An MboI Two-Allele Polymorphism May Implicate the Human Renin Gene in Primary Hypertension

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As a key enzyme of the renin-angiotensin-aldosterone system, the renin gene (REN) is a good candidate quantitative trait locus that may be implicated in the molecular etiology of essential hypertension. Among mixed reports on the subject, a REN MboI restriction fragment length polymorphism has been shown to be significantly associated with a family history of hypertension in a Japanese population. We show here that the REN MboI dimorphic site is located in the ninth intron of the gene, and we describe a polymerase chain reaction-based assay for detection of this site. We investigated MboI genotype distributions in 331 hypertensive and 279 normotensive subjects from the United Arab Emirates (UAE), a genetically homogeneous ethnic population with no history of smoking or alcohol consumption. A statistically significant association was found between alleles on which the MboI site is present and clinical diagnosis of essential hypertension, indicating that 1) the presence of the MboI site is a marker for susceptibility to hypertension in the UAE (the associated odds ratio is 3.16); and 2) variations of the REN (or of a nearby) gene that may be in linkage disequilibrium with this marker play a role in the development of essential hypertension in the UAE. (Hypertens Res 1998; 21: 221-225)

Key Words: genetics, hypertension, polymerase chain reaction, renin

Human essential (idiopathic or primary) hypertension is hypertension of no definable cause. It is a heterogeneous group of disorders ranging from very rare forms of Mendelian hypertensive syndromes to multifactorial disorders in which contributing gene effects exhibit low penetrance (1, 2). The challenge facing molecular geneticists is to unravel the molecular and genetic architectures of blood pressure regulation, with the aim of being able to classify hypertension into subtypes based on the identification of specific combinations of underlying mechanisms. This will in turn lead to optimal management of the disorder and will allow health care professionals to design appropriate preventive strategies (3).

Epidemiological, clinical, and experimental evidence has shown that the renin-angiotensin-aldosterone system (RAAS) plays a crucial role in blood pressure regulation (4-7). For this reason, the genes whose products are involved in the RAAS pathway represent good candidates for putative identification of quantitative trait loci (QTLs) implicated in hypertension (4-7). Among these, the human renin (REN) gene has been studied extensively since its characterization (8), and mixed results have been generated with respect to its involvement in the development of hypertension (for reviews, see 5, 6, 9). Amongst the abundant literature on the subject, Okura et al. (10) have reported that a REN MboI restriction fragment length polymorphism (RFLP) (11) was significantly associated with a family history of hypertension in a Japanese population.

As data from genetically isolated populations is of utmost importance to resolve issues of contention related to studies based on the concept of linkage disequilibrium (12-15), we studied the REN MboI two-allele polymorphism in a population from the United Arab Emirates (UAE). We developed a polymerase chain reaction (PCR)-based restriction endonuclease assay for detection of the MboI dimorphism and we compared the corresponding genotype frequencies in unrelated normotensive and hypertensive UAE subjects.

Subjects and Methods

Subjects

The United Arab Emirates (UAE) is a Federation of seven Emirates (the Abu Dhabi Emirate being the largest) with an indigenous population comprising UAE nationals, who are Gulf Arabs of Bedouin descent. In this pilot, retrospective case-control...
study, we investigated a sample population of 610 UAE nationals (Emirati) from the Abu Dhabi Emirate for putative associations between the REN MboI dimorphism and essential hypertension in a genetically homogeneous ethnic group. This project was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences (UAE University, Al Ain, UAE).

The sample population of 610 unrelated subjects (316 men, 294 women) had a mean age (± standard deviation) of 52.5 ± 13.1 yr and was composed of the two following groups: 331 patients with essential hypertension (EHT; mean age = 53.0 ± 12.3 yr), and 279 healthy “controls,” used as a comparison group (mean age = 51.7 ± 13.5 yr).

Patients were classified as having EHT if they met the following conditions: a systolic blood pressure above 160 mmHg and a diastolic blood pressure above 95 mmHg on at least three separate occasions; no clinical signs, symptoms, or laboratory findings suggestive of secondary hypertension; and a positive family history of hypertension (as assessed by direct questioning of the patient as to a history of hypertension in any close relative). All hypertensive subjects were receiving antihypertensive medications, and therefore neither their blood pressure nor renin values could be taken into account in this study.

“Control” (comparison) subjects were healthy individuals who had no personal history of hypertension (documented by individual medical records as a resting systolic blood pressure below 140 mmHg and a diastolic blood pressure below 90 mmHg on at least three separate occasions) and no family history of hypertension. No control subject was receiving any antihypertensive or other medication that could affect blood pressure.

Great care was taken to ensure that the subjects (both hypertensive and normotensive) were not affected by confounding clinical phenotypes, including non-insulin dependent diabetes mellitus (which is otherwise quite prevalent in this population) and ischemic heart disease. Moreover, all had normal hearts as assessed by echocardiography.

Total serum cholesterol levels were taken from the subjects’ medical charts. In patients who were receiving lipid-lowering medication, the value before the start of such treatment was used. Individual height and weight values were used to calculate body mass index (BMI) as [weight (in kg)]/[height in m]² (Table 1). No member of the study group admitted to alcohol intake or had a history of smoking.

DNA Analysis
DNA was extracted from 5-ml blood samples according to usual methods (16).

We have previously established that the dimorphic MboI site is located in the ninth intron of the REN gene (Frossard, unpublished observations, and 17). In the present study, we developed a polymerase-chain-reaction (PCR)-based assay for detection of this dimorphism. From sequence data published by Hardman et al. (8), we designed the following oligonucleotide primers targeted to the first 250 base pairs (bp) of intron 9 of the REN gene: forward primer, 5’-TGAAGTTCCAGTGGGCCCC-T-3’; reverse primer, 5’-TGCCCAAAACATGGGCC-ACACAT-3’.

PCR reactions were routinely carried out in a final volume of 5 μl, using a Perkin Elmer GeneAmp PCR system 2400 (Perkin Elmer Corp., Applied Biosystems Division, Foster City, CA, USA). PCR conditions in each assay were as follows: 100 ng genomic DNA, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM dATP, dCTP, dGTP, dTTP, 2 mM MgCl₂, 0.1 μM of each of the two primers, 0.25 Unit Taq Polymerase. PCR cycling variables were as follows: an initial denaturation step of 5 min at 94°C followed by 30 cycles of denaturation, annealing, and extension steps, which were, respectively, 30 s at 94°C, 30 s at 68°C, and 30 s at 72°C; and a final step of 5 min at 72°C before storage of the samples at 4°C.

The 5-μl PCR product samples were then submitted to restriction endonuclease digestion for 2 h in a final volume of 10 μl, using either restriction enzyme MboI (New England Biolabs, Beverly, MA, USA), whose recognition site is ↓GATC, or its isoschizomer Sau3AI (Pharmacia Biotech, Pharmacia Export S.A., Glyfada, Greece). Digested PCR products were then submitted to 3% agarose gel electrophoresis (Gibco BRL, Life Technologies, Gaithersburg, MD, USA) at 80 Volts for 45 min, using a Pharmacia LKB mini-gel system GNA 100 (Pharmacia Biotech, Pharmacia Export S.A., Glyfada, Greece). DNA fragments were visualized by staining with ethidium bromide.

Data Analyses
Statistical determinations were carried out with an SPSS® version 6.1 for Windows® software package (Gorinchem, the Netherlands). Differences in the distributions of MboI genotypes according to clinical phenotypes (normotensive vs. hypertensive) were assessed using 3 × 2 tables of association and chi-square procedures. Odds ratios were determined directly from data stratified from the 3 × 2 tables.

<table>
<thead>
<tr>
<th>Sample Size</th>
<th>Gender (M/F)</th>
<th>Age (yr)</th>
<th>Total Chol (mmol/l)</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>279</td>
<td>141/138</td>
<td>51.7±13.5</td>
<td>5.3±0.5</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>331</td>
<td>167/164</td>
<td>53.0±12.3</td>
<td>6.9±2.9*</td>
</tr>
</tbody>
</table>

* indicates a significant difference at p<0.05 from control. Means±SD for age, total serum cholesterol level, and BMI.
Hardy-Weinberg proportions of allele distributions were investigated by chi-square analyses. For all analyses, differences were considered statistically significant when p values were less than 0.05.

Results

Double-digestions using a combination of MboI and any of several other restriction endonucleases allowed us to localize the mutated MboI site in the ninth intron of the REN gene (unpublished observations, and 17). By reference to the REN gene sequence (5), the first nucleotide of the dimorphic MboI site is located 80 bp 3' to the end of exon 9 (Fig. 1).

Under the PCR-restriction endonuclease conditions described in the Subjects and Methods section, the altered MboI site leads to the detection of a two-allele polymorphism, where the absence of MboI sites \([MboI(\neg)]\) is visualized as a 250 bp fragment, and the presence of MboI sites \([MboI(+)]\) as two fragments of 171 bp and 79 bp (Fig. 2).

This study included 610 UAE national (Emirati) subjects (316 men, 294 women) with complete phenotypic and genotypic data. Table 1 summarizes the demographic and clinical characteristics of the two study groups. The normotensive and hypertensive subjects were age- and sex-matched, and there was no difference in BMI values between the two groups. Total serum cholesterol levels were significantly higher in the hypertensive group.

Table 2 shows the data pertaining to both genotype and allele distributions in the two groups of subjects. MboI genotypes occur in Hardy-Weinberg proportions in both groups (normotensive, \(\chi^2 = 0.13, 2 \text{ degrees of freedom (df)}, p = 0.935\); hypertensive, \(\chi^2 = 0.61, 2 \text{ df}, p = 0.74\)). MboI (+) allele frequencies were 0.36 and 0.51 in the normotensive and hypertensive subjects, respectively. Differences in the distributions of the three genotypes according to clinical phenotype were highly significant (\(\chi^2 = 24.3; 2 \text{ df}; p = 5 \times 10^{-6}\)). As compared with the normotensive subjects, the hypertensive subjects had a higher incidence of MboI (+)/MboI (+) genotypes (27.5% vs. 14%) and a lower incidence of MboI (−)/MboI (−) genotypes (25.7% vs. 41.2%).

The association of the MboI (+) allele with an increased risk for essential hypertension is reflected by the values of the retrospective odds ratios (OR). Indeed, the odds ratio for \([MboI(\neg)/MboI(\neg)]\) vs. \([MboI(\neg)/MboI(\neg)]\) genotypes was 1.68 (95% confidence interval: 1.14-2.46), and the odds ratio for \([MboI(\neg)/MboI(\neg)]\) vs. \([MboI(+)/MboI(+)]\) genotypes was 3.16 (95% confidence interval, 1.93-5.19).

Discussion

Studies of animal (such as rat) models have clearly demonstrated that genetically determined variations in the REN gene affect blood pressure and that the REN gene is directly involved in the development
of hypertension (18, 19). However, molecular genetic studies of humans have led to mixed reports. Indeed, linkage and sib-pair linkage analyses as well as some association studies have failed to identify involvement of the \textit{REN} gene in EHT (20–24). Positive correlations, however, have been evidenced by other association studies (10, 25–27), and associations between \textit{REN} gene polymorphisms and intermediate phenotypes of EHT have also been reported (28).

Variations in the amount of linkage disequilibrium have been observed at the human \textit{REN} gene locus (21, 29), indicating the importance of investigating several \textit{REN} gene polymorphisms in different ethnic groups. Results of previous studies indicate that positive associations with susceptibility to EHT have been identified with the HindIII and \textit{MboI} two-allele polymorphisms (10, 26, 27), \textit{i.e.}, causal genetic variations may be located in the 3' portion of the human \textit{REN} gene. We have thus designed a PCR assay for the detection and study of the \textit{MboI} marker in UAE nationals.

The original report describing the \textit{REN} gene \textit{MboI} RFLP in a North American population indicated frequencies of 0.80 and 0.20 for \textit{MboI} (−) and \textit{MboI} (+) alleles, respectively (11). Similar frequencies (0.76 and 0.24) were reported by Okura et al. (10) amongst Japanese. In the UAE nationals in our study, the frequencies were 0.64 and 0.36 in normotensive subjects, and the difference with previously studied populations was accentuated in EHT subjects, in whom allelic frequencies were shifted to 0.49 and 0.51, \textit{i.e.}, \textit{MboI} (+) was the most common allele.

Genotype frequency distribution in the normotensive subjects occurred in Hardy-Weinberg proportions, which indicates that the extensive level of consanguinity frequently advocated in Gulf populations does not affect genotypic heterozygosities—at least not at the \textit{REN} gene locus. This is in agreement with our previous findings at the angiotensin-converting enzyme and atrial natriuretic factor gene loci (30, 31). Interestingly, Hardy-Weinberg equilibrium was also found in the hypertensive subjects, although deviations from Hardy-Weinberg proportions are expected in a group of patients, especially when there is positive association with a clinical phenotype, such as hypertension.

Distributions of \textit{MboI} genotypes significantly differ between normotensive and hypertensive UAE subjects, and the presence of \textit{MboI} (+) alleles is associated with a 3.16-fold increase in the odds of presenting with EHT before or at age 53 (mean age of the hypertensive group).

Association (retrospective case-control) studies using candidate genes are gaining wide acceptance, as this approach offers great power for detecting QTLs of reduced or low penetrance (12–15). Such methods, however, are subject to the effects of selection bias, population stratification, confounding by other variables, and clinical criteria used to define patient groups. As for the first two, it is of utmost importance to explore the nature of reported associations in various ethnic groups that may be more genetically homogeneous. The Emirati population in this investigation offered another advantage—abstinence from alcohol intake and smoking, which usually are confounding environmental factors.

"Control" individuals included in this investigation constituted a "comparative" rather than a "control" group. They were indeed free of disease up to the time of investigation and were also chosen because their BMIs, age, and sex matched with those of the patients. Their serum cholesterol levels, however, were significantly lower than those of the patients (Table 1). As the control subjects had neither a personal nor a family history of hypertension, however, they represented a valid comparison group for these association studies. BMI values (Table 1) indicate that all subjects were overweight (26 < BMI < 30), and that the hypertensive subjects were even slightly obese (BMI > 30).

We have therefore shown an association between a genetic marker in the ninth intron of the human \textit{REN} gene and a clinical diagnosis of EHT in a genetically homogeneous population whose members tend to be overweight. As the \textit{MboI} polymorphism is located in an intron, it is probably not the causative mutation of the effect uncovered here. However, our results together with those of Okura et al. (10) suggest that genetic variations in linkage disequilibrium with this site (either in the \textit{REN} gene itself or in a nearby gene in linkage disequilibrium with it) may be directly implicated in an individual's genetic susceptibility to blood pressure dysregulation.

\textbf{References}


