Association of a polymorphism of *BTN2A1* with chronic kidney disease in community-dwelling individuals

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Abstract. Results of recent studies have shown that the C→T polymorphism (rs6929846) of the butyrophilin, subfamily 2, member A1 gene (*BTN2A1*) was significantly associated with myocardial infarction. The aim of the current study was to examine the association of rs6929846 of *BTN2A1* with chronic kidney disease (CKD) in community-dwelling individuals. Study subjects comprised 1,709 community-dwelling individuals, including 435 subjects with CKD [estimated glomerular filtration rate (eGFR) <60 ml/min per 1.73 m^2] and 1,274 controls (eGFR≥90 ml/min per 1.73 m^2) who were recruited to a population-based cohort study. Genotype distributions (P=0.0010) and allele frequencies (P=0.0002) of rs6929846 were significantly associated with CKD. Multivariate logistic regression analysis with adjustment for covariates revealed that the rs6929846 of *BTN2A1* was significantly (P=0.0002; odds ratio, 2.02; dominant model) associated with CKD, with the minor T allele representing a risk for this condition. The serum concentrations of creatinine were significantly (P=0.0107) higher for all the individuals, whereas eGFR was significantly (P=0.0468) lower for individuals in the combined group of CT and TT genotypes compared to those with the CC genotype. *BTN2A1* may therefore be a susceptibility gene for CKD.

Introduction

Chronic kidney disease (CKD) is a growing public health concern worldwide, considering that individuals with CKD are at increased risk for end-stage renal disease as well as for cardiovascular diseases and premature death (1-3). For the purpose of improvement of prognosis and cost-effectiveness, aggressive prevention and early detection is imperative as a global approach to CKD (4).

In Japan, a nationwide health screening program was begun in order to control lifestyle-related diseases, particularly metabolic syndrome in 2008 (5). Results of previous studies showed that use of dipstick urinalysis to detect proteinuria but was inadequate in the routine screening for the detection of early stage CKD due to insufficient sensitivity and specificity (6). In the general population, screening combined with serum concentrations of creatinine or other biomarkers is therefore likely to be appropriate and cost-effective for the stratification of high-risk individuals (7). Identification of genetic variants that confer susceptibility to CKD may thus be important in determining which patients are recommended for frequent checkup.

Mounting evidence indicates that hypertension, diabetes mellitus, and dyslipidemia are major risk factors for the development and progression of CKD (8-11). In addition to these conventional risk factors, genetic epidemiological studies have demonstrated that genetic factors are key in the development of CKD in the general population (12,13). Although recent genome-wide association studies (GWASs) identified various loci and genes indicating a predisposition to CKD (14,15), the genes that contribute to genetic susceptibility to CKD in Japanese individuals remain to be identified definitively.

In a recent study, we showed that the C→T polymorphism (rs6929846) in the butyrophilin, subfamily 2, member A1 gene (*BTN2A1*) was significantly associated with myocardial infarction in Japanese individuals by a GWAS (16). Since CKD is considered an important risk factor for coronary heart disease, we hypothesized that the association of rs6929846 of *BTN2A1* with myocardial infarction might be partially attributable to its effects on susceptibility to CKD. Therefore, an association study for rs6929846 of *BTN2A1* and CKD was performed in community-dwelling Japanese individuals to provide a basis for the personalized prevention of this condition.

Materials and methods

Study population. The study population comprising 1,709 community-dwelling Japanese individuals (435 subjects

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Table I. Characteristics of study subjects.

Characteristics	CKD	Controls	P-value
No. of subjects	435	1,274	
Age (years)	65.5±9.6	46.4±11.5	<0.0001
Gender (male/female, %)	66.2/33.8	49.1/50.9	<0.0001
Body mass index (kg/m^2)	23.4±3.1	22.7±3.7	<0.0001
Current or former smoker (%)	32.9	41.4	0.0017
Hypertension (%)	57.5	19.2	<0.0001
Systolic blood pressure (mmHg)	125±17	117±15	<0.0001
Diastolic blood pressure (mmHg)	76±12	72±12	<0.0001
Dyslipidemia (%)	61.2	39.1	<0.0001
Serum total cholesterol (mmol/l)	5.16±0.91	5.02±0.84	0.0475
Serum triglycerides (mmol/l)	1.38±0.81	1.20±0.99	<0.0001
Serum HDL-cholesterol (mmol/l)	1.54±0.43	1.68±0.45	<0.0001
Serum LDL-cholesterol (mmol/l)	3.17±0.79	3.08±0.83	0.0157
Diabetes mellitus (%)	16.6	8.7	<0.0001
Fasting plasma glucose (mmol/l)	5.69±1.03	5.58±1.52	<0.0001
Blood glycosylated hemoglobin (%)	5.9±0.6	5.7±0.8	<0.0001
Blood urea nitrogen (mmol/l)	6.32±1.81	4.50±1.18	<0.0001
Serum creatinine (μmol/l)	79.3±20.8	45.3±7.9	<0.0001
eGFR (ml/min per 1.73 m^2)	52.8±7.5	100.6±9.7	<0.0001

Quantitative data are means ± standard deviation. CKD, chronic kidney disease HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate.

with CKD and 1,274 controls) were recruited to a population-based cohort study in Inabe City (Mie Prefecture, Japan) during the period of March 2010 and September 2012.

Estimated glomerular filtration rate (eGFR) was calculated with the use of the simplified prediction equation derived from the modified version of that described in the Modification of Diet in Renal Disease (MDRD) study as proposed by the Japanese Society of Nephrology (17): eGFR (ml/min per 1.73 m^2) = 194 x [age (years)]$^{-0.287}$ x [serum creatinine (mg/dl)]$^{-1.094}$ x [0.739 if female]. The National Kidney Foundation-Kidney Disease Outcomes Quality Initiative guidelines recommend a diagnosis of CKD if eGFR is <60 ml/min per 1.73 m^2 (18). On the basis of this criterion, 435 subjects (288 males and 147 females) were diagnosed with CKD. The control subjects comprised 1,274 individuals (625 males and 649 females) whose eGFR was ≥90 ml/min per 1.73 m^2. Control individuals had no renal disease or major health problems. Subjects with CKD and controls had or did not have conventional risk factors for CKD, including hypertension (systolic blood pressure of ≥140 mmHg, diastolic blood pressure of ≥90 mmHg, or taking antihypertensive medication), diabetes mellitus (fasting plasma glucose of ≥6.93 mmol/l, blood hemoglobin A1c content of ≥6.9%, or taking antidiabetes medication) or dyslipidemia (a serum concentration of triglycerides of ≥1.65 mmol/l, a serum high-density lipoprotein (HDL)-cholesterol of <1.04 mmol/l, a serum low-density lipoprotein (LDL)-cholesterol of ≥3.64 mmol/l or had taken antidyslipidemic medication).

The study protocol complied with the Declaration of Helsinki and was approved by the ethics committees of Human Research of Mie University Graduate School of Medicine and Inabe General Hospital. Written informed consent was obtained from each subject.

Genotyping of a polymorphism. Venous blood (5 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), peripheral blood leukocytes were isolated, and genomic DNA was extracted from these cells using a DNA extraction kit (SMITEST EX-R&D; Medical & Biological Laboratories Co., Ltd., Nagoya, Japan). Genotypes of rs6929846 of *BTN2A1* were determined at G&G Science Co., Ltd. (Fukushima, Japan) by the multiplex bead-based Luminex assay, a method that combines polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX, USA). Genotyping involved PCR amplification, hybridization, streptavidin-phycoerythrin reaction, and measurement of fluorescence. The detailed genotyping methodology was described in previous studies (16,19).

Statistical analysis. Quantitative data were compared between subjects with CKD and controls using the unpaired Student's t-test. The Chi-square test was used to compare categorical data. Allele frequencies were estimated by the gene counting method. The Chi-square test was used to identify deviations from the Hardy-Weinberg equilibrium as well as genotype distributions and allele frequencies of rs6929846 of *BTN2A1* between subjects with CKD and controls. Multivariate logistic regression analysis was carried out with CKD as a dependent variable. Independent variables including age, gender

Table II. Comparison of genotype distributions and allele frequencies of rs6929846 in butyrophilin, subfamily 2, member A1 gene by the Chi-square test between subjects with chronic kidney disease (CKD) and controls.

Genotypes	CKD (%)	Controls (%)	P-value (genotype)	P-value (allele)
rs6929846			0.0010	0.0002
CC	327 (75.2)	1,056 (82.9)		
CT	100 (23.0)	208 (16.3)		
TT	8 (1.8)	10 (0.8)		
Hardy-Weinberg P-value	0.9112	0.9449		

Table III. Multivariate logistic regression analysis of rs6929846 in butyrophilin, subfamily 2, member A1 gene and chronic kidney disease.

	Dominant		Recessive		Additive 1		Additive 2	
Genotype	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
rs6929846	**0.0002**	2.02 (1.40-2.92)	0.2918		**0.0003**	2.00 (1.38-2.91)	0.2184	

OR, odds ratio; CI, confidence interval. Multivariate logistic regression analysis was performed with adjustment for age, gender, body mass index, smoking status, and the prevalence of hypertension, diabetes mellitus and dyslipidemia. P<0.05 is shown in bold.

Table IV. Association of rs6929846 in butyrophilin, subfamily 2, member A1 gene (*BTN2A1*) to the serum concentration of creatinine and estimated glomerular filtration rate (eGFR) in all subjects.

	BTN2A1 genotype		
Parameters	CC	CT + TT	P-value
Serum creatinine (μmol/l)	53.3±18.2	56.9±23.6	0.0107
eGFR (ml/min per 1.73 m^2)	89.3±22.3	85.0±24.5	0.0468

(0, female; 1, male), body mass index (BMI), smoking status (0, non-smoker; 1, current or former smoker), the prevalence of hypertension, diabetes mellitus, or dyslipidemia (0, no history of these conditions; 1, positive history), and *BTN2A1* genotype, as well as P-values, odds ratios, and 95% confidence intervals were also calculated. The *BTN2A1* genotype was assessed according to dominant, recessive, and additive genetic models. Additive models included additive 1 (heterozygotes vs. wild-type homozygotes) and additive 2 (variant homozygotes vs. wild-type homozygotes) models, which were analyzed simultaneously using a single statistical model.

P<0.05 was considered to indicate a statistically significant difference. Statistical significance was examined by two-sided tests performed with JMP Genomics version 6.0 software (SAS Institute, Cary, NC, USA).

Results

The baseline characteristics of the subjects are shown in Table I. Age, the frequency of being male, BMI, the prevalence of hypertension, dyslipidemia, and diabetes mellitus, as well as serum concentrations of blood urea nitrogen and creatinine were higher, whereas the prevalence of smoking and eGFR was lower, in subjects with CKD than in controls.

Using the Chi-square test, a comparison of genotype distributions and allele frequencies was performed, revealing that rs6929846 of *BTN2A1* was significantly (P<0.05) associated with CKD (Table II). The frequencies of the risk T allele were 0.133 and 0.089 in subjects with CKD and controls, respectively. Genotype distributions were in the Hardy-Weinberg equilibrium for subjects with CKD and controls.

Multivariate logistic regression analysis was performed with adjustment for age, gender, BMI, smoking status, and the prevalence of hypertension, diabetes mellitus, and dyslipidemia. The results demonstrated that rs6929846 of *BTN2A1* was significantly associated with CKD (dominant and additive 1 models), with the minor T allele representing a risk factor for CKD (Table III).

The association of rs6929846 of *BTN2A1* to the serum concentration of creatinine and eGFR was examined for all the individuals (Table IV). The serum concentration of creatinine was significantly higher, whereas eGFR was significantly

lower for individuals in the combined group of CT and TT genotypes than for those with the CC genotype.

Discussion

Results of the present study have shown that rs6929846 of *BTN2A1* was significantly associated with the prevalence of CKD in community-dwelling Japanese individuals, with the minor T allele representing a risk factor for this condition. Previously, we showed that rs6929846 of *BTN2A1* was significantly associated with CKD in a different hospital-based study population (20). The present results in the population-based study were consistent with the previous observations in the hospital-based study (20) and validate the association of rs6929846 in *BTN2A1* with CKD.

The *BTN2A1* belongs to the BTN superfamily, and is a cell surface transmembrane glycoprotein that has roles in lipid, fatty acid, and sterol metabolism (Entrez Gene, NCBI). Although the butyrophilin family was originally identified on the basis of its ability to promote the production of milk fat globules (21), many butyrophilin and butyrophilin-like family of proteins were shown to regulate immune function, and polymorphisms in the coding sequences were related to predisposition to inflammatory diseases (22). In a previous study, we showed that the T allele of rs6929846 of *BTN2A1* was associated with an increased risk for myocardial infarction (16). The T allele of rs6929846 was associated with an increased transcription activity of *BTN2A1*, and the serum concentrations of high sensitivity C-reactive protein were significantly greater in individuals in the combined group of CT and TT genotypes than in those with the CC genotype among healthy individuals without neoplastic, infectious, or inflammatory disease (23). These observations suggest that the T allele of rs6929846 of *BTN2A1* may accelerate inflammatory processes.

Renal tubulointerstitial damage is considered a common feature in CKD (24). Findings of previous studies have shown that chronic inflammation is crucial in the promotion of interlinked fibrosis and cell injury within the tubulointerstitium, and macrophages initially mediate this inflammatory process (25). In addition, infiltrating macrophages in response to glomerular and tubular injury lead to the generation of proinflammatory cytokines (interleukin 1, β; tumor necrosis factor; and transforming growth factor, β 1), vasoactive eicosanoids, and reactive oxygen species (26,27). This vicious cascade accelerates structural and functional damage, leading to the deterioration of renal function. Concerning the role of chronic inflammation in the pathogenesis of CKD, the association of rs6929846 of *BTN2A1* with CKD may be attributable to acceleration of the inflammatory process by the T allele of this polymorphism.

There are some limitations to our study: i) given that the study subjects comprised only Japanese individuals, validation of our findings will required in other ethnic groups; ii) we used eGFR instead of a directly measured rate to define CKD. Although the equation of eGFR has not been validated in individuals >70 years, the best estimate of GFR for elderly Japanese individuals has not been determined; iii) we were not able to obtain information on the pathological cause of CKD in a substantial proportion of the subjects with this condition. Such information can be obtained by detailed clinical examination, including renal biopsy, however, such diagnostic procedures are not considered feasible for a genetic epidemiological study; and iv) the molecular mechanisms underlying the effects of rs6929846 of *BTN2A1* on the development of CKD have not been determined.

In conclusion, the present results suggest that *BTN2A1* is a susceptibility gene for CKD in community-dwelling Japanese individuals. Determination of the genotype for rs6929846 of *BTN2A1* may prove informative for assessment of the genetic risk for CKD in the Japanese population.

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