DETERMINATION OF C1R TYPES IN BLOODSTAINS

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Introduction

Genetic polymorphism of the C1R subcomponent was first discovered by Kamboh and Ferrell (1986). Using isoelectric focusing and immunoblotting, they described 2 common alleles C1R*1 and C1R*2 in native plasma samples from US whites and US blacks. Besides these 2 common alleles, several further variant alleles have been detected in a variety of ethnic groups by treating plasma samples with neuraminidase (Kamboh et al. 1988, 1989; Nakamura et al. 1988).

We have recently reported that the Japanese population has a large genetic variation in C1R as compared with other ethnic groups, carrying 3 common alleles C1R*1, C1R*2 and C1R*5 (Kido et al. 1991).

In the present study the distribution of C1R allele frequencies was examined in desialylated plasma samples from a rather large size of Japanese population and phenotyping of C1R was investigated in bloodstains for medicolegal purpose. We have followed the C1R nomenclature proposed by Kamboh et al. (1989).

Materials and Methods

Blood samples were collected from 1000 unrelated Japanese individuals in Yamanashi Prefecture, a central part of Japan. EDTA plasma samples were separated by centrifigation at 2000 rpm for 5 min and stored at -20°C until use. Ten µl of plasma was treated with 2 µl 1 M potassium phosphate buffer (pH 7.0) containing 50 U/ml neuraminidase from *Clostridium perfringens* (type V, Sigma, USA) for 24 h at 4°C. The samples were applied to the gel using 5 x 6 mm filter paper strips (Whatman No. 3, UK).

Venous blood from 29 donors with known phenotypes was dropped on filter paper (Whatman No. 3) and dried at room temperature. The bloodstains thus made were stored in a thermostatic chamber at 37°C, at room temperature and in a refrigerator at 4°C and examined after different time intervals. The stains were cut in 5 x 5 mm pieces and soaked in 20 µl 1 M potassium phosphate buffer (pH 7.0) containing 10 U/ml neuraminidase (type V, Sigma) for 24 h at 4°C. The extracts were

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absorbed onto 10 x 5 mm filter paper strips (Whatman No. 3) and applied to the gel.

Isoelectric focusing and electroblotting were performed as described previously (Kido et al. 1991). C1R patterns were detected by the method of Kamboh and Ferrell (1986) with minor modifications.

Results and Discussion

In our population sample 6 phenotypes associated with 3 common alleles, C1R*1, C1R*2 and C1R*5, and 5 variant types were observed (Fig. 1). Table 1 shows the distribution of C1R types and allele frequencies in 1000 Japanese individuals. The population data fitted the Hardy-Weinberg equilibrium.

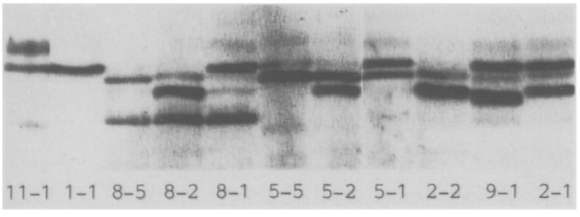

Fig. 1. Isoelectric focusing pattern of C1R types observed in the present study. The anode is at the top

Table 1. Distribution of C1R types in a Japanese population

Phenotype	No. observed	%	No. expected
1-1	229	22.9	226.6
2-1	305	30.5	307.0
2-2	102	10.2	104.0
5-1	178	17.8	184.2
5-2	133	13.3	124.8
5-5	37	3.7	37.4
8-1	9	0.9	6.7
8-2	3	0.3	4.5
8-5	2	0.2	2.7
8-8	0	0	0.0
9-1 1 / 11-1 1	2	0.2	1.0
Others	0	0	1.0
Total	1000	100.0	999.9

Allele frequencies: C1R*1 = 0.4760, C1R*2 = 0.3225, C1R*5 = 0.1935, C1R*8 = 0.0070, C1R*R (the combined frequency of C1R*9 and C1R*11) = 0.0010. χ^2= 4.299; d.f. = 10; 0.95 > p > 0.90

Comparison of C1R allele frequencies in different populations indicates that the Japanese population was a large genetic variation in C1R in the following respects: (1) the C1R polymorphism in Japanese is controlled by 3 common alleles C1R*1, C1R*2 and C1R*5; (2) the C1R*2 allele frequency in

Japanese is considerably higher than that in other ethnic groups; (3) C1R*8 is observed at an almost polymorphic level exclusively in Japanese. Our results disagree with the geographical cline in the Japanese main islands postulated by Nakamura et al. (1990).

By the present isoelectric focusing and subsequent electroblotting, fairly clear C1R patterns were observed also from dried and stored bloodstains (Fig. 2). Our sample included 8 C1R 1-1, 8 C1R 2-1, 3 C1R 2-2, 6 C1R 5-1 and 4 C1R 5-2. All the bloodstains examined were correctly typed for C1R at 37°C for up to 3 weeks, at room temperature for up to 5 weeks and at 4°C even over 10 weeks. The products of C1R*5 stained fainter and more indistinct than those of the other 2 common alleles.

The present method permits C1R phenotyping from dried bloodstains for at least 3 weeks of storage. The C1R would therefore provide a new useful genetic marker for the medicolegal grouping of bloodstains.

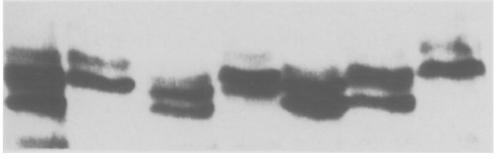

Fig. 2. Isoelectric focusing pattern of C1R types in bloodstains stored at room temperature for 1 week. The anode is at the top

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