 ± 11.2 |
| Sex, male | 72% | 64% |
| Diabetes | 38% | 34% |
| Hyperlipidemia | 80% | 81% |
| Hypertension | 70% | 76% |
| Ejection Fraction | 54.3 ± 12.7% | 57.4 ± 13.3% |
| Current Smoker | 58% | 62% |
| Body Mass Index, kg/m$^2$ | 29.4 ± 5.1 | 29.5 ± 6.1 |
| Number of Vein Grafts, median | 2 | 2 |



Figure 1. Q-Q Plot.



Figure 2. Manhattan Plot

had a subsequent coronary angiography performed for clinically indicated reasons between 30 days and 2 years after CABG.

Demographics, medical history, angiographic data and longitudinal follow-up for CATHGEN are collected through the Duke Databank for Cardiovascular Disease (DDCD), which has archived this information on all patients undergoing cardiac procedures at DUMC since 1969. Follow-up includes annual determination of mortality, myocardial infarction, hospitalizations and coronary revascularization procedures. Vital status is further confirmed through the National Death Index and the Social Security Death Index.

The CATHGEN biorepository and this substudy are approved by the Duke University Institutional Review Board (IRB). Prior to collection of blood samples, all study subjects provided written informed consent to participate.

### Genotyping

Genotyping was done using the Illumina Human Omni1-Quad Bead Chip. After calling the genotypes, we applied the following set of QC filters to the autosomal SNPs: we excluded SNPs with call frequency <98%, minor allele frequency ≤1 and HWE $P$ value <10$^{-6}$. We also applied standard QC methods to the samples genotyped on the array and removed samples based on call rate (<98%), mismatch between self-reported gender and genotypic gender, and cryptic relatedness between samples. 905,781 [89%] single nucleotide polymorphisms [SNPs] passed quality-control measures.

### Statistics

Cases were defined as individuals with ≥50% stenosis in any vein graft on cardiac catheterization after CABG (n = 361), and controls were defined as those who did not have vein graft stenosis on subsequent coronary angiography (n = 160). Given the small number of non-white patients in our cohort, this study included white patients only. A GWAS was performed using a multivariable logistic regression (additive model) to assess the association between SNPs which had minor allele frequencies (MAF) >0.01 and vein graft stenosis
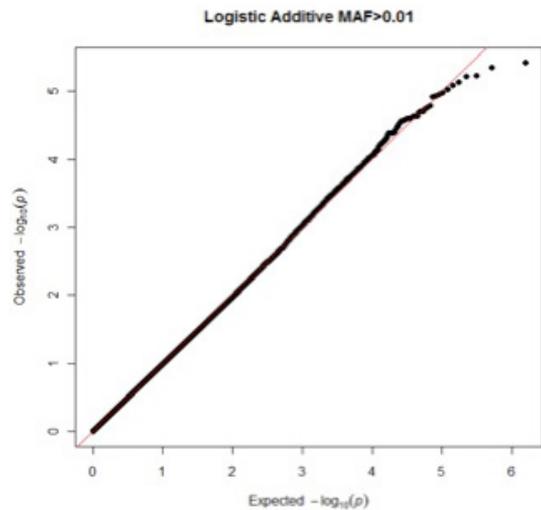
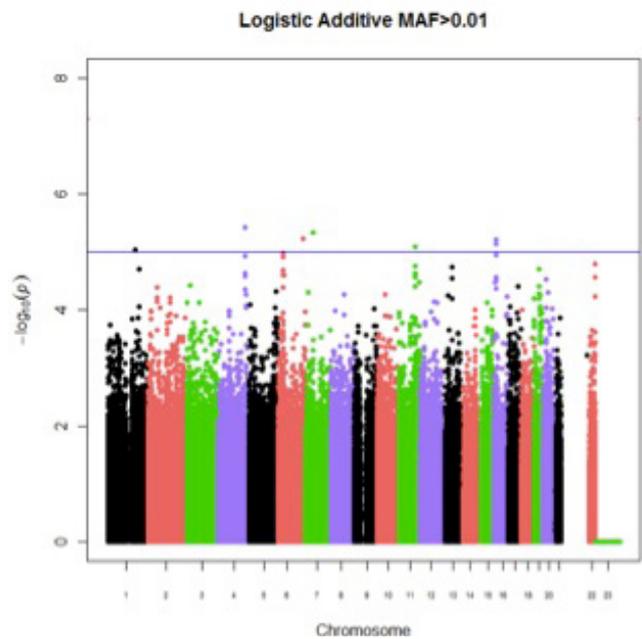(≥50% stenosis). This model was adjusted for the clinical variables sex, diabetes, hypertension, hyperlipidemia, and smoking. Statistics were performed by D.M.C., C.H., and S.H.S. using SAS Version 9.1 (Cary, NC). All authors had access to the data used for this study and take responsibility for the published results.

### RESULTS

There were 521 patients who underwent coronary angiography after CABG. Patient characteristics are presented in

Table 2. Genetic Variants Associated with Vein Graft Stenosis

| SNP | MAF (Allele) | Gene | Name | Location | Function | P* |
|---|---|---|---|---|---|---|
| Rs6854137 | 0.44 (A) | PALLD | Palladin, cytoskeletal associated protein | Intronic | A cytoskeletal protein involved in the control of cell shape, adhesion, and contraction. Associated with a risk for myocardial infarction | $3.77 \times 10^{-6}$ |
| Rs10232860 | 0.17 (G) | ABCA13 (downstream) | ATP-binding cassette, sub-family A (ABC1), member 13 | Intergenic | Transmembrane transporter | $4.54 \times 10^{-6}$ |
| Rs184074 | 0.45 (A) | ARID1B | AT rich interactive domain 1B (SWI1-like) | Intronic | Involved in transcriptional activation and repression of select genes by chromatin remodeling | $5.97 \times 10^{-6}$ |
| Rs9921338 | 0.27 (A) | RMI2 (upstream) | RecQ mediated genome instability 2 | Intergenic | A component of the BLM complex, which plays a role in homologous recombination-dependent DNA repair and is essential for genome stability | $6.15 \times 10^{-6}$ |
| Rs7198849 | 0.27 (A) | PRM2 (upstream) | Protamine 2 | Intergenic | The major DNA-binding proteins in the nucleus of sperm, and package the DNA in a volume less than 5% of a somatic cell nucleus | $7.27 \times 10^{-6}$ |
| Rs11225247 | 0.08 (C) | TMEM123 | Transmembrane protein 123 | Intronic | A transmembrane protein that functions as a cell surface receptor that mediates cell death | $8.25 \times 10^{-6}$ |
| Rs17346536 | 0.42 (G) | TNFSF4 (downstream) | TNFSF4 tumor necrosis factor (ligand) superfamily, member 4 | Intergenic | Encodes a cytokine involved in T cell-antigen-presenting cell interactions. Directly mediates adhesion of activated T cells to vascular endothelial cells | $9.33 \times 10^{-6}$ |

SNP indicates single nucleotide polymorphism; MAF, minor allele frequency.

Table 1. Median follow-up for controls was 4.9 (25th, 75th percentile: 1.7, 9.0) years and 5.8 (1.7, 10.4) years for cases. All SNPs were in Hardy-Weinberg equilibrium. A Q-Q plot and Manhattan plot demonstrating results of the GWAS are presented in Figures 1 and 2, respectively.

Sixty-nine percent of patients had vein graft failure at a median of 371 (215,474) days after CABG. Seven SNPs were associated with vein graft failure ($P < 1 \times 10^{-5}$) (Table 2) using an additive model: (1) Rs6854137 (allele A) located in *PALLD* (Palladin, cytoskeletal associated protein), a cytoskeletal protein involved in the control of cell shape, adhesion, and contraction ($P = 3.77 \times 10$-6); (2) Rs10232860 (allele G) located downstream from ABCA13 (ATP-binding cassette, sub-family A [ABC1], member 13), a transmembrane transporter ($P = 4.54 \times 10^{-6}$); (3) Rs184074 (allele A) located in the gene *ARID1B* (AT rich interactive domain 1B [SWI1-like]), which is involved in transcriptional activation and repression of select genes by chromatin remodeling ($P = 5.97 \times 10^{-6}$); (4) Rs9921338 (allele A) located upstream from *RMI2* (RecQ mediated genome instability 2), which is a component of the BLM complex that plays a role in homologous recombination-dependent DNA repair and DNA stability ($P = 6.15 \times 10^{-6}$); (5) Rs7198849 (allele A) located upstream from *PRM2* (protamine 2) a gene which produces major DNA binding proteins involved in DNA packaging ($P = 7.27 \times 10^{-6}$); (6) Rs1125247 (allele C) located in the gene *TMEM123* (transmembrane protein 123), which encodes a transmembrane protein that functions as a cell surface receptor that mediates cell death ($P = 8.25 \times 10^{-6}$); and (7) Rs17346536 (allele G) located downstream of *TNFSF4* (TNFSF4 tumor necrosis factor [ligand] superfamily, member 4), which encodes a cytokine involved in T-cell-antigen presenting cell interactions and directly mediates adhesion of activated T cells to vascular endothelial cells ($P = 9.33 \times 10^{-6}$).

## DISCUSSION

Despite superior patency of the left internal mammary artery, vein grafts remain the most commonly utilized conduits during CABG for a variety of reasons, including mixed results with other arterial conduits [Khot 2004; Goldman 2011]. Unfortunately, vein graft failure remains a major cause of morbidity and mortality after CABG. In this exploratory analysis using genome-wide association, we have identified novel genetic variants that may predispose to risk of vein graft failure after CABG, many within biologically plausible

pathways. These polymorphisms merit further investigation, as they could assist in stratifying patients with multi-vessel coronary artery disease, which could lead to alterations in management and revascularization strategy. Additionally, further exploration of the molecular pathways resulting from genetic variants we identified may elucidate novel mechanisms of vein graft failure.

Specific genetic variants have been shown to be independently associated with overall mortality after CABG, even after adjusting for clinical variables known to be associated with outcome [Muehlschlegel 2010; Lobato 2011]. However, the mechanisms and biological pathways that result in this increased mortality are not well understood. Vein graft failure is strongly predictive of mortality after CABG [Lytle 1992]. Thus, vein graft failure may be a more specific phenotype on which to base genetic association studies both for mechanistic and clinical management purposes.

A genetic component to the pathophysiology of vein graft stenosis has been shown. Using a candidate gene study, Ellis and colleagues identified five genes associated with saphenous vein graft patency [Ellis 2007]. The genes identified through our unbiased, genome-wide approach did not overlap with these results. However, the top genes in both studies identified genes involved in endothelial cell function.

The top SNP in our GWAS was Rs6854137, located in the gene *PALLD*. This gene encodes Palladin, a cytoskeletal protein involved in the control of cell shape, adhesion, and contraction. It is involved in organizing the actin cytoskeleton and is a component of actin-containing myofilaments [The NCBI Handbook 2002]. It has been associated with a risk for myocardial infarction [Shiffman 2005] and coronary heart disease [Bare 2007]. Palladin is believed to be involved in the early development of atherosclerotic lesions and neointima formation following vascular injury [Jin 2009]. Thus, *PALLD* represents a biologically plausible gene involved in vein graft failure after CABG.

Rs17346536 is located 100kb downstream from *TNFSF4* (tumor necrosis factor [ligand] superfamily, member 4). The protein encoded by this gene is a cytokine member of the tumor necrosis factor (TNF) ligand family [The NCBI Handbook 2002]. It is a ligand for receptor TNFRSF4/OX4 and is involved in T-cell antigen-presenting cell (APC) interactions. This protein and its receptor have been shown to directly mediate adhesion of activated T-cells to vascular endothelial cells [Imura 1996]. This gene represents another biologically plausible mediator of vein graft stenosis after CABG. Polymorphisms in this gene are associated with increased risk of myocardial infarction [Ria 2011] and increased risk of venous thromboembolism [Mälarstig 2008].

Rs11225247 is located in *TMEM123*, which encodes transmembrane protein 123. This protein, similar to the class of proteins termed mucins, is highly glycosylated with a high content of threonine and serine residues in its extracellular domain. It is proposed to function as a cell surface receptor that mediates the oncosis type of cell death [Ma 2001; Zhang 1998]. Its potential link to vein graft stenosis in unclear.

Rs7198849 is located 80kbp upstream from *PRM2* (protamine 2). Protamines are the major DNA-binding proteins in the nucleus of sperm, and package the DNA in a volume less than 5% of a somatic cell nucleus [Cho 2001]. Rs10232860 is located 200kbp downstream from *ABCA13* (ATP-binding cassette, sub-family A [ABC1], member 13). This membrane transporter has 12 or more transmembrane alpha-helix domains that likely arrange to form a single central chamber with multiple substrate binding sites [The NCBI Handbook 2002]. It is believed to carry out energy-dependent transport of substrate molecules [OMIM 2014]. The association of both of these genes with vein graft stenosis or cardiovascular disease has not been demonstrated.

Rs9921338 is located 20kbp upstream from *RMI2* (RecQ mediated genome instability 2), a component of the BLM complex. It plays a role in homologous recombination-dependent DNA repair and is essential for genome stability [Xu 2008]. Rs184074 is located in *ARID1B* (AT rich interactive domain 1B [SWI1-like]), which is involved in transcriptional activation and repression of select genes by chromatin remodeling. It plays a role in cell cycle control [Blais 2007]. A link between these genes and cardiovascular disease or vein graft function has not yet been demonstrated.

Limitations of this study include its retrospective nature. Additionally, we excluded patients who did not receive post-CABG coronary angiographies. Thus, there is likely a subset of patients without vein graft disease and without symptoms who did not undergo coronary angiography and were not included as non-events in this study. However, we believe our approach is the more conservative and appropriate approach, as including these asymptomatic patients may place some subjects with >50% asymptomatic vein graft disease into the non-event group. Lastly, further confirmatory studies in other large population(s) are needed for further validation of this exploratory analysis.

In conclusion, we have identified a novel set of genetic variants that are associated with vein graft failure after CABG. These polymorphisms merit further investigation, as they may potentially assist clinicians in identifying patients at high-risk for vein graft failure and in elucidating novel mechanisms of disease.

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