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Genome-wide association study of acute kidney injury after coronary bypass graft surgery identifies susceptibility loci

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Abstract

Acute kidney injury (AKI) is a common, serious complication of cardiac surgery. Since prior studies have supported a genetic basis for postoperative AKI, we conducted a genome-wide association study (GWAS) for AKI following coronary bypass graft (CABG) surgery. The discovery dataset consisted of 873 non-emergent CABG surgery patients with cardiopulmonary bypass (PEGASUS), while a replication dataset had 380 cardiac surgical patients (CATHGEN). Single nucleotide polymorphism (SNP) data were based on Illumina Human610-Quad (PEGASUS) and OMNI1-Quad (CATHGEN) BeadChips. We used linear regression with adjustment for a clinical AKI risk score to test SNP associations with the postoperative peak rise relative to preoperative serum creatinine concentration as a quantitative AKI trait. Nine SNPs meeting significance in the discovery set were detected. The *rs13317787* in *GRM7|LMCD1-AS1* intergenic region (3p21.6) and *rs10262995* in *BBS9* (7p14.3) were replicated with significance in the CATHGEN data set and exhibited significantly strong overall association following meta-analysis. Additional fine-mapping using imputed SNPs across these two regions and meta-analysis found genome wide significance at the *GRM7|LMCD1-AS1* locus and a significantly strong association at *BBS9*. Thus, through an unbiased GWAS approach, we found two new loci associated with post-CABG AKI providing new insights into the pathogenesis of perioperative AKI.

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Disclosure

The authors declare that they have no competing interest.

Keywords

acute kidney injury; coronary artery bypass graft surgery; GWAS; BBS9

Introduction

Acute kidney injury (AKI), as reflected by systemic accumulation of nitrogenous waste products due to impaired plasma filtration (e.g., creatinine and blood urea nitrogen), occurs in a variety of clinical scenarios where it is consistently associated with poor outcome.[1] The postoperative period represents an ideal setting for epidemiological investigation of AKI, since up to 47% of all in-hospital episodes follow surgery.[2] Particularly, cardiac surgery is the most common etiology of postoperative AKI,[3] with an incidence ranging between 5 and 30% following coronary artery bypass graft (CABG) surgery.[4–6] Escalating degrees of AKI are closely associated with more complicated postoperative courses, including increased in-hospital mortality rates, and (for survivors) needs for intensive and post-discharge supportive care, hospital readmissions, and poorer subsequent quality of life and long-term survival.[7–10]

Numerous AKI risk factors have been identified in cardiac surgery cohorts, including advanced age, obesity, chronic kidney disease (CKD), diabetes, poor ventricular function, hypertension, embolic and inflammatory processes, and specific surgery-related interventions (e.g., intra-aortic balloon counter pulsation or the use of cardiopulmonary bypass).[11–17] Nonetheless, current risk models poorly explain observed variability in AKI occurrence.[18]

Beyond traditional clinical risk factors, a genetic predisposition for postoperative AKI has been suggested by previous candidate gene studies.[19] To date, association studies of post-CABG AKI have mostly focused on selected candidate genes that modulate inflammatory and vasomotor responses to injury, including functional alleles influencing cytokine production that can cause renal tubular and microvascular damage,[20–27] but are limited by marked heterogeneity of AKI phenotype definitions, lack of power, and poor reproducibility. Family and linkage studies, although impractical as tools for the study of perioperative AKI, demonstrate impaired glomerular filtration (GFR) to be a heritable trait,[28, 29] supporting the heritability of renal dysfunction in general. However, a heritability index has not been specifically assessed for AKI. Similarly, although several genome-wide association studies (GWAS) have identified susceptibility loci for indices of renal function (estimated GFR) and CKD,[30–32] comparable studies are lacking for AKI in general and following cardiac surgery in particular. We therefore conducted a GWAS among participants from Perioperative Genetics and Safety Outcome Study (PEGASUS), followed by independent replication using data from CATHeterization GENetics (CATHGEN) study at Duke Heart Center to identify common genetic variants that show association with risk of developing AKI following cardiac surgery with cardiopulmonary bypass (CPB).

Results

Descriptive statistics for demographic and clinical variables and comparisons between the two datasets are presented in Table 1. The discovery (PEGASUS) and replication (CATHGEN) datasets consisted of 873 and 380 subjects of self-reported European ancestry, respectively; CATHGEN had more females than PEGASUS (38.4% vs. 23.6%, p<0.001). Postoperative AKI was common and occurred at similar rates in both cohorts, as reflected by the relative increase in serum creatinine concentration from baseline (preoperative) to peak values within the first ten days after surgery expressed as a percentage rise (%ΔCr),[14] which averaged 22.5 (standard deviation, SD=35.9) for PEGASUS and 23.6 (SD=37.0) for CATHGEN, respectively (p=0.6). This is further supported by similar AKI case rates as defined by AKIN, RIFLE, and KDIGO criteria. Finally, severe AKI (KIDGO stage 3) complicated the postoperative courses of 16 (1.2%) patients in the PEGASUS and 6 (1.6%) in the CATHGEN cohort.[33] Although the prevalence of baseline CKD was marginally higher in the PEGASUS cohort, serum creatinine concentrations and estimated glomerular filtration rates (eGFR) were similar between groups both at baseline (preoperative) and postoperatively. Notably, the types of cardiac surgical procedures were different between the two datasets (p<0.001), with all patients in the discovery cohort undergoing isolated CABG, whereas 29% of patients in the replication cohort had concomitant valve surgery. Additional differences in comorbidities between the two cohorts included higher prevalence of congestive heart failure, hypertension, and hypercholesterolemia in CATHGEN. Consequently, the average clinical AKI risk score was significantly higher in the replication than discovery cohort (32.1±6.8 vs. 26.3±12.6, p< 0.001, Table 1).

Association results

The genome wide association results from the discovery cohort are depicted as Manhattan and quantile-quantile (QQ) plots, which showed good adherence to null expectations (Figure S1-A and -B). Nine single nucleotide polymorphisms (SNPs), located in seven loci, showed promising association with %ΔCr ($p<10^{-5}$) from the GWAS and were brought forward for replication (Table 2). Two of these SNPs showed nominal significant associations with %ΔCr in the replication cohort - rs13317787 (p=0.02) at 3p21.6 (intergenic region between *GRM7|LMCD1-AS1*), and rs10262995 (p=0.03) at 7p14.3 (located in *BBS9*), with allelic effects on %ΔCr in the same direction as observed in the discovery cohort. The overall association results derived from meta-analysis of both datasets revealed strong association with AKI for both rs13317787 (meta-$p=5.35\times10^{-7}$) and rs10262995 (meta-$p= 2.24\times10^{-7}$), close to commonly accepted genome wide significance levels ($p< 5\times10^{-8}$). The heterogeneity I^2 between the two datasets at these two SNPs was not significant (Table 2: $I^2=0$).

To provide a high-resolution overview of the association signal across the 3p31.6 and 7p14.3 loci, we performed *in silico* fine-mapping by imputing the untyped SNPs on chr3: 6,907,193–8,537,944 (for the *GRM7* to *LMCD1-AS1* region), and chr7: 33,173,404–33,639,870 (for the *BBS9* region), respectively. Among 2029 genotyped and imputed SNPs at 3p31.6, 44 including the initially identified rs13317787 (spanning from chr3: 8,099,146–8,161,987) met discovery criteria ($p<10^{-5}$), and 17 of these reached genome-wide

significance in meta-analysis (meta-p < 5×10^{-8}). The most significant SNP (meta-p=2.49×10^{-11}) is an un-named SNP located at chr3:8,119,772 (SNP 3-8119772; Figure 1A; Table S1), which is also in strong linkage disequilibrium (LD) (r^2=0.97) with rs1488349 (chr3: 8,153,260), the second most significant SNP in meta-analysis (meta-p=5.41×10^{-10}) (Table S1). Since minor allele frequencies for 3-8119772 and rs1488349 are relatively low (between 1% and 3%), we also conducted permutation tests with 10^6 repeats to obtain empirical p-values (min empirical p=4.07×10^{-5} for 3-8119772, Table S1). Furthermore, all other top SNPs (43 SNPs) at 3p31.6 were highly correlated with SNP 3-8119772 (r^2=0.52–0.77), including rs13317787 (r^2=0.65) the initial SNP identified from GWAS (Table S1). Fine-mapping of the *BBS9* region identified one additional imputed SNP (rs28619003; chr7:33548225), in complete LD with the original top SNP rs10262995 (r^2=1), which also approached genome-wide significance after meta-analysis (meta-p=6.51×10^{-8}) (Figure 1B, Table S1).

Further analysis of the relationship of identified loci with AKI

To further assess the clinical relevance of the identified loci, we estimated the AKI incidence and severity observed with variation in the chromosomal regions of interest using the original genotyped SNPs rs13317787 and rs10262995 as representative tag SNPs in the combined dataset (N=1,253). For both SNPs, AKI incidence increased with each additional copy of the minor allele (Figure 2). Average %ΔCr (SD) for rs13317787 was 21.8% (0.34) for the CC genotype, 40.5% (0.63) for CA, and 108.0% (0.90) for AA. Similarly, for rs10262995, average %ΔCr (SD) was 20.6% (0.32), 32.4% (0.49), and 62.1% (0.57) for CC, CA, and AA genotypes, respectively.

We also evaluated the ability of two SNPs with strongest association signals (rs1488349 in *GRM7|LMCD1-AS1* and rs28619003 in *BBS9* regions) to predict inter-individual variability in %ΔCr. When jointly added to the patient-specific clinical AKI risk score, the two loci explain roughly double the %ΔCr variance (r^2: 9.7% vs. 4.9% in the discovery cohort, and 9% vs 3.6% in the replication cohort, Table 3). The improved r^2, corroborated by two commonly used global measures of relative model fit like the Akaike information criterion (AIC) and Bayesian information criterion (BIC), both demonstrating reduced (albeit modestly) values (differences of 39.5 and 39.5 for AIC and BIC, respectively, Table 3) support the superior performance of the clinical-genomic model as a postoperative AKI risk stratification tool to potentially individualize reno-protective interventions.

Discussion

In this study, we present a genome-wide analysis to screen genetic variants associated with AKI following CABG surgery with CPB. Using a discovery-replication analysis approach involving independent cardiac surgical cohorts and a continuous variable (%ΔCr) to reflect AKI severity, we describe two novel susceptibility loci: the first with genome-wide significant association is located in the intergenic region *GRM7|LMCD-AS1* (chr3p21.6; lowest meta-p= 2.49×10^{-11}), and the second at the boundary of genome wide significance in the Bardet-Biedl syndrome 9 (*BBS9*) gene (chr7p14.3; min meta-p=6.51×10^{-8}). Patients carrying one or both of the minor alleles at these loci show an incremental increase in risk of

incident AKI, even after accounting for currently known clinical AKI risk factors. We believe this is the first such analysis and speculate that our findings have uncovered a novel predictive tool to improve individualized AKI risk stratification, which may also provide pathophysiologic clues to better investigate and prevent postoperative AKI.

Although no previous GWAS for postoperative AKI is available for comparison, a survey of the two risk loci for links with renal disorders is warranted. At the 3p21.6 locus, an intergenic region bounded by *GRM7* (glutamate receptor, metabotrophic 7) and LMCD1 (LIM and cysteine rich domains protein 1, dyxin) genes, our study identified a 62.8kb peak region highly associated with AKI (17 genome wide significant SNPs in Table S1, Figure 1). No direct functional roles are currently attributed to this intergenic region. However, SNPs in this region were located within active regulatory elements based on ENCODE ChIP-Seq and DNase-Seq data in RegulomeDB[34]. Additionally, HaploReg[35] lists *rs1488349* as located within a hypothetical gene, AC018832.1 (based on GENCODE data), or at the 3' end of LMCD1 antisense RNA 1 (LMCD1-AS1), a non-coding RNA (based on the RefSeq data). LMCD1 is a member of the LIM-domain family of zinc finger proteins, abundantly expressed in kidney tissue, and functionally involved in protein-protein interactions with transcriptional co-repressor activity (MIM*604859) (http://www.ncbi.nlm.nih.gov/omim), including regulation of the calcineurin-NFAT signaling cascade known to play a critical role in recovery from AKI.[36] Our literature review did not identify direct functional links between the *GRM7|LMCD-AS1* intergenic region and AKI pathophysiology, thus future studies are needed to uncover its potential regulatory roles.

Most interestingly, our second risk locus involves a peak 1.8kb region in *BBS9* (MIM*607968), also known as parathyroid hormone-responsive B1 gene (*PTHB1*), named for its relationship with Bardet-Biedl syndrome (BBS, MIM*209900). Kidney disease is a key feature and major source of early mortality with BBS.[37, 38] Approximately 10% of children and adolescents with BBS have end-stage renal disease, and 25% of surviving patients have CKD by their fifth decade. Almost all BBS patients have renal structural defects, and while renal glomerular abnormalities are rare, one-third of patients have vasopressin-resistant urinary concentration defects. A *BBS9* translocation is also associated with the most common pediatric renal malignancy, Wilm's tumor.[39]

BBS is a genetically heterogeneous multiorgan ciliopathy of non-motile cilia that includes mutant variants of their anchoring structure or "BBSome" (also known as the basal body).[40] In the kidney, BBS9 proteins are expressed in focal adhesions and play a central role in controlling cilia length through regulation of actin cytoskeleton polymerization.[41] While the exact role of BBS9 within the BBSome remains unknown, the protein is conserved across species, highly expressed in adult human kidney,[38] and approximately 6% of BBS cases involve *BBS9* mutations.[42–44] Although these mutations are not available in our SNP panels, the two *BBS9* intronic variants identified in this study (rs10262995 and rs28619003) were part of an LD block located immediately upstream of a recombination hotspot in intron 20 (Figure S2).

Non-motile or primary cilia (containing BBS9) are solitary apical appendages found on most cells in the body that function as signal transduction antennae. *Renal primary cilia* act as

mechanosensors that protrude into the nephron lumen from tubule and collecting duct epithelial cells, and are necessary for water absorption in the kidney.[45] Critical drops in tubular flow, such as occur with AKI, are sensed by the cilia and activate cell proliferation, presumably to promote renal recovery.[46, 47] Following ischemia/reperfusion induced AKI, renal primary cilia undergo predictable morphologic changes, as observed in kidney transplant patients and animal experiments; these include a doubling in length over the first 7 days, and return to normal size over weeks as recovery occurs.[48] Collectively, these observations suggest that further investigation of the potential mechanistic involvement of BBS9 in postoperative AKI pathogenesis is warranted.

While this report expands the investigation of cardiac surgery-associated AKI from candidate gene to an unbiased GWAS approach, several limitations remain. First, although power estimates indicate that our sample sizes (N=873 in discovery alone or N=1,253 for the combined two datasets) can reach 80% power to detect SNPs in similar ranges of MAF (0.03–0.09) and proportion of %ΔCr variation ($R^2_G = 3.1\%$ to 4.4%) as observed in this study (see Supplementary Materials), we may still miss potential susceptibility variants with smaller effect sizes. A more powerful study could also have been achieved through refinements to our AKI phenotype and clinical risk score incorporating additional variables such as preoperative albuminuria, acute decline in renal function over the months prior to and following surgery, and perioperative use renin-angiotensin system inhibitors, data which was not available for all patients. Further, our results would have been bolstered if a validation cohort from another institution were included in the analysis. Nonetheless, baseline to peak postoperative serum creatinine concentration increase is highly validated as a marker of adverse outcome following cardiac surgery in studies from numerous institutions, and variables identified for inclusion in our clinical renal risk score are similar to those from previous studies.[23] Of note, sensitivity analyses revealed that the top two variants identified (rs13317787 and rs10262995, Table 2) remain associated with postoperative AKI as defined using the standard KDIGO criteria, albeit at nominal significance levels (p=0.05 and 0.03, respectively). The weaker association signal, likely reflecting a limited number of cases and controls (294 vs. 579) available for association analysis using the dichotomous KDIGO AKI phenotype, supports however our primary findings using %ΔCr as a continuous phenotype.

Second, markers at the 3p21.6 locus are rare (minor allele frequencies between 1–3%) and, although supported by empirical p-values, a larger sample size would increase confidence in this finding. Combining strict genotype QC criteria with visual inspection of cluster plots for rs13317787 revealed well-separated genotype clusters (Figure S3), thus confirming accurate genotype calling for this rare maker. Although we used an imputation method to refine the two most significant association loci, this strategy is not designed toward rare functional variants. These concerns notwithstanding, our findings could form the basis of a genetic pre-operative risk stratification tool which, by individually assessing risk alleles of rs13317787 and rs10262995 in the current samples, would have identified 2.3% and 7.4% of cardiac surgery patients, respectively, to have considerably elevated AKI risk (1.5–5 fold greater rise in serum creatinine) relative to non-carriers. As preliminary evidence for increased predictive ability, genotype information at the two loci improves the performance of a

patient-specific clinical risk score for postoperative AKI, with the clinico-genomic model explaining a higher proportion of variability in the primary AKI phenotype (%ΔCr) and showing improved (albeit modestly) relative fit as evidenced by lower AIC and BIC. Such a genetic susceptibility biomarker for postoperative AKI would be useful not only to assist clinical decision-making, but also to aid researchers in identifying candidates for evaluation of promising reno-protective interventions.

Finally, although we provide intriguing indirect evidence for possible mechanistic roles of the observed risk loci, our study offers no direct functional analysis to validate the GWAS hits. Such interpretation of these putative noncoding regulatory variants could entail gene expression, eQTL, and allelic imbalance analyses in animal models. The limited availability of well-characterized animal models of post-cardiac surgery AKI represents a possible obstacle, and may require the use of experimental models of acute renal ischemia-reperfusion injury, which in addition to the significant pathobiological differences reported between common forms of human AKI and rodent models, would limit clinical relevance and translatability to a cardiac surgical population.

As AKI is a significant complication of cardiac surgery that contributes to perioperative mortality and medical cost, a better understanding of its risk factors is important. Our comprehensive study design presented here has pinpointed two novel susceptibility loci (chr3p21.6 and *BBS9*) for AKI after cardiac surgery with CPB. The conclusion of this report will provide candidate regions for future genetic research on cardiac surgery-associated AKI, and may eventually lead to improved preoperative screening, novel prevention and intervention options to reduce AKI and associated morbidity and mortality.

Materials and Methods

Study design

We utilized two independent cohorts, from the PEGASUS and the CATHGEN studies at Duke Heart Center, to conduct initial common variant discovery by GWAS followed by replication analysis of top candidate single nucleotide polymorphisms (SNPs), respectively. Both PEGASUS and CATHGEN studies were approved by the Duke University School of Medicine Institutional Review Board, and all patients provided informed consent. Our study was performed in accordance with the Declaration of Helsinki and followed the "Strengthening the Reporting of Genetic Association Studies" (STREGA) recommendations.[49] For this AKI substudy, patients in the discovery cohort were participants in the ongoing PEGASUS longitudinal study, and underwent isolated non-emergent CABG surgery with CPB between 1997 and 2006.[50] Patients were excluded from enrollment in PEGASUS if they had a history of end-stage renal disease, active liver disease, bleeding disorders, autoimmune diseases, or immunosuppressive therapy. An *a priori* decision was made to limit the analyses to subjects of self-reported European ancestry, justified by the limited number of non-Caucasian patients in the PEGASUS GWAS dataset and to avoid potential confounding from population admixture given previous reports identifying self-reported race as an independent predictor of postoperative AKI.[7, 23] After applying quality control criteria (see below) and excluding patients with

missing genotypes or phenotypic information, 873 subjects of self-reported European ancestry met eligibility for genome-wide association analysis.

The replication dataset consists of 380 subjects of self-reported European ancestry from the CATHGEN study,[51, 52] who underwent non-emergent CABG with or without concomitant valve surgery using CPB between 2006 and 2010. Similarly, CATHGEN enrollees with end-stage renal disease prior to surgery were excluded from this substudy. Patient and procedural characteristics for both cohorts were collected and curated from the Duke Information System for Cardiovascular Care, an integral part of the Duke Databank for Cardiovascular Disease.

Primary phenotype and clinical data

Daily serum creatinine concentrations considered in this study were those routinely measured at baseline (preoperative) and up to 10 postoperative days at a single core hospital laboratory for both the discovery and validation datasets. The primary study outcome was a continuous AKI endophenotype, the percentage change of the highest postoperative serum creatinine from the baseline preoperative concentration (%ΔCr).[14] For each patient, %ΔCr reflects a gross approximation of the maximum relative loss of renal function. For example, a postoperative serum creatinine doubling (100% rise) approximates a 50% acute functional nephron loss. Notably, in this study we selected %ΔCr (as with our previous studies[23, 53]) in preference to standard dichotomous definitions of AKI (e.g., KDIGO, AKIN and RIFLE criteria[54–56]), which include thresholds for relative creatinine rise (e.g., 50%) that closely resemble %ΔCr. Considering that dichotomous outcomes are known not to be as informative as continuous outcomes, the rationale for using %ΔCr as a quantitative AKI trait was to enhance the ability and power to identify risk variants,[57] as evidenced by previously reported GWAS of other continuous renal traits such as eGFR[30, 58] and serum creatinine[59] in ambulatory populations. Importantly, %ΔCr reflects a spectrum of injury to the kidneys that often does not meet the dichotomous AKI threshold criteria; even small relative rises in serum creatinine are associated with substantial reductions in post-operative event-free survival.[60, 61]

Multiple preoperative and postoperative clinical measures were collected including patient characteristics, procedural variables, and information related to renal function (Table 1). Descriptive statistics for serum creatinine concentrations, estimated glomerular filtration rate (eGFR) based on the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation[62], as well as alternative definitions of AKI (KDIGO, AKIN, RIFLE) are provided for both baseline (preoperative) and peak postoperative measures. Further, a patient-specific clinical AKI risk score was computed based on a previously developed multivariable model that used traditional clinical and procedural risk factor in a large contemporaneous consecutive cohort (N=10,708) of non-emergent CABG surgery with CPB procedures between July 2000–July 2010, as (−2.59207 − 7.72486 × (*preoperative creatinine*) + 0.30737 × (*weight*) + 0.14174 × (*aortic cross − clamp time*) + 16.35924 × (*transfusion*) − 9.06373 ×(*hypertension*)). The clinical risk score was *a priori* confirmed to be independently predictive of AKI in the PEGASUS study cohort, and thus incorporated as a covariate in regression models to adjust the SNP associations with %ΔCr.

Genotyping and quality controls (QCs)

Genomic DNA was isolated from whole blood using standard procedures. Genotyping the PEGASUS discovery cohort used the Illumina Human610-Quad BeadChip at the Duke Genomic Analysis Facility for a total of 1004 samples. The Illumina GenomeStudio program (Illumina Inc., San Diego CA) was used for genotype calling. Markers with low GenCall score (≤ 0.15) or call frequency < 98% were filtered out. Samples with call rate < 98% or gender specification errors were also excluded in this initial QC. Additional QCs, conducted using PLINK software,[63] included checks for cryptic relatedness and duplications. For a pair of samples with identity by descent (IBD) >0.1875 (between 2nd and 3rd degree relative), one sample was excluded from further analysis. Population structure was investigated using EigenSoft program[64]. All 15 principal components (PCs) were computed for each sample, and multiple PC plots were generated to determine whether any obvious outliers deviated from the main cluster and hence should be excluded. In total, we filtered out 44 samples (14 with call rate < 0.98, 3 with gender errors, 21 due to their relatedness with other samples, and 6 outliers from PC analysis). The QC'ed genotype dataset consisted of 960 subjects with 561,091 markers. Additionally, 86 subjects missing %ΔCr data, and one outlier with extreme high %ΔCr (outside of 3 SD from the mean %ΔCr) were excluded. Therefore, the final PEGASUS analysis dataset consists of 873 patients, all of European descent, with both genotype and phenotype data available.

All CATHGEN samples were genotyped using Illumina OMNI1-Quad BeadChip, and subjected to the same marker and sample QC criteria described above for PEGASUS. Following QC, a subset of CATHGEN samples was selected based on availability of %ΔCr data. Only SNPs identified in the discovery dataset were tested in the CATHGEN cohort for replication purposes.

Imputation of untyped markers

To increase overlap in SNP coverage between the genotyping platforms used for PEGASUS and CATHGEN cohorts for both replication analysis and meta-analysis, as well as to improve coverage of top candidate regions for fine-mapping associations, imputation of untyped autosomal SNPs was conducted in the post-QCed PEGASUS genotype dataset (960 samples with 561,091 markers) using a hidden Markov Model algorithm implemented in IMPUTE v2 software[65] and phased haplotypes from the 1000 Genomes CEU reference panel. The best-guess imputed genotype for any untyped SNP per sample was chosen as the genotype with the highest imputation probability (imputation score). If the highest imputation score for an imputed SNP of a sample was less than 90%, a missing imputed genotype was assigned.

Statistical Analysis

Descriptive statistics of clinical variables are presented as frequency (percentage) for categorical variables and mean (standard deviation) for continuous variables. None of the principal components (PCs) derived from population structure analysis were significantly associated with %ΔCr in univariate linear regression tests, suggesting a lack of population stratification in our ethnically homogenous patient cohorts. As such, no ancestry covariates were included in the final multivariable linear regression model, which therefore tested SNP

allelic association with %ΔCr adjusting only for the patient-specific AKI clinical risk score. An additive genetic model was used for coding each SNP genotype (0 for common homozygous, 1 for heterozygous, and 2 for rare homozygous).

Association analyses were carried out in the discovery cohort for all variants that passed QCs. Additional marker exclusion criteria – significant deviations from Hardy-Weinberg equilibrium (HWE) ($P<10^{-6}$), genotype missingness rate < 10%, heterozygous haploid, minor allele frequency (MAF) < 1% - were applied to all association tests throughout the study. For the discovery dataset, no markers were excluded based on the 10% missingness threshold due to our prior stringent QC criteria to choose markers with >98% call frequencies. In total, 30,375 SNPs were excluded, leaving 530,716 SNPs to be tested in the final dataset. We used an *a priori* defined significance threshold of $p<10^{-5}$ to select candidate SNPs for replication in the CATHGEN cohort, as a balance between the overly conservative Bonferroni correction and type II error, given that we had an *a priori* defined replication dataset to obviate type I error.

Association tests for the replication dataset were performed based on the same regression model with an additional covariate indicating patient-specific heart valve surgery status; statistically significant replication was defined as nominal significance in the replication cohort ($p<0.05$) with the same direction of allelic effect. All analyses were conducted using PLINK.

Finally, meta-analyses were performed to assess the overall effect of SNPs tested in both cohorts (meta-p values) using z-scores weighted by the inverse variance of effect size of each study, implemented in METAL[66](http://www.sph.umich.edu/csg/abecasis/metal). The weighted z-score method allows for an overall p-value (meta-p) to be computed, by taking into account the beta-estimates and their standard errors from both datasets. The top genomic regions with SNPs meeting meta-p value $<10^{-6}$ were further investigated (fine-mapped) by adding all imputed markers within the region in discovery dataset, followed by replication and meta-analysis as described above. Same marker exclusion criteria described for HWE, genotype missingness rate, and MAF were also applied to imputed markers prior to association analysis.

Linkage disequilibrium (LD) block information was computed and displayed using HaploView[67](version 4.2) for top candidate regions. To evaluate whether genetic information independently adds prognostic value for postoperative AKI above traditional risk factors, we contrasted multiple regression models of AKI clinical risk score alone (*clinical model*) and with the addition of most significant independent SNPs (defined by LD) in top associated regions (*clinical-genomic model*). To facilitate model performance comparison, we present p-values, r^2, and two commonly used information criteria (AIC and BIC) for each model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix: Duke Perioperative Genetics and Safety Outcomes (PEGASUS) Investigative Team Members

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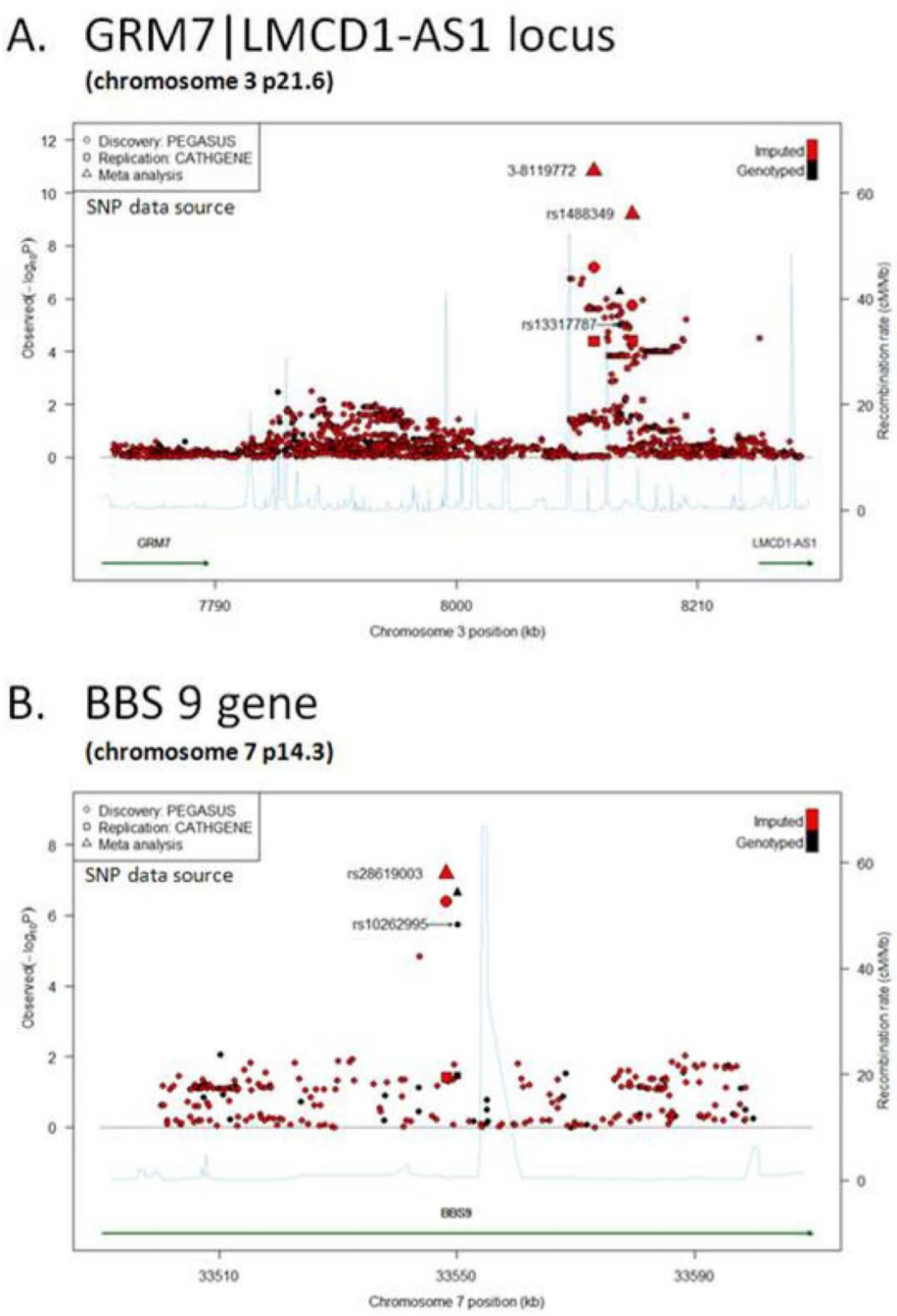

Figure 1.

Regional association plot for (A) chr3p21.6 locus (*GRM7|LMCD1-AS1*) and (B) *BBS9* gene, presenting – $\log_{10}$(p-values) from the discovery (PEGASUS) and replication (CATHGEN) datasets, as well as the meta-analysis. Directly genotyped SNPs are plotted in black; imputed markers are plotted in red.

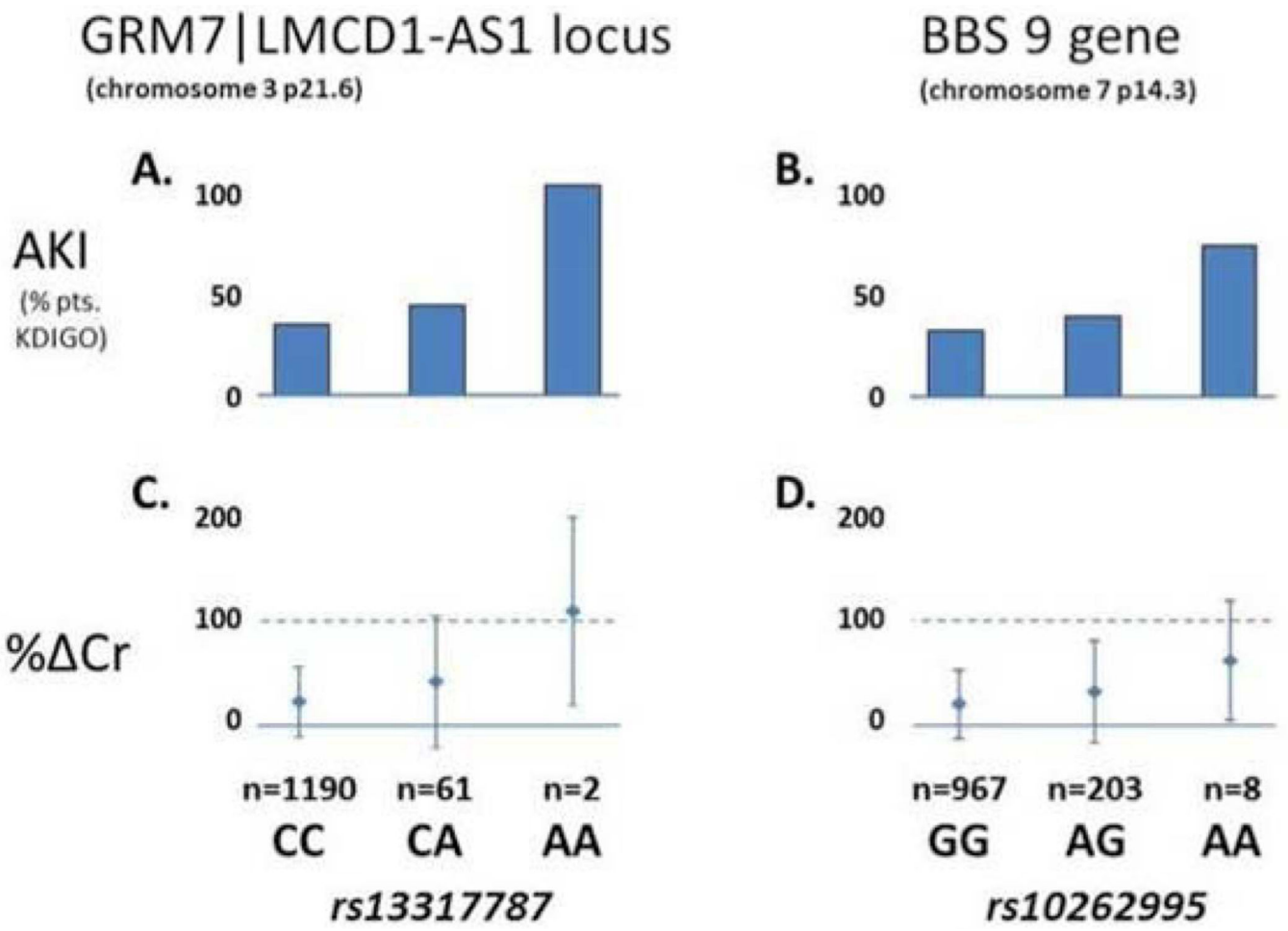

Figure 2.

Comparative graphical representation of genotypic effects of rs13317787 at the chr3p21.6 locus (A&C) and rs10262995 in *BBS9* (B&D) on incident post-cardiac surgery acute kidney injury (AKI) – defined using either the KDIGO criteria[56] or peak postoperative serum creatinine increase (%ΔCr). The dashed line represents a 2-fold (100%) increase in serum creatinine from baseline, approximately equivalent to a 50% reduction in glomerular filtration rate.

Table 1

Patient, Renal and Procedural Characteristics

	Discovery cohort (PEGASUS N=873)	Replication cohort (CATHGEN N=380)	p-value
Patient characteristics			
Demographic variables			
Age (years)	63.8 (10.1)	62.9 (11.0)	0.175
Body weight (kg)	86.7 (18.7)	87.2 (20.2)	0.689
Female sex	206 (23.6%)	146 (38.4%)	<0.001
Comorbidities			
Left ventricular ejection fraction (%)	55.3 (14.3)	55.6 (17.7)	0.776
Angina	495 (58.0%)	138 (42.6%)	<0.001
Arrhythmia	189 (22.0%)	52 (16.1%)	0.023
Congestive heart failure	97 (11.3%)	111 (29.5%)	<0.001
Chronic obstructive lung disease	45 (6.6%)	31 (8.2%)	0.341
Diabetes	266 (30.7%)	136 (36.0%)	0.078
History of hypertension	438 (50.2%)	288(75.8%)	<0.001
Hypercholesterolemia	488 (57.2%)	267 (70.8%)	<0.001
Previous myocardial infarction	381 (43.9%	135 (36.2%)	0.011
Peripheral vascular disease	87 (10.2%)	38 (10.0%)	0.915
Smoking history	394(46.2%)	220 (58.1%)	<0.001
Chronic kidney disease[a,b]	199 (22.8%)	107 (28.2%)	0.042
Procedural variables			
Duration of CPB (min)	114.7 (36.3)	141.5 (51.8)	<0.001
Duration of aortic cross-clamping (min)	64.0 (26.7)	79 (34.2)	<0.001
Blood transfusion[c]	368 (42.2%)	241 (63.4%)	<0.001
Intraoperative balloon counterpulsation	32 (3.8%)	31 (9.6%)	<0.001
Concomitant valve surgery	0	110 (29.0%)	<0.001
Clinical AKI risk score[d]	26.3 (12.6)	32.1 (6.8)	<0.001
Markers of renal function			
Serum creatinine concentrations (mg/dL)			
baseline preoperative	1.06 (0.46	1.06 (0.32)	0.893
peak postoperative	1.28 (0.57)	1.30 (0.51)	0.711
$eGFR_{crea}$ (ml/min/1.73m^2)[b]			
baseline preoperative	73.7(18.1)	71.9 (19.9)	0.142
nadir postoperative	62.0 (19.6)	60.2 (20.7)	0.144
AKI criteria[e]			
Peak relative to baseline	22.5 (35.9)	23.6 (37.0)	0.621
creatinine %ΔCr (%)			

	Discovery cohort (PEGASUS N=873)	**Replication cohort (CATHGEN N=380)**	**p-value**
KDIGO	294 (33.7%)	119(381.3%)	0.139
AKIN	290 (33.2%)	115 (30.3%)	0.221
RIFLE	149 (17.1%)	64(16.8%)	0.333
KDIGO AKI Stage			0.612
Stage 1	69 (7.9%)	29 (7.6%)	
Stage 2	29 (3.3%)	11 (2.9%)	
Stage 3	10 (1.2%)	6 (1.6%)	

Results presented as mean (standard deviation) for continuous variables, and frequency (percentage) for categorical variables.

Abbreviations: CPB – cardiopulmonary bypass; CABG – coronary artery bypass grafting; AKI – acute kidney injury; $eGFR_{crea}$ – estimated glomerular filtration rate (creatinine) based on the CKD-EPI equation; AKIN – Acute Kidney Injury Network;[37] RIFLE - risk, injury, failure, loss, end-stage kidney disease;[38] KDIGO - Kidney Disease: Improving Global Outcomes.[39]

[a] Chronic kidney disease defined as baseline eGFR < 60 ml/min/1.73m^2 (modified KDIGO criteria, lacking the 3 month preoperative window)

[b] eGFR was computed based on CKD-EPI equation

[c] Transfusion defined as receipt of any blood transfusion perioperatively.

[d] Clinical AKI risk scores for individual subjects computed as:

−2.59207 −7.72486 (Preop creatinine) + 0.30737(weight) + 0.14174 (cross-clamp time) + 16.35924 (transfusion) −9.06373 (hypertension).

[e] Reflect only serum creatinine criteria (i.e., lack oliguria criteria).

Table 2

Summary of SNPs Selected in the Discovery Cohort for Replication

					Discovery cohort: PEGASUS				Replication cohort: CATHGEN				Combined dataset			
Chr	**SNP**	**Base Pair**	**Gene** *	**allele**	**MAF**	**Effect**	**SE**	**P**	**MAF**	**Effect**	**SE**	**P**	**Effect**	**SE**	**I^2**	**meta-P**
1	rs2352039	78819945	MGC27382	A	0.156	10.07	2.26	9.78×10^{-6}	0.168	6.23	3.43	0.070	8.90	1.89	0	2.45×10^{-6}
3	**rs13317787**	**8141952**	**GRM7 | LMCD1-AS1**	**A**	**0.028**	**21.56**	**4.84**	**9.67×10^{-6}**	**0.02**	**22.04**	**9.56**	**0.022**	**21.66**	**4.32**	**0**	**5.35×10^{-7}**
7	**rs10262995**	**33550041**	**BBS9**	**A**	**0.087**	**14.33**	**2.98**	**1.83×10^{-6}**	**0.099**	**9.51**	**4.47**	**0.034**	**12.84**	**2.48**	**0**	**2.24×10^{-7}**
12	rs2248098 **	48253356	VDR	A	0.488	−7.48	1.60	3.60×10^{-6}	0.474	−0.21	2.83	0.942	−5.71	1.40	80	4.22×10^{-5}
16	rs1109836	57660346	GPR56	A	0.013	37.57	7.46	5.67×10^{-7}	0.012	−11.32	12.33	0.36	24.48	6.38	91.3	0.0001
18	rs8086030 **	9750395	RAB31	A	0.420	7.64	1.69	7.33×10^{-6}	0.438	−1.07	2.83	0.705	5.35	1.45	85.7	0.0002
18	rs8099036 **	9756056		G	0.389	8.47	1.71	9.01×10^{-7}	0.397	−0.44	2.92	0.880	−6.19	1.48	85.5	2.74×10^{-5}
21	rs2831026	28969040	LOC100288252	G	0.289	8.95	1.82	1.11×10^{-6}	0.263	−5.39	3.08	0.081	−5.22	1.57	93.8	0.0009
21	rs1551588	28982407	| NCRNA00113	A	0.165	11.86	2.20	9.14×10^{-8}	0.147	−7.52	3.73	0.044	6.85	1.90	95	0.0003

Abbreviations: SNP - single nucleotide polymorphism; Chr – chromosome; MAF – minor allele frequency; Effect – the beta coefficient of linear regression; SE – standard error; I^2 – heterogeneity; PEGASUS, CATHGEN – see text.

* Intergenic regions are identified by the two flanking genes separated by "|".

** Imputed SNPs in the CATHGEN cohort.

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Table 3

Comparison of Clinical and Clinico-genomic AKI Predictive Models with and without Inclusion of the top SNP in each region (rs1488349 and rs28619003) for two datasets

Dataset	Model variables	beta(SE)	P	Model p	r^2	AIC	BIC
Discovery Cohort: PEGASUS	Clinical Model			<0.0001	0.049	6029.55	6031.37
	AKI risk score	0.64 (0.10)	7.3×10^{-11}				
	Clinico-Genomic Model			<0.0001	0.097	5990.02	5992.06
	AKI risk score	0.60(0.09)	2.43×10^{-10}				
	rs1488349	28.81(6.24)	4.50×10^{-6}				
	rs28619003	13.37(3.05)	1.34×10^{-5}				
Replication Cohort: CATHGEN	Clinical Model			0.001	0.036	2706.96	2708.70
	AKI risk score	1.13(0.31)	3.2×10^{-4}				
	Valve procedure	10.83(4.64)	0.02				
	Clinico-Genomic Model			<0.0001	0.090	2691.26	2708.77
	AKI risk score	1.22(0.30)	6.76×10^{-5}				
	Valve procedure	10.84(4.52)	0.017				
	rs1488349	50.58(11.98)	3.06×10^{-5}				
	rs28619003	9.14(4.43)	0.04				

Abbreviations: SNP - single nucleotide polymorphism; AKI – acute kidney injury, Chr – chromosome; MAF – minor allele frequency; Beta – regression coefficient; SE – standard error; Model p – p-value for the corresponding model; AIC -- Akaike information criterion; BIC -- Bayesian information criterion; PEGASUS, CATHGEN – see text.