

Blood pressure and urinary sodium excretion in relation to the A–1984G adrenomedullin polymorphism in a Chinese population

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Adrenomedullin (ADM) is a vasodilator and inhibits salt appetite. An A-to-G substitution at position –1984 in the promoter region of the ADM gene likely increases transcription. We therefore investigated this polymorphism in relation to blood pressure and urinary sodium in a Chinese population. We genotyped 427 Chinese enrolled in a family-based population study. We measured blood pressure by conventional sphygmomanometry and ambulatory monitoring. The frequencies of the ADM AA, AG, and GG genotypes were 50.6, 38.2, and 11.2%, respectively. In adjusted analyses, G allele carriers, compared to AA homozygotes, had significantly lower conventional (45.3 versus 48.5 mm Hg, $P = 0.004$) and 24-h (42.6 versus 44.3 mm Hg, $P = 0.03$) pulse pressures and urinary sodium excretion (143.8 versus 159.4 mmol/day, $P = 0.03$). In parents, but not offspring, both systolic pressure and pulse pressure were significantly ($P < 0.01$) lower in G allele carriers. The genotypic difference in sodium excretion was consistent across the age range. In 68 informative offspring, transmission of the G allele was associated with lower urinary sodium excretion (effect size, 40.1 mmol/day, $P = 0.01$). In 81 healthy volunteers, the plasma ADM concentration was 15.2% higher in GG homozygotes than in sex- and age-matched AA subjects (11.4 versus 9.9 pmol/l, $P = 0.10$). In conclusion, in Chinese, the ADM –1984G allele is associated with lower sodium excretion and in older subjects also with lower systolic pressure and narrower pulse pressure.

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Adrenomedullin (ADM) is a peptide consisting of 52 amino acids, first isolated from human pheochromocytoma in 1993.¹ It is expressed in various tissues.² According to a recent review of the literature,³ ADM acts as an autocrine, paracrine, or endocrine modulator of biologically important functions, including the endothelial regulation of blood pressure, protection of organ damage in sepsis or hypoxia, and the control of blood volume through the regulation of thirst. In healthy subjects, ADM circulates at low picomolar concentrations. In pathological conditions, such as for instance heart failure, sepsis, and renal impairment, the plasma concentration of ADM substantially increases.

Experimental studies showed that ADM is a vasodilator, and via its expression in the brain and adrenals inhibits salt appetite, thirst, and sympathetic activity.^{4–8} In humans, the plasma ADM concentration rises with higher blood pressure and associated complications.^{9,10} In rodent models, knockout of the ADM gene increases blood pressure,¹¹ whereas ADM infusion or ADM gene transfer attenuates hypertension.^{12–14} In humans, the gene encoding preproadrenomedullin maps to chromosome 11p15.4. After posttranslational modification, the peptides proadrenomedullin and ADM are generated.¹⁵ A genetic polymorphism (A/G) in the ADM promoter region at position –1984 has been reported (rs3814700, <http://www.ncbi.nlm.nih.gov>). The A to G substitution leads to the appearance of a binding site for the glucocorticoid receptor (<http://www.gene-regulation.com/pub/programs/alibaba2>), which stimulates the transcription of several genes.¹⁶ We therefore hypothesized that the ADM A–1984G polymorphism might be functional. The frequency of the minor G allele is approximately 25% in Japanese, but only 5% in Caucasians (<http://www.ncbi.nlm.nih.gov>). Given that ADM might be an interesting candidate gene for blood pressure regulation and sodium homeostasis, we investigated the association between blood pressure, urinary sodium excretion, and the ADM A–1984G polymorphism in a Chinese population.

RESULTS

Characteristics of the participants

The 427 participants included 223 (52.2%) women and 113 (26.5%) hypertensive patients, of whom 47 (11.0%) were taking antihypertensive drugs. Age ranged from 12 to 85

Table 1 | Characteristics of the study population

Characteristic	Men (N=204)	Women (N=223)
Age (years)	44.9±15.2	43.3±13.7
Body mass index (kg/m ²)	22.0±2.7	22.9±3.2 ^a
<i>Conventional blood pressure (mm Hg)</i>		
Systolic	126.9±22.6	124.6±23.7
Diastolic	79.2±10.8	78.2±11.9
Pulse pressure	47.8±16.2	46.4±15.6
<i>24-h ambulatory blood pressure (mm Hg)</i>		
Systolic	122.4±14.3	119.5±16.8
Diastolic	78.0±9.6	76.4±10.0
Pulse pressure	44.4±7.9	43.1±9.5
Plasma renin activity (ng/ml/h)	1.44 (1.24–1.68)	1.13 (0.95–1.34) ^a
<i>Questionnaire data N (%)</i>		
Current smoking	122 (59.8)	0 ^a
Alcohol intake	152 (74.5)	46 (20.6) ^a
Taking antihypertensive drugs	22 (10.8)	25 (11.2)
<i>24-h urinary excretion</i>		
Volume (l)	1.14±0.54	0.98±0.44 ^a
Creatinine (mmol)	7.50±2.89	6.32±2.16 ^a
Sodium (mmol)	147.9±75.6	158.7±71.5
Potassium (mmol)	24.7±11.8	26.8±12.9
Na ⁺ /K ⁺ ratio	6.37±2.55	6.43±2.56 ^a

Values are arithmetic mean±s.d., geometric mean (95% CI), or number of subjects (%).

Ambulatory recordings and urinary measurements were available in 314 subjects (138 men and 176 women) and 384 subjects (185 men and 199 women), respectively.

^a*P*<0.05 versus men.

years. Overall, the conventional and 24-h ambulatory blood pressures averaged 125.7/78.7 and 120.8/77.1 mm Hg, respectively. Table 1 provides further characteristics of the population sample by gender. Compared to women, men more frequently reported smoking and alcohol intake, and had higher (*P*<0.05) daytime systolic blood pressure (124.1 versus 127.6 mm Hg), plasma renin activity, urinary volume, and creatinine excretion. With cumulative adjustment applied for sex, age, and systolic blood pressure, plasma renin activity was inversely associated with the 24-h urinary sodium excretion (*P*=0.05).

Genotype and allele frequencies

The frequencies of the ADM genotypes (AA 50.6%, AG 38.2%, and GG 11.2%) did not deviate from the Hardy–Weinberg equilibrium (*P*=0.11). The genotype and allele frequencies were similar in parents and offspring (*P*>0.36), and did not differ according to the presence or absence of hypertension (*P*>0.74).

Population-based analyses

With adjustment applied for sex, age, body mass index (BMI), current smoking, alcohol intake, and the use of antihypertensive drugs, pulse pressure measured at the subjects' homes as well as on 24-h and daytime ambulatory monitoring was significantly lower in G allele carriers than AA homozygotes (*P*≤0.03, Table 2). This was mainly due to a consistently lower systolic pressure in G allele carriers, although the difference from AA homozygotes did not reach statistical significance (*P*≥0.22). The night-time pulse pressure adjusted for the same covariates was similar in G allele carriers and AA homozygotes (41.6 versus 42.2 mm Hg, *P*=0.43). Furthermore, both before and after adjustment for sex, age, and BMI, G allele carriers had a significantly lower

Table 2 | Blood pressure and sodium excretion by adrenomedullin genotype in all subjects

	Unadjusted			Adjusted ^a		
	AA (N=216)	AG+GG (N=211)	<i>P</i>	AA (N=216)	AG+GG (N=211)	<i>P</i>
<i>Conventional blood pressure (mm Hg)</i>						
Systolic	127.4±1.7	124.0±1.4	0.13	126.4±1.4	123.5±1.3	0.09
Diastolic	78.6±0.8	78.7±0.8	0.94	78.2±0.7	78.7±0.7	0.64
Pulse pressure	48.8±1.2	45.3±0.9	0.02	48.5±1.0	45.3±0.8	0.004
<i>24-h ambulatory blood pressure (mm Hg)</i>						
Systolic	121.4±1.3	120.1±1.2	0.46	120.7±1.1	119.0±0.9	0.22
Diastolic	77.1±0.8	77.1±0.8	0.99	76.7±0.7	76.8±0.7	0.99
Pulse pressure	44.3±0.7	43.0±0.7	0.18	44.3±0.6	42.6±0.6	0.03
<i>Daytime ambulatory blood pressure (mm Hg)</i>						
Systolic	126.4±1.3	124.8±1.2	0.38	126.0±1.1	124.1±1.0	0.19
Diastolic	81.4±0.8	81.6±0.8	0.84	80.9±0.7	81.3±0.7	0.70
Pulse pressure	45.1±0.8	43.2±0.7	0.07	45.0±0.6	43.1±0.6	0.02
Urinary sodium excretion ^b (mmol/day)	159.8±5.6	144.9±4.8	0.05	159.4±5.7	143.8±4.9	0.03
Plasma renin activity (ng/ml/h)	1.21 (1.02 to 1.42)	1.34 (1.14 to 1.58)	0.35	1.27 (1.09 to 1.47)	1.31 (1.14 to 1.51)	0.70

Values are arithmetic mean±s.e. or geometric mean (95% CI). Ambulatory recordings and urinary measurements were available in 314 subjects (161 AA homozygotes and 153 G allele carriers) and 384 subjects (202 AA homozygotes and 182 G allele carriers), respectively.

^aAdjusted for sex, age, BMI, current smoking, alcohol intake, and use of antihypertensive drugs.

^bCovariates in the adjusted analysis were sex, age, and BMI.

($P=0.03$) urinary sodium excretion than AA homozygotes with no difference in plasma renin activity according to genotype ($P=0.70$, Table 2). In further analyses, we did not detect a significant gene dose effect in any of the phenotype-genotype relations (data not shown).

Sensitivity analyses revealed significant genotype-by-age interactions in relation to systolic pressure and pulse pressure (Figure 1). In the parent generation, G allele carriers compared to AA homozygotes had a consistently lower systolic pressure and pulse pressure ($P\leq 0.01$, Figure 2). On conventional measurement, the differences amounted to 9.8 mm Hg (95% confidence interval (CI), 2.4–17.3 mm Hg; $P=0.009$) and 6.8 mm Hg (95% CI, 1.7–11.9 mm Hg; $P=0.009$), respectively. On 24-h ambulatory measurement, the corresponding estimates were 5.5 mm Hg (95% CI, 0.3–10.7 mm Hg; $P=0.04$) and 3.9 mm Hg (95% CI,

0.9–6.9 mm Hg; $P=0.01$). Among offspring, systolic pressure and pulse pressure were similar across the ADM genotypes. On the other hand, the 24-h urinary sodium excretion adjusted for sex, age, and BMI was consistently lower in parents (118.6 versus 138.1 mmol/day; $P=0.07$) and offspring (152.5 versus 174.7 mmol/day; $P=0.04$) carrying the G allele. This explained why the genotype-by-age interaction for sodium excretion was not significant ($P=0.76$).

Family-based analyses

Our study sample included 48 one-parent families with two ($N=35$) or more ($N=13$) offspring and 25 two-parent families with one ($N=3$), two ($N=16$), or more ($N=6$) offspring. We excluded seven subjects because of errors in Mendelian inheritance. The orthogonal model did not reveal significant population stratification ($P>0.12$).

In 85 informative offspring with adjustments applied as before, the orthogonal model did not show any significant association between the blood pressure phenotypes and the transmission of G allele ($P > 0.21$). However, in 68 informative offspring, transmission of the G allele was associated with lower urinary sodium excretion (effect size, 40.1 mmol/day; $P=0.01$).

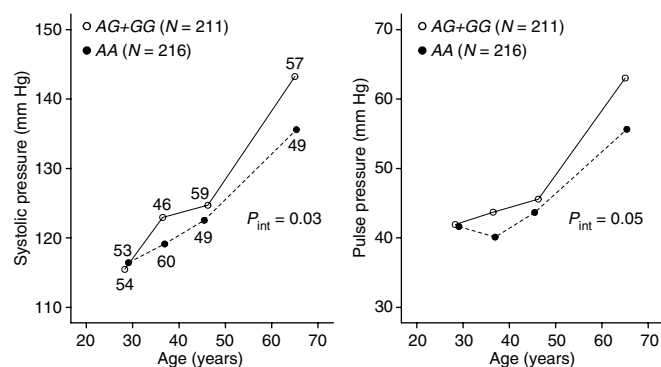


Figure 1 | Conventional systolic pressure and pulse pressure by ADM genotype and quartiles of age in the population study. Values are means adjusted for sex, age, BMI, current smoking, alcohol intake, and use of antihypertensive drugs. The P -value for interaction (P_{int}) between ADM genotypes and age, analyzed as a continuous variable, was derived from the GEE analysis.

Plasma ADM concentration in healthy volunteers

The 81 volunteers, 51.9% female, had a mean age of 52.7 ± 17.2 (s.d.) years. The geometric mean plasma concentration of ADM was 9.9 pmol/l (95% CI, 8.8–11.2) in AA homozygotes, 10.1 pmol/l (95% CI, 9.0–11.4) in AG heterozygotes, and 11.4 pmol/l (95% CI, 10.1–12.9) in GG homozygotes (Figure 3). The phenotypic difference between GG and AA homozygotes tended to be significant ($P=0.10$).

DISCUSSION

To our knowledge, our study is the first to examine whether at the level of the general population blood pressure and urinary sodium excretion are related to variation in the promoter of the ADM gene. In a Chinese population sample,

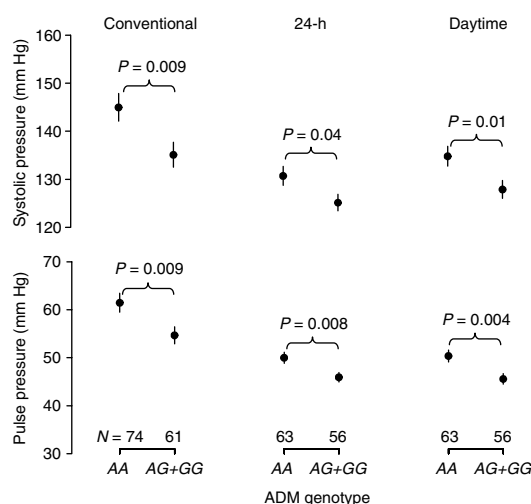


Figure 2 | Conventional and ambulatory blood pressure by ADM genotype in parents recruited from the population. Means were adjusted for sex, age, BMI, current smoking, alcohol intake, and use of antihypertensive drugs. (I) denote s.e.'s bar. The number of subjects contributing to each mean is given. P -values were derived from the GEE analysis.

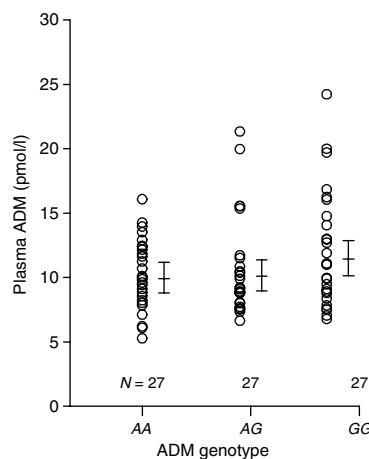


Figure 3 | Plasma ADM concentration by ADM genotype in 81 healthy volunteers. (O) represent individual measurements. (I) represent the geometric means with 95% CI.

we found that pulse pressure and urinary sodium excretion were lower in $-1984G$ allele carriers than AA homozygotes. We also noticed an interaction between the $ADM A-1984G$ polymorphism and age in relation to systolic blood pressure and pulse pressure, but not sodium excretion. Indeed, among parents, but not offspring, G allele carriers had significantly lower systolic pressure and pulse pressure, whereas the urinary sodium excretion was consistently lower in G allele carriers across the age range. Furthermore, in healthy Chinese volunteers, the plasma ADM concentration tended to increase with the number of $-1984G$ alleles.

There is abundant experimental evidence that ADM plays an important role in blood pressure regulation and sodium homeostasis.^{3–14,17} The targeted null mutation of the ADM gene was lethal, but heterozygous $ADM^{+/-}$ mice were hypertensive.^{11,18} Transgenic mice overexpressing the ADM gene in the cardiovascular system had lower blood pressure than controls.¹⁸ Chronic administration of ADM to salt-sensitive rats significantly attenuated the development of hypertension and prolonged survival.^{19,20} In hypertensive patients, infusion of ADM at a physiological dose (2.9 and 5.8 pmol/kg/min) for 2 h significantly reduced systolic and diastolic blood pressure.²¹ In addition, injection of ADM into the central nervous system dose-dependently inhibited sodium appetite and water drinking in rats.⁵ The intrarenal infusion of ADM in rats enhanced the glomerular filtration rate and the fractional sodium excretion and decreased the distal tubular sodium reabsorption.²² Thus, our present epidemiological findings concur with the evidence from previous experimental studies.

Irrespective of age, we observed that G allele carriers had a lower urinary sodium excretion than AA homozygotes. However, the difference in blood pressure associated with the ADM polymorphism was only significant in parents, but not offspring. Although these findings must be cautiously interpreted, they might indicate that in $ADM -1984G$ allele carriers, a lower salt intake early in life and continuing throughout the age range might in the long run lead to a lower systolic blood pressure and a narrower pulse pressure. Indeed, age is an important determinant of the penetrance of genetic variants.²³ Older age increases sodium sensitivity, steepens the relation between blood pressure and exchangeable body sodium, decreases the gain of the baroreceptor reflex, reduces renal perfusion, and compromises the buffering effects of the large arteries on both systolic and diastolic pressure.²² At younger age, compensatory feed back loops have a greater potential to maintain the homeostasis of the sodium balance. These age-related mechanisms might also contribute to the presently observed genotype-by-age interaction in relation to blood pressure.

The present study should be interpreted within the context of its limitations. First, our epidemiological study demonstrated association of blood pressure and sodium excretion with variation in the promoter of the ADM gene in Chinese, but did not provide direct information on the mechanisms underlying these phenotype-genotype relations.

Second, we did not measure the plasma concentration of ADM in the JingNing study participants, but in 81 age- and sex-matched healthy volunteers, equally distributed over the three ADM genotypes. Although we found a weak association between the plasma concentration of ADM and variation in the ADM gene, the functionality of the $A-1984G$ polymorphism remains to be tested in transfected cell models. Third, we did not assess the reproducibility of the 24-h urinary sodium excretion. A single 24-h urine collection might be insufficient to characterize an individual's habitual sodium intake, but it does reproducibly reflect the average salt consumption of groups of subjects.²⁴ Fourth, our sample size was relatively small, and hence the possibility of a chance finding cannot be entirely ruled out. On the other hand, our epidemiological observations are consistent with the large body of evidence supporting the hypothesis that a higher salt intake superimposed on genetic predisposition plays a pivotal role in the pathogenesis of essential hypertension.

Conclusion

In our Chinese population sample, the $ADM -1984G$ allele was associated with a lower 24-h urinary sodium excretion and in middle-aged and older subjects also with a lower systolic pressure and a narrower pulse pressure. If replicated in other populations, our results highlight the need for further experimental and clinical studies to elucidate the role of ADM in regulating salt appetite and in the pathogenesis of primary hypertension.

MATERIALS AND METHODS

Study population

In 2003, we visited all homes in six villages randomly selected from the JingNing County, a rural area approximately 500 km south of Shanghai.²⁵ Approximately 90% of the inhabitants are SHE Chinese, and the remainder belong to the HAN ethnicity. The literacy rate among adults is 65.5% and life expectancy is 74.7 years.²⁵ The Ethics Committee of Shanghai Second Medical University approved the study.

We invited families to take part, if at least two offspring with a minimum age of 12 years and one parent were available for examination. Of 839 eligible individuals, 509 (61.7%) participated after having given informed written consent. We excluded 42 subjects from the present analysis because of missing information on genotype ($N=18$) or phenotype ($N=24$). There is no generally agreed algorithm to construct the variance-covariance matrix for correlated data within complex pedigrees using generalized estimating equations (GEE). We therefore removed 40 participants from the analysis, keeping within each extended pedigree the most informative nuclear unit. These nuclei spanned two generations and included the largest possible number of informative relatives with all phenotypes and the ADM genotype available for analysis. Thus, the number of subjects statistically analyzed totalled 427.

Field work

Five experienced observers visited the subjects at their homes. They measured each participant's blood pressure five times consecutively by conventional sphygmomanometry, after the subjects had rested for at least 5 min in the sitting position. These five readings were

averaged for analysis. During the home visit, the observers administered a standardized questionnaire to collect information on smoking habits, alcohol consumption, and use of medications. Hypertension was defined as a blood pressure of at least 140 mm Hg systolic or 90 mm Hg diastolic, or as the use of antihypertensive drugs.

We programmed oscillometric SpaceLabs 90207 monitors (SpaceLabs Inc., Redmond, WA, USA) to obtain ambulatory blood pressure measurements with an interval of 20 min from 0800 until 2200 hours and every 45 min from 2200 to 0800 hours. The calibration of these devices was checked monthly against a mercury column. If the ambulatory recordings were longer than 24 h, only the first day was used for analysis. Intraindividual means of the ambulatory measurements were weighted by the time interval between successive readings. We defined daytime and night-time as the intervals ranging from 0800 to 1800 hours and from 2200 to 0400 hours, respectively.²⁵

Within 2–3 weeks of the home visit, the participants collected a 24-h urine sample in a wide-neck plastic container for measurement of electrolytes and creatinine. We sampled venous blood to measure plasma renin activity and the ADM genotype.

Determination of genotype

Genomic DNA was extracted from white blood cells. From the sequence of the human ADM gene, we amplified a 377 base pair fragment incorporating the A–1984G polymorphic site. The forward and reverse primers were 5'-CAAGTGGGAAGCTGGCGA CAAG-3' and 5'-CGGACCTGAATTCATCTGAGG-3', respectively. The PCR mixture (20 μ l) contained 100 ng DNA, 0.5 nmol/l primers, 1.5 mmol/l MgCl₂, 0.2 mmol/l dNTPs, and 1 U *Taq* polymerase. The amplification conditions were 95°C for 5 min, followed by 32 cycles at 94, 68 and 72°C, each for 45 s, and termination at 72°C for 10 min. We determined the ADM genotype by 2.5% agarose gel electrophoresis after digestion of 5 μ l PCR product by 2.5 U HpyCH4III (New England Biolabs, Beverly, MA, USA) at 37°C for 4 h. One DNA band of 377 base pairs and two DNA bands of 241 and 136 base pairs indicate AA and GG homozygosity, respectively. The AG heterozygotes have the combination of the three DNA bands.

Measurement of plasma ADM in healthy volunteers

To study the plasma concentration of ADM in relation to the ADM A–1984G polymorphism, we recruited and genotyped 454 healthy and untreated subjects, aged 17–84 years, whose blood pressure on conventional measurement (average of five consecutive readings) was less than 140 mm Hg systolic and 90 mm Hg diastolic. We selected all 27 GG homozygotes in the sample and we matched them for sex and age (within 0.8 years) with 27 AG heterozygotes and 27 AA homozygotes.

From the 81 volunteers, we collected a venous blood sample in the morning in chilled EDTA tubes. We measured the plasma concentration of ADM by radioimmunoassay as described by Ohta and co-workers.²⁶ We obtained monoclonal antibodies from the Navy Radioimmunoassay Centre (Beijing, China). The detection limit of our assay was 2 pmol/l. The working range was from 2 to 72 pmol/l. The intra- and interassay coefficients of variations were less than 10 and 15%, respectively.

Statistical analysis

For database management and statistical analysis, we used SAS software, version 8.2 (SAS Institute, Cary, NC, USA). Continuous

measurements with a skewed distribution were normalized by logarithmic transformation and represented by the geometric mean and 95% CI. Means and proportions were compared by the standard normal z-test and Fisher's exact test, respectively. We identified covariates of the phenotypes under study using stepwise multiple regression with the *P*-value for independent variables to enter and stay in the model set at 0.10. To account for the non-independence of the observations within families, while controlling for covariates and confounders, we studied genetic associations using GEE,²⁷ as implemented in the PROC GENMOD procedure of the SAS package.

In family-based analyses, with similar adjustments applied as in GEE, we performed a transmission disequilibrium test for quantitative traits (QTDT). We evaluated the within- and between-family components of phenotypic variance, using the orthogonal model as implemented by Abecasis *et al.*²⁸ in the disequilibrium test for quantitative traits software, version 2.4 (<http://www/sph.umich.edu/cgs/abecasis/QTDT>).

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REFERENCES

1. Kitamura K, Kangawa K, Kawamoto M *et al.* Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 1993; **192**: 553–560.
2. Sugo S, Minamino N, Kangawa K *et al.* Endothelial cells actively synthesize and secrete adrenomedullin. *Biochem Biophys Res Commun* 1994; **201**: 1160–1166.
3. Bunton DC, Petrie MC, Hillier C *et al.* The clinical relevance of adrenomedullin: a promising profile? *Pharmacol Ther* 2004; **103**: 179–201.
4. Nakamura M, Yoshida H, Makita S *et al.* Potent and long-lasting vasodilatory effects of adrenomedullin in humans. Comparisons between normal subjects and patients with chronic heart failure. *Circulation* 1997; **95**: 1214–1221.
5. Samson WK, Murphy TC. Adrenomedullin inhibits salt appetite. *Endocrinology* 1997; **138**: 613–616.
6. Samson WK, Bode AM, Murphy TC, Resch ZT. Antisense oligonucleotide treatment reveals a physiologically relevant role for adrenomedullin gene products in sodium intake. *Brain Res* 1999; **818**: 164–167.
7. Murphy TC, Samson WK. The novel vasoactive hormone, adrenomedullin, inhibits water drinking in the rat. *Endocrinology* 1995; **136**: 2459–2463.
8. Fujita M, Kuwaki T, Ando K, Fujita T. Sympatho-inhibitory action of endogenous adrenomedullin through inhibition of oxidative stress in the brain. *Hypertension* 2005; **45**: 1165–1172.

9. Ishimitsu T, Nishikimi T, Saito Y *et al.* Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. *J Clin Invest* 1994; **94**: 2158–2161.
10. Kohno M, Hanehira T, Kano H *et al.* Plasma adrenomedullin concentrations in essential hypertension. *Hypertension* 1996; **27**: 102–107.
11. Shindo T, Kurihara Y, Nishimatsu H *et al.* Vascular abnormalities and elevated blood pressure in mice lacking adrenomedullin gene. *Circulation* 2001; **104**: 1964–1971.
12. Yoshihara F, Suga S, Yasui N *et al.* Chronic administration of adrenomedullin attenuates the hypertension and increases renal nitric oxide synthase in Dahl salt-sensitive rats. *Regul Pept* 2005; **128**: 7–13.
13. Dobrzynski E, Wang C, Chao J, Chao L. Adrenomedullin gene delivery attenuates hypertension, cardiac remodeling, and renal injury in deoxycorticosterone acetate-salt hypertensive rats. *Hypertension* 2000; **36**: 995–1001.
14. Wei X, Zhao C, Jiang J *et al.* Adrenomedullin gene delivery alleviates hypertension and its secondary injuries of cardiovascular system. *Hum Gene Ther* 2005; **16**: 372–380.
15. Ishimitsu T, Kojima M, Kangawa K *et al.* Genomic structure of human adrenomedullin gene. *Biochem Biophys Res Commun* 1994; **203**: 631–639.
16. Lee HC, Shibata H, Ogawa S *et al.* Transcriptional regulation of the mouse IL-7 receptor-alpha promoter by glucocorticoid receptor. *J Immunol* 2005; **174**: 7800–7806.
17. Swynghedauw B. Adrenomedullin: a new member of the vasoactive peptide family with trophic action. *J Hypertens* 2003; **21**: 1085–1086.
18. Imai Y, Shiindo T, Maemura K *et al.* Evidence for the physiological and pathological roles of adrenomedullin from genetic engineering in mice. *Ann N Y Acad Sci* 2001; **947**: 26–33.
19. Yoshihara F, Suga S, Yasui N *et al.* Chronic administration of adrenomedullin attenuates the hypertension and increases renal nitric oxide synthase in Dahl salt-sensitive rats. *Regul Pept* 2005; **128**: 7–13.
20. Mori Y, Nishikimi T, Kobayashi N *et al.* Long-term adrenomedullin infusion improves survival in malignant hypertensive rats. *Hypertension* 2002; **40**: 107–113.
21. Troughton RW, Lewis LK, Yandle TG *et al.* Hemodynamic, hormone, and urinary effects of adrenomedullin infusion in essential hypertension. *Hypertension* 2000; **36**: 588–593.
22. Jougasaki M, Wei CM, Aarhus LL *et al.* Renal localization and actions of adrenomedullin: a natriuretic peptide. *Am J Physiol* 1995; **268**: F657–F663.
23. Staessen JA, Wang J, Bianchi G, Birkenhager WH. Essential hypertension. *Lancet* 2003; **361**: 1629–1641.
24. Luft FC, Fineberg NS, Sloan RS. Estimating dietary sodium intake in individuals receiving a randomly fluctuating intake. *Hypertension* 1982; **4**: 805–808.
25. Li Y, Wang JG, Gao P *et al.* Are published characteristics of the ambulatory blood pressure generalizable to rural Chinese? The JingNing population study. *Blood Press Monit* 2005; **10**: 125–134.
26. Ohta H, Tsuji T, Asai S *et al.* One-step direct assay for mature-type adrenomedullin with monoclonal antibodies. *Clin Chem* 1999; **45**: 244–511.
27. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. *Biometrics* 1988; **44**: 1049–1060.
28. Abecasis GR, Cardon LR, Cookson WO. A general test of association for quantitative traits in nuclear families. *Am J Hum Genet* 2000; **66**: 279–292.