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ORIGINAL ARTICLE

Influence of adrenomedullin 2/intermedin gene polymorphism on blood pressure, renal function and silent cerebrovascular lesions in Japanese: the Ohasama study

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Adrenomedullin 2/intermedin (AM2/IMD) is a novel vasodilator peptide with various effects on the renal function and cardiovascular system. An exonic insertion (I)/deletion (D) polymorphism (rs3840963) may influence generation of AM2/IMD-53, due to its location within the N-terminal sequence. We investigated the association of this polymorphism with blood pressure, renal function and the risk of silent cerebrovascular lesions in a Japanese population recruited from the Ohasama study. We recorded 24 h ambulatory blood pressure (ABP), estimated glomerular filtration rate (eGFR) and proteinuria of 1073 individuals over 40 years of age. Silent cerebrovascular lesions (lacunar infarction and white matter hyperintensity (WMH)) were recorded in 794 individuals over 55 years of age. Chronic kidney disease (CKD) was diagnosed in individuals with proteinuria and/or decreased eGFR $\leqslant 60\,ml\,min^{-1}$ per $1.73\,m^2$. DD carriers, compared with II and ID carriers, displayed significantly higher 24 h ABP (127.4 *vs.* 122.0 and 122.9 mm Hg, respectively, in systolic ABP, $P=0.009$; and 74.8 *vs.* 71.3 and 72.5 mm Hg, respectively, in diastolic ABP, $P=0.002$), and lower eGFR (75.4 *vs.* 82.6 and 82.9 $ml\,min^{-1}$ per $1.73\,m^2$, respectively, $P=0.04$). DD carriers also had a significantly higher odds ratio (OR) for prevalence of CKD (OR: 2.7, $P=0.003$), presence of lacunar infarction (OR: 2.4, $P=0.01$) and WMH (OR: 2.7, $P=0.003$), compared with II carriers. The AM2/IMD I/D polymorphism is associated with renal dysfunction, blood pressure regulation and asymptomatic cerebrovascular diseases in the Japanese general population.
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Keywords: adrenomedullin 2/intermedin; chronic kidney disease; polymorphism; silent cerebrovascular lesions

INTRODUCTION

Adrenomedullin 2 (AM2), also called intermedin (IMD), is a novel vasodilator peptide belonging to the calcitonin/calcitonin gene-related peptide (CGRP) family. The CGRP family includes calcitonin, CGRP, amylin and adrenomedullin (AM), and has multiple biological effects involving potent vasodilation (CGRP and AM), reduced nutrient intake (amylin) and decreased bone resorption (calcitonin).[1] Recently, two research groups concurrently discovered AM2/IMD by searching the genome database, and named it AM2[2,3] and IMD,[4] respectively. The human AM2/IMD gene encodes a prepro-AM2/IMD of 148 amino acids, with a signal peptide for secretion at the C-terminus (Figure 1). Proteolytic processing of human prepro-AM2/IMD yields three types of biologically active C-terminal fragments: AM2/IMD-53 (prepro-AM2/IMD$_{95-147}$), AM2/IMD$_{1-47}$

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  1 CCGCGACTCC GCTCCAGGCA GGACCCCCAA CCCGCCCAGC CCGCTCCGCC TTGCGCCCCG
 61 GACCCGCGGC CGACCCCAGA CCCGCTGCCC GCTTCGCGCC CGAGGCCTGC GCCCCGACGG
121 ACGCCCGTGC CCAGCTTGCC ACGCCCACGC CCGGCGCCCC GACCGCGGAG GACTCCCCGA
181 GGTGCCGGCG GAGGGGGTGG CTCGCGGCTC AGGCTGCCCC CGACGTGCCC GGCTCACCGC
241 CCCCTCCCCT GCAGCCCCGC CCGCCATGGC CCGGATCCCG ACGGCCGCCC TGGGTTGCAT
                            M  A  R  I  P  T  A  A  L  G  C  I
301 CAGCCTCCTC TGCCTGCAGC TCCCTGGCTC GCTGTCCCGC AGCCTGGGCG GGGACCCGCG
     S  L  L  C  L  Q  L  P  G  S  L  S  R  S  L  G  G  D  P  R
361 ACCCGTCAAA CCCAGGGAGC CCCCAGCCCG GAGCCCTTCC AGCAGCCTGC AGCCCAGGCA
     P  V  K  P  R  E  P  P  A  R  S  P  S  S  S  L  Q  P  R  H
421 CCCCGCACCC CGACCTGTGG TCTGGAAGCT TCACCGGGCC CTCCAGGCAC AGAGGGGTGC
     P  A  P  R  P  V  V  W  K  L  H  R  A  L  Q  A  Q  R  G  A
481 CGGCCTGGCC CCTGTTATGG GTCAGCCTCT CCGGGATGGT GGCCGCCAAC ACTCGGGCCC
     G  L  A  P  V  M  G  Q  P  L  R  D  G  G  R  Q  H  S  G  P
541 CCGAAGACAC TCGGGCCCCC GCAGGACCCA AGCCCAGCTC CTGCGAGTGG GCTGTGTGCT
     R  R  H  S  G  P  R  R  T  Q  A  Q  L  L  R  V  G  C  V  L
601 GGGCACCTGC CAGGTGCAGA ATCTCAGCCA CCGCCTGTGG CAACTCATGG GACCGGCCGG
     G  T  C  Q  V  Q  N  L  S  H  R  L  W  Q  L  M  G  P  A  G
661 CCGGCAGGAC TCAGCTCCTG TGGACCCCAG CAGCCCCCAC AGCTATGGCT GAGGTGGGGC
     R  Q  D  S  A  P  V  D  P  S  S  P  H  S  Y  G  X
721 CGGGCCACAC CCCTGCCCAT CCCAGCCAGG GTGCTGTGCC CCCGTCCAGA GCTGCAGCTG
    ......
4201 GCTCCTCTCA CCACTCTCTT GAATAAAGCT TTTCTCACCA TTTTAA
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b

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AM2/IMD-53 :HSGPRRTQAQLLRVGCVLGTCQVQNLSHRLWQLMGPAGRQDSAPVDPSSPHSY
AM2/IMD1-47 :      TQAQLLRVGCVLGTCQVQNLSHRLWQLMGPAGRQDSAPVDPSSPHSY
AM2/IMD8-47 :            VGCVLGTCQVQNLSHRLWQLMGPAGRQDSAPVDPSSPHSY
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Figure 1 (**a**) Nucleotide sequence of the human adrenomedullin 2/intermedin (AM2/IMD) mRNA (NCBI Reference Sequence: NM_024866). Black box with white graph, AM2/IMD insertion/deletion polymorphism (rs3840963); boxes, primers used for genotyping; *underlined*, ATG initiation codon and TGA stop codon; double underlined, putative basic cleavage sites; italic, AM2/IMD-53; *closed triangle*, exon–exon junction. (**b**) Three isoforms of putative mature AM2/IMD peptide (AM2/IMD-53, $AM2/IMD_{1-47}$ and $AM2/IMD_{8-47}$). In the deletion allele sequence of the AM2/IMD gene, AM2/IMD-53 is not generated because the N-terminal amino acid is missing (bold and underlined).

(prepro-$AM2/IMD_{101-147}$) and $AM2/IMD_{8-47}$ (prepro-$AM2/IMD_{108-147}$).[4,5]

CGRP, AM and AM2/IMD activate the calcitonin receptor-like receptor (CRLR)/receptor activity-modifying protein (RAMP) system to transduce their signals. AM preferentially interacts with CRLR/RAMP2 or 3, and CGRP with CRLR/RAMP1, whereas AM2/IMD interacts with CRLR/RAMP1, 2 or 3 non-selectively.[4] AM2/IMD stimulates cAMP production and has a potent vasodilator action, similar to AM and CGRP.[3,4] Intrarenal infusion of AM2/IMD causes diuresis and natriuresis without a significant decrease in systemic blood pressure in rats.[6] Moreover, intravenous injection of AM2/IMD decreases arterial blood pressure,[3,4,7] and this effect is partially blocked by CGRP and AM receptor antagonists.[4]

It has been reported that a certain AM gene polymorphism is significantly involved either in the pathogenesis of hypertension or in the initiation, and in the progression of diabetic renal damage.[8–10] However, there are no databases or reports assessing an association of AM2/IMD polymorphisms with clinical phenotypes. The AM2/IMD gene has an 18 base pair (bp) I/D polymorphism in one of its exons (NCBI dbSNP accession no. rs3840963), which results in an amino-acid I/D (RHSGPR/−) at amino acids 94–99 (Figure 1). This I/D polymorphism may influence the generation of AM2/IMD-53, because of its location within the N-terminal sequence. Hence, we investigated the association of a genetic polymorphism in the AM2/IMD gene with blood pressure, renal function and the risk of silent cerebrovascular lesions in a Japanese general population that was recruited from the Ohasama study.

METHODS

Study population

This study is a part of the Ohasama study and is based on the data obtained from subjects who participated in our blood pressure monitoring and genetic analysis project in a rural community of Ohasama, Iwate Prefecture, Japan. The characteristics of this area and details of the study design have been described previously.[11,12] The study protocol, including the genetic analysis, was approved by the Institutional Review Board of Tohoku University School of Medicine and by the Department of Health of the Ohasama Town Government.

The total population of Ohasama in 1998 was 7202. Of these, 1826 individuals who were over 40 years of age gave written informed consent for blood pressure measurements and genetic analysis. Subjects who were inpatients, mentally ill or bedridden were excluded. Of them, 388 were excluded because their ambulatory blood pressure (ABP) was not measured. Of the remaining 1438 individuals, 365 were also excluded as they lacked full data on clinical characteristics or genotypes. As a result, 1073 individuals were included in the analysis. Subsequently, 794 individuals who were older than 55 years of age without a previous history of stroke or transient ischemic attack participated in the magnetic resonance imaging examination, and were included in the analysis of silent cerebrovascular lesions.

Determination of genotype

Genomic DNA samples were extracted from peripheral leukocytes of participants. We genotyped an 18 bp I/D polymorphism in an exon of the AM2/IMD gene (NCBI dbSNP accession no. rs3840963; Figure 1). The following primers were designed for PCR of the flanking region of the I/D polymorphism in AM2/IMD: forward, 5′-GGTGGCCGCCAACACTCG-3′; reverse, 5′-CAGGCG GTGGCTGAGATTCTG-3′. PCR was performed in a 20 μl volume of 10 ng genomic DNA, Colorless GoTaq Reaction Buffer, 0.2 mM each of deoxynucleotide triphosphate, 0.25 μM each of primer and 0.5 units of GoTaq DNA polymerase (Promega, Madison, WI, USA). The amplification conditions were as follows: initial denaturation at 94 °C for 2 min followed by 32 cycles of 94 °C for 15 s, 64 °C for 15 s, 72 °C for 1 min and a final cycle at 72 °C for 5 min. The I allele had 120 bp, while the D allele had 102 bp. Genotyping was repeated in a random 50% of the participants as quality control. All repeated experiments revealed identical results when compared with the initial genotyping.

Blood pressure measurement

Details of ABP monitoring have been described previously.[11,13] In brief, ABP was monitored every 30 min using a fully automatic device (ABPM 630: Nippon Colin, Komaki, Japan). Mean 24 h, daytime and night time values for ABP were calculated for each participant. 'Daytime' and 'night time' were determined according to each participant's diary. Thus, we analyzed ABP data obtained during $\geqslant$6 h period of daytime and $\geqslant$3 h period of night time. The mean number of measurements was 44.4 ± 5.1 per subject.

Casual blood pressure (CBP), measured by public health nurses or technicians using an automatic device (HEM 907: Omron Healthcare, Kyoto, Japan), was measured twice consecutively with individuals in the sitting position, with a minimum 2 min rest between measurements. The mean of the two measured values was used for analysis.

Devices used to measure ABP or CBP met the criteria of the Association for the Advancement of Medical Instrumentation.[13–15] Hypertension was defined as 24 h systolic blood pressure (SBP) $\geqslant$130 mm Hg and/or 24 h diastolic blood pressure (DBP) $\geqslant$80 mm Hg and/or use of antihypertensive medications.[16]

Biochemical measurement

Study nurses administered a standardized questionnaire, inquiring into medical history, medications and smoking and drinking habits of each patient. Previous cardiovascular disease included stroke, transient ischemic attack, coronary heart disease and atrial fibrillation. Biochemical parameters were measured with an autoanalyzer by SRL (Tokyo, Japan). Diabetes mellitus was defined as a fasting or random blood glucose level of $\geqslant$7.0 or $\geqslant$11.1 mmol l^{-1}, respectively, or as the use of antidiabetic drugs.[17] Proteinuria was defined as urine protein $\geqslant$1+ by tape method. Estimated glomerular filtration rate (eGFR) was calculated using the following formula: eGFR=194×serum

creatinine$^{-1.094}$×Age$^{-0.287}$ (×0.739, if women).[18] We defined chronic kidney disease (CKD) as the presence of kidney damage (positive proteinuria) or eGFR < 60 ml min^{-1} per 1.73 m^2.[19]

Silent cerebrovascular lesions

The evaluation of silent cerebrovascular lesions using magnetic resonance imaging has been reported previously.[20,21] A lacunar infarction was defined as an area of low signal intensity measuring ⩽15 and ⩾3 mm in diameter on T1-weighted images, and visible as a hyperintense lesion on T2-weighted images. White matter hyperintensities (WMHs) were defined as hyperintensities visible only on T2-weighted images, and were graded according to Fazekas *et al*.[22] WMH of grade 1 or more was defined as the presence of WMH.

Statistical analysis

Statistical analysis was performed with the JMP 5.0.1 statistical software package (SAS Institute, Cary, NC, USA). Student's *t*-test, χ^2-text, analysis of variance and analysis of covariance were used where appropriate. The associations of the AM2/IMD I/D polymorphism with prevalence of CKD, lacunar infarction and WMH were examined using multiple logistic regression analysis. Continuous values were expressed as mean values ± s.d. Differences of $P<0.05$ were considered statistically significant.

RESULTS

Genotype and allele frequencies, and characteristics of the participants

The frequencies of the II, ID and DD genotypes were 649/1073 (60.4%), 365/1073 (34.0%) and 59/1073 (5.5%), respectively. The allele frequencies of the I and D alleles were 1663/2146 (77.5%) and 483/2146 (22.5%), respectively. The genotype frequencies were in Hardy–Weinberg equilibrium (P=0.4). Although the prevalence of hypertension (P=0.08) and use of antihypertensive medications (P=0.06) tended to be higher in the DD genotype than in the other genotypes, no significant differences were observed between the genotypes and clinical characteristics (Table 1).

Blood pressure

DD carriers showed significantly higher ABP levels in 24 h SBP, 24 h DBP, daytime SBP, daytime DBP and night time DBP, but not in night time SBP, as compared with the II homozygous and the ID heterozygous carriers (Table 2). With adjustment applied for age, sex, body mass index, serum creatinine, use of antihypertensive medications, prevalence of diabetes mellitus, history of previous cardiovascular disease, smoking status and alcohol intake, the association of these with 24 h SBP, 24 h DBP, daytime SBP and daytime DBP remained significant. CBP did not differ among the genotypes.

Renal function

Homozygous carriers of the DD genotype had significantly higher serum creatinine levels and significantly lower eGFR compared with II and ID carriers (Table 3). The adjusted odds ratio (OR) and 95% confidence intervals (CIs) for CKD in the I/D genotype, by multiple logistic regression analysis, are shown in Figure 2. After adjusting for age, sex, body mass index, 24 h SBP, use of antihypertensive

Table 1 Clinical characteristics of adrenomedullin 2/intermedin insertion (I)/deletion (D) genotype in all subjects (*n*=1073)

	Genotype				
	All	*II*	*ID*	*DD*	P-*value*
Number of subjects	1073	649	365	59	
Age (years)	59.6 ± 9.4	60.0 ± 9.4	59.1 ± 9.5	59.6 ± 8.9	0.4
Sex (% men)	32.0	32.2	31.8	30.5	1
Body mass index (kg m^{-2})	23.5 ± 3.1	23.6 ± 3.1	23.7 ± 3.1	24.4 ± 2.9	0.2
Smoking status (%)	15.7	15.3	17.0	11.9	0.5
Alcohol intake (%)	16.1	15.3	17.8	15.3	0.6
Hypertension (%)	44.8	42.2	48.2	52.5	0.08
Antihypertensive medications (%)	31.8	29.4	34.3	42.4	0.06
Cardiovascular disease (%)	8.8	8.9	8.2	10.2	0.9
Diabetes mellitus (%)	14.9	15.3	15.3	8.5	0.3

Continuous values are shown as mean values ± s.d.

Table 2 Blood pressure stratified by the adrenomedullin 2/intermedin insertion (I)/deletion (D) genotype (*n*=1073)

	II (n=*649*)	*ID* (n=*365*)	*DD* (n=*59*)	P-*value*	*Adjusted* P-*value*
Ambulatory blood pressure					
24 h systolic (mm Hg)	122.0 ± 12.6	122.9 ± 12.5	127.4 ± 15.0	0.006	0.009
24 h diastolic (mm Hg)	71.3 ± 7.5	72.5 ± 7.3	74.8 ± 8.3	<0.001	0.002
Daytime systolic (mm Hg)	127.7 ± 13.4	128.7 ± 13.6	134.6 ± 16.0	0.001	0.002
Daytime diastolic (mm Hg)	75.4 ± 8.2	76.7 ± 8.1	79.8 ± 8.7	<0.001	<0.001
Night time systolic (mm Hg)	110.4 ± 13.6	111.2 ± 13.0	113.6 ± 15.5	0.2	
Night time diastolic (mm Hg)	63.1 ± 7.8	64.1 ± 7.4	65.3 ± 8.7	0.03	0.08
Casual blood pressure					
Systolic (mm Hg)	132.4 ± 17.2	132.6 ± 17.1	135.1 ± 16.1	0.5	
Diastolic (mm Hg)	74.4 ± 9.9	74.8 ± 10.2	75.9 ± 9.9	0.5	

Continuous values are shown as mean values ± s.d. Adjusted *P*-values: adjusted for age, sex, body mass index, serum creatinine, use of antihypertensive medications, prevalence of diabetes mellitus, history of previous cardiovascular disease, smoking status (current or not) and alcohol intake (current or not).

Table 3 Renal features stratified by the adrenomedullin 2/intermedin insertion (I)/deletion (D) genotype (*n*=1073)

	II (n=*649*)	*ID* (n=*365*)	*DD* (n=*59*)	P-*value*	*Adjusted* P-*value*
Serum uric acid (mg dl^{-1})	4.4 ± 1.3	4.6 ± 1.3	4.4 ± 1.7	0.2	
Blood urea nitrogen (mg dl^{-1})	15.7 ± 3.5	15.1 ± 3.9	14.6 ± 3.6	0.1	
Serum creatinine (mg dl^{-1})	0.65 ± 0.15	0.65 ± 0.15	0.74 ± 0.33	<0.001	<0.001
eGFR (ml min^{-1} per 1.73 m^2)	82.6 ± 20.6	82.9 ± 19.0	75.4 ± 20.8	0.02	0.04
Urinary protein, *n* (%)	14 (2.2)	10 (2.7)	6 (10.2)	0.03	
eGFR<60, *n* (%)	47 (7.2)	33 (9.0)	12 (20.3)	0.009	
CKD, *n* (%)	59 (9.1)	41 (11.2)	14 (23.7)	0.007	

Continuous values are shown as mean values ± s.d. Adjusted *P*-values: adjusted for age, sex, body mass index, 24 h ambulatory systolic blood pressure, use of antihypertensive medications, prevalence of diabetes mellitus, history of previous cardiovascular disease, smoking status (current or not) and alcohol intake (current or not). Estimated glomerular filtration rate (eGFR) was calculated using the following formula: eGFR=194×serum creatinine$^{-1.094}$×age$^{-0.287}$ (×0.739, if women). Chronic kidney disease (CKD) was defined as positive for urinary protein or eGFR <60 ml min^{-1} per 1.73 m^2.

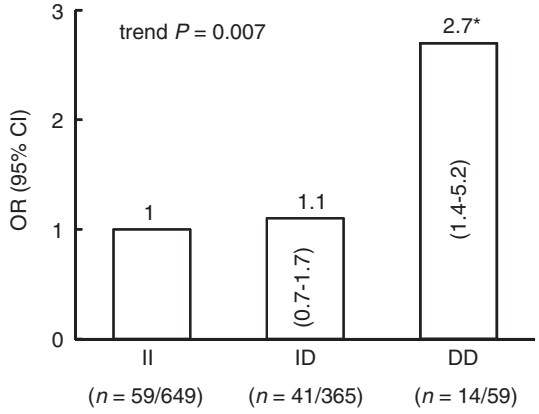

Figure 2 Adjusted odds ratio (OR) and 95% confidence intervals (CIs) for the presence of chronic kidney disease in individuals with the adrenomedullin 2/intermedin insertion (I)/deletion (D) polymorphism, by multiple logistic regression analysis (*n*=1073). Adjusted for age, sex, body mass index, 24 h ambulatory systolic blood pressure, use of antihypertensive medications, prevalence of diabetes mellitus, history of previous cardiovascular disease, smoking status (current or not) and alcohol intake (current or not). **P*<0.05 *vs.* II carriers.

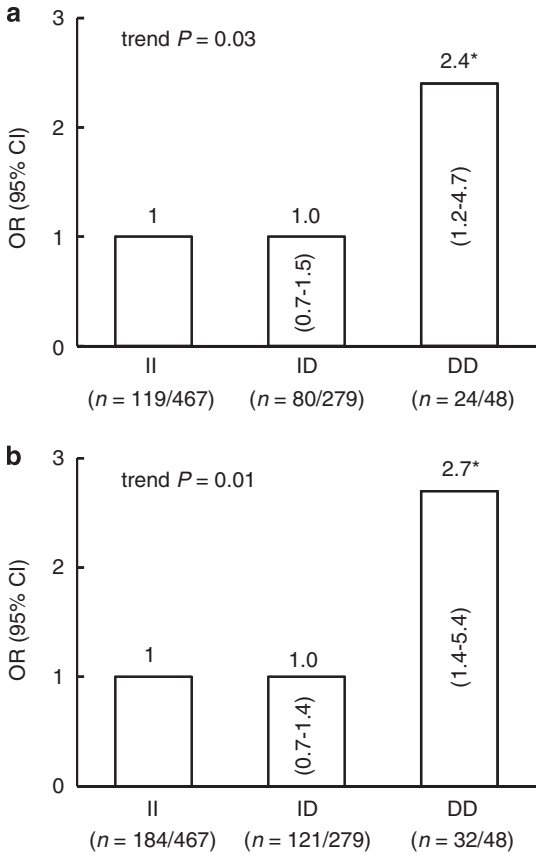

Figure 3 Adjusted odds ratio (OR) and 95% confidence intervals (CIs) for the presence of (**a**) lacunar infarction and (**b**) white matter hyperintensity in individuals with adrenomedullin 2/intermedin insertion (I)/deletion (D) polymorphism, by multiple logistic regression analysis (*n*=794). Adjusted for age, sex, body mass index, 24 h ambulatory systolic blood pressure, use of antihypertensive medications, prevalence of diabetes mellitus, prevalence of chronic kidney disease, history of previous heart disease, smoking status (current or not) and alcohol intake (current or not). **P*<0.05 *vs.* II carriers.

medications, prevalence of diabetes mellitus, history of previous cardiovascular disease, smoking status and alcohol intake, the DD genotype was significantly and independently related to CKD risk (trend *P*=0.007; Figure 2). The adjusted OR for ID carriers was 1.1 (95% CI 0.7–1.7, *P*=0.6) and for DD carriers 2.7 (95% CI 1.4–5.2, *P*=0.003), compared with II carriers.

Silent cerebrovascular lesions

The prevalence of lacunar infarction and WMH was significantly higher in DD carriers than in II and ID carriers (lacunar infarction, 50.0, 25.5 and 28.7%, respectively, *P*=0.003; WMH, 66.7, 39.4 and 43.4%, respectively, *P*=0.001). After adjusting for age, sex, body mass index, 24 h SBP, use of antihypertensive medications, prevalence of diabetes mellitus, prevalence of CKD, history of heart disease, smoking status and alcohol intake, the DD genotype was significantly and independently related to the risk for lacunar infarction (trend *P*=0.03; Figure 3a) and WMH (trend *P*=0.01; Figure 3b). The adjusted OR for lacunar infarction in ID carriers was 1.0 (95% CI 0.7–1.5, *P*=0.9) and in DD carriers was 2.4 (95% CI 1.2–4.7, *P*=0.01), compared with II carriers. The adjusted OR for WMH in ID carriers was 1.0 (95% CI 0.7–1.4, *P*=0.9) and in DD carriers was 2.7 (95% CI 1.4–5.4, *P*=0.003), compared with II carriers.

DISCUSSION

This is the first study to report an association of the AM2/IMD gene DD genotype with blood pressure, renal dysfunction and silent cerebrovascular lesions. DD carriers of the AM2/IMD I/D polymorphism were associated with a significantly higher risk of CKD and values of ABP than II or ID carriers. In contrast to ABP, the AM2/IMD I/D polymorphism was not associated with CBP. Pickering *et al.*[23] described that ABP may reflect basal blood pressure better than CBP, and is an indicator of a patient's 'true' blood pressure.

CBP usually does not reflect basal blood pressure, but is susceptible to physical or psychological stress and environmental factors. Thus, the AM2/IMD I/D polymorphism may affect basal blood pressure regulation and be related to hypertensive organ damage. Moreover, the relationship between the AM2/IMD I/D polymorphism and silent cerebrovascular lesions remained significant after adjustment for various cardiovascular risk factors, including 24 h ABP and CKD. These findings suggest that the DD genotype of the I/D polymorphism in the exon of the AM2/IMD gene may be a new genetic risk factor for hypertension, renal dysfunction and asymptomatic cerebrovascular diseases.

AM2/IMD may directly affect the small vessels to prevent organ damage. AM2/IMD is reportedly expressed in vascular endothelial and smooth muscle cells of the small vessels in human heart and kidney.[24] Expression of AM2/IMD is decreased in the kidney with chronic renal failure due to 5/6 nephrectomy or hypertension.[25] Furthermore, AM2/IMD is abundantly expressed in the brain as well as in the heart and kidney.[24,26] In this study, the AM2/IMD I/D polymorphism was significantly and independently associated with blood pressure, CKD and silent cerebrovascular lesions. Although these phenotypes are highly related, it has been recognized that both hypertension and CKD are independent risk factors for all-cause mortality, including cardiovascular disease events and stroke, in the general population.[27–34] Therefore, our results support the hypothesis that cerebral, cardiac and renal damage is crosslinked via injury of the microvasculature of the vital organs, such as juxtamedullary afferent arterioles in the kidney, perforating arteries in the central nervous system, and coronary arteries.[35]

AM and CGRP are potent endogenous cardio- and reno-protective substances. Exogenous administration of AM and CGRP peptides or their gene delivery is a new preventive and therapeutic strategy for cardiovascular diseases such as hypertension, myocardial ischemia, heart failure and renal failure.[36–38] Because AM2/IMD shares the receptors consisting of CRLR/RAMP complexes and the cAMP signaling pathway,[4,39,40] AM2/IMD may have cerebro-, cardio- and reno-protective effects similar to CGRP and AM. Moreover, AM2/IMD gene delivery significantly lowered blood pressure, increased urine volume and restored creatinine clearance in deoxycorticosterone acetate (DOCA)-salt hypertensive rats.[41]

AM2/IMD-53 has the most potent cardio-protective effects among the three endogenously degraded fragments of prepro-AM2/IMD (AM2/IMD-53, AM2/IMD$_{1-47}$ and AM2/IMD$_{8-47}$) both *in vitro* and *in vivo*.[42–45] The D type of the AM2/IMD gene sequence, lacking amino acids 94–99 (Figure 1), may be unable to generate AM2/IMD-53, because of its location in the N-terminus of AM2/IMD-53. Taking together our results and previous findings, we suggest that the protective effects of AM2/IMD might be lowered in DD carriers by loss of AM2/IMD-53 generation. On the other hand, AM2/IMD$_{1-47}$ and AM2/IMD-53 act within the central nervous system to elevate blood pressure and inhibit food and water intake,[43,46] whereas both peptides act as potent vasodilators in the peripheral administration. The activity of AM2/IMD-53 and its ability to elevate blood pressure is more potent than AM2/IMD$_{1-47}$ and AM.[43] The association of the AM2/IMD I/D polymorphism with blood pressure, renal dysfunction and silent cerebrovascular lesions shown in this study may, therefore, be caused by loss of the local effects of AM2/IMD-53 on vascular tissues rather than its central effects on the brain.

This study has some limitations. First, we have not yet confirmed that AM2/IMD-53 is absent in the plasma, kidney or vascular tissues of the DD carriers of the AM2/IMD I/D polymorphism. Two microliters of human plasma is required to measure the plasma concentration of total AM2/IMD immunoreactivity.[24] In the present study, it was ethically not permitted to obtain a larger amount of plasma or tissues from the subjects to analyze AM2/IMD$_{1-47}$ and AM2/IMD-53 levels separately. Second, it remains to be clarified why AM2/IMD$_{1-47}$, AM or CGRP cannot compensate for the actions of AM2/IMD-53 if AM2/IMD-53 is not generated from the precursor in DD carriers with the AM2/IMD I/D polymorphism. The possible presence of a novel, unique AM2/IMD receptor other than the CRLR/RAMP system has been suggested by Taylor *et al.*,[47] who showed an inhibition of growth hormone release by AM2/IMD in pituitary cells. We cannot deny the possibility that such an unidentified AM2/IMD receptor is related to our findings in the AM2/IMD gene polymorphism. Third, the possibility of selection bias needs to be considered before generalizing the present finding. Marked differences exist in the environmental factors and genetic factors between different ethnicities. Therefore, more sophisticated study designs, including different ethnic groups, and longitudinal surveillance, should help to clarify the role of the AM2/IMD gene polymorphism in blood pressure regulation, renal dysfunction and asymptomatic cerebrovascular diseases.

To the best of our knowledge, this is the first report that presents evidence of the association of AM2/IMD with blood pressure, renal function and silent cerebrovascular lesions in humans. Our results suggest that AM2/IMD has a role in organ protection, at least in the Japanese population, and is a candidate target for the treatment of hypertension, kidney dysfunction and asymptomatic cerebrovascular diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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