Tumor Necrosis Factor Alpha Promoter Polymorphism and Severity of Acute Kidney Injury

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Key Words

TNFA gene · Polymorphism · Acute kidney injury · KIM-1 · NAG · α -GST

Abstract

Background: Tumor necrosis factor- α is a proinflammatory cytokine that has been implicated in the pathobiology of acute kidney injury (AKI). **Methods:** We explored the association of a functional polymorphism in the promoter region (rs1800629) of the *TNFA* gene with severity of AKI, as defined by level of glomerular filtration (serum cystatin C and creatinine) and tubular injury (urinary NAG, KIM-1, α -GST, and π -GST) markers, in 262 hospitalized adults. **Results:** In unadjusted analyses, compared with the GG genotype, the *TNFA* GA and AA genotype groups tended to have higher enrollment ($p = 0.08$), peak ($p = 0.004$), and discharge ($p = 0.004$) serum creatinine levels, and the AA genotype tended to have a higher enrollment serum cystatin C level ($p = 0.04$). Compared with the GG genotype, the *TNFA* GA and AA genotype groups tended to have a higher urinary KIM-1 level ($p = 0.03$), and the AA genotype group tended to have a higher urinary π -GST level ($p = 0.03$). After adjust-

ment for sex, race, age, baseline estimated glomerular filtration rate, sepsis, and dialysis requirement, compared with the GG genotype, the *TNFA* minor A-allele group had a higher peak serum creatinine of 1.03 mg/dl (0.43, 1.63; $p = 0.001$) and a higher urinary KIM-1 (relative ratio: 1.73; 95% CI: 1.16, 2.59; $p = 0.008$). The *TNFA* minor A-allele group also had a higher Multiple Organ Failure score of 0.26 (95% CI: 0.03, 0.49; $p = 0.024$) after adjustment for sex, race, age, and sepsis. **Conclusions:** The *TNFA* rs1800629 gene polymorphism is associated with markers of kidney disease severity and distant organ dysfunction among patients with AKI. Larger studies are needed to confirm these relationships.

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Introduction

Acute kidney injury (AKI) is a powerful predictor of in-hospital death and is associated with resource utilization, including prolonged hospital length of stay and increased healthcare expenditures [1–3]. AKI has also been variably linked to long-term risk of chronic kidney disease, kidney failure, and death [4, 5]. In recent years,

there has been interest in deciphering the role of genetic polymorphisms as potential determinants of adverse outcomes in patients with AKI [6–13].

In experimental settings, ischemia-reperfusion and nephrotoxic injury induce the generation of proinflammatory cytokines, which result in morphological and functional changes in glomerular endothelial and tubular epithelial cells [14–16]. Moreover, cytokines can also mediate distant organ injury [17, 18]. High circulating levels of tumor necrosis factor- α (TNF- α) have been associated with adverse clinical outcomes in patients with AKI [19]. Functionally relevant polymorphisms within the promoter region of the TNF- α (*TNFA*) gene, which affect transcriptional activity [20], have previously been linked to adverse clinical outcomes in critically ill patients [21–23], including those with AKI requiring dialysis [6]. In the present study, in a cohort of hospitalized adults with AKI, we explore the association of a functional polymorphism in the promoter region (position –308) of the *TNFA* gene (rs1800629) with kidney disease severity, including glomerular filtration markers and urinary tubular injury markers.

Methods

Study Population

Hospitalized patients with AKI were recruited from two acute care hospitals (Boston, Mass., USA) between November 2003 and January 2007. All eligible patients were 18 years or older and received in-hospital nephrology consultation for AKI. AKI was defined as a rise in serum creatinine by 0.5, 1.0, or 1.5 mg/dl from a baseline level of ≤ 1.9 , 2.0–4.9, or ≥ 5.0 mg/dl, respectively [24]. This definition was adopted prior to the development of the AKI network consensus definition [3]. Exclusion criteria were age < 18 years, pregnancy, chronic dialysis, organ transplantation within the prior year, and urinary obstruction. Institutional review board approval was granted and informed consent was obtained for each subject.

Data Collection

Medical records were reviewed prospectively to retrieve data on each subject, including demographic characteristics, coexisting conditions, hospitalization course, and outcomes. Sepsis was ascertained using the systemic inflammatory response syndrome criteria [25]. Two severity-of-illness scores were calculated, the Acute Physiology and Chronic Health Evaluation (APACHE) II score [26] and the Multiple Organ Failure (MOF) score [27]. Preexisting chronic kidney disease was defined on the basis of a baseline estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m², which was calculated using the Modification of Diet in Renal Disease study equation [28]. At enrollment, AKI was reclassified according to the AKI network staging system, using serum creatinine-based criteria [3]. For biogeographic ancestry, self-identified ethnicity was used.

DNA Extraction and Genotyping Analyses

At enrollment, EDTA-anticoagulated whole blood was collected, aliquoted into cryotubes and stored at -80°C . Genomic DNA was extracted from leukocytes using a spin column method (Qiagen Inc., Valencia, Calif., USA). A commercially available polymerase chain reaction (PCR) technique (One Lambda Inc., Canoga Park, Calif., USA) was used to analyze single nucleotide allelic variations in the promoter region of the *TNFA* gene at position –308, using sequence-specific oligonucleotide primers [29]. In brief, a Perkin-Elmer 9600 thermocycler (Perkin-Elmer-Cetus, Norwalk, Conn., USA) was used to amplify the promoter regions by PCR. An internal control primer pair was included in every PCR reaction to exclude nonspecific DNA amplification. Amplified DNA fragments were separated by agarose gel electrophoresis and stained with ethidium bromide, and the bands were visualized under ultraviolet light.

Two blinded investigators classified the *TNFA* genotypes as low (GG), intermediate (GA), and high (AA) producer genotypes, based on in vitro transfection studies using constructs of the minor allele and human studies [30].

Serum Measurement of Glomerular Filtration Markers

Creatinine was measured by a modified Jaffé method, using a Beckman DxC 800 analyzer (Beckman Coulter, Brea, Calif., USA). Plasma cystatin C was measured by immunonephelometry using the BN II System (Siemens Healthcare Diagnostics, Deerfield, Ill., USA).

Urinary Measurement of Tubular Injury Markers

Urinary NAG (N-acetyl- β -D-glucosaminidase) activity was measured by a colorimetric assay (Boehringer Mannheim, Mannheim, Germany). The inter- and intra-assay coefficient of variation was 4.3 and 6.0%, respectively. As part of a previously reported study, urinary KIM-1 was measured in a subset of patients ($n = 204$) by a microsphere-based Luminex assay [31]. The inter- and intra-assay coefficient of variation was $< 10.0\%$. α -GST and π -GST were measured by sandwich ELISA (Argutus Medical Ltd., Dublin, Ireland). The inter- and intra-assay coefficient of variation was 9.1 and 8.0% for α -GST and 7.5 and 2.0% for π -GST, respectively. All measurements were performed in duplicate. All urinary biomarker levels were normalized to urinary creatinine and expressed as mU/mg (for NAG) or ng/mg (for KIM-1, α -GST, and π -GST).

Statistical Analysis

The genotype frequencies were tested for Hardy-Weinberg equilibrium using a standard χ^2 test for any deviation of the observed frequencies. Comparisons between genotype groups were made by ANOVA and the Kruskal-Wallis test for continuous variables, and by χ^2 or Fisher's exact test for categorical variables. Continuous variables are reported as means (SD) or medians (with 25th and 75th percentile) according to their distribution. Categorical variables are reported as counts (%).

Multiple linear regression analyses were used to evaluate the association of the *TNFA* gene polymorphism with filtration (serum creatinine and cystatin C) and tubular injury markers (NAG, KIM-1, α -GST and π -GST), using dominant (i.e. one or two copies of the minor allele) and additive (per 1-allele copy increase) genetic models. NAG, KIM-1, α -GST and π -GST were log-transformed because of their skewed distribution. To account for the high proportion of levels below the detection limit for α -GST and π -GST, Tobit regression with left censoring and the log-normal distribution was used.

Table 1. Characteristics of the cohort according to the *TNFA* rs1800629 genotypes

Variable	GG (n = 182)	GA (n = 66)	AA (n = 14)	p
Age, years	65±16	66±15	70±12	0.485
Men	92 (50.6)	38 (57.6)	8 (57.1)	0.583
White ethnicity	161 (88.5)	62 (93.9)	14 (100)	0.198
BMI, kg/m ²	30±9	30±7	33±7	0.432
Coexisting conditions				
Diabetes mellitus	79 (43.4)	30 (45.5)	7 (50.0)	0.870
Heart failure	30 (16.5)	9 (13.6)	3 (21.4)	0.736
Sepsis	76 (41.8)	30 (45.5)	6 (42.9)	0.874
Baseline eGFR <60 ml/min/1.73 m ²	118 (65.9)	44 (69.8)	12 (85.7)	0.291
AKI stage				
Stage 1	85 (46.7)	23 (35.4)	4 (28.6)	0.268
Stage 2	6 (3.3)	5 (7.7)	1 (7.1)	
Stage 3	91 (50.0)	37 (56.9)	9 (64.3)	
Cause of AKI				
Ischemic	64 (35.4)	23 (34.9)	7 (50.0)	0.794
Nephrotoxic	30 (16.6)	11 (16.7)	0 (0.0)	
Sepsis	19 (10.5)	4 (6.1)	1 (7.1)	
Atheroembolic	9 (5.0)	4 (6.1)	1 (7.1)	
Other/unknown/multifactorial	59 (32.6)	24 (36.4)	5 (35.7)	
ICU admission	133 (73.1)	48 (72.7)	11 (78.6)	0.898
Assisted mechanical ventilation	46 (25.3)	13 (19.7)	3 (21.4)	0.646
APACHE II score	20±7	19±5	21±8	0.428
Enrollment serum cystatin C, mg/l	3.0±1.2	3.1±1.1	4.0±1.7	0.039
Serum creatinine, mg/dl				
Enrollment	3.4±1.6	3.9±2.0	4.0±1.6	0.084
Peak	4.0±1.9	5.2±3.9	4.8±1.9	0.004
Discharge	2.3±1.4	3.1±2.3	2.0±0.9	0.004
Urinary tubular injury marker				
KIM-1, ng/mg	2.9 (1.2, 7.4)	4.9 (2.4, 9.3)	6.7 (3.3, 9.3)	0.034
NAG, mU/mg	39.9 (17.0, 87.6)	29.6 (12.1, 76.3)	32.7 (15.7, 114.9)	0.333
α-GST, ng/mg	15.6 (6.0, 35.7)	10.7 (3.7, 29.8)	19.3 (7.9, 50.1)	0.458
π-GST, ng/mg	274.0 (97.0, 826.9)	274.5 (61.8, 1,062.5)	1,397.3 (1,164.7, 2,440.9)	0.031
Oliguria, %	33 (18.4)	14 (21.9)	4 (28.6)	0.589
Dialysis requirement	68 (37.4)	27 (40.9)	8 (57.1)	0.329
In-hospital death	39 (21.4)	13 (19.7)	5 (35.7)	0.411

Continuous variables are presented as means (SD) or medians (25th and 75th percentile), and categorical variables as n (%).

[32]. All the analyses were adjusted for sex, race, age, baseline eGFR, sepsis, and dialysis requirement. Results from the regression models are provided as a parameter estimate or relative ratio with 95% CI. All statistical analyses were performed using the SAS software (version 9.2, SAS Institute, Cary, N.C., USA). Differences were considered statistically significant at $p > 0.05$.

Results

Characteristics of the Cohort Stratified by TNFA Genotypes

Genotyping was performed on a total of 262 subjects. The test for Hardy-Weinberg equilibrium showed deviation

from expected genotype frequencies ($\chi^2 = 5.46$; $p = 0.02$). The *TNFA* gene polymorphism had a minor allele frequency of 18%. The mean age of the cohort was 66 years, 53% were men, 91% were of white ethnicity, 73% were in the intensive care unit, and 43% had sepsis. The mean baseline eGFR was 53 ml/min/1.73 m², and the mean APACHE II score was 20. At enrollment, 53% of patients had stage 3 AKI and 20% had oliguria.

The characteristics of the cohort stratified by the *TNFA* rs1800629 genotypes are shown in table 1. In brief, demographic characteristics, coexisting conditions, and disease severity measures did not differ significantly among the genotype groups, except for a higher number of failed

Table 2. Association of the *TNFA* rs1800629 polymorphism with filtration markers

Genetic model	Enrollment serum cystatin C estimate mg/l (95% CI)	p	Enrollment serum creatinine estimate mg/dl (95% CI)	p	Peak serum creatinine estimate mg/dl (95% CI)	p	Discharge serum creatinine estimate mg/dl (95% CI)	p
<i>Dominant model (A-allele vs. GG)</i>								
Unadjusted	0.23 (−0.12, 0.58)	0.193	0.52 (0.06, 0.98)	0.026	1.14 (0.47, 1.81)	0.001	0.58 (0.14, 1.03)	0.010
Adjusted age, sex, race, and baseline eGFR	0.22 (−0.12, 0.57)	0.209	0.48 (0.05, 0.91)	0.028	1.09 (0.43, 1.75)	0.001	0.44 (0.03, 0.85)	0.034
Adjusted age, sex, race, baseline eGFR, and sepsis	0.22 (−0.13, 0.56)	0.221	0.49 (0.06, 0.92)	0.025	1.10 (0.44, 1.75)	0.001	0.45 (0.04, 0.86)	0.030
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.18 (−0.15, 0.50)	0.281	0.45 (0.05, 0.86)	0.028	1.03 (0.43, 1.63)	0.001	0.43 (0.03, 0.83)	0.034
<i>Additive model (per 1 A-allele copy increase)</i>								
Unadjusted	0.29 (0.01, 0.57)	0.046	0.40 (0.04, 0.76)	0.032	0.78 (0.25, 1.32)	0.004	0.29 (−0.06, 0.65)	0.104
Adjusted age, sex, race, and baseline eGFR	0.26 (−0.02, 0.53)	0.070	0.36 (0.02, 0.69)	0.039	0.74 (0.22, 1.26)	0.006	0.18 (−0.14, 0.51)	0.263
Adjusted age, sex, race, baseline eGFR, and sepsis	0.25 (−0.03, 0.53)	0.075	0.36 (0.02, 0.70)	0.036	0.74 (0.22, 1.27)	0.005	0.19 (−0.13, 0.51)	0.244
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.20 (−0.06, 0.46)	0.127	0.31 (−0.01, 0.63)	0.055	0.65 (0.17, 1.13)	0.008	0.16 (−0.15, 0.48)	0.316

Sample size on enrollment cystatin C (n = 233), enrollment serum creatinine (n = 262), peak serum creatinine (n = 262), and discharge serum creatinine (n = 262).

organs, as defined by the MOF score, in the *TNFA* rs1800629 minor A allele (GA/AA genotype) group ($p = 0.01$; figure 1).

As shown in table 1, with regard to the filtration markers, compared to the GG genotype group, carriers of the *TNFA* rs1800629 GA and AA genotype tended to have a higher enrollment ($p = 0.08$), peak ($p = 0.004$), and discharge ($p = 0.004$) serum creatinine. Compared to the GG and GA genotype groups, carriers of the *TNFA* rs1800629 AA genotype tended to have a higher enrollment cystatin C ($p = 0.04$). In terms of urinary markers, compared to the GG genotype group, carriers of the *TNFA* rs1800629 GA and AA genotype tended to have a higher urinary KIM-1 level ($p = 0.03$), and the AA genotype group tended to have a higher urinary π -GST level ($p = 0.03$).

Association of *TNFA* Genotypes with Filtration and Tubular Injury Markers

The results of the multivariable dominant and additive genetic models are displayed in tables 2 and 3. There were some significant associations between the *TNFA* rs1800629 minor A allele and both filtration and tubular injury markers. Indeed, in the dominant models, after adjustment for sex, race, age, baseline eGFR, sepsis, and dialysis requirement, compared with the GG genotype, the *TNFA* rs1800629 minor A allele group had a higher en-

Fig. 1. MOF score stratified by the *TNFA* rs1800629 gene polymorphism. $p = 0.012$ by χ^2 test.

rollment serum creatinine ($p = 0.028$), a higher peak serum creatinine ($p = 0.001$), and a higher serum creatinine at hospital discharge ($p = 0.034$). Similar but weaker associations were observed with serum creatinine in the multivariable additive models. There was a weak association between the *TNFA* rs1800629 AA genotype group and higher enrollment serum cystatin C ($p = 0.039$) in the unadjusted additive model, which became nonsignificant in the multivariable analysis.

Table 3. Association of the *TNFA* rs1800629 polymorphism with tubular injury markers

Genetic model	Urinary NAG relative ratio (95% CI)	p	Urinary α -GST relative ratio (95% CI)	p	Urinary π -GST relative ratio (95% CI)	p	Urinary KIM-1 relative ratio (95% CI)	p
<i>Dominant model (A-allele vs. GG)</i>								
Unadjusted	0.87 (0.61, 1.25)	0.459	0.64 (0.29, 1.39)	0.259	1.21 (0.58, 2.51)	0.618	1.75 (1.16, 2.65)	0.008
Adjusted age, sex, race, and baseline eGFR,	0.86 (0.61, 1.20)	0.365	0.70 (0.32, 1.54)	0.372	1.26 (0.60, 2.64)	0.544	1.77 (1.18, 2.65)	0.006
Adjusted age, sex, race, baseline eGFR, and sepsis	0.84 (0.60, 1.17)	0.299	0.70 (0.32, 1.55)	0.378	1.24 (0.59, 2.61)	0.565	1.76 (1.18, 2.64)	0.006
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.81 (0.59, 1.12)	0.205	0.70 (0.32, 1.55)	0.381	1.23 (0.59, 2.56)	0.587	1.73 (1.16, 2.59)	0.008
<i>Additive model (per 1 A-allele copy increase)</i>								
Unadjusted	0.94 (0.70, 1.26)	0.669	0.79 (0.42, 1.47)	0.452	1.30 (0.72, 2.35)	0.384	1.61 (1.14, 2.26)	0.007
Adjusted age, sex, race, and baseline eGFR,	0.93 (0.71, 1.23)	0.625	0.83 (0.44, 1.56)	0.562	1.39 (0.77, 2.52)	0.278	1.62 (1.16, 2.26)	0.005
Adjusted age, sex, race, baseline eGFR, and sepsis	0.92 (0.70, 1.20)	0.542	0.83 (0.44, 1.57)	0.572	1.38 (0.76, 2.50)	0.294	1.61 (1.16, 2.25)	0.005
Adjusted age, sex, race, baseline eGFR, sepsis, and dialysis requirement	0.88 (0.68, 1.14)	0.347	0.84 (0.44, 1.58)	0.584	1.35 (0.74, 2.43)	0.325	1.59 (1.14, 2.21)	0.006
Sample size on urinary NAG (n = 234), α -GST (n = 243), π -GST (n = 246), and KIM-1 (n = 204).								

Similarly, in the dominant model, after adjustment for sex, race, age, baseline eGFR, sepsis, and dialysis requirement, compared with the GG genotype, the *TNFA* rs1800629 minor A allele group had a higher urinary KIM-1 ($p = 0.008$). A similar association was observed in the multivariable additive models.

In the dominant models, after adjustment for sex, race, age, and sepsis, compared with the GG genotype, the *TNFA* rs1800629 minor A-allele group had a higher MOF score of 0.26 (95% CI: 0.03, 0.49; $p = 0.024$). This association persisted in the additive model (data not shown).

Finally, sensitivity analyses restricted to white subjects did not affect any of the adjusted analyses.

Discussion

In the present study, we examined in a cohort of hospitalized adults with AKI the association between a functional polymorphism (at position -308; rs1800629) located in the promoter region of the *TNFA* gene, which is a pivotal proinflammatory cytokine, and kidney disease severity, including levels of glomerular filtration and tubular injury markers. We found that carriers of the *TNFA* rs1800629 minor A allele had higher levels of filtration markers, including higher serum creatinine and cystatin C, and higher urinary tubular injury markers, including

KIM-1 and π -GST. Carriers of the *TNFA* rs1800629 minor A allele also experienced more organ system dysfunction, as evidenced by a higher MOF score.

Studies have previously evaluated the relationship of the *TNFA* rs1800629 polymorphism with adverse outcomes in several acute clinical settings [33], including acute myocardial infarction, acute pancreatitis, and sepsis [34–36]. In a study of 603 patients, carriers of the rs1800629 *TNFA* minor A allele had significantly higher levels of biomarkers of cardiac injury, including troponin I, creatine kinase-MB, and lactate dehydrogenase [34]. These findings are consistent with our results demonstrating an independent association between carriers of the *TNFA* rs1800629 minor A allele and higher levels of filtration (serum creatinine and cystatin C) and tubular injury (KIM-1 and π -GST) markers in a cohort of patients with AKI.

In our study, we demonstrated that carriers of the *TNFA* rs1800629 minor A allele also had higher serum creatinine at hospital discharge. We can only speculate as to whether carriers of this genetic marker are at an increased long-term risk of developing chronic kidney disease. In a cohort of 231 patients with chronic kidney failure and 180 healthy matched control subjects, the *TNFA* rs1800629 minor A allele was a strong risk modifier for development of kidney failure [37]. In kidney transplant recipients, a similar association has been observed

whereby the *TNFA* rs1800629 minor A allele was associated with an increased risk of chronic allograft nephropathy [38].

We observed an association between the *TNFA* rs1800629 minor A allele and organ system dysfunction, as defined by the MOF score. A similar association has previously been observed in patients with acute pancreatitis [35], sepsis [21], and community-acquired pneumonia [36]. A recent meta-analysis demonstrated an association between the *TNFA* rs1800629 minor A allele carrier and development of sepsis [39]. The association between the *TNFA* rs1800629 minor A allele and a higher MOF score is consistent with a similar association observed in a cohort of patients with chronic kidney disease and a higher burden of comorbidities [40]. Although prior studies of patients with sepsis or AKI requiring dialysis demonstrated an association between the *TNFA* rs1800629 minor A allele carrier and an adjusted increased risk of death [6, 21], we were unable to demonstrate a similar association.

To our knowledge, this is the first study that examines the association between a functionally relevant polymorphism in the *TNFA* gene and AKI disease severity, as measured by levels of filtration and tubular injury markers. The heterogeneity of the cohort was offset by the selective inclusion of subjects with more advanced AKI requiring formal nephrology consultation. Although sizeable, our cohort was relatively small for the purpose of genetic studies; however, the participants were 91% white, which reduced the potential impact of race and ethnicity on genotype prevalence. There are, however, several study limitations that should be noted. The *TNFA* rs1800629 polymorphism did not fulfill the Hardy-Weinberg equilibrium, which might be due in part to the relatively modest sample size of our study or natural selection. We did not have a second cohort to validate our findings in a replication cohort. Of the 103 patients (39%) who required renal replacement therapy, 36 initiated dialysis prior to study enrollment (median of 1 day prior to study enrollment), which might have confounded the association of the *TNFA* rs1800629 polymorphism with serum creatinine and cystatin C. To account for this confounding effect, we adjusted our analyses for dialysis requirement, which did not markedly affect the effect sizes. In our cohort, we had a higher prevalence rate of preexisting CKD, as defined by $\text{eGFR} < 60 \text{ ml/min/1.73 m}^2$ due to the selection criteria used to identify eligible patients with AKI, mainly the criteria of Hou et al. [24], which require higher absolute rises in serum creatinine according to the baseline serum creatinine value. All our multivariable

analyses, however, are adjusted for the baseline eGFR, which did not markedly attenuate the effect sizes. Finally, although we found an association with AKI-related surrogate endpoints, we were unable to demonstrate an association with hard clinical endpoints.

In conclusion, the present study explores the complex nature of how a functionally relevant genetic variant in the *TNFA* gene might influence severity of kidney injury in patients with AKI, and supports the hypothesis that the study of polymorphisms of host genes as genetic risk markers in the setting of acute illnesses such as AKI has merit. Additional studies are needed to establish the mechanisms underlying the influence of the identified *TNFA* gene polymorphism on AKI disease severity traits.

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Disclosure Statement

Dr. Bonventre is a coinventor on KIM-1 patents that are assigned to Partners Healthcare and licensed by Partners to Johnson and Johnson, Sekisui, Biogen Idec, R&D, and BioAssayWorks. Dr. Bonventre is also a consultant for Sekisui. The other authors declare that they have no financial interests relevant to this work.

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