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Association of genetic variants of *CELSR1* and 3q28 with hypertension in community-dwelling individuals

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Abstract. Findings of previous studies demonstrated that rs6007897 (C→T, Ala2268Thr) of the cadherin, epidermal growth factor (EGF) laminin A G-type repeats (LAG) seven-pass G-type receptor 1 gene (*CELSR1*) and rs9846911 (A→G) at chromosome 3q28 were significantly associated with ischemic stroke and chronic kidney disease, respectively. Given that hypertension is a risk factor for both ischemic stroke and chronic kidney disease, it was hypothesized that the association of rs6007897 with ischemic stroke or of rs9846911 with chronic kidney disease might be attributable, at least in part, to their effects on genetic susceptibility to hypertension. The purpose of the present study was to examine a possible association of rs6007897 of *CELSR1* or rs9846911 at 3q28 with hypertension in community-dwelling individuals. Study subjects comprised 5,959 community-dwelling individuals (1,670 subjects with hypertension and 4,289 controls) who were recruited to a population-based cohort study. Comparisons of allele frequencies by the Chi-square test revealed that rs6007897 of *CELSR1* (P=0.0280) and rs9846911 at 3q28 (P=0.0171) were significantly associated with the prevalence of hypertension. Multivariate logistic regression analysis with adjustment for age, gender, body mass index (BMI), smoking status, the serum concentration of creatinine and the prevalence of dyslipidemia and diabetes mellitus revealed that rs6007897 (P=0.0308; recessive model; odds ratio, 1.56) and rs9846911 (P=0.0353; dominant model; odds ratio, 1.22) were significantly associated with hypertension with the T allele rs6007897 and the G allele rs984691 representing risk factors for this condition. *CELSR1* and 3q28 may thus be susceptibility loci for hypertension.

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Introduction

Hypertension is a complex multifactorial disorder that is thought to result from an interaction between an individual's genetic background and various environmental factors (1). The genetic effect on blood pressure (BP) variability has been suggested to be between 30 and 50% for each individual (2). Given that hypertension is a major risk factor for coronary heart disease, stroke, and chronic kidney disease, the personalized prevention of hypertension is an important public health objective (3,4). Previous genome-wide association studies (GWASs) have suggested that various loci and genes are responsible for the predisposition to hypertension in Caucasian or African American populations (5-9). Although the polymorphism rs3755351 in the adducin 2 gene was shown to be a susceptibility locus for hypertension in Japanese individuals (10), the genes that confer susceptibility to this condition in Japanese individuals remain to be identified definitively.

In a previous study, we showed that rs6007897 (C→T, Ala2268Thr) of the cadherin, epidermal growth factor (EGF) laminin A G-type repeats (LAG) seven-pass G-type receptor 1 gene (*CELSR1*) was significantly associated with ischemic stroke in Japanese individuals by a GWAS (11). We also identified rs9846911 (A→G) at chromosome 3q28 as a susceptibility locus for chronic kidney disease in Japanese individuals by a GWAS (12). Considering that hypertension is a significant risk factor for ischemic stroke and chronic kidney disease, we hypothesized that the association of rs6007897 of *CELSR1* with ischemic stroke or of rs9846911 at 3q28 with chronic kidney disease might be attributable, at least in part, to their effects on genetic susceptibility to hypertension. The aim of the present study was to examine a possible association of rs6007897 of *CELSR1* or of rs9846911 at 3q28 with hypertension in community-dwelling Japanese individuals.

Materials and methods

Study population. Study subjects comprised 5,959 community-dwelling Japanese individuals (1,670 subjects with hypertension and 4,289 controls) who were recruited to a

Table I. Characteristics of 5,959 study subjects.

Characteristic	Hypertension	Controls	P-value
No. of subjects	1,670	4,289	
Age (years)	60.3±10.3	49.6±12.3	<0.0001
Gender (male/female, %)	63.2/36.8	52.3/47.7	<0.0001
Body mass index (kg/m^2)	24.2±3.5	22.4±3.1	<0.0001
Current or former smoker (%)	39.2	39.3	0.9368
Dyslipidemia (%)	62.1	43.6	<0.0001
Diabetes mellitus (%)	17.2	4.8	<0.0001
Coronary heart disease (%)	2.5	1.1	<0.0001
Stroke (ischemic and hemorrhagic)	3.3	0.8	<0.0001
Systolic blood pressure (mmHg)	134±15	113±11	<0.0001
Diastolic blood pressure (mmHg)	83±12	70±9	<0.0001
Serum total cholesterol (mmol/l)	5.17±0.85	5.17±0.84	0.8762
Serum triglycerides (mmol/l)	1.48±1.03	1.17±0.78	<0.0001
Serum HDL-cholesterol (mmol/l)	1.57±0.43	1.68±0.44	<0.0001
Serum LDL-cholesterol (mmol/l)	3.20±0.80	3.23±0.82	0.256
Fasting plasma glucose (mmol/l)	5.90±1.33	5.41±0.94	<0.0001
Blood glycosylated hemoglobin (%)	5.89±1.00	5.62±0.79	<0.0001
Serum creatinine (μmol/l)	69.0±19.2	63.7±13.7	<0.0001

Quantitative data are means ± standard deviations. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

population-based cohort study in Inabe (Inabe Health and Longevity Study), Mie Prefecture, Japan between 2010 and 2012. The subjects with hypertension either had a systolic BP of ≥140 mmHg or diastolic BP of ≥90 mmHg (or both) or had taken antihypertensive medication. The control individuals had systolic BP of <140 mmHg and diastolic BP of <90 mmHg and no history of hypertension or of taking antihypertensive medication. BP was measured at least twice with subjects having rested in the sitting position for >5 min. The measurements were taken by a skilled physician or a nurse according to the guidelines of the American Heart Association (13). Individuals with dyslipidemia either had 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. Diabetes mellitus was defined as individuals who either had fasting plasma glucose level of ≥6.93 mmol/l or blood glycosylated hemoglobin content of ≥6.9%, or had taken antidiabetes medication. The study protocol complied with the Declaration of Helsinki and was approved by the Ethics Committees of the Human Research of Mie University Graduate School of Medicine and Inabe General Hospital. Written informed consent was obtained from all 5,959 subjects.

Genotyping of polymorphisms. Venous blood (5 ml) was collected into tubes containing 50 mmol/l ethylenediaminetetraacetic acid (disodium salt), the peripheral blood leukocytes were isolated, and genomic DNA was extracted from these cells with a DNA extraction kit (SMITEST EX-R&D; Medical & Biological Laboratories, Nagoya, Japan). Genotypes of rs9846911 and rs6007897 were determined at G&G Science Co., Ltd. (Fukushima, Japan) by a method that combines polymerase chain reaction and sequence-specific oligonucleotide probes with suspension array technology (Luminex Corporation, Austin, TX, USA) as described previously (11,12). Detailed genotyping methodology was also described previously (14).

Statistical analysis. Quantitative data were compared between subjects with hypertension and controls by the unpaired Student's t-test. Categorical data were compared by the Chi-square test. Allele frequencies were estimated by the gene counting method, and the Chi-square test was used to identify departure from the Hardy-Weinberg equilibrium. Multivariate logistic regression analysis was performed with hypertension as a dependent variable and independent variables including age, gender (0, woman; 1, man), body mass index (BMI), smoking status (0, non-smoker; 1, current or former smoker), the serum concentration of creatinine, and the prevalence of dyslipidemia and diabetes mellitus (0, no history of these conditions; 1, positive history), and genotype of rs6007897 or rs9846911. The P-value, odds ratio, and 95% confidence interval were also calculated. Genotype of rs6007897 or rs9846911 was assessed according to dominant (0, wild-type homozygotes; 1, the combined group of variant homozygotes and heterozygotes) and recessive (0, the combined group of wild-type homozygotes and heterozygotes; 1, variant homozygotes) genetic models. P<0.05 was considered to indicate a statistically significant difference. Statistical tests were performed with JMP 5.1 software (SAS Institute, Inc., Cary, NC, USA).

Results

Characteristics of the 5,959 study subjects are shown in Table I. Age, gender, BMI, the prevalence of dyslipidemia,

Table II. Comparison of genotype distributions and allele frequencies of rs6007897 of *CELSR1* or of rs9846911 at 3q28 by the chi-square test between subjects with hypertension and controls.

Gene/locus	Polymorphism	Hypertension[a]	Controls[a]	P-value (genotype)	P-value (allele)
CELSR1	rs6007897			0.0267	0.0280
	CC	0 (0)	0 (0)		
	CT	40 (2.4)	151 (3.5)		
	TT	1630 (97.6)	4138 (96.5)		
Hardy-Weinberg P-value	0.6204	0.2406			
3q28	rs9846911			0.0592	0.0171
	AA	1416 (84.8)	3732 (87.0)		
	AG	240 (14.4)	533 (12.4)		
	GG	14 (0.8)	24 (0.6)		
Hardy-Weinberg P-value	0.2815	0.2957			

[a]Numbers in parentheses are percentages.

Table III. Multivariate logistic regression analysis of rs6007897 of *CELSR1* or of rs9846911 at 3q28 and hypertension.

		Dominant		Recessive	
Gene/locus	Polymorphism	P-value	OR (95% CI)	P-value	OR (95% CI)
CELSR1	rs6007897			0.0308	1.56 (1.05-2.36)
3q28	rs9846911	0.0353	1.22 (1.01-1.47)	0.4659	

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 dyslipidemia and diabetes mellitus.

Table IV. Association of combined genotypes of rs9846911 and rs6007897 with hypertension.

rs9846911 (0, AA; 1, AG + GG)	rs6007897 (0, CT; 1, TT)	No. of subjects	OR (95% CI)	P-value
0	0	165	1.0	
0	1	4983	1.7 (1.1-2.7)	0.0143
1	0	26	2.7 (0.8-8.6)	0.0809
1	1	785	2.1 (1.3-3.4)	0.0025

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 diabetes mellitus and hypercholesterolemia.

diabetes mellitus, coronary heart disease, and stroke, and serum concentrations of creatinine were greater in subjects with hypertension than in controls.

Comparisons of genotype distributions and allele frequencies between subjects with hypertensive and controls by the Chi-square test revealed that rs6007897 of *CELSR1* was significantly ($P<0.05$) associated with hypertension (Table II). Allele frequencies of rs9846911 at 3q28 were also significantly associated with hypertension. The genotype distributions of rs6007897 and rs9846911 were in Hardy-Weinberg equilibrium among subjects with hypertension and controls (Table II).

Multivariate logistic regression analysis with adjustment for age, gender, BMI, smoking status, the serum concentration of creatinine, and the prevalence of dyslipidemia and diabetes mellitus revealed that rs6007897 (recessive model) and rs9846911 (dominant model) were significantly associated with hypertension with the T allele rs6007897 and the G allele rs9846911 representing risk factors for this condition (Table III). We performed multivariate logistic regression analysis of the combined genotypes of rs9846911 and rs6007897 to assess the genetic risk for hypertension. The combined genotype analysis revealed that the highest odds ratio of 2.1 was obtained with

Table V. Association with rs6007897 or rs9846911 to systolic or diastolic blood pressure.

A, rs6007897

Variables	Genotype CC	CT	TT	P-value (dominant)	P-value (recessive)
All individuals					
No. of subjects	0	191	5768		
Systolic blood pressure (mmHg)		120±15	119±15		0.8075
Diastolic blood pressure (mmHg)		74±11	73±12		0.5599
Individuals without antihypertensive medication					
No. of subjects	0	166	4662		
Systolic blood pressure (mmHg)		117±13	117±15		0.8107
Diastolic blood pressure (mmHg)		73±11	72±11		0.4351

B, rs9846911

Variables	Genotype AA	AG	GG	P-value (dominant)	P-value (recessive)
All individuals					
No. of subjects	5148	773	38		
Systolic blood pressure (mmHg)	119±15	119±16	122±16	0.8224	0.3217
Diastolic blood pressure (mmHg)	73±12	73±12	74±11	0.4835	0.8745
Individuals without antihypertensive medication					
No. of subjects	4186	614	28		
Systolic blood pressure (mmHg)	117±15	117±15	117±15	0.7664	0.9372
Diastolic blood pressure (mmHg)	72±11	72±12	71±10	0.3877	0.8119

Data for blood pressure are means ± standard deviations.

the combined genotype of AG or GG for rs9846911 and TT for rs6007897 compared to the combined genotype of AA for rs9846911 and CT for rs6007897 (Table IV).

The association of rs9846911 or rs6007897 with systolic or diastolic BP among all individuals or individuals not taking antihypertensive medication was examined. No significant differences were observed in systolic or diastolic BP between genotypes of rs9846911 or rs6007897 (Table V).

Discussion

Given that genetic factors and interactions between multiple genes and environmental factors are important in the development of hypertension (1), prediction of the risk for hypertension on the basis of genetic variants would be beneficial for the personalized prevention of this condition. Results of the present study have shown that rs6007897 of *CELSR1* and rs9846911 at 3q28 were significantly associated with the prevalence of hypertension in community-dwelling Japanese individuals, with the T and G alleles, respectively, representing risk factors for this condition. A combined genotype analysis of two polymorphisms revealed that the highest odds ratio of 2.1 was obtained for individuals with the high-risk genotype compared to those with the low-risk genotype.

CELSR1 is a member of the flamingo subfamily of cadherin proteins (15-17). We previously showed that rs6007897 of *CELSR1* was associated with the prevalence of ischemic stroke (11). The relationship of this polymorphism to ischemic stroke was replicated in a Portuguese case-control cohort (18). Human *CELSR1*, *CELSR2* and *CELSR3* are planar cell polarity signaling molecules that are involved in the regulation of cell polarity, convergent extension, and invasion. Activation of the planar cell polarity signaling pathway controls tissue polarity and cell movement through the activation of ras homolog gene family, member A, mitogen-activated protein kinase 8, and nemo-like kinase (19). The rs6007897 of *CELSR1* might influence this signaling pathway, although the effect of this polymorphism on the protein structure or function remains to be determined. The molecular mechanism underlying the role of rs6007897 of *CELSR1* in the pathogenesis of hypertension thus remains unclear.

The rs9846911 is located in a non-gene region at 3q28, which is located downstream of the genes for receptor transporter protein 4 (~130k bp), somatostatin (~170k bp), receptor

transporter protein 2 (~200k bp), or B-cell CLL/lymphoma 6 (~220 kbp), and upstream from the mannan-binding lectin serine peptidase 1 gene (~210k bp). Previously, we showed that rs9846911 at 3q28 was associated with the prevalence of chronic kidney disease (12). Given that there is a large linkage disequilibrium block in the 3q28 region, it is likely that rs9846911 is in linkage disequilibrium with other polymorphisms in the nearby genes that are actually responsible for the development of hypertension. The functional relevance of the association of rs9846911 with the pathogenesis of hypertension thus remains unclear.

Although rs6007897 of *CELSR1* and rs9846911 at 3q28 were significantly associated with the prevalence of hypertension, genotypes of these polymorphisms were not associated with systolic or diastolic BP. Although the reason for this discrepancy remains to be elucidated, there are several possibilities: i) the number of hypertensive subjects without taking antihypertensive medication was small; ii) information for drug compliance obtained by a questionnaire was incomplete; iii) a substantial proportion of the subjects had white-coat hypertension; and iv) two polymorphisms were not linked to systolic or diastolic BP among individuals without hypertension.

The present study had several limitations including the fact that: i) given that the results of the present study were not replicated, validation of our findings require replication of the results with other independent subject panels or ethnic groups; ii) it is possible that rs6007897 or rs9846911 is in LD with other polymorphisms in the same gene or in other nearby genes that are actually responsible for the development of hypertension; iii) although rs6007897 and rs9846911 were significantly associated with the prevalence of hypertension, these polymorphisms were not linked to systolic or diastolic BP; and iv) the functional relevance of rs6007897 or rs9846911 to pathogenesis of hypertension remains to be determined.

In conclusion, results of the present study suggest that rs6007897 of *CELSR1* and rs9846911 at 3q28 may be susceptibility loci for hypertension in Japanese individuals. Determination of genotypes for these polymorphisms may prove informative for assessment of the genetic risk for hypertension in Japanese individuals.

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