Association of Functional Kallikrein-1 Promoter Polymorphisms and Acute Kidney Injury: A Case-Control and Longitudinal Cohort Study

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Abstract

Background—Kallikrein-1 (KLK1) is a highly conserved serine protease that is expressed in the kidney and involved in blood pressure regulation. The activity of this enzyme is diminished in acute kidney injury (AKI).

Methods—We first evaluated the potential role of functional multiallelic *KLK1* promoter gene polymorphisms in a case-control study of 481 subjects (214 hospitalized patients with AKI of mixed causes and 267 healthy subjects). The complex, multiallelic G/C-rich repeat region of the proximal *KLK1* promoter was determined by direct Sanger/capillary resequencing.

Results—16 alleles were identified in a complex, polymorphic G/C-rich region of the *KLK1* proximal promoter; 5 of these alleles (F, G, H, I, and K) were associated with development of AKI. Alleles I and G were classified as risk-alleles (unadjusted OR 1.86; 95% CI 1.23, 2.81; p = 0.003), whereas alleles F, H, and K were classified as protective-alleles (unadjusted OR 0.32; 95% CI 0.22, 0.46; p < 0.001) according to their directional association with development of AKI. After adjustment for sex, race, preexisting chronic kidney disease and APACHE II score, the *KLK1* risk-allele (I or G) carrier state was associated with the composite of   2-fold increase in serum creatinine, oliguria, or dialysis requirement (adjusted OR 2.71; 95% CI 1.14, 6.44; p = 0.02). The *KLK1* risk-allele carrier state was also marginally associated with the composite of   2-fold increase in serum creatinine, oliguria, dialysis requirement, or in-hospital death (adjusted OR 2.33; 95% CI 0.98, 5.52; p = 0.06).

Conclusions—*KLK1* promoter polymorphisms are associated with development of AKI and adverse outcomes. Further studies are needed to validate these findings.

Keywords

Kallikrein-1; Gene; Polymorphism; Promoter; Acute kidney injury

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Introduction

Acute kidney injury (AKI) is an increasingly common and life-threatening complication in hospitalized patients [1, 2], conferring an increased short-term mortality risk, and a long-term risk of development of chronic kidney disease (CKD), kidney failure, and death [3, 4]. Unfortunately, AKI therapies that have been shown to be beneficial in experimental studies have often failed to demonstrate clinical efficacy [5, 6], due in part to untimely initiation of treatment. The identification of genetic risk markers that might influence the susceptibility to and severity of AKI, and improve individualized treatment is an emerging concept [7, 8]. Several polymorphisms of genes encoding cytokines, oxidant stress enzymes, hypoxia-inducible factor-α, and catecholamines have previously been shown to be associated with disease severity and adverse outcomes in patients with AKI [9–13].

Kallikreins are a subgroup of serine proteases with diverse physiological functions. Kallikrein-1 (KLK1), one of 15 kallikrein family members, is the major kallikrein expressed in the kidney, where it generates kinins, including lysyl-bradykinin and bradykinin [14]. KLK1 is involved in kidney function and blood pressure regulation through vasodilatory and natriuretic effects. Kidney tissue levels of KLK1 are decreased in experimental settings of hypertension [15], diabetic nephropathy [16], high salt- and aminoglycoside-induced kidney injury [17, 18].

KLK1 is encoded by the *KLK1* gene, which is located on chromosome 19q13.3. In experimental models of AKI, *KLK1* gene transfer can attenuate the severity of kidney injury [19–21]. In human AKI, a biphasic response of urinary KLK1 excretion has been observed, with an initial decrease followed by an increase, as a result of activation of the adrenergic pathway [22]. There is a complex, highly polymorphic (~16-allele) G/C-rich region of approximately 120 base pairs upstream of the transcription initiation site in the human *KLK1* promoter, whose multiple alleles influence promoter activity of the gene [23]. Polymorphisms at this site have been identified in the general population [23] and subsequently associated with CKD [24, 25].

In the present study, using a case-control design and a candidate allele approach, we first examine the association of the multiallelic *KLK1* promoter region polymorphisms with the development of hospital-acquired AKI in an adult population, identifying both risk- and protective-alleles for susceptibility to AKI. We next explore the association of the *KLK1* polymorphic risk-alleles with kidney-related adverse outcomes in patients with AKI.

Methods

Study Populations

Cases consisted of hospitalized patients with AKI recruited from two acute care facilities (Boston, Mass., USA) between November 2003 and January 2007. All eligible patients were 18 years of age and received in-hospital nephrology consultation for AKI of mixed causes. Exclusion criteria were pregnancy and long-term dialysis dependence. Control subjects consisted of non-hospitalized healthy adults 18 years of age with no known kidney disease who were also genotyped for the same *KLK1* promoter allele polymorphisms. Informed consent was obtained for each subject and institutional review board approval was granted.

Data Collection

For the AKI cohort, data collection included baseline demographic characteristics, comorbid conditions, 24-hour urine output at enrollment, and serial serum creatinine values. Sepsis was ascertained using the systemic inflammatory response syndrome criteria [26], and two

severity-of-illness scores were calculated, the Acute Physiology and Chronic Health Evaluation (APACHE) II score [27], and the Multiple Organ Failure (MOF) score [28]. The baseline serum creatinine was defined as a stable value prior to the acute rise representing the AKI event under study. Using this baseline serum creatinine value, preexisting CKD was defined on the basis of an estimated glomerular filtration rate <60 ml/min/1.73 m^2, which was calculated using the Modification of Diet in Renal Disease study equation [29]. Oliguria at enrollment was defined as a 24-hour urine output <400 ml. At enrollment, AKI was reclassified according to the AKI network staging system, using serum creatinine-based criteria [30]. For biogeographic ancestry, self-identified ethnicity was used.

Genotyping Analyses

For both cohorts, EDTA-anticoagulated whole blood was collected and aliquoted for subsequent DNA extraction. Genomic DNA was prepared using a QiAmp DNA blood kit or Gentra Pure-gene columns (both from Qiagen, Valencia, Calif., USA).

The complex, multiallelic, G/C-rich polymorphic region of the human *KLK1* promoter [23] was evaluated by resequencing of the isolated genomic DNA. In brief, PCR amplification of the polymorphic region was performed as previously described [31] using forward primer hKPM6 and reverse primer hKPM7 [23]. PCR products were treated with exonuclease I and shrimp alkaline phosphatase to remove primers and dNTPs prior to cycle sequencing with BigDye terminators (Applied Biosystems, Foster City, Calif., USA). Sequence was determined from each direction (both sense and antisense strands) on an ABI-3100 automated sequencer, with visualization of sequence by system software as well as visual inspection of sequence tracings in each subject, to assign diploid genotypes.

Statistical Analysis

Genotype frequencies for each *KLK1* promoter polymorphism in the control subjects and the AKI cohort were examined for deviation from Hardy-Weinberg equilibrium using the χ^2 test. Additive genetic logistic regression models (i.e. per allele copy increase) were used to assess the association of the *KLK1* promoter polymorphic risk- and protective-alleles with development of AKI, after adjustment for age, sex, race, and presence of hypertension, diabetes mellitus, and preexisting CKD.

For the AKI cohort, comparisons of baseline characteristics and outcomes between the *KLK1* polymorphic risk- and non-risk-allele groups were performed using Student's t test or analysis of variance for normally distributed continuous variables, and non-parametric Wilcoxon rank sum or Kruskal-Wallis tests for non-normally distributed variables. χ^2 or Fisher exact tests were used for categorical variables. In consideration of testing the effects of multiple correlated alleles (or diploid genotypes) at a locus on a trait, we employed estimation of the false discovery rate (FDR) in order to minimize false negative results while maximizing false positive results, using the Excel calculator of FDRs from p values (http://www.rowett.ac.uk/gwh/fdr.html).

Additive genetic (per 1-allele copy increase) logistic regression models were also used to assess the association of *KLK1* promoter polymorphic alleles with the composite kidney-related outcome of 2-fold increase in serum creatinine representing stage 2 and 3 AKI according to the AKI network classification [30], oliguria, or dialysis requirement. The models were adjusted for sex, race, preexisting CKD and APACHE II score.

Sensitivity analyses restricted to white subjects were performed in both the case-control study and the AKI cohort. The data are presented as frequencies with proportions, and means with standard deviations. The results of the logistic regression analyses are displayed as odds ratios (OR) with 95% confidence interval (CI). All the statistical analyses were

performed using the SPSS statistical package (version 11.5 for Windows; SPSS, Inc., Chicago, Ill., USA). Differences were considered statistically significant at a p value <0.05.

Results

Characteristics of the Control Subjects and the AKI Cohort

A total of 481 subjects were included for the case-control analysis, of whom 267 were healthy non-hospitalized control subjects and 214 hospitalized patients with a diagnosis of AKI (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/ 10.1159/000350733). The control subjects had a mean age of 41.5 ± 16.5 years, 26% were men, 82% were white, 13% had hypertension, 2% had diabetes, and 8% had preexisting CKD. The mean age of the AKI cohort was 64.5 ± 15.9 years, 54% were men, and 91% were white. The prevalence of hypertension, diabetes, and preexisting CKD was 75, 44, and 73%, respectively, which was significantly different from control subjects ($p < 0.001$ for all analyses). The biogeographic ancestry (ethnicity) distribution also differed between the two groups ($p < 0.001$).

92 (43%) patients with AKI required dialysis and 51 (24%) died. The majority of patients had severe AKI as defined by the AKI network criteria. In brief, 66 (31%) patients suffered from stage 1, 12 (6%) from stage 2, and 136 (63%) from stage 3 AKI.

Association of KLK1 Promoter Polymorphisms with Development of AKI

A total of 16 *KLK1* alleles were identified and the observed allele distribution in the control subjects and AKI cohort is shown in table 1 with discernable differences (global $p < 0.001$ by χ^2 test). We identified 5 *KLK1* alleles, including alleles F, G, H, I, and K, to be significantly associated with development of AKI (table 2). The I and G alleles were classified as risk-alleles, whereas the F, H, and K alleles were classified as protective-alleles, according to their directional association with development of AKI. Among the significant ($p < 0.05$) risk-alleles (I and G), allele I had a relatively common frequency of 9.0–13.8%, and among the protective-alleles (F, H, and K), allele H had a common frequency of 11.2–22.9%. Tests for Hardy-Weinberg equilibrium showed no significant deviation from expected frequencies ($p = 0.15$) for all alleles (online suppl. table 2).

The results of the unadjusted and adjusted genetic additive models for individual and combined polymorphic alleles are displayed in table 2. Each copy increase in the *KLK1* protective-allele (F, H, or K) was significantly associated with a *lower* unadjusted and adjusted OR for development of AKI. By contrast, each copy increase in the *KLK1* risk-allele (I or G) was significantly associated with a *higher* unadjusted OR for development of AKI, which became non-significant in the adjusted analysis (table 2). In sensitivity multivariable analyses restricted to the subset of white subjects (n = 413), increasing copies of both *KLK1* protective- and risk-alleles remained significantly associated with development of AKI (online suppl. table 3).

Association of KLK1 Risk-Alleles with Kidney-Related Endpoints in the AKI Cohort

We next examined the association of the risk-allele (I or G) with individual kidney-related endpoints, and the composite of 2-fold increase in serum creatinine, oliguria, or dialysis requirement. As shown in table 3, the risk-allele was associated with an adjusted OR for the composite kidney-related endpoint of 2-fold increase in serum creatinine, oliguria or dialysis requirement, after adjustment for sex, race, preexisting CKD, and APACHE II score (adjusted OR 2.71; 95% CI 1.14, 6.44; $p = 0.02$). Although there was no association between the risk-allele and the individual components of the composite endpoint (table 4), the risk-

allele group was associated with an adjusted OR of 2.3 for the composite outcome, which reached near significance (p = 0.06).

In sensitivity multivariable analyses restricted to the subset of white subjects (n = 195), increasing copies of the *KLK1* risk-allele remained associated with the two composite outcomes (online suppl. table 4).

Discussion

In the present study, we examined the association of functional *KLK1* promoter polymorphisms with the development of AKI in a hospitalized adult population. Five alleles, including alleles F, G, H, I, and K, were found to be associated with development of AKI, and confirmed by FDR analysis; I and G were classified as risk-alleles, while F, H, and K were classified as protective-alleles, according to their directional association with development of AKI. In patients with AKI, the risk-alleles were found to be associated with disease severity, including the composite of 2-fold increase in serum creatinine, oliguria, or dialysis requirement.

There are only a limited number of studies of the KLK1 system in human AKI. An early report found that patients with AKI after kidney transplantation exhibited diminished urinary KLK1 excretion [32]. More recently, patients with AKI were found to have a biphasic response in urinary KLK1 enzymatic activity excretion, likely the result of activation of the adrenergic system in the setting of volume contraction [22]. In another study of 6 patients with ischemic acute tubular necrosis, urinary KLK1 excretion reached the highest level before resolution of oliguria [33]. In an animal model of AKI, renal KLK1 expression was persistently reduced in the kidney after functional recovery from ischemic injury [34]. In our study, carriers of the *KLK1* risk-allele displayed significantly decreased urine output, suggesting indirectly that this allele might be associated with lower kidney KLK1 enzymatic activity.

In a recent study, the experimental implantation of KLK1-modified mesenchymal stem cells into the kidney provided apparent protection against ischemia-induced AKI, as evidenced by reduced blood urea nitrogen, serum creatinine, and tubular injury [35]. *KLK1* gene transfer has also been shown to attenuate the decline in kidney function in a rodent model of CKD involving 5/6 nephrectomy [36]. The KLK1 system has been proposed to play a role in hypertension, but its role in human AKI has thus far been incompletely explored. Evaluation of additional candidate genes associated with KLK1 biosynthesis, catabolism, and function could be undertaken in this setting. Potential novel interventions into the KLK1 system might provide alternative treatment options for AKI management in the future.

To our knowledge, this is the first study to test the hypothesis of whether these functional *KLK1* promoter polymorphisms associate with development of AKI and adverse clinical outcomes. Strengths of the analyses include the demonstrable associations between *KLK1* promoter polymorphisms and development of AKI and adverse outcomes. The AKI cohort was well characterized with prospectively collected data and ascertainment of clinically meaningful kidney-related endpoints, and no attrition or loss to follow-up. There are several limitations however that should be noted. For control subjects, we used a non-hospitalized adult population with no known kidney disease; although we adjusted for potentially important factors such as age, sex, ethnicity, hypertension, diabetes, and preexisting CKD, we could not control for factors associated with hospitalization per se, which might alter KLK1 activity. In addition, the biogeographic ancestry distribution differed between cases and control subjects. Although we had ancestry-informative markers on the control subjects, we did not perform ancestry-informative markers on the AKI cohort. To overcome this

important limitation, we adjusted our analyses for race and performed sensitivity analyses restricted to white subjects. Based on the inclusion criteria used in the study, we included patients with more severe stages of AKI and a higher percentage of preexisting CKD, which likely resulted in a higher percentage (43%) of patients requiring dialysis. These selection biases likely reduce the generalizability of our findings. Another important limitation is the relatively small sample size for a gene association study of a very complex trait, as well as a multiallelic promoter polymorphism with both common and infrequent alleles, likely enhancing the chance of false negatives (or type II error) for some of our attempted allelic associations. We also acknowledge that dialysis initiation and management of oliguria is subject to variability among physicians and was likely influenced by patient and physician factors. We enrolled subjects with AKI at the time of nephrology consultation rather than at its onset. Finally, we did not link the *KLK1* alleles to intermediate phenotypes, such as promoter transcriptional activity or urinary enzymatic activity of KLK1.

In conclusion, in the present study, we provide support for the viewpoint that *KLK1* genetic variation plays a role in the development of AKI as well as its associated outcomes. These associations require further exploration, with external validation in more diverse populations. Nonetheless, the KLK1 system may provide valuable genomic biomarkers for the study of AKI, including risk stratification, prognosis, and possible response to therapeutic interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Observed *KLK1* allele distribution in healthy control subjects and the AKI cohort

KLK1 allele	Healthy control subjects (n = 267)		AKI cohort (n = 214)		χ^2 p value	FDR p value
	number observed	allele frequency	number observed	allele frequency		
GGAGGGGGGGGGGGGC = A	203	0.380	183	0.430	0.136	0.102
GGAGGCGGGGGGGGC = B	106	0.199	111	0.257	0.084	0.084
GGGGGGGGGGGGGGC = C	4	0.008	1	0.002	0.269	0.179
GGGGCGGGGGGGGGC = D	2	0.004	2	0.005	0.824	0.412
GGAGGGCGGGGGGGC = E	1	0.002	2	0.005	0.439	0.263
GGAGCGGGGGGGGGC = F	15	0.028	3	0.007	0.017	0.025
GGAGCGGGGGGGGGC = G	2	0.004	9	0.021	0.012	0.024
GGAGGGGGGGGGGGC = H	122	0.229	48	0.112	<0.001	0.001
GGAGGGGGGGGGGC = I	48	0.090	59	0.138	0.019	0.022
GGAGGGGGGGC = J	1	0.002	1	0.002	0.875	0.404
GGAGGGGGGGGGGGGGC = K	23	0.043	2	0.005	<0.001	0.001
GGAGGCGGGGGGC = P	0	0.000	0	0.000	–	–
GGAGGGGGGGGGGGGGC = M	1	0.002	0	0.000	–	–
GGAGCGGGGGGGGC = O	0	0.000	2	0.005	0.114	0.098
GGAGGCGGGGGGGGGC = Q	2	0.004	0	0.000	–	–
GGAGGCGGGGGGGC = S	3	0.006	3	0.007	0.785	0.428

A global χ^2 test on this 2 × 16 (healthy control subjects *vs.* AKI cohort; 16 alleles) contingency table yielded a χ^2 value = 60.97 and p value < 0.001.

Table 2

Association of *KLK1* alleles with development of AKI (n = 481)

KLK1 allele	OR (95% CI)	p value
Allele I (per allele copy ↑)		
Unadjusted	1.68 (1.10, 2.56)	0.016
Allele G (per allele copy ↑)		
Unadjusted	4.48 (0.92, 21.80)	0.063
Risk (I or G)-allele (per allele copy ↑)		
Unadjusted	1.86 (1.23, 2.81)	0.003
Adjusted	1.86 (0.95, 3.66)	0.072
Allele F (per allele copy ↑)		
Unadjusted	0.26 (0.07, 0.91)	0.035
Allele H (per allele copy ↑)		
Unadjusted	0.34 (0.23, 0.51)	<0.001
Allele K (per allele copy ↑)		
Unadjusted	0.10 (0.02, 0.43)	0.002
Protective (F, H, or K)-allele (per allele copy ↑)		
Unadjusted	0.32 (0.22, 0.46)	<0.001
Adjusted	0.34 (0.19, 0.62)	<0.001

Analysis adjusted for age, sex, race, hypertension, diabetes, and pre-existing CKD.

Table 3

Characteristics and outcomes of the AKI cohort stratified according to *KLK1* polymorphic risk- vs. non-risk-alleles (n = 214)

Variable	Risk-allele (allele I or G) (n = 63)	Non-risk-allele (other alleles) (n = 151)	p value
Age, years	62.9±14.5	65.2±16.5	0.31
Men, n	34 (54)	81 (54)	0.97
White ethnicity, n	57 (90)	138 (91)	0.87
Cause of AKI, n			
Ischemic	13 (21)	46 (31)	0.14
Nephrotoxic	12 (19)	20 (13)	0.28
Sepsis	9 (14)	11 (7)	0.11
Multifactorial/other	29 (46)	74 (49)	0.69
Coexisting conditions, n			
Diabetes mellitus	28 (44)	66 (44)	0.92
Hypertension	46 (73)	114 (75)	0.70
Cardiovascular disease	36 (57)	91 (60)	0.67
Liver cirrhosis	7 (11)	11 (7)	0.39
Chronic lung disease	14 (22)	21 (14)	0.13
Chronic kidney disease	45 (71)	111 (74)	0.47
Sepsis, n	30 (48)	67 (44)	0.66
APACHE II score	19.7±6.8	20.0±6.4	0.84
MOF score	1.2±1.0	1.1±0.9	0.56
AKI network stage, n			
Stage 1	16 (25)	50 (33)	0.27
Stage 2	6 (10)	6 (4)	0.10
Stage 3	41 (65)	95 (63)	0.76
Enrollment urine output, ml/day	979±827	1,274±1,063	0.03
Oliguria at enrollment, n	18 (29)	31 (21)	0.21
2-Fold increase in serum creatinine, n	47 (75)	98 (65)	0.26
Dialysis requirement, n	30 (48)	62 (41)	0.38
In-hospital mortality, n	17 (27)	34 (23)	0.48

Values in parentheses are percentage.

Table 4

Association of the *KLK1* risk-allele (I or G) with adverse outcomes in the AKI cohort (n = 214)

Outcome variable	OR (95% CI)	p value
2-Fold increase in serum creatinine		
Unadjusted	1.38 (0.73, 2.60)	0.318
Adjusted	1.56 (0.78, 3.12)	0.209
Oliguria		
Unadjusted	1.49 (0.80, 2.77)	0.206
Adjusted	1.50 (0.77, 2.90)	0.233
Dialysis requirement		
Unadjusted	1.44 (0.83, 2.50)	0.195
Adjusted	1.54 (0.85, 2.81)	0.155
2-Fold increase in serum creatinine, oliguria, or dialysis requirement		
Unadjusted	2.09 (0.94, 4.68)	0.072
Adjusted	2.71 (1.14, 6.44)	0.024
In-hospital death		
Unadjusted	1.26 (0.68, 2.35)	0.459
Adjusted	1.38 (0.64, 2.98)	0.406
2-Fold increase in serum creatinine, oliguria, dialysis requirement, or in-hospital death		
Unadjusted	1.88 (0.84, 4.21)	0.126
Adjusted	2.33 (0.98, 5.52)	0.056

Adjustment variables were sex, race, preexisting CKD and the APACHE II score.