PRELIMINARY STUDIES ON THE POPULATION SUBSTRUCTURE FOR C1R PROTEIN IN CHINESE POPULATIONS

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Introduction

Human complement subcomponent C1R is one of three distinct glycoproteins that constitute the macromolecular complex of the first complement component. It consists of two identical subunits with 170-180 kd molecular weight. Its normal concentration in human plasma is about 3-11 mg/dL. It plays an important role in activating the classical pathway of complement. The complete amino acid and nucleotide sequences of C1R have been determined (Journet and Tosi, 1986). The structural gene of C1R has been mapped on chromosome 12 (Leppert et al, 1987). The genetic polymorphism of C1R was originally described by Kamboh and Ferrell (1986). Two common alleles, C1R*1 and C1R*2, with some variants (C1R*3, C1R*4, C1R*5, C1R*6 and C1R*7) have been reported in several populations. The purposes of the present study are to investigate the polymorphism of C1R in 5 Chinese populations and to observe the genetic microdifferentiation of Chinese population by comparing allele distribution.

Materials and Methods

EDTA-blood samples were collected from 552 unrelated, healthy Chinese individuals living in 5 different geographic areas of China (fig.1). Plasma were separated within 12 h and store at -20°C until use.

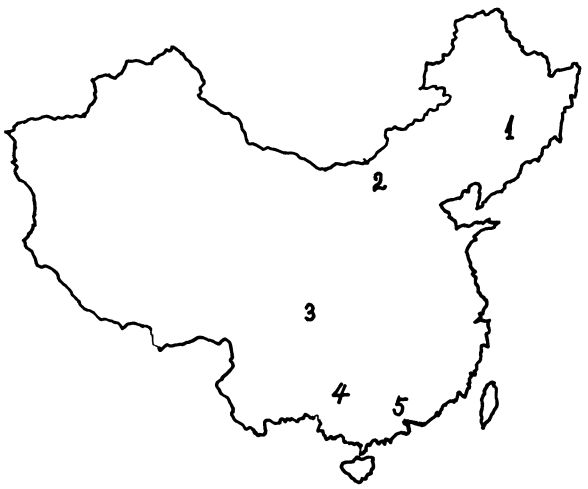

Fig.1. The geographic locations of five Chinese populations investigated.
1=Jilin 2=Inner Mongolia 3=Chengdu 4=Nanning 5=Guangzhou

Isoelectric focusing

Isoelectric focusing was carried out in 0.4mm thick 5%polyacrylamide gels containing 6mol/L urea and 2.5 (w/v) Pharmalyte pH4-6.5 (Pharmacia) as described by Kamboh. The electrode solutions were 0.1 mol/L sodium hydroxide for cathode and 0.1 mol/L phosphoric acid for anode. After 30 min prefocusing, native plasma were applied on the surface of gel near the anode. The electrofocusing was run for 5 hr at the maximum setting of 2000V with cooling temperature of 8°C.

Immunoblotting

A nitrocellulose membrane (0.45*um*, China) was used for passive transfer of proteins. This membrane was incubated for 60 min in 3% BSA, followed by exposure to goat antihuman C1R antiserum (Atlantic, USA) at a 1:1000 dilution for 12 hr at 4°C. After washing in PBS-Tween, the membrane was incubated for 4 hr in rabbit anti-goat IgG conjugated with peroxidase (Dako) at 1:500 dilution. The specific bands were developed by the solution of DAB.

Results and Discussion

Two common alleles, C1R*1 and C1R*2, were observed in all the studied Chinese populations. Some variants, C1R*5, C1R*6 and C1R*7 were encountered and designated according to the nomenclature recommended by Kamboh et al (1989).The distribution of C1R phenotypes and allele frequencies in 5 Chinese populations are presented in table 1 and table 2, respectively. The observed and expected values based upon Hardy-Weinberg equilibrium showed good agreement in all populations (table 2). For the purpose of studying the population substructure of C1R, two statistical analyses were conducted. Firstly, all comparisons of allele frequencies between these 5 groups were evaluated using a chi-test for significance and no significant differences were observed (P>0.05 for each pair). Secondly, a test for Hardy-Weinberg equilibrium was conducted when all the individuals studied were pooled together and no deviations from this equilibrium (x^2=10.7487, df=6, P>0.05) and no loss of heterozygotes were observed. All these data suggested that there was no population substructure for C1R in these 5 Chinese populations.

Table 1. Distributions of C1R phenotypes in 5 Chinese populations

		C1R phenotypes									
Population	N	1-1	2-2	5-5	5-1	2-1	5-2	5-V	2-V	1-V	1-6	1-7
Jilin	105	28	7	4	20	35	5	2	1	1	1	1
Inner Mongolia	142	42	10	7	20	46	13	2	1	1	0	0
Chengdu	111	34	13	1	12	45	5	0	0	1	0	0
Nanning	93	26	15	0	5	39	6	0	0	2	0	0
Guangzhou	101	24	10	1	12	46	8	0	0	0	0	0
pooled	552	154	55	13	69	211	37	4	2	5	1	1

Table 2. C1R allele frequencies in 5 Chinese populations

Population	N	C1R allele frequencies				x^2	df	P
		C1R*1	C1R*2	C1R*5	C1R*Var§			
Jilin	105	0.5381	0.2619	0.1714	0.0286	4.78	6	>0.05
Inner Mongolia	142	0.5317	0.2817	0.1725	0.0141	6.93	6	>0.05
Chengdu	111	0.5676	0.3423	0.0856	0.0045	1.45	6	>0.05
Nanning	93	0.5269	0.4032	0.0591	0.0108	2.77	6	>0.05
Guangzhou	101	0.5248	0.3663	0.1089	-	2.83	3	>0.05
Pooled	552	0.5380	0.3261	0.1241	0.0118	10.75	6	>0.05

§: C1R*Var=C1R*V+C1R*6+C1R*7

There are two interesting features which make C1R a useful marker in population genetics and anthropological genetics studies. First, the allele distributions of C1R showed bimodal in Mongoloid populations, while it showed unimodal in Caucasoid and Negroid populations (table3). Second, the widespread distribution of C1R*5 among all Mongoloid populations and its low frequencies in Caucasoid populations suggested that it was a gene which characterizes the gene of Mongoloid populations.

Table 3. C1R allele frequencies in various populations

Population	N	C1R allele frequencies			
		C1R*1	C1R*2	C1R*5	C1R*Var
U.S. Whites	133	0.8910	0.1090	-	-
U.S. Blacks	109	0.8990	0.1010	-	-
Dogrib Indians	95	0.8320	-	0.1680	-
Aleuts	187	0.8930	0.0670	0.0400	-
Japanese	1000	0.4760	0.3260	0.1940	0.0080
Chinese	552	0.5380	0.3261	0.1241	0.0118

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