Original Article

Interaction of Angiotensin I–Converting Enzyme Insertion-Deletion Polymorphism and Daily Salt Intake Influences Hypertension in Japanese Men

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The contribution of angiotensin I–converting enzyme insertion-deletion polymorphism (ACE I/D) to salt-sensitivity hypertension has been extensively studied by means of salt-loading tests, but whether or not the interaction with daily salt intake affects blood pressure still remains to be clarified. We therefore conducted a cross-sectional study of 284 Japanese male workers (age range, 20–64 years) to examine the effect of ACE I/D genotype and daily salt intake on hypertension. Blood pressure was measured and the ACE I/D was identified by polymerase chain reaction (PCR). Daily salt intake was calculated from a food frequency questionnaire (FFQ). In multivariate analyses, we explored the interaction of ACE I/D and salt intake by means of logistic regression analysis and multiple linear regression analysis. ACE I/D *per se* was not associated with blood pressure levels or hypertension. ACE I/D interacted with daily salt intake and correlated with hypertension (p for interaction=0.047). In the ID+II genotype, hypertension was increased by high salt intake (p=0.005), while in the DD genotype it was not (p=0.257). The interaction was more prominent in the overweight group (p=0.039) than in non-overweight group. In the overweight group, high salt intake induced a 10.5 mmHg higher diastolic blood pressure in the ID+II genotype than in the DD genotype (p=0.042). Our results suggest that ACE I/D and daily salt intake constitute a gene-environment interaction, which may be further modulated by overweight. (*Hypertens Res* 2006; 29: 751–758)

***Key Words*: angiotensin I–converting enzyme, polymorphism, salt sensitivity, hypertension, gene-environment interaction**

Introduction

Hypertension is a complex disease caused by genetic and environmental factors. Among the dietary factors, salt intake is one of the most important (*1–3*). Blood pressure usually responds to high salt intake, but the magnitude of response varies considerably among individuals (*3–5*). Genetic factors are likely to be involved in this difference in response (*6*, *7*). The renin-angiotensin-aldosterone system (RAAS), which has a central role in the maintenance of body fluid and blood pressure, is involved in the development of hypertension, and several genes in this system, such as the angiotensinogen (AGT), angiotensin I–converting enzyme (ACE), angiotensin II type 1 receptor (AT_1-R) and angiotensin II type 2 receptor (AT_2-R) genes, have been reported to have an association with hypertension (*8–12*). ACE is the key enzyme of the RAAS, and its insertion-deletion polymorphism (ACE I/D) in intron 16 is the most studied polymorphism in the RAAS. Physiologically, it has been shown that ACE I/D accounts for about 50% of the interindividual variability of plasma ACE concentration (*13*). ACE I/D has been recurrently studied in

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Table 1. Clinical Characteristic of the Study Participants According to Hypertension Status

Characteristics	Hypertensive subjects	Normotensive subjects	*p*
n	75	209	
Age (years)	52.6±5.4	43.7±12.2	<0.001
BMI (kg/m^2)	24.1±3.5	23.0±3.2	0.011
SBP (mmHg)	154.0±14.4	127.5±13.4	<0.001
DBP (mmHg)	96.0±8.2	76.7±9.6	<0.001
TC (mg/dl)	218.8±33.6	206.6±37.2	0.001
TG (mg/dl)	139.9±77.3	126.9±80.4	0.357
HDL-C (mg/dl)	56.0±15.7	55.1±13.2	0.615
Salt intake (g)	10.4±3.5	9.5±2.9	0.032
Family history of hypertension (%)	57.7	31.4	<0.001

Values are mean±SD or percentage. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, plasma total cholesterol; TG, plasma triglyceride; HDL, high-density–lipoprotein cholesterol.

hypertension (*14–18*) as well as various other conditions, particularly cardiovascular diseases (*19–23*). ACE I/D has also been shown to be associated with salt-sensitivity hypertension (*24–26*). These studies were conducted as salt-loading tests, and whether ACE I/D is effective in the context of daily salt intake still remains to be determined.

In the present study we examined the interaction of daily salt intake and ACE I/D in the regulation of blood pressure in Japanese men. Daily salt intake was determined by a food frequency questionnaire (FFQ), such as those used for assessing the composition of ingredients from food intake in large epidemiological studies (*27*). Thus our initial goal was to investigate whether interventional salt-loading studies and studies measuring salt intake in an *ad libitum* diet would yield similar results. Our results indicated that the latter type of study may more faithfully reflect the role of habitual salt intake in inducing hypertension. Since overweight is a risk for hypertension, and obesity is known to be associated with higher serum ACE levels (*28*, *29*), we also considered this factor in our analysis.

Methods

Subjects

The present study was untaken as a part of a larger study being carried out to investigate the association among lifestyle factors, hypertension, and various genetic factors in Japanese workers. Three hundred and fifty-eight Japanese working for a company in Kanagawa Prefecture, including 321 men and 37 women, participated in this study. Because there was a relatively low percentage of women (10.2%), because the number of women was insufficient to divide them into three salt intake groups by genotype, and because ACE I/D has been reported to be associated with hypertension in men but not in women, we selected only male subjects (n=284) who replied to the FFQ for the present study. The mean (±SD) age and body mass index (BMI) of the 284 enrolled subjects were 46.0±11.5 years and 23.3±3.3 kg/m^2, respectively. These values are typical for Japanese male workers.

Height, weight, serum lipid levels, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by a medical check-up. BMI was calculated (weight in kg divided by height in m squared). Overweight was defined as BMI≥23.0 kg/m^2 according to the criteria of the Regional Office for the Western Pacific Region of WHO (WPRO), which define adult overweight in Asians as a BMI over 23.0 kg/m^2, and obesity as a BMI over 25.0 kg/m^2 (*30–32*). Blood pressure was measured twice by well-trained nurses using a form PWV/ABI device (Nippon Colin, Komaki, Japan) with the subjects resting in a supine position. This device has been approved by the United States Food and Drug Administration (VP-2000/1000). The blood pressure data were then applied to the following study. Hypertension was defined as an SBP≥160 mmHg and/or DBP≥95 mmHg and/or current treatment for hypertension. The daily salt intake of each subject was calculated from a FFQ, a method that has been validated by Takahashi *et al*. (*33*). The sodium intake level determined by the FFQ method has been shown to be in good correlation with the urinary sodium level in man (correlation coefficient=0.66) (*34*). The FFQ used in this study contained questions about the consumption of food items in 29 food groups over the previous 1 or 2 months. The daily nutrient intake was calculated by multiplying the frequency of each food item consumed by the nutrient content of the portion size and summing the products for all food items. All subjects gave written informed consent before participating in the study, and the study was approved by the ethical committees of the Tokyo Medical and Dental University and Keio University School of Medicine.

Determination of ACE I/D

DNA was extracted from whole blood using a classical phenol/chloroform method. The I and D alleles were identified using the method of Lindpaintner *et al*. (*35*) with some modi-

Table 2. Clinical Characteristics According to the ACE I/D Genotypes

Characteristics	DD	ID	II	*p*
Genotype frequency (% (*n*))	9.5 (27)	46.1 (131)	44.4 (126)	0.398
Age (years)	43.5±12.4	45.5±12.0	47.1±10.7	0.248
BMI (kg/m^2)	22.4±3.0	23.2±3.4	23.6±3.3	0.177
SBP (mmHg)	132.96±19.2	134.0±17.4	135.2±18.4	0.801
DBP (mmHg)	78.9±13.8	81.0±12.7	83.1±12.1	0.184
TC (mg/dl)	213.0±38.8	201.1±34.6	211.4±38.2	0.054
TG (mg/dl)	118.3±53.6	119.8±67.5	142.0±93.5	0.158
HDL-C (mg/dl)	55.5±12.9	56.4±15.3	54.3±12.5	0.489
Salt intake (g)	10.3±3.1	10.0±3.3	9.4±2.8	0.161
Family history of hypertension (%)	46.2	36.1	39.3	0.612
Ambulatory patients (%)	14.8	13.0	11.1	0.828
Hypertension (%)	29.6	26.0	26.2	0.923

Values are mean±SD or percentage. *p* values are calculated from ANOVA or χ^2 test. ACE I/D, angiotensin I–converting enzyme insertion-deletion polymorphism. Other abbreviations are the same as in Table 1. TG was used as the log-transformed value.

fications. The upstream primer was 5′-GCCCTGCAG GTGTCTGCAGCATGT-3′ and the downstream primer was 5′-GAGACAAGGCGGGGAGAGCCATCC-3′. The polymerase chain reaction (PCR) mixture contained 17.5 ng genomic DNA, 0.2 μmol/l of each primer, 0.2 mmol/l of each dNTP, 2 mmol/l $MgCl_2$ and 0.5 U Ex Taq DNA polymerase (TaKaRa Japan, Tokyo, Japan) in a final volume of 10 μl. The amplification cycle was performed on a GeneAmp PCR System9700 (Applied Biosystems, Foster City, USA). After initial denaturation at 95°C for 5 min, the DNA was amplified by 35 PCR cycles of denaturation at 95°C for 45 s, annealing at 62°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 4 min. After addition of 2 μl of a 10× loading buffer, 5 μl of the mixture was loaded onto a 2% submarine agarose slab. Homozygotes produced either a single 597-bp band (II) or a 319-bp band (DD) (*28*); heterozygotes (ID) produced both bands. Because the D allele in heterozygous samples was preferentially amplified, each sample found to have the DD genotype was subjected to a second, independent PCR amplification with a primer pair that recognized an insertion-specific sequence (upstream primer, 5′-TGGGACCACAGCGCCCGCCACTAC-3′; downstream primer, 5′-TCGCCAGCCCTCCCATGC CCATAA-3′) using the following PCR conditions: 30 PCR cycles of denaturation at 95°C for 30 s and annealing at 68°C for 60 s, followed by a final extension at 68°C for 4 min. The reaction yielded a 335-bp amplicon only in the presence of an I allele, and no product in samples homozygous for DD.

Statistical Analyses

The statistical analyses were carried out using the Statistical Package for the Social Sciences for Windows, version 11.0 (SPSS Inc., Chicago, USA). The differences of frequencies or means were compared by χ^2 analysis or unpaired Student's *t*-test, respectively. Hardy-Weinberg equilibrium was determined by χ^2 test. In order to examine the degree to which the interaction between the ACE I/D and salt intake level contributed to hypertension, logistic regression analysis was performed both with unadjusted results and results adjusted for age and BMI. Multiple linear regression analysis was employed to test the effect of the interaction between ACE genotype and salt intake level on SBP or DBP. Since the mode of inheritance of the D allele has not been clarified, we examined three possible models of inheritance: dominant (II *vs*. ID+DD), additive (II *vs*. ID *vs*. DD), or recessive (ID+II *vs*. DD) (*14*). Values of $p<0.05$ were considered to indicate statistical significance for all analyses.

Results

Subject Characteristics

The subjects' characteristics are shown in Table 1. Age, BMI, SBP, DBP, plasma total cholesterol (TC), salt intake and family history of hypertension were significantly higher in hypertensive subjects. No difference was detected in plasma triglycerides (TG) or high-density–lipoprotein cholesterol (HDL-C) between the two subgroups (Table 1).

Frequency of ACE I/D

In all 284 Japanese male subjects the frequencies of DD, ID and II were 9.5%, 46.1% and 44.4%, respectively. The allele frequencies were 32.6% and 67.4% for the D and I alleles, respectively. These results are consistent with the Hardy-Weinberg equilibrium ($p=0.398$) (Table 2).

Association of ACE I/D with Phenotypes

The characteristics of subjects according to ACE I/D are given in Table 2. There were no significant differences in

Table 3. Logistic Regression Analysis for the Association between the Interaction of ACE I/D with Salt Intake Level and Hypertension

Parameters	Unadjusted OR (95% CI)	*p*	Adjusted OR (95% CI)	*p*
ACE (II+ID *vs*. DD)	0.1 (0.01–0.9)	0.038	0.1 (0.003–0.7)	0.024
ACE/II+ID × salt intake level (*vs*. DD × salt intake level)	3.0 (1.0–9.0)	0.047	3.6 (1.0–12.5)	0.047
Salt intake level	0.6 (0.2–1.5)	0.257	0.4 (0.1–1.3)	0.138
Age	—	—	1.1 (1.1–1.2)	<0.001
BMI	—	—	1.1 (1.0–1.2)	0.024

The DD genotype is the reference group. The interaction between the salt intake level and DD genotype is the reference group. The odds ratios (OR) were derived from a logistic regression model unadjusted or adjusted for age and body mass index (BMI) as indicated. Salt intake was divided into 3 groups by category and was coded into a single variable as salt intake level. Angiotensin I–converting enzyme insertion-deletion polymorphism (ACE I/D) was assigned as: DD=0; ID+II=1. CI, confidence interval.

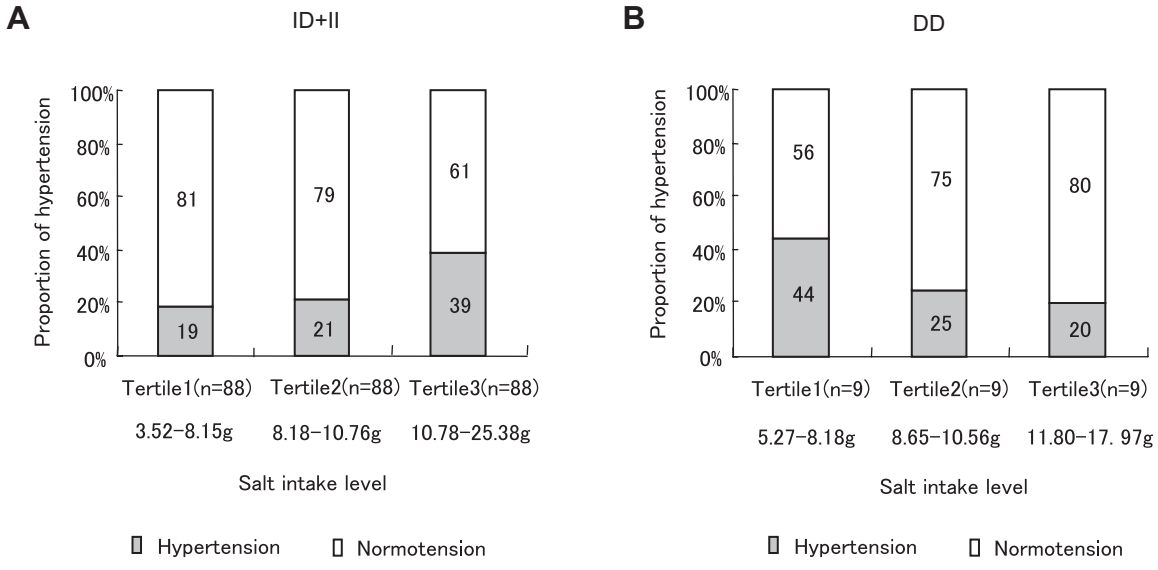

Fig. 1. *Proportion of hypertension and normotension according to salt intake level in the ACE genotypes of ID+II (A) and DD (B). Trend tests were done from the contingency table. A positive linear trend was detected in the ID+II genotype (*p*=0.005), but not in the DD genotype (*p*=0.257).*

SBP, DBP, hypertension or other parameters among the ACE genotype groups (DD *vs*. ID *vs*. II). There were also no significant differences in the dominant model (ID+II *vs*. DD) or in the recessive model (II *vs*. ID+DD) (data not shown). After adjusting for age, salt intake level and BMI by a multiple linear regression analysis, there was no association between ACE I/D and SBP or DBP in any genetic models.

Effect of the Interaction between ACE I/D and Salt Intake Level on Hypertension

In order to determine the effect of ACE I/D and salt intake on hypertension, we conducted multiple logistic regression analysis. There was no association between ACE genotype and hypertension adjusted for age, BMI and salt intake level in any genetic models. However, in the dominant model (ID+II *vs*. DD) (Table 3), when an ACE I/D × salt intake level interaction term was included in the model there was a significant association between this interaction and hypertension (unadjusted odds ratio [OR]=3.0, $p=0.047$; adjusted OR=3.6, $p=0.047$). An association between ACE I/D and hypertension was also detected (unadjusted OR=0.1, $p=0.038$; adjusted OR=0.1, $p=0.024$). Moreover, age and BMI also increased the risk of hypertension (age: OR=1.1, $p<0.001$; BMI: OR=1.1, $p=0.024$). We did not observe any association between hypertension and the ACE I/D × salt intake interaction in either the additive or recessive model.

We also examined the interaction between ACE I/D and salt intake level by a χ^2 trend test (Fig. 1). Our results showed that the prevalence of hypertension tended to increase in the ID+II genotype group with the increasing of salt intake (Fig. 1A, $p=0.005$); but not in the DD genotype group (Fig. 1B,

Table 4. Logistic Regression Analysis for the Association between the Interaction of ACE I/D Variant with Salt Intake Level and Hypertension Stratified by BMI

Parameters	Total (n=284)		BMI≥23.0 kg/m^2 (n=142)		BMI<23.0 kg/m^2 (n=142)	
	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
ACE (II+ID *vs*. DD)	0.1 (0.003–0.7)	0.024	0.003 (0.003–0.6)	0.033	0.2 (0.004–16.1)	0.507
ACE/II+ID × salt intake level (*vs*. DD × salt intake level)	3.6 (1.0–12.5)	0.047	11.4 (1.1–14.2)	0.039	1.6 (0.3–10.3)	0.624
Salt intake level	0.4 (0.1–1.3)	0.138	0.1 (0.01–1.2)	0.072	0.9 (0.1–5.0)	0.853
Age	1.1 (1.1–1.2)	<0.001	1.1 (1.0–1.1)	0.002	1.1 (1.1–1.2)	<0.001
BMI	1.1 (1.0–1.2)	0.024	1.2 (1.0–1.4)	0.067	1.0 (0.7–1.3)	0.881

The DD genotype is the reference group. The interaction between the salt intake level and DD genotype is the reference group. The odds ratios (OR) were derived from a logistic regression model unadjusted or adjusted for age and body mass index (BMI) as indicated. Salt intake was divided into 3 groups by category and was coded into a single variable as salt intake level. Angiotensin I–converting enzyme insertion-deletion polymorphism (ACE I/D) was assigned as: DD=0; ID+II=1. CI, confidence interval.

Table 5. Regression Analysis for the Association between the Interaction of ACE I/D Genotype with Salt Intake, Adjusted SBP/DBP and Stratified by BMI

Parameters	Total (n=284)				BMI≥23.0 kg/m^2 (n=142)				BMI<23.0 kg/m^2 (n=142)			
	SBP		DBP		SBP		DBP		SBP		DBP	
	β	p	β	p	β	p	β	p	β	p	β	p
ACE (II+ID *vs*. DD)	−5.8	0.499	−4.7	0.399	−26.3	0.064	−23.0	0.014	7.1	0.513	6.1	0.379
ACE/II+ID × salt intake level (*vs*. DD × salt intake level)	2.3	0.558	2.7	0.286	11.4	0.058	10.5	0.008	−3.9	0.450	−2.3	0.471
Salt intake level	0.7	0.045	0.3	0.159	0.7	0.167	0.7	0.039	4.9	0.310	1.8	0.566
Age	0.6	<0.001	0.5	<0.001	0.7	<0.001	0.5	<0.001	0.5	<0.001	0.5	<0.001
BMI	1.2	<0.001	0.9	<0.001	1.7	0.006	0.9	0.022	0.3	0.635	0.6	0.268

β values were derived from multiple regression analysis models adjusted for age and body mass index (BMI). ACE I/D, angiotensin I–converting enzyme insertion-deletion polymorphism; SBP, systolic blood pressure; DBP, diastolic blood pressure.

p=0.257). In the individuals with the ID+II genotype the prevalence of hypertension rose significantly in each of the low, middle and high salt intake subgroups (by 19%, 21% and 39%, respectively).

Effect of BMI on the Association between Hypertension and the ACE I/D × Salt Intake Level Interaction

We next analyzed the effect of BMI on the association between hypertension and the interaction between ACE I/D and salt intake. We found lower p values in overweight subjects (unadjusted OR=13.4, p=0.023; adjusted OR=11.4, p=0.039) than in all subjects (unadjusted OR=3.0, p=0.047; adjusted OR=3.6, p=0.047). This association was not observed in non-overweight subjects (unadjusted OR=1.4, p=0.678; adjusted OR=1.6, p=0.624) (Table 4). Additionally, we detected that the interaction between ACE I/D and salt intake was associated with DBP (p for β=0.008) in overweight subjects but not in non-overweight subjects or all subjects (Table 5). In the high salt intake subgroup, subjects with the ID+II genotype had a 10.5 mmHg higher blood pressure than those with the DD genotype (p=0.042).

Discussion

The present study showed that, while ACE I/D was not associated with hypertension by itself, the interaction between ACE polymorphism and salt intake level was correlated with hypertension. In other words, subjects with the ID+II genotype may be more susceptible to developing hypertension as a result of high daily salt intake than those with the DD genotype. The ACE I/D is the most studied polymorphism in regard to hypertension and cardiovascular diseases. Our analysis shows that ACE I/D is not likely to be an independent risk factor for hypertension. A lack of association between ACE I/D and hypertension has been reported in several previous studies (*36–39*), indicating that the effect of this polymorphism may not be straightforward. Indeed, several reports have suggested that ACE I/D might play a different role in the etiology of hypertension according to gender, race or age (*40–42*). Interactions between ACE I/D and smoking (*43*) or between ACE I/D and other gene polymorphisms, such as α-adducin (G460W) (*44*) or AT_1R-$A^{1166}C$ (*15*), have also been

correlated with hypertension. Thus, the effect of ACE I/D on hypertension may be more evident when considered in relation to other genetic and non-genetic factors.

Salt sensitivity–induced hypertension is highly dependent on genetic factors (*6*, *45–47*). Several previous studies have investigated the relation between ACE I/D and salt sensitivity, and our results are consistent with these earlier reports. In their study on Japanese hypertensive patients, Hiraga *et al*. (*26*) reported that the frequency of the I allele in the salt-sensitivity group was significantly higher than that in the salt-resistant group. Giner *et al*. (*24*) and Poch *et al*. (*25*) reported the same tendency in Spanish hypertensives, and further demonstrated that patients with the ID+II genotype had a significantly greater blood pressure rise during high salt diet load than patients with the DD genotype. This salt sensitivity is partially explained by blunted RAAS response to high salt intake (*48*). It can be speculated that the ID+II genotype may lead to a decreased response to high salt intake, and thus the effects of a high salt diet may not be suppressed as efficiently as in patients with the DD genotype.

In addition to salt intake, we found that overweight may be a confounding factor. The effect of the interaction between salt intake level and ACE I/D on hypertension was more significant in overweight subjects. Overweight individuals with ID+II had significantly higher risk for hypertension (OR=11.4, $p=0.039$) compared with the complete subject group. This result suggests an interaction among ACE I/D, salt intake, and overweight.

In fact, a relation among overweight, salt intake and the RAAS has already been established, as follows. Adipose tissue has its own local RAAS (*49*). The plasma ACE level has been positively correlated with BMI (*28*). High salt intake has been strongly and independently associated with an increased risk of cardiovascular disease and all-cause mortality in overweight persons, but not in non-overweight persons (*50*). Furthermore, current studies suggest that ACE I/D might underlie obesity-associated hypertension (*51*, *52*). In the light of these observations, our results might be attributable to the effect of ACE I/D becoming more overt in overweight individuals who were under high salt intake. Alternatively, it may simply reflect the fact that salt and calorie intake in the food are correlated (*53*), and hypertension and overweight may occur simultaneously. The effect of the interaction between ACE I/D and salt intake on hypertension may thus be over-represented in the overweight group. The effect of the interaction among ACE I/D, salt intake level, and overweight on the regulation of blood pressure warrants further investigation.

In the present study we used two definitions of hypertension: the older, more conservative criteria (SBP≥160 mmHg and/or DBP≥95 mmHg and/or current treatment for hypertension.); and the more recent criteria (SBP≥140 mmHg and/or DBP≥90 mmHg and/or current treatment for hypertension). Our results showed that the interaction between ACE I/D and salt intake level was correlated with hypertension only as defined according to the previous criteria, and not by the more recent criteria. This suggests that the effect of the interaction between ACE I/D and salt intake is stronger in moderate hypertension. Moreover, we did not measure the serum renin activity or ACE activity, and could not identify cases of salt-sensitivity hypertension.

In conclusion, ACE I/D and daily salt intake might be an example of a gene-environment interaction leading to hypertension. Overweight might also be a modulator of this interaction.

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