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Interaction and Relationship Between Angiotensin Converting Enzyme Gene and Environmental Factors Predisposing to Essential Hypertension in Mongolian Population of China[1]

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Objective To investigate the association of specific functional gene ACE (I/D) variants of the renin-angiotensin system with essential hypertension (EH) and interaction between ACE (I/D) gene and risk factors for EH in a genetically homogenous Mongolia rural population of China. **Methods** Individuals (n=1099) were recruited from general population of Kezuohouqi Banner in Inner Mongolian Autonomous Region. **Results** The association was found between ACE genotype DD plus ID and EH, with an interaction between ACE genotype DD plus ID and cigarette smoking in an additive model. Cigarette smoking index and ACE gene showed a low exposure-gene (LEG) effect on EH, with interaction indices from 7.10 to 1.16. Interaction between ACE genotype DD plus ID and alcohol drinking on EH appeared an additive model. Alcohol drinking index and ACE gene showed a low exposure-gene (LEG) effect on EH, with interaction indices from 1.66 to 1.09. BMI and ACE gene showed a low exposure-gene (LEG) effect on EH, with interaction indices from 6.15 to 2.49. Interactions between ACE genotype and WHR on EH showed a multiplicative model. In a short, there was an interaction between ACE gene and cigarette smoking, alcohol drinking and BMI on EH,

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especially in a low dose-exposure effect. **Conclusion** It is important for individuals who carry ACE D allele gene to prevent EH, and furthermore, to prevent and control coronary heart disease, in a view of population-based prevention.

Key words: Angiotensin-converting enzyme; Hypertension; Genetic; Risk factors; Interaction; Exposure-gene effect

INTRODUCTION

Essential hypertension (EH) is a polygenetic complex trait, as well as one of the main risk factors for coronary heart disease[1], which is resulted from interaction between environmental and genetic factors. Its etiology is complicated because of its onset at late age, genetic heterogeneity, incomplete penetrance, and quantitative variability of blood pressure phenotype[2]. Traditional epidemiologic methods explore etiology of EH only from environmental risk factors exposure[3-6]. In recent years, with progress in Human Genome Project, it becomes possible to explore etiology from many of the genes susceptible to EH, as illustrated angiotensin-converting enzyme (ACE) gene, angiotensinogen gene, and other environmental risk factors[7-9].

In this study, we investigated the association of specific functional ACE insertion/deletion (I/D) gene polymorphism in intron 16 of the renin-angiotensin system with EH, and interaction between ACE genotypes I/D polymorphism and risk factors of EH in a homogenous Mongolian rural population of China.

MATERIALS AND METHODS

Selection of Study Subjects

A cross-sectional study of environmental exposure factors for essential hypertension was performed in rural population of Kezuohouqi Banner, Tongliao, Inner Mongolian Autonomous Region using a self-designed questionnaire from May to June 2002. A sample of 1099 individuals aged more than or equal to 20 years were recruited.

Blood Pressure Measurements

Standard procedure for measurement of blood pressure was performed in the morning for all the subjects fasted in the study. Three blood pressure measurements were taken on their right arm of each participant at sitting position by a trained personnel. Standard mercury sphygmomanometers were used with appropriate cuff sizes. Systolic blood pressure (SBP) was measured at the first appearance of a pulse sound (Korotkoff phase 1) and diastolic blood pressure (DBP) at the disappearance of the pulse sound (Korotkoff phase 5). An average of the three measurements was calculated. Hypertension was defined as mean systolic blood pressure greater than or equal to 140 mmHg and/or mean diastolic blood pressure greater than or equal to 90 mmHg and no-antihypertensive medication taken currently.

Body Measurements

Height was measured in centimeters for each participant in an upright standing position without shoes, and weight was measured in kilograms for the participant standing without shoes and in light clothing. Body mass index (BMI) was calculated as weight in kilograms

over height in meters squared. Waist circumference was measured in centimeters at the midpoint between the bottom of the ribs and the top of the iliac crest. Hip circumference was measured at the largest posterior extension of the buttocks. Waist-hip ratio (WHR) was calculated as waist over hip circumference in centimeters.

Alcohol and Cigarette Consumption

Alcohol consumption was assessed by a questionnaire, in which the subject reported his daily average amount of consumption (in a drink unit) and duration of consumption. Alcohol consumption equaled to multiplication of daily average consumption (in a drink unit) by duration of consumption. Cigarette consumption equaled to multiplication of cigarettes smoked per day and duration of smoking.

Identification of Diallelic Polymorphisms

The leukocytes from 5 mL peripheral blood were digested with proteinase K and genomic DNA was extracted using phenol and chloroform, followed by ethanol precipitation. Polymerase chain reaction (PCR) was performed in a 25 μL reaction volume containing 100 ng of genomic DNA.

The PCR primers were as follows[10]: sense oligo 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and anti-sense oligo: 5′-GATGTGGCCATCACATTCGTCAGAT-3′. PCR amplification was carried out in a PTC-200 MJ research peltier thermal cycler (Perkin-Elmer Corp., Foster City, USA) and followed by a prior denaturation at 94℃ for 3 min. This was followed by 30 cycles of denaturation at 94℃ for 1 min, annealing at 58℃ for 1 min, and extension at 72℃ for 1 min with a final extension at 72℃ for 5 min. The PCR product was a 190 bp fragment (DD) in the absence of the insertion and a 490 bp fragment (II) in the presence of the insertion. A third fragment (ID) with an intermediate molecular weight was present in PCR from heterozygotes, the products were visualized with ethidium bromide after electrophoresis on 3% agarose gel.

Statistical Analysis

Difference in categorical data between the hypertensive and normotensive subjects was examined by χ^2 test and difference in quantitative data between the hypertensive and normotensive subjects was examined by student's *t*-test. A trend test was performed for exposure dose and risk of EH. All statistical procedures were performed with SAS version 6.12.

Interaction Between Gene and Environmental Factors

A common way to describe interaction between the effects of an environmental risk factor (ERF) and a genetic risk factor (GRF) was to use both terms in a multiple regression model and to include a term that multiplied the GRF by the ERF. Coefficient of an interactive item determined whether interaction was present: $G(Y)= \alpha+\beta_e E+\beta_g G+\beta_{eg} EG$, where Y is the odds of disease, α is a constant, E is environmental exposure, G is the GRF, and EG is interaction term. Coefficients β_e, β_g, and β_{eg} were determined by regression analysis. Interaction index $\gamma=\beta_{eg}/\beta_g$.

When dose of environmental exposure was analyzed with respect to genotype of a susceptible gene, two apparently divergent patterns were seen. A low or high exposure-gene (LEG and HEG) effect was defined when there is a decreased or increased degree of

interaction occurred as a function of exposure dose, respectively[11-14].

The study was approved by the Institute Committee for Biomedical Ethics. All subjects gave informed consent before the study.

RESULTS

Study Population

A total of 1099 rural residents were recruited from Kezuohouqi Banner of Tongliao, Inner Mongolian Autonomous Region, China, with 448 hypertensive subjects met the diagnostic criteria described above. Comparison of clinical characteristics between the hypertensive and normotensive (NH) subjects revealed that age, proportion of men, smoking, alcohol drinking, BMI, WHR, serum levels of total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), and fasting plasma glucose (FPG), were significantly higher in hypertensive subjects than those in normotensive subjects. But, there was no difference in level of LDL-cholesterol (LDL-C) between the hypertensive and normotensive subjects (Table 1).

TABLE 1

Characteristics of Study Participants ($\bar{x} \pm s$)

Characteristics	EH	NH	*P*
No. of Subjects	448	651	
Age (yr.)	52.30±12.15	42.16±11.69	<0.0000
Sex (% men)	46.88	34.87	<0.001
Smoking (%)	48.21	38.10	<0.001
Drinking (%)	40.18	21.97	<0.001
BMI (kg/m^2)	23.15±3.96	22.01±3.08	<0.0001
WHR (cm/cm)	0.88±0.07	0.85±0.06	<0.0001
TC (mmol/L)	3.94±1.26	3.67±1.26	<0.0004
TG (mmol/L)	1.36±1.01	1.16±1.01	<0.0011
HDL-C (mmol/L)	1.15±0.39	1.09±0.38	<0.0201
LDL-C (mmol/L)	2.20±1.24	2.09±1.19	0.1467
FPG (mmol/L)	5.79±1.38	5.48±0.96	<0.0001

Distribution of ACE I/D Polymorphism

ACE genotype distribution was deviated from Hardy-Weinberg's expectation only in women (χ^2=4.08, P<0.04), but not in men (χ^2=0.02, P=0.89), or in both men and women combined (χ^2=2.73, P=0.10). No significant difference in ACE genotype distribution (χ^2=1.39, P=0.50) was observed between men and women in Mongolian population.

ACE Genotype and EH

Relationship between ACE genotype and EH is shown in Table 2. There was a significant difference in varied ACE genotype between hypertensive (EH) and normotensive (NH) subjects (χ^2=7.15, P=0.028). We compared the frequencies of ACE genotypes of DD plus ID and II in EH and NH subjects, with an estimated odds ratio (OR) of 1.42 (95% CI 1.10–0.84). ACE genotype DD plus ID was significantly associated with EH, indicating that ACE I/D genotype polymorphism would be a genetic factor predisposing to EH.

TABLE 2

ACE Genotype and EH in Mongolian Population

ACE Genotype	EH	NH	OR	OR 95% CI
II	130	239	1.00	–
ID	225	287	1.44	1.09–1.90
DD	93	125	1.37	0.97–1.93
	χ^2=7.15, P=0.028			
II	130	239	1.00	–
DD+ID	318	412	1.42	1.10–1.84
	χ^2=7.05, P=0.008			

Interactions Between ACE Genotype and Cigarette Smoking

Results of interaction between ACE and smoking are shown in Table 3. OR_e for smokers alone was 1.54 (95% CI 1.00–2.37), as compared with non-smokers and OR_g for ACE (DD+ID) alone was 1.43 (95% CI 1.01–2.02), as compared with ACE II. OR_{eg} for joint ACE (DD+ID) genotype and smoker was 2.14 (95% CI 1.49–3.06), as compared with ACE II genotype and no-smoker. There was an interaction between ACE (DD+ID) genotype and cigarette smoking, in an additive pattern. Attributable fraction (AF) was 35.06% for cigarette smoking, 30.07% for ACE (DD+ID) and 53.27% for both ACE (DD+ID) and cigarette smoking, respectively.

TABLE 3

Interactions Between ACE and Cigarette Smoking

ACE	Cigarette Smoking	EH (n=448)	NH (n=651)	OR	OR 95% CI	AF*(%)
II	No	68	150	1.00	–	–
II	Yes	62	89	1.54	1.00–2.37	35.06
DD+ID	No	164	253	1.43	1.01–2.02	30.07
DD+ID	Yes	154	159	2.14	1.49–3.06	53.27

Note. * AF (%): Attributable fraction (percent) in exposed cases.

Cigarette Smoking Index-Gene Effect on EH

Table 4 shows that ACE genotype DD plus ID increased with cigarette smoking index in a low exposure-gene (LEG) effect, with interaction indices from 7.10 to 1.16.

TABLE 4

Cigarette Smoking Index-Gene Effect on EH

ACE Genotype	Cigarette Smoking Index (pack-year)	EH (n=448)	NH (n=651)	OR*	OR 95% CI	β †		γ ‡
						β_e	β_{eg}	
II	0	68	150	1.00	–	0.0000		
II	<30	35	72	1.07	0.65–1.75	0.0698		
II	⩾30	27	17	3.50	1.81–6.96	1.2538		
DD+ID	0	164	253	1.43	1.01–2.03	0.0000	0.3576	
DD+ID	<30	96	129	1.64	1.11–2.43	0.0698	0.4957	7.10
DD+ID	⩾30	58	30	4.26	2.54–7.29	1.2538	1.4504	1.16

Note. *P trend=0.001, † β: Coefficient of regression, ‡ $\gamma=\beta_{eg}/\beta_e$.

Interactions Between ACE Genotype and Alcohol Drinking

Results of interaction between ACE and alcohol drinking are shown in Table 5. OR_e for alcohol drinkers alone was 2.40 (95% CI 1.52–3.77), as compared with non-drinkers. OR_g for ACE genotype DD plus ID alone was 1.45 (95% CI 1.05–2.00), as compared with ACE II. OR_{eg} for joint ACE (DD+ID) genotype and alcohol drinker was 3.51 (95% CI 2.40–5.11), as compared with ACE II genotype and no-drinker. There was an interaction between ACE genotype DD plus ID and alcohol drinking, in an additive pattern. AF was 58.33% for alcohol drinking, 31.03% for ACE genotype DD plus ID, and 71.51% for both alcohol drinking and genotype DD plus ID, respectively.

TABLE 5

Interactions Between ACE and Alcohol Drinking

ACE Genotype	Alcohol Drinking	EH (n=448)	NH (n=651)	OR	OR 95% CI	AF* (%)
II	No	75	183	1.00	–	–
II	Yes	55	56	2.40	1.52–3.77	58.33
DD+ID	No	193	325	1.45	1.05–2.00	31.03
DD+ID	Yes	125	87	3.51	2.40–5.11	71.51

Note. *AF (%): Attributable fraction (percent) in exposed cases.

Alcohol Drinking Index-Gene Effect on EH

Table 6 shows that risk for EH increased with alcohol index in subjects with ACE (DD+ID) genotype in a LEG effect, with interaction indices from 1.66 to 1.09.

TABLE 6

Alcohol Drinking Index-gene Effect on EH

ACE Genotype	Alcohol Drinking Index (Year Drink-unit)	EH (n=448)	NH (n=651)	OR*	OR 95% CI	β†		γ‡
						β_e	β_{eg}	
II	0	75	183	1.00	–	0.0000		
II	<192	37	47	1.92	1.15–3.19	0.6528		
II	⩾192	18	9	4.88	2.15–11.85	1.5851		
DD+ID	0	193	326	1.44	1.05–2.00	0.0000	0.3678	
DD+ID	<192	81	67	2.95	1.94–4.51	0.6528	1.0818	1.66
DD+ID	⩾192	44	19	5.65	3.14–10.51	1.5851	1.7317	1.09

Note. *P trend=0.001, † β: Coefficient of regression, ‡ $\gamma=\beta_{eg}/\beta_e$.

Interactions Between ACE and BMI

Table 7 shows interaction between ACE and BMI on EH. Risk for EH increased with BMI in subjects with ACE (DD+ID) genotype in a LEG effect, with interaction indices from 6.15 to 2.49.

TABLE 7

Interactions Between ACE and BMI on EH

ACE Genotype	BMI	EH (n=448)	NH (n=651)	OR*	OR 95% CI	β†		γ‡
						β_e	β_{eg}	
II	<23	75	155	1.00	–	0.0000		
II	23–	23	43	1.10	0.61–1.95	0.1002		
II	25–	32	41	1.61	0.94–2.76	0.4781		
DD+ID	<23	161	288	1.15	0.83–1.62	0.0000	0.1444	
DD+ID	23–	52	58	1.85	1.16–2.95	0.1002	0.6167	6.15
DD+ID	25–	105	66	3.29	2.18–4.99	0.4781	1.1902	2.49

Note. *P trend=0.001, † β: Coefficient of regression, ‡ $\gamma=\beta_{eg}/\beta_e$.

Interactions Between ACE Genotype and WHR

Results of interaction between ACE and WHR are shown in Table 8. Median WHR was 0.85. OR_e for subjects with WHR⩾0.85 alone was 3.66 (95% CI 2.35–5.70), as compared

with those with WHR<0.85. OR_g for subjects with ACE (DD+ID) alone was 1.68 (95% CI 1.11–2.52), as compared with those with ACE II. OR_{eg} for subjects with both ACE (DD+ID) genotype and WHR⩾0.85 was 4.22 (95% CI 2.88–6.18), as compared with those with ACE II genotype and WHR<0.85. There was an interaction between ACE (DD+ID) genotype and WHR in a multiplicative model. AF was 72.68% for WHR, 40.48% for ACE genotype (DD+ID), and 76.30% for ACE genotype (DD+ID) and WHR, respectively.

TABLE 8

Interaction Between ACE and WHR on EH

ACE Genotype	WHR*	EH (*n*=448)	NH (*n*=651)	OR	OR 95% CI	AF† (%)
II	<0.85	42	152	1.00	–	–
II	⩾0.85	88	87	3.66	2.35–5.70	72.68
DD+ID	<0.85	107	231	1.68	1.11–2.52	40.48
DD+ID	⩾0.85	211	181	4.22	2.88–6.18	76.30

Note. * Median WHR as cut-off value. †AF (%): Attributable fraction (percent) in exposed cases.

DISCUSSION

Advances in medical genetics and the Human Genome Project will play a central role in medical care and public health practice in the 21-century by predicting and preventing disease and promoting health[15]. It is known that humans have not only a great deal of gene polymorphism, but also expansive phenotype to environmental risk factors[11]. It is the most effective intervention or prevention for hypertension with a target at changing environmental factors matched to an individual's specific genetic susceptibility and understanding the interaction and relationship between gene and environmental[14].

Mongolian population of China, both farmers and herdsmen, have lived in Inner Mongolian Autonomous Region over thousands of years. There, individuals recruited in this study were relatively homogeneous with regard to ethnicity, socioeconomic status, and occupational and dietary exposures. Thus, a study with genetic homogeneous populations may increase statistical power for identification of relevant gene[16], and results from population-based studies could help medical and public health professionals better their target medical, behavioral, and environmental interventions[17].

It was reported that alcohol consumption was positively associated with blood pressure[3]. In this study, interaction between ACE (DD+ID) genotype and alcohol drinking showed an additive interaction model. The alcohol drinking index and ACE (DD+ID) genotype showed a LEG effect.

To explain existing interaction of drinking-hypertension, we need to consider three possible explanations. Firstly, alcohol drinking would directly increase blood pressure levels. Secondly, it is possible that alcohol drinking might be associated with an increased consumption of animal fat or salted food, which could lead to an increase in fibrinogen levels. Mongolians are traditional to consume more animal fat and salt (to preserve vegetables), and less unsaturated fatty acid and fresh vegetables. Generally speaking, drinkers consume more

animals' fat and salt, as compared with non-drinkers. Thirdly, there might be interactions between alcohol drinking and over-consumption of animal fat and salt in an additive or synergistic manner. Another dietary custom in Mongolians is frequently drinking strong spirit, as well as consumption of more animal fat and salt, which might be a major cause of higher prevalence of EH in the study.

BMI is positively and independently associated with morbidity and mortality caused by hypertension. Positive association between BMI and blood pressure has been well documented in Caucasian population and East Asian populations, but not in Filipinos[4]. In this study, The ACE (DD+ID) genotype and BMI showed a LEG effect. Interactions between ACE (DD+ID) genotype and WHR displayed a multiplicative model. To explain why these ethnic differences in strength of association between BMI or WHR and hypertension exist, we need to consider genetically determined differences in body composition and metabolic response, as well as clustering of risk factors due to difference in social and environmental factors.

A consensus has not been reached regarding the association between cigarette smoking and blood pressure. The association between smoking habits and BP in normotensive Japanese men[18], negative dose-effect relationships between the amount of smoking and systolic[19], diastolic blood pressure[20] or both[21] were reported. On the other hand, some studies failed to observe a significant dose-effect relationship[22,23]. In this study, interaction between ACE (DD+ID) genotype and cigarette smoking showed an additive interaction model. The cigarette smoking index and ACE (DD+ID) genotype showed a LEG effect. The mechanism of cigarette smoking and ACE gene on EH is unclear.

It is reported that there is a LEG effect between cigarette smoking index, alcohol drinking index, BMI and ACE gene on EH. We speculate that their biological mechanisms may be similar in the pathophysiology of EH. It is possible to explore the interaction between gene-environmental factors on EH and pathophysiology of EH under new progress in EH susceptible genes[17]. It is important for individuals who carry ACE D allele gene to prevent EH and coronary heart disease, in a view of population-based prevention.

PERSPECTIVES

The interaction between genetic and environmental risk factors highlights the etiological study of EH. It is the most effective intervention or prevention for hypertension with a target at changing environmental factors matched to an individual's specific genetic susceptibility and understanding the interaction and relationship between genes and environment.

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