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Association of a polymorphism of *BCHE* with ischemic stroke in Japanese individuals with chronic kidney disease

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Abstract. Although chronic kidney disease (CKD) is an important risk factor for ischemic stroke, the genetic variants that confer susceptibility to ischemic stroke in individuals with CKD remain largely unknown. We performed an association study for candidate gene polymorphisms and ischemic stroke in individuals with CKD. The study population comprised 1041 Japanese individuals with CKD, including 228 subjects with ischemic stroke and 813 controls. The genotypes of 150 polymorphisms of 127 candidate genes were determined by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology. An initial χ^2 test (false discovery rate <0.05) and subsequent multivariate logistic regression analysis with adjustment for covariates (P<0.05) revealed that the 1615G→A (Ala539Thr) polymorphism (rs1803274) of *BCHE* (OR=3.33; 95% CI 1.32-8.28) and the 2445G→A (Ala54Thr) polymorphism (rs1799883) of *FABP2* (OR=1.66; 95% CI 1.01-2.70) were significantly associated with ischemic stroke. The variant alleles of *BCHE* and *FABP2* were risk factors for ischemic stroke. A stepwise forward selection procedure demonstrated that the *BCHE* genotype was a significant (P<0.05) and independent determinant of ischemic stroke. Genotyping for *BCHE* may prove informative for the assessment of the genetic risk of ischemic stroke in Japanese individuals with CKD.

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Introduction

The prevalence of chronic kidney disease (CKD) is gradually increasing due to population aging, and is predicted to affect as much as 20% of the general adult population of Japan (1). CKD is well documented as a striking risk factor for cardiovascular disease, including ischemic stroke-related mortality, in selected and general populations (2-6). In a cohort study, at least 35% of subjects with CKD had cardiovascular disease at the time of presentation to a nephrologist (7). Even during the early stages of CKD, approximately 10-20% of subjects die due to cardiovascular disease (8). Although genetic epidemiological studies have identified several genetic variants as potential risk factors for ischemic stroke (9-14), genetic factors underlying the predisposition to ischemic stroke in individuals with CKD remain largely unknown. The identification of genetic markers of disease may thus be essential for the risk prediction of future cardiovascular events and the reduction of the mortality rate.

We performed an association study for 150 polymorphisms of 127 candidate genes and ischemic stroke in 1041 Japanese individuals with CKD. The aim of the study was to identify genetic variants that confer susceptibility to ischemic stroke in individuals with CKD, and thereby to provide a basis for the personalized prevention of this condition in such individuals.

Materials and methods

Study population. The study population comprised 1041 unrelated Japanese individuals (566 men, 475 women) who visited the outpatient clinics of or were admitted to one of five participating hospitals (Gifu Prefectural General Medical Center, Gifu Prefectural Tajimi Hospital, Gifu, Japan; Hirosaki University Hospital, Reimeikyo Rehabilitation Hospital and Hirosaki Stroke Center, Aomori, Japan) between October 2002 and March 2008. Patients were seeking medical treatment for various symptoms or were admitted for an annual health

checkup, or were recruited to a population-based prospective cohort study of aging and age-related diseases in Gunma and Tokyo, Japan. The estimated glomerular filtration rate (eGFR) was calculated using the simplified prediction equation proposed by the Japanese Society of Nephrology as previously described in a study on the modification of diet in renal disease (15): eGFR (ml min^{-1} 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's Kidney Disease Outcomes Quality Initiative guidelines recommend a diagnosis of CKD if the eGFR is <60 ml min^{-1} 1.73 m^{-2} (16). On this basis, all 1041 subjects in the present study were diagnosed with CKD.

Among the 1041 subjects, 228 individuals (157 men, 71 women) were additionally diagnosed with ischemic stroke. The diagnosis of ischemic stroke was based on the occurrence of a new and abrupt focal neurological deficit with neurological symptoms and signs persisting for >24 h, and was confirmed by positive findings in computed tomography or magnetic resonance imaging (or both) of the head. The type of stroke was determined according to the Classification of Cerebrovascular Diseases III (17). Individuals with cardiogenic embolic infarction, lacunar infarction alone, transient ischemic attack, moyamoya disease or cerebral venous sinus thrombosis were excluded from the study, as were those with atrial fibrillation in the absence or presence of valvular heart disease.

The 813 control subjects (409 men, 404 women) were recruited from community-dwelling individuals or patients that regularly visited outpatient clinics for the treatment of various common diseases. They had no history of ischemic or hemorrhagic stroke or other cerebral diseases, of coronary heart disease, aortic disease including thoracic or abdominal aortic aneurysm, peripheral arterial occlusive disease or other atherosclerotic diseases, or of other thrombotic, embolic or hemorrhagic disorders. The study protocol complied with the Declaration of Helsinki and was approved by the Committees on the Ethics of Human Research of Mie University Graduate School of Medicine, Hirosaki University Graduate School of Medicine, Gifu International Institute of Biotechnology, Tokyo Metropolitan Institute of Gerontology, and participating hospitals. Written informed consent was obtained from each participant.

Selection and genotyping of polymorphisms. By referring to public databases including PubMed (NCBI), we selected 127 candidate genes that were characterized and suggested to be associated with ischemic stroke. On the basis of published studies or by searching PubMed and single nucleotide polymorphism (SNP) databases [dbSNP (NCBI) and Japanese SNP (JSNP)], we further selected 150 polymorphisms of these genes, most located in the promoter region or exons, that might be expected to result in changes in the function or expression of the encoded protein (data not shown).

Venous blood (7 ml) was collected into tubes containing 50 mmol/l EDTA (disodium salt), and genomic DNA was isolated with a Genomix kit (Talent, Trieste, Italy). Genotypes of the 150 polymorphisms were determined at G&G Science (Fukushima, Japan) by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex, Austin, TX). Primers, probes, and other conditions for the genotyping of polymorphisms related (with a P-value for allele frequency of

Table I. Primers, probes, and other conditions for the genotyping of polymorphisms related to ischemic stroke in individuals with chronic kidney disease.[a]

Gene	Polymorphism	Sense primer (5'→3')	Antisense primer (5'→3')	Probe 1 (5'→3')	Probe 2 (5'→3')	Annealing (°C)	Cycles
BCHE	1615G→A (Ala539Thr)	TACAACTTATTCCATATTTTACAGGA	TGTAATTGTTCCAGCGATGGAATC	CACTCCCATTCTGCTTCATCA	CACTCCCATTCTGTTTCATCAAT	60	50
FABP2	2445G→A (Ala54Thr)	AGCTGACAATTACACAAGAAGGAA	GTTGTAATTAAAGGTGACACCAAG	AATGTTTCGAAAAGCGCTTGATT	TCAAAGAATCAAGCACTTTTCGA	60	50
COL1A2	G→C (Ala459Pro)	GGTGTTCAAGGTGGAAAAGGTGA	GTATATGCTTGAGTTGACTTACCTG	CTGGAGGACCAGGGGGACCC	CTGGAGGACCAGCGGGAC	60	50
IL6	-572G→C	GGAGACGCCTTGAAGTAACTGC	GAAGGTAATACTACCAGTCATCTG	ACAACAGCCGCTCACAGGGA	TCTACAACAGCCCCTCACAGG	60	50
AKAP10	2073A→G (Ile646Val)	GGCCCAGGAAGAGCTAGCTTG	GTAGATTTCTCTAACGGTTGATCAT	GATAGTCAGTGACATTATGCAG	CCTGCTGCATAACGTCACTG	60	50
TNFSF4	A→G	TAATTGCCTGATCAAACACATTAC	ACTTTGAAGCTTTGAGTCACTGAT	CTGGTCTACCCATTGTGATAG	CTGGTCTACCCACTGTGATAG	60	50
F3	-603A→G	TCTCCTGTGCGACCCGCTAAG	AGCCACGGTGGCTTCTTCTAC	GTGGGCAGGCCAAGTATTCT	AGGTCAAGAATACCTGGCCT	60	50
STX1A	T→C (Asp68Asp)	AAGCGGAAGCACAGTGCCATC	GAGGCTTGTGGGGCCTGAAAC	CACACTCACTCTCATCGGG	CCAACCCCGACGAGAGTGA	60	50
PPP1R3A	G→T (Try905Asp)	AACAGACTCGGATGCCATTGTG	TTGACACTGAAATTTCAGTATGATG	ATTAGTGTCTGAGTTAAAAGCA	CTCTATTAGTGTATGAGTTAAA	60	50

[a]Allele frequency determined by the χ^2 test, P<0.01.

Table II. Characteristics of subjects with ischemic stroke and controls among individuals with chronic kidney disease.

Characteristic	Ischemic stroke	Controls	P-value
No. of subjects	228	813	
Age (years)	72.6±7.5	70.5±9.1	0.0006
Gender (male/female, %)	68.9/31.1	50.3/49.7	<0.0001
BMI (kg/m^2)	23.3±3.3	23.6±3.5	0.1980
Current or former smoker (%)	17.1	24.9	0.0143
Hypertension (%)	85.1	50.7	<0.0001
Systolic blood pressure (mmHg)	153±28	138±22	<0.0001
Diastolic blood pressure (mmHg)	83±16	78±13	0.0004
Hypercholesterolemia (%)	45.6	30.6	<0.0001
Serum total cholesterol (mmol/l)	5.34±1.12	5.17±0.96	0.0494
Serum triglyceride (mmol/l)	1.74±1.14	1.65±1.02	0.3133
Serum HDL-cholesterol (mmol/l)	1.30±0.39	1.45±0.41	<0.0001
Diabetes mellitus (%)	49.1	18.9	<0.0001
Fasting plasma glucose (mmol/l)	7.21±2.85	6.43±2.87	0.0008
Glycosylated hemoglobin (%)	6.12±1.40	5.54±1.15	0.0002
Serum creatinine (μmol/l)	96.9±88.9	81.2±58.2	0.0126
eGFR (ml min^{-1} 1.73 m^{-2})	47.5±10.8	50.9±8.8	<0.0001
Myocardial infarction (%)	20.6	0	<0.0001

Quantitative data are the means ± SD. Hypertension: systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or use of antihypertensive medication. Hypercholesterolemia: serum total cholesterol ≥5.72 mmol/l (220 mg/dl), or use of lipid-lowering medication. Diabetes mellitus: fasting blood glucose ≥6.93 mmol/l (126 mg/dl), glycosylated hemoglobin (hemoglobin A1c) content ≥6.5%, or use of antidiabetic medication. BMI, body mass index; HDL, high density lipoprotein; eGFR, estimated glomerular filtration rate.

<0.01) to ischemic stroke are shown in Table I. Genotyping methodology was as previously described (18).

Statistical analysis. Quantitative data were compared between subjects with ischemic stroke and controls by the unpaired Student's t-test. Categorical data were compared by the χ^2 test. Allele frequencies were estimated by the gene counting method, and the χ^2 test was used to identify departures from Hardy-Weinberg equilibrium. In an initial screen, the genotype distributions (3x2) and allele frequencies (2x2) of each polymorphism were compared between subjects with ischemic stroke and controls by the χ^2 test. Given the multiple comparison of genotypes, the false discovery rate (FDR) was calculated from the distribution of P-values for allele frequencies of the 150 polymorphisms (19,20). Polymorphisms with an FDR of <0.05 were further examined by multivariate logistic regression analysis with adjustment for covariates that differed significantly between subjects with ischemic stroke and controls. Multivariate logistic regression analysis was thus performed with ischemic stroke as a dependent variable and with independent variables including age, gender (0 = woman, 1 = man), smoking status (0 = non-smoker, 1 = smoker), the serum concentration of creatinine, the prevalence of hypertension, diabetes mellitus and hypercholesterolemia (0 = no history of these conditions, 1 = positive history) and the genotype of each polymorphism. The P-value, odds ratio (OR) and 95% confidence interval (CI) were then calculated. Each genotype was assessed according to dominant, recessive and additive genetic models. Additive models included the additive 1 model (heterozygotes vs. wild-type homozygotes) and additive 2 model (variant homozygotes vs. wild-type homozygotes), and were analyzed simultaneously using a single statistical model. A stepwise forward selection procedure was also performed to examine the effects of genotypes and other covariates on ischemic stroke. In the stepwise forward selection procedure, each genotype was examined according to a dominant or recessive model on the basis of statistical significance determined in the multivariate logistic regression analysis. With the exception of the initial screen by the χ^2 test (FDR <0.05), a P-value of <0.05 was considered statistically significant. Statistical significance was examined by two-sided tests performed using JMP version 5.1 software and JMP Genomics version 3.2 software (SAS Institute, Cary, NC).

Results

The characteristics of the 1041 study subjects are shown in Table II. Age, the number of men, prevalence of hypertension, diabetes mellitus and hypercholesterolemia, myocardial infarction, systolic and diastolic blood pressure, serum concentration of total cholesterol and creatinine, fasting plasma glucose levels and blood glycosylated hemoglobin content were greater, whereas the prevalence of smoking, the serum concentration of HDL-cholesterol, and eGFR were lower, in subjects with ischemic stroke compared to the controls.

Evaluation of allele frequencies by the χ^2 test revealed that nine polymorphisms were related to the prevalence of ischemic

Table III. Genotype distributions of polymorphisms related to ischemic stroke in individuals with chronic kidney disease.[a]

Gene symbol	Polymorphism	dbSNP	Ischemic stroke (%)	Controls (%)	P-value (genotype)	P-value (allele frequency)	FDR (allele frequency)
BCHE	1615G→A (Ala539Thr)	rs1803274			0.0024	0.0004	0.0475
	GG		143 (62.7)	600 (73.8)			
	GA		73 (32.0)	192 (23.6)			
	AA		12 (5.3)	21 (2.6)			
FABP2	2445G→A (Ala54Thr)	rs1799883			0.0031	0.0006	0.0475
	GG		74 (32.4)	346 (42.6)			
	GA		108 (47.4)	363 (44.6)			
	AA		46 (20.2)	104 (12.8)			
COL1A2	G→C (Ala459Pro)	rs42524			0.0006	0.0034	0.1463
	GG		202 (88.6)	764 (94.0)			
	GC		25 (11.0)	49 (6.0)			
	CC		1 (0.4)	0 (0)			
IL6	-572G→C	rs1800796			0.0047	0.0039	0.1463
	GG		11 (4.8)	47 (5.8)			
	GC		61 (26.8)	307 (37.8)			
	CC		156 (68.4)	459 (56.4)			
AKAP10	2073A→G (Ile646Val)	rs203462			0.0194	0.0050	0.1487
	AA		160 (70.2)	496 (61.0)			
	AG		63 (27.6)	274 (33.7)			
	GG		5 (2.2)	42 (5.2)			
TNFSF4	A→G	rs3850641			0.0266	0.0064	0.1492
	AA		169 (74.1)	667 (82.1)			
	AG		54 (23.7)	136 (16.7)			
	GG		5 (2.2)	10 (1.2)			
F3	-603A→G	rs1361600			0.0227	0.0070	0.1492
	AA		133 (58.3)	540 (66.4)			
	AG		79 (34.7)	243 (29.9)			
	GG		16 (7.0)	30 (3.7)			
STX1A	T→C (Asp68Asp)	rs2293485			0.0120	0.0080	0.1505
	TT		77 (33.8)	322 (39.6)			
	TC		102 (44.7)	380 (46.7)			
	CC		49 (21.5)	111 (13.7)			
PPP1R3A	G→T (Tyr905Asp)	rs1799999			0.0316	0.0096	0.1603
	GG		13 (5.7)	82 (10.1)			
	GT		91 (39.9)	356 (43.8)			
	TT		124 (54.4)	375 (46.1)			

[a]Allele frequency determined by the χ^2 test, P<0.01. FDR, false discovery rate.

stroke (P-value for allele frequency <0.01). Among these polymorphisms, the 1615G→A (Ala539Thr) polymorphism (rs1803274) of the butyrylcholinesterase gene (*BCHE*) and the 2445G→A (Ala54Thr) polymorphism (rs1799883) of the fatty acid binding protein 2, intestinal gene (*FABP2*) were significantly (FDR for allele frequency <0.05) associated with the prevalence of ischemic stroke (Table III). The genotype distributions for all nine polymorphisms related to ischemic stroke are also shown in Table III, and were in Hardy-Weinberg equilibrium in subjects with ischemic stroke and in controls (Table IV).

Table IV. Hardy-Weinberg P-values in subjects with ischemic stroke and controls.

Gene	Polymorphism	Ischemic stroke	Controls
BCHE	1615G→A (Ala539Thr)	0.5057	0.2361
FABP2	2445G→A (Ala54Thr)	0.5648	0.5647
COL1A2	G→C (Ala459Pro)	0.8114	0.3756
IL6	-572G→C	0.1253	0.6442
AKAP10	2073A→G (Ile646Val)	0.6779	0.6039
TNFSF4	A→G	0.7800	0.3089
F3	-603A→G	0.3705	0.6825
STX1A	T→C (Asp68Asp)	0.1668	0.9472
PPP1R3A	G→T (Try905Asp)	0.4853	0.8531

Table VI. Effects of genotypes and other characteristics on ischemic stroke in individuals with chronic kidney disease.[a]

Variable	P-value	R^2
Hypertension	<0.0001	0.0877
Diabetes mellitus	<0.0001	0.0491
Gender	<0.0001	0.0179
Smoking	0.0002	0.0127
Age	0.0004	0.0115
Hypercholesterolemia	0.0004	0.0113
BCHE (*AA* vs. *GG* + *GA*)	0.0192	0.0050

[a]P<0.05, determined by a stepwise forward selection procedure. R^2, contribution rate.

Multivariate logistic regression analysis with adjustment for age, gender, smoking status, serum concentration of creatinine, and the prevalence of hypertension, diabetes mellitus and hypercholesterolemia revealed that the 1615G→A (Ala539Thr) polymorphism of *BCHE* (dominant, recessive and additive 2 models) and the 2445G→A (Ala54Thr) polymorphism of *FABP2* (additive 2 model) were significantly (P<0.05) associated with ischemic stroke (Table V). The variant alleles of *BCHE* and *FABP2* were risk factors for ischemic stroke.

For the polymorphisms associated with ischemic stroke by multivariate logistic regression analysis, a stepwise forward selection procedure was performed to examine the effects of genotypes and of age, gender, smoking status, the serum concentration of creatinine, and the prevalence of hypertension, diabetes mellitus and hypercholesterolemia on ischemic stroke (Table VI). Hypertension, diabetes mellitus, gender, smoking, age, hypercholesterolemia and *BCHE* genotype (recessive model) were, in descending order, statistically significant (P<0.05) and independent determinants of ischemic stroke.

Finally, the effects of the 1615G→A (Ala539Thr) polymorphism of *BCHE* on intermediate phenotypes, including serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides, fasting plasma glucose levels, blood glycosylated hemoglobin content and systolic and diastolic blood pressure (Table VII) were examined. Analysis revealed the *BCHE* genotype to be significantly associated with systolic blood pressure, while the AA genotype was related to lower blood pressure.

Discussion

We examined the possible relationship between 150 polymorphisms in 127 candidate genes and the prevalence of ischemic stroke in 1041 Japanese individuals with CKD. This association study revealed that the 1615G→A (Ala539Thr) polymorphism of *BCHE* was significantly associated with the prevalence of ischemic stroke in such individuals.

Butyrylcholinesterase (BCHE) is a serine hydrolase that has key biological functions. It is synthesized in the liver, and is widely expressed in several organs and tissues, including the nervous system (21). Recent studies have shown serum BCHE activity to be increased in individuals with diabetes mellitus, hypertension, hypercholesterolemia and hypertriglyceridemia, which are constituents of metabolic syndrome (22-25). As evidenced by enhanced inflammation due to the inactivation of acetylcholine, increased BCHE activity is also found in individuals with Alzheimer's disease, and could serve as a marker for the prediction of disease prognosis (26,27). Although serum BCHE activity was positively related to cardiovascular risk factors, it was inversely related to cardiovascular mortality, suggesting that this relationship

Table V. Multivariate logistic regression analysis of polymorphisms associated with ischemic stroke in individuals with chronic kidney disease.[a]

Gene	Polymorphism	Dominant		Recessive		Additive 1		Additive 2	
		P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
BCHE	1615G→A (Ala539Thr)	0.0179	1.53 (1.07-2.18)	0.0170	3.03 (1.20-7.48)	0.0717	-	0.0098	3.33 (1.32-8.28)
FABP2	2445G→A (Ala54Thr)	0.0736	-	0.1042	-	0.1825	-	0.0424	1.66 (1.01-2.70)

[a]Association determined by the χ^2 test. OR, odds ratio; CI, confidence interval. Multivariate logistic regression analysis was performed with adjustment for age, gender, smoking status, the serum concentration of creatinine and the prevalence of hypertension, hypercholesterolemia and diabetes mellitus.

Table VII. Effects of the *BCHE* genotype on intermediate phenotypes.

Intermediate phenotype	*GG*	*GA*	*AA*	P-value (dominant model)	P-value (recessive model)
Serum total cholesterol (mmol/l)	5.23±1.01	5.17±1.00	5.04±0.83	0.3070	0.2736
Serum HDL-cholesterol (mmol/l)	1.42±0.41	1.41±0.40	1.34±0.32	0.4472	0.2303
Serum LDL-cholesterol (mmol/l)	2.93±0.76	2.87±0.75	2.92±0.66	0.5005	0.9583
Serum triglycerides (mmol/l)	1.64±0.90	1.77±1.39	1.56±0.72	0.2218	0.4112
Fasting plasma glucose (mmol/l)	6.54±2.95	6.77±2.76	6.11±1.84	0.4041	0.1710
Glycosylated hemoglobin (%)	5.67±1.33	5.58±0.91	5.68±1.01	0.4665	0.8695
Systolic blood pressure (mmHg)	142±24	142±26	131±20	0.5628	0.0151[a]
Diastolic blood pressure (mmHg)	80±14	80±14	76±13	0.7375	0.1162

HDL, high density lipoprotein; LDL, low density lipoprotein. Data are the means ± SD. [a]P<0.05.

is not traceable to the association with cardiovascular risk factors (28).

BCHE is located on 3q26.1-q26.2 and comprises one non-coding and three coding exons spanning at least 73 kilobases (29). Several variants of this gene affecting cholinesterase activity have been reported, including the 1615G→A (Ala539Thr) polymorphism, which produces an ~30% decrease in enzyme activity (+177400, Online Mendelian Inheritance in Man). This polymorphism was shown to be associated with type 2 diabetes mellitus (30,31). In addition, the A allele of the 1615G→A (Ala539Thr) polymorphism of *BCHE* significantly increased the risk of coronary heart disease in subjects with or without diabetes mellitus (32). We previously reported that the 1616G→A (Ala539Thr) polymorphism of *BCHE* was associated with restenosis after coronary stenting, with the A allele representing a risk factor for this condition (33). We have now shown that the 1615G→A (Ala539Thr) polymorphism of *BCHE* is significantly associated with the prevalence of ischemic stroke in individuals with CKD, with the A allele representing a risk factor for this condition. In the present study, systolic blood pressure was significantly decreased in individuals with the AA genotype. This is consistent with previous observations regarding decreased serum BCHE activity (24,28). Our results thus suggest that the effect of the 1615G→A (Ala539Thr) polymorphism of *BCHE* on ischemic stroke is not attributable to its effect on blood pressure. A recent study (34) showed that serum BCHE activity was lower in subjects with ischemic stroke than in controls, supporting our observation. However, the molecular mechanism underlying the association of the 1615G→A (Ala539Thr) polymorphism of *BCHE* with ischemic stroke remains to be elucidated.

There are several limitations to the present study: i) given that the association of the *BCHE* polymorphism with ischemic stroke was not replicated in independent subject panels, our study can only be considered hypothesis generating. ii) It is possible that the polymorphism found to be associated with ischemic stroke in the present study is in linkage disequilibrium with other polymorphisms in the same or other nearby genes that are actually responsible for the development of the condition. iii) The functional relevance of the association of the identified polymorphism with ischemic stroke remains to be determined. iv) We used eGFR instead of a directly measured GFR to define CKD. v) We were not able to obtain information about underlying renal disease in each subject.

In conclusion, the present results suggest that *BCHE* may be a susceptibility locus for ischemic stroke in Japanese individuals with CKD. Determination of the genotype for the polymorphism of this gene may prove informative for the assessment of the genetic risk of ischemic stroke in such individuals. Validation of our findings will require their replication in independent subject panels.

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