A STUDY OF POLYMORPHISM OF ANTITHROMBIN III AT THE LEVEL OF BOTH PROTEIN AND DNA IN A CHINESE POPULATION

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Introduction

A systematical study of structure and genomic organization of antithrombin III (ATIII) (Lane and Caso, 1989) indicated that there are two polymorphisms: an intragenic polymorphism arising from a translationally silent A to G transition in condon 305, and a length polymorphism arising from the presence of 32 BP or 108 BP non-homologous sequences 345bp upstream from the translation intiation condon. Products of three ATIII codominant autosomal alleles have been revealed by IEF from Caucasoid and Negroid (Kambol and Ferrell, 1988). On the other hand, the fragment length polymorphism of ATIII has been studied by Southern blotting (Bock and Levitan, 1983) and by PCR (Seino, 1989) in Caucasian population.The purpose of this study is to reveal the genetic polymorphism of ATIII in a Chinese population at the level of both protein and DNA and to explore the relationship between these two kinds of polymorphisms.

Materials and Methods

EDTA-blood specimens were collected from 51 healthy unrelated Chinese individuals living in the Inner Mongolia antonomous Region of China. For family studies, the samples were collected from the members of two families residing in Chengdu, China.

Isoelectric focusing and immunoblotting for products of ATIII alleles

IEF was carried out in ultra-thin layer polyacrylamide gels containing 6M urea as described by Kambol and Ferrell (1988). An optimal pH gradient was obtained by mixing Pharmalyte of pH4.2-4.9 and of pH 4.5-5.4 at a ratio of 1:1. The power supply settings were 1500V, 5W, 6hr (gel dimension 120x80x0.4mm). Immunoblotting was achieved by diffusion blotting for 45 min onto nitrocellulose filter. The ATIII proteins on this filter were probed by rabbit anti-human ATIII serum . Horseradish-peroxidase-labeled goat anti-rabbit IgG was used as the second antibody and the DAB served as a detecting agent. The alleles for ATIII were named according to Kambol and Ferrell(1988).

Polymerase chain reaction for ATIII 5'locus

The oligonucleotides with sequences of 5'-CCACAGGTGAACATTGTGT-3' and 5'-GAGATAGTGTGATCTGAGGC-3' were used as the PCR primers. DNA was extracted using Chelex100 method. Reaction system of the PCR contained 2-100ng genomic DNA, 1xTaq buffer, 2 mM $MgCl_2$, 200uM each of dNTP, 40 pM each of primers and 1U Taq polymerase (Gibco BRL) in 25 ul. The reactions were subjected to 30 cycles consisting of 1min at 94°C for denature, 1min at 54°C for annealing and 4min at 71°C for extension. The PCR products combined with the size marker of PBR322/HaeIII were electrophoresed on a

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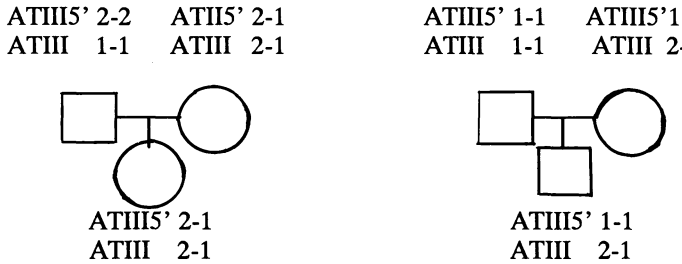
2.5% agarose gel containing ethidene bromide. The ATIII 5' alleles were identified relative to this marker and named according to Seino (1989).

Results

The IEF pattern of ATIII revealed in this study is similar to that reported by Kambol and Ferrell except that the ATIII*3 product was not encountered. Using the primers described above, two fragments (496 bp and 572 bp) were successfully amplified and named as ATIII5'*2 and ATIII5'*1 accordingly.

The pedigrees below show the distributions of ATIII genotypes at the level of both protein and DNA in two families. The codominant segregations of both ATIII alleles and ATIII5' alleles were observed, suggesting that their inheritance are in accordance with Mendelian law. Secondly, the ATIII5' polymorphism doesn't correlate with any specific one of the allelic IEF variations, implying that these two polymorphisms are independent.



The distributions of genotypes and allele frequencies for ATIII and ATIII5' loci in Chinese population are given in table 1. The genotypes in each locus are in Hardy-Weinberg equilibrium. The PICs (Botstein et al, 1980) for ATIII5' locus and ATIII locus were 0.6018 and 0.0782, respectively. This indicated that the ATIII5' locus was more useful for genetic linkage analysis.

Table 1. Distributions of ATIII5' and ATIII genotypes in a Chinese population

Genotypes		No. observed	No. expected	Allele frequencies
ATIII5'	2-1	24	24.71	ATIII5'*1=0.4118
	1-1	9	8.65	ATIII5'*2=0.5882
	2-2	18	17.64	x^2=0.0419
				df=1
	total	51	51.00	P>0.05
ATIII	1-1	47	47.08	ATIII*1=0.9608
	2-1	4	3.83	ATIII*2=0.0391
	2-2	0	0.08	x^2=0.0877
				df=1
	total	51	51.00	P>0.05

The results of the estimation of pairwise haplotype frequencies and the linkage disequilibrium coefficient (Ott, 1985) between ATIII and ATIII5' are shown in table 2. The observed frequencies of both ATIII*1-ATIII5'*2 and ATIII*2-ATIII5'*1 were higher than those

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