Renin-Angiotensin System Gene Polymorphisms, Blood Pressure, Dyslipidemia, and Diabetes in Hong Kong Chinese

A significant association of the ACE insertion/deletion polymorphism with type 2 diabetes

G. Neil Thomas, PhD  
Brian Tomlinson, FRCP  
Juliana C.N. Chan, FRCP  
John E. Sanderson, FRCP  
Clive S. Cockram, FRCP  
Julian A.J.H. Critchley, FRCP

OBJECTIVE — In Chinese populations, hypertension is common and is a major risk factor for cerebrovascular and coronary heart disease, particularly when associated with diabetes. The clustering of these disorders and dyslipidemia and obesity is termed the metabolic syndrome and is increasing in prevalence in the populations of modernizing Asian nations. The renin-angiotensin system (RAS) helps maintain blood pressure and salt homeostasis and may play a role in the pathogenesis of aspects of the metabolic syndrome. We investigated three RAS gene polymorphisms—the ACE insertion/deletion (I/D), angiotensinogen (AGT) M235T, and angiotensin II type 1 receptor (AT1, R) A1166C polymorphisms—for a possible role in modulating these disorders in 853 Chinese subjects with varying components of the metabolic syndrome.

RESEARCH DESIGN AND METHODS — The three gene polymorphisms of this cross-sectional study were detected using polymerase chain reaction–based protocols. The genotype frequencies were compared between the controls (n = 119) and both overlapping and nonoverlapping groups of patients with type 2 diabetes, hypertension, and dyslipidemia using χ² test. Differences in levels of the biochemical parameters between the genotypes were determined using analysis of variance.

RESULTS — No significant association of the ACE insertion/deletion polymorphism and blood pressure in this population. Although the AT1 R A1166C polymorphism was not associated with any aspect of the metabolic syndrome examined, there was limited evidence to suggest that the AGT M235T polymorphism may be associated with cholesterol levels. The ACE I allele was significantly more frequent in each group comprising subjects with type 2 diabetes/glucose intolerance (GIT), and the I allele was associated with higher fasting plasma glucose levels.

CONCLUSIONS — These findings suggest that these polymorphisms are unlikely to be involved in the pathogenesis of hypertension. The ACE I/D polymorphism was associated with the metabolic syndrome, having a higher frequency of I allele–containing genotypes in those groups, but this appeared to result predominantly from the relationship with type 2 diabetes/GIT in this population of Chinese subjects.

tions between the AGT M235T polymorphism and hypertension (7,8,10) and the ACE I/D with coronary heart disease (CHD) and stroke (13,14). However, the associations with these gene polymorphisms and cardiovascular disease are otherwise generally negative (7,9,13,21). In addition to the regulation of blood pressure, the RAS may also affect other aspects of the pathogenesis of the metabolic syndrome, such as the development of atherosclerosis and insulin resistance. ACE inhibitor therapy has been reported to reduce the rates of both of these disorders (22,23). Activation of the RAS through insulin resistance may promote the development of dyslipidemia and diabetes. This possibility is supported by the finding that ACE inhibitors reduced the increased lipolysis in adipose tissue associated with insulin resistance in centrally obese hypertensive subjects; however, it was not determined whether this was a result of diminished levels of angiotensin II or elevated levels of bradykinin (24). There has been some evidence to support an association between the AGT polymorphism and insulin resistance (11). In addition, the I allele of the ACE I/D polymorphism has been associated with increased indexes of insulin resistance in nondiabetic Caucasians (15) and African-Americans (16). Another report, however, found a similar relationship in type 2 diabetic patients but not in nondiabetic subjects (17). In Japanese subjects, the ACE I/D polymorphism I allele was associated with higher 2-h insulin levels after a 75-g oral glucose tolerance test in both type 2 diabetic and nondiabetic subjects (18). Despite these positive associations with insulin resistance, the literature also comprises many other studies not reporting such associations (7,13).

We have previously reported that these RAS gene polymorphisms are not associated with nondiabetic essential hypertension in our Hong Kong Chinese population (9), although the ACE I/D gene polymorphism may contribute to inter-ethnic differences in CHD mortality rates (25). To extend our previous findings, we investigated the relationship between the three RAS gene polymorphisms and components of the metabolic syndrome in 853 Chinese subjects.

**RESEARCH DESIGN AND METHODS** — The Clinical Research Ethics Committee of the Chinese University of Hong Kong approved the study protocol. All 853 unrelated subjects gave written informed consent. They were of Han Chinese origin, without any known ancestors of other ethnic origin, and were living in the Hong Kong Special Administrative Region of China at the time of the study. The catchment area of the Prince of Wales Hospital has been developed only since the 1960s and serves a population exceeding 1 million people. The majority of its inhabitants are a typical socioeconomic representation of first- or second-generation migrants from southern China now living in a Westernized environment.

The Prince of Wales Hospital is a teaching hospital and tertiary referral center. However, like other public hospitals in Hong Kong, because the system of government-funded primary care is not developed to the same extent as in most Western countries, many of the patients attending the clinics use the facility as their only source of subsidized medical care for management of their chronic diseases. The patients involved therefore do not represent a highly selected group of the most severe cases, but are a typical cross-section of patients with these conditions from this region of Hong Kong. Subjects seen by the study physicians in the medical outpatient clinics at the Prince of Wales Hospital who met the selection criteria described below were consecutively invited to participate in the study. Those patients who expressed willingness to participate were then recruited.

Measurement of seated blood pressure and anthropometric (waist circumference and BMI) and plasma biochemical (renal and liver function tests as well as lipid and glycemic profiles) parameters taken after an overnight fast have been described in detail previously (26). The anthropometric parameters required to calculate the BMI and waist-to-hip ratio (WHR) parameters were measured.

Subjects with impaired fasting glucose (IFG) and diabetes were diagnosed based on fasting plasma glucose (FPG) levels. An FPG level <6.0 mmol/l was considered normoglycemic, an FPG level of 6.0–7.0 mmol/l was considered to indicate IFG, and an FPG level ≥7.0 mmol/l was indicative of diabetes (27). If the subjects had no diabetes-related complaints, then two elevated FPG readings were required for diagnosis. However, if the subject was symptomatic, a single elevated reading was sufficient. In subjects with equivocal plasma glucose levels who underwent a 75-g oral glucose tolerance test, a 2-h postglucose level of 7.8–11.1 mmol/l was considered indicative of impaired glucose tolerance (IGT) (27). In this study, subjects with IFG, IGT, and type 2 diabetes were grouped together and considered to have glucose intolerance (GIT), of which 95.9% had type 2 diabetes. Indexes of insulin resistance were available in 491 subjects, including a subset of 89 control subjects and 402 patients. The index that we used is equivalent to that derived from the homeostasis model assessment (HOMA) (28). However, the relatively complex equation generated by this model [i.e., insulin resistance = insulin/(22.5e-in glucose)] can be mathematically rearranged to the following conceptually simpler equation: fasting insulin-glucose product (FIGP) divided by 22.5 (i.e., insulin × glucose/22.5). This adjusted FIGP (FIGPa) is numerically identical to the originally published equation. We present the FIGPa so that our data can be compared with those in previous publications in other ethnic groups. However, division by a constant is not necessary for analysis of data within our study, so we also present the simpler FIGP values, which have not been divided by 22.5. As the FIGP is the product of picamoles per liter and millimoles per liter, we have omitted its units. The HOMA-derived index has been shown to correlate well with the results of the euglycemic-hyperinsulinemic clamp in population-based studies (29).

Subjects were defined as hypertensive, if, after 5 min of rest, their seated systolic blood pressure (sBP) was ≥140 mmHg and/or diastolic blood pressure (dBP) ≥90 mmHg on at least two occasions while off antihypertensive treatment (after a 4-week wash-out period) (30). A mean of three readings taken 1 min apart was used. No subjects had a history of significant renal, hepatic, or cardiac disease. Normotensive normoglycemic Chinese control subjects were recruited from hospital staff. Obesity was defined as a BMI ≥25.0 or ≥27.0 kg/m² and/or WHR ≥0.85 or ≥0.90 in women and men, respectively (27). Dyslipidemia was classified as either fasting plasma total cholesterol ≥6.2 mmol/l or between 5.2 and 6.2 mmol/l, with the ratio of total cholesterol to HDL cholesterol being ≥5.0, and/or fasting plasma triglycerides ≥2.3 mmol/l (31).

The subjects were classified either as control subjects or into seven nonoverlapping groups based on whether they had one of the three conditions of GIT, hypertension, or dyslipidemia alone or a combination of the conditions (Table 2). Additionally, overlapping groups of sub-
RAS and the metabolic syndrome

RESULTS — The demographic characteristics of the Chinese subjects are described in Table 1. Compared with the control subjects, the subjects with components of the metabolic syndrome had a similar sex distribution, but were slightly older and—as expected—were more obese, more dyslipidemic, and had higher plasma sodium and urate levels. Plasma glucose levels were also elevated relative to those of the control subjects.

Data from normally distributed parameters are presented as means ± SD, whereas skewed data were logarithmically transformed and expressed as geometric means with 95% CIs. Differences in the genotype and allele frequencies between the hypertensive and normotensive populations and between normotensive Chinese and Caucasian populations were analyzed using the χ² test and odds ratios with Cornfield 95% CIs (EpiInfo version 5.0 Statcalc, 1990). Differences in anthropometric and fasting plasma biochemical parameters between the hypertensive and normotensive cohorts and between the genotypes were examined using the Student’s t test (Statistics Package for the Social Sciences, version 7.5.1; SPSS, Chicago).

Table 1—Demographic characteristics of the sex-matched control and affected Chinese subjects

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Affected subjects</th>
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<tbody>
<tr>
<td>n</td>
<td>119</td>
<td>734</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.6 ± 9.2</td>
<td>48.5 ± 12.3</td>
</tr>
<tr>
<td>Sex (% Male)</td>
<td>36.1</td>
<td>42.5*</td>
</tr>
<tr>
<td>sBP (mmHg)</td>
<td>113 ± 9</td>
<td>139 ± 23</td>
</tr>
<tr>
<td>dBP (mmHg)</td>
<td>66 ± 10</td>
<td>83 ± 14</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>82 ± 9</td>
<td>101 ± 15</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
<td>140.5 ± 1.5</td>
<td>141.4 ± 1.9</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>3.9 ± 0.4</td>
<td>4.0 ± 0.5*</td>
</tr>
<tr>
<td>Urate (mmol/l)</td>
<td>0.26 (0.25–0.28)</td>
<td>0.32 (0.31–0.33)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.0 (4.9–5.1)</td>
<td>7.9 (7.6–8.1)</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>5.3 (5.1–5.5)</td>
<td>7.4 (7.2–7.6)</td>
</tr>
<tr>
<td>Insulin (mmol/l)</td>
<td>39.4 (34.8–44.8)</td>
<td>71.3 (66.4–76.6)</td>
</tr>
<tr>
<td>Insulin-glucose product</td>
<td>195 (171–237)</td>
<td>513 (468–558)</td>
</tr>
<tr>
<td>HOMA</td>
<td>8.7 (7.6–9.9)</td>
<td>22.8 (20.8–24.9)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.6 ± 0.7</td>
<td>5.9 ± 1.5</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.4 ± 0.4</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>2.8 ± 0.7</td>
<td>3.7 ± 1.3</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>0.75 (0.67–0.85)</td>
<td>1.71 (1.60–1.83)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4 ± 3.5</td>
<td>25.2 ± 4.0</td>
</tr>
<tr>
<td>WHR</td>
<td>0.80 ± 0.06</td>
<td>0.88 ± 0.07</td>
</tr>
<tr>
<td>Subjects with hypertension (%)</td>
<td>0</td>
<td>64.5</td>
</tr>
<tr>
<td>Subjects with GIT (%)</td>
<td>0</td>
<td>57.0</td>
</tr>
<tr>
<td>Subjects with dyslipidemia (%)</td>
<td>0</td>
<td>62.1</td>
</tr>
<tr>
<td>Subjects with obesity (%)</td>
<td>31.6</td>
<td>57.9</td>
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All P < 0.001, except *sex and plasma potassium levels (NS); HOMA = (insulin × glucose)/22.5.

There was limited evidence suggesting that the M235T polymorphism was associated with dyslipidemia in the overlapping groups, with fewer subjects carrying the M allele with dyslipidemia than those without (2.2, 20.2, and 77.5%, n = 399 vs. 3.4, 28.1, and 68.5%, and n = 454 for the MM, MT, and TT genotypes, respectively, P = 0.009). However, no difference was identified when the control subjects (4.2, 26.1, and 69.7% for the MM, MT, and TT genotypes, respectively) were compared with either the overlapping group of dyslipidemic subjects or the nonoverlapping groups with components of the metabolic syndrome. In the total population, however, the subjects carrying the M allele (MM/MT) had significantly lower total cholesterol (5.4 ± 1.4 vs. 5.7 ± 1.4 mmol/l, P = 0.005) and LDL cholesterol (3.3 ± 1.1 vs. 3.6 ± 1.3 mmol/l, P = 0.001) than those with the TT genotype. Furthermore, there was a linear dose-dependent relationship between the genotypes for these parameters (5.2 ± 1.2, 5.4 ± 1.4, and 5.7 mmol/l, analysis of variance [ANOVA] P = 0.016, and 3.27 ± 1.10, 3.34 ± 1.11, and 3.64 ± 1.30 mmol/l, ANOVA P = 0.004, for MM, MT, and TT genotypes, respectively). There was no significant difference in blood pressure levels between the subjects with the TT genotype compared with those carrying the M allele for sBP (135 ± 23 vs. 136 ± 23 mmHg) and dBP (81 ± 14 vs. 81 ± 13 mmHg), respectively.

The genotype and allele frequencies of the ACE I/D are shown in Table 2. There was an apparent association between the ACE I/D polymorphism and the GIT and dyslipidemic groups, with a lower proportion of the D allele–containing genotypes in groups containing subjects with these disorders (Table 2). Furthermore, the D allele frequency was lower in each group containing patients with GIT (Table 2). The findings were similar when those with only type 2 diabetes were examined. There was a trend toward an increase in FPG levels, with ACE genotypes of increasing proportions of the D allele (6.8 [6.5–7.0], 6.5 [6.3–6.7], and 6.2 [5.8–6.6] mmol/l for the II, ID, and DD genotypes, respectively, ANOVA P = 0.081), but not with insulin or the insulin resistance index. The difference between the two
homozgyous genotypes reached significance using the t test \( (P = 0.045) \). None of the gene polymorphisms showed any relationship with indexes of obesity. There was only limited evidence to support the involvement of the ACE I/D polymorphism in the modulation of blood pressure, with the combined hypertensive group having a higher I allele frequency relative to the control group investigated \( (P = 0.037) \), but not in any other hypertensive group (Table 2). The association between the ACE I/D polymorphism and GIT affects this relationship in these subjects, with 51\% of the combined hypertensive subjects also having GIT. When we compared normotensive non-GIT control subjects with non-GIT hypertensive subjects and GIT hypertensive subjects, no differences in the ACE I/D genotype distributions were observed. Furthermore, when we compared blood pressure levels in the total population between the subjects carrying the homozgyous genotypes, despite a 5-mmHg rise in sBP in those carrying the II genotype, only a possible trend was detected \( (137 \pm 23 \text{ vs. } 132 \pm 22 \text{ mmHg), } P = 0.08) \).

**CONCLUSIONS** — As found in previous studies, the hypertensive subjects were generally more obese and dyslipidemic than the normotensive control subjects \( (2,32) \). The sex-matched hypertensive subjects were also slightly older, although it is unlikely that this would account for all of the differences in anthropometric and biochemical parameters seen between the groups.

ACE inhibitor therapy has been shown to induce improvements in atherosclerosis and insulin resistance, which suggests that activation of the RAS may promote the development of dyslipidemia and diabetes \( (22,23) \). Therefore, the association identified between the ACE I/D polymorphism and GIT, with a higher I allele frequency observed in the groups containing subjects with GIT, is feasible. Furthermore, the I allele–containing genotypes presented with higher FPG levels, but not insulin or the insulin-glucose product. The D allele has been associated with cardiovascular disease in other studies \( (13,14) \); therefore, the current observations may appear counterintuitive. However, the association between increased insulin resistance and the I allele is supported by a limited number of association studies \( (15–18) \). Furthermore, the ACE I allele has also been shown to be associated with hypertension \( (33) \), although such observations may not be representative of the literature in general, for which association studies are predominantly negative \( (9,13,21) \). In the current study, the association between diabetes and the I allele are similar for each group examined.

Treatment with ACE inhibitors improves indexes of insulin resistance \( (22) \), possibly through a reduction in bradykinin degradation \( (34,35) \). Conditions in which ACE activity is reduced, such as in subjects carrying the ACE I allele \( (12) \), may increase bradykinin levels reducing insulin resistance, which would appear contradictory to the current findings. However, ACE is involved in the formation of the vasoconstrictor angiotensin II as well as the degradation of the vasodilator bradykinin. Low-dose angiotensin II infusion has been reported to reduce insulin resistance either through redistribution of renal blood flow to skeletal muscle, which increases glucose uptake and reduces insulin excretion \( (36,37) \) directly via diacylglycerol-mediated protein kinase C activation, or by increasing hepatic carbohydrate metabolism \( (38) \). Although the specific mechanisms by which this polymorphism may modulate these parameters remain to be determined \( (24) \), the physiological relationship between the polymorphism and diabetes is possible.

Insulin resistance has been reported to reduce insulin-mediated suppression of adipocyte lipolysis to which activation of the RAS in adipose tissue may contribute \( (24) \). The association between cholesterol levels and the AGT M235T polymorphism observed in the current study has also been reported in Caucasian populations \( (39) \). Further studies are required to confirm the current relationship between this polymorphism and lipid metabolism.

Positive associations in cross-sectional studies may arise from the unintentional stratification of the subjects, such as disproportionate ethnic backgrounds in case subjects and control subjects with alleles that show a differential ethnic distribution, for which the RAS genes are no exception \( (9) \). Despite this population being recruited from a relatively monoehtnic society near the Mainland Chinese border, when compared to societies with high degrees of ethnic admixture, it is important to recognize the constraints of case-control studies. As such, the data from the present study need to be confirmed in other populations of southern Chinese origin.

The RAS is clearly involved in the maintenance of blood pressure \( (4–6,19) \); as such, mutations in the genes of RAS components might be expected to contribute to the pathogenesis of hypertension. On the whole, the literature supports...
the association of the T allele of the AGT M235T polymorphism with hypertension (7) and the ACE I/D polymorphism D allele with a predisposition to the development of vascular disease rather than to hypertension in Caucasian and Japanese populations (13). A recent systematic review of the ACE I/D polymorphism suggested an excess risk of hypertension with the D allele in Asians (13). This conclusion was drawn from only three studies, two of which were in Japanese populations. Furthermore, in Chinese subjects, previous reports describing the involvement of the AGT polymorphism in hypertension have been conflicting (9,10), indicating that further studies in Asian populations are still necessary. We found that the ACE D allele frequency was significantly lower in the nondiabetic subjects than in the nonhypertensive subjects (3). We have previously shown that the Chinese type 2 diabetic subjects are sodium replete, with low plasma renin activity and aldosterone levels and high atrial natriuretic peptide levels (40). The sodium-mediated suppression of the RAS in the diabetic subjects helps explain the reduced efficacy of losartan. Furthermore, it may also account for the lack of a relationship between the ACE gene polymorphisms and blood pressure in the current study.

In summary, there was no evidence that any of these genes affected the clustering of the components of the metabolic syndrome. Nor, despite the efficacy of drugs such as AT₁R antagonists in lowering blood pressure, was there any evidence to confirm that these gene polymorphisms modulate blood pressure or are involved in the pathogenesis of hypertension in either the nondiabetic or diabetic Chinese subjects. However, there was some evidence that these polymorphisms may be associated with individual components of the metabolic syndrome, because a weak association was observed between cholesterol levels and the AGT M235T polymorphism. Furthermore, there was consistent evidence to support an association between the ACE I/D polymorphism and IGT, including an increased frequency of the I allele-containing genotypes in the metabolic syndrome group, all of whom had hypertension, type 2 diabetes, and dyslipidemia combined.

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References


