Symposium

Alu-Repeat Polymorphism in the Gene Coding for Tissue-Type Plasminogen Activator and the Risk of Hypertension in a Chinese Han Population

Bing WANG, Xin ZHOU*, Aiming DANG, Guozhang LIU, Ran HE**

Accumulating data support an association between hypertension and impaired fibrinolytic potential abnormalities in endogenous fibrinolysis. The present study examined whether there was an association between essential hypertension and either a polymorphism in the gene coding for t-PA or the plasma concentration of t-PA antigen. Chinese hypertensive subjects (n = 126) and normotensive controls (n = 102; sex- and age-matched with hypertensives) were recruited from among the outpatients of FuWai Hospital. The distributions of the II, ID, and DD genotypes of the t-PA gene in hypertensive patients (0.15, 0.49, 0.36) were similar to those in control subjects (0.11, 0.51, 0.38; p = 0.626). No significant difference in overall allele frequencies was found between the hypertension and control groups (p = 0.656). The allelic frequencies were in Hardy-Weinberg equilibrium. There was no evidence of an association between the level of t-PA antigen and risk of hypertension. Thus, in this case control study, neither the presence of the insertion allele of the Alu-repeat polymorphism of the t-PA nor the level of t-PA antigen were associated with the risk of essential hypertension. (Hypertens Res 2002; 25: 949–953)

Key Words: essential hypertension, tissue plasminogen activator, polymorphism

Introduction

Hypertension is an important risk factor for myocardial infarction and stroke (1) that may be due to the direct physical effects of increased blood pressure, as well as the active promotion of atherosclerosis and thrombogenesis. Accumulating data supports an association between hypertension and the impaired fibrinolytic potential abnormalities in endogenous fibrinolysis. In particular, elevations in t-PA antigen have emerged as an important marker of increased risk for cardiovascular diseases (2).

A polymorphism consisting of the presence or absence of a 311-bp Alu sequence in intron 8 has been identified at the t-PA locus on chromosome 8 (3). It was recently reported that t-PA genotypes influence plasma protein level, but due to the diverse populations and cardiovascular diseases tested, different investigators have reported conflicting results in regard to this association. At this time, therefore, the prevalence of the Alu-repeat insertion polymorphism in China is unknown. The aim of the present was to explore whether plasma levels of t-PA antigen and activity are associated with hypertension, and whether there exists a variant of the Alu II/D polymorphism between Chinese hypertensives and normals.

Methods

Population

Subjects with essential hypertension and normotensive controls were recruited from the outpatient population of FuWai...
Hospital. All cases and controls were Chinese. The study was approved by the Medical Ethics Committee of FuWai Hospital, and written informed consent for genetic analysis was obtained from all participants. All hypertensive subjects (n = 126) were diagnosed as having mild or moderate primary hypertension according to the criteria of the 1999 Chinese Hypertension Guidelines (4). Subjects with secondary hypertension, diabetes mellitus, apparent ischemic heart diseases, or liver or kidney dysfunction were excluded. Normotensive controls (n = 102) without a history of hypertension and with no clinically or biologically abnormal data were recruited from the same population and matched to the experimental subjects for sex and age; their systolic and diastolic blood pressures were < 140 mmHg and < 90 mmHg, respectively. Individuals with first degree relatives or immediate family members having hypertension were also excluded from the control group. Blood pressure values were the means of 2 measurements recorded 5 min apart with subjects seated after 15 min of rest. Blood samples were obtained after a fast of at least 12 h.

Extraction and Amplification of the I/D Polymorphism of the t-PA Gene

The DNA of subjects was extracted from leukocytes according to the standard protocol (phenol/trichloromethane method) (5). The primers for polymerase chain reaction (PCR) amplification were F (5 ʻCATCCGTAACAGGACAGCTCA-3 )` and R (5 ʻACCGTGCCCTGAGTATGGA-3 ′) respectively. The total reaction volume was 50 µl in a mixture containing 50–100 ng genomic DNA, 50 ng of each primer, 200 µmol of each dNTP, 1.5 mmol/l MgCl2, and 0.5 units of Taq DNA polymerase. PCR conditions consisted of a single cycle of 4 min at 94 °C, followed by 32 cycles of 1 min at 94 °C, 1 min at 62 °C, and 2 min at 72 °C, and a final extension of 4 min at 72 °C. A 2 µl sample was analyzed by agarose gel electrophoresis and visualized with ethidium bromide and UV transillumination. The amplification yielded a 966-bp fragment from chromosomes with the insertion (I allele) and a 655-bp fragment from those without (D allele).

To avoid misclassification of ID genotypes as DD, an independent PCR with a forward primer that recognizes an insertion-specific sequence (5 ʻATCACGAGCTCAGGAGAT-3 ′) was performed on each sample classified as DD by initial PCR. The reverse-primer and PCR conditions were as described above, with the exception that the annealing temperature was slightly higher (62 °C). The reaction yielded a 850-bp amplicon only in the presence of the I allele (6).

Blood Sampling and Analysis

To minimize circadian variations, blood samples were collected between 8 and 9 AM from an antecubital vein with subjects in the supine position after an overnight fast. To determine the plasma level of t-PA antigen, blood was anticoagulated with 3.8 % trisodium citrate (9:1, vol/vol) and kept on crushed ice until centrifugation. Plasma was separated by centrifugation at 2,500 g for 30 min at 4 °C. Plasma aliquots were quickly frozen and stored at -70 °C for subsequent analysis. Levels of t-PA antigen were obtained by means of an ELISA according to the procedure described by Ranby et al. (7). The intra-assay coefficient of variation in our laboratory was 5.1% for t-PA.

Statistical Analysis

All values were expressed as the means ± SD unless otherwise specified. A foxpro database was established. Allele frequencies were calculated from the genotypes of all subjects. Hardy-Weinberg equilibrium was assessed by chi-square (χ2) analysis. Significant differences in the total number of alleles on all chromosomes between the hypertensive and normotensive group were assessed by χ2 analysis with one degree of freedom. Differences in clinical data between the hypertensive and normotensive group were assessed by χ2 analysis with one degree of freedom or analysis of variance (ANOVA) followed by a Fisher’s protected least significance (PLSD) test. SPSS 8.0 software was used for the statistical analysis. Values of p < 0.05 were considered to indicate statistical significance.

Results

The clinical characteristics of hypertensive and control subjects are shown in Table 1. Body mass index (BMI), smoking, total cholesterol, triglyceride and fasting blood glucose levels were higher in hypertensive subjects than in normotensive subjects, but HDL-C was higher in normotensive subjects than in hypertensive subjects. There were no differences in the plasma levels of t-PA antigen between these two groups. When samples classified as DD by the initial PCR were subjected to a second amplification with an insertion-specific primer, it was determined that 6 of the samples would have been mistyped if the additional PCR had not been applied. Among the 102 control subjects, 38% were homozygous for the Alu deletion allele, 51% were heterozygous carriers of the Alu insertion (ID), and 11% were homozygous for the Alu insertion (II); the allelic frequencies of I and D were 0.36 and 0.64, respectively. The genotypes and allelic frequencies followed Hardy-Weinberg equilibrium. A virtually identical genotype distribution was observed among the hypertensive subjects (DD = 36%, ID = 49%, II = 15%, p = 0.626), and no significant difference in overall allele frequencies was found between the hypertension and control groups (p = 0.656).

As shown in Table 3, there was no significant association between hypertension and the II genotype in analyses assuming either allelic recessive or allelic dominant modes of inheritance. To examine whether the results of the study were confounded by other risk factors for the different genotypes,
The means and proportions of these risk factors are presented in Table 4. No significant differences in cardiovascular risk factors were found among the genotypes. There were also no differences in the mean plasma levels of t-PA antigen among the three genotypes.

**Discussion**

The objective of the present study was to assess the association between hypertension and both the I/D polymorphism of the *t-PA* gene and the plasma concentration of t-PA antigen.
The nature of the vantage that intersubject variability can be measured irresociated with the risk of hypertension. We chose to assess hypertensive patients often have a decreased fibrinolytic capacity. The present study, the genotype distributions of DD, ID, II were 38%, 51%, and 11%, and the allele frequencies were 0.36 for the I allele and 0.64 for the D allele in a Chinese Han population. It is thus clear there are remarkable racial differences in the genotype distribution of the t-PA gene.

This research was conducted in response to the fact that hypertensive patients often have a decreased fibrinolytic capacity. Plasma levels of the fibrinolytic factors have been associated with the risk of hypertension. We chose to assess the only polymorphism of the t-PA gene that has been identified to date. Assessment of genetic parameters has the advantage that intersubject variability can be measured irrespective of highest, namely, in the phases of hypertension. The nature of the I/D polymorphism, which consists of an insertion of an Alu repeat in an intron in a non-translated region, makes it unlikely, though not impossible, that the I allele has a direct functional effect on the t-PA protein (9). In this respect, the Alu insertion in the t-PA gene shows a similarity with the Alu I/D polymorphism present in the gene for ACE. Our results suggest that the Alu I/D event can alter mRNA stability and/or splicing. In contrast to the ACE I/D polymorphism, the Alu polymorphism in the t-PA gene was not associated with t-PA plasma levels in our study. However, and frequency of polymorphic Alu insertion at the t-PA locus and the release rate of t-PA. Further studies in this field will be needed to help clarify the mechanism of impaired endogenous fibrinolysis in patients with essential hypertension.

References


