C4 PHENOTYPE AND GENE DISTRIBUTION IN A POPULATION OF EASTERN LOMBARDY (ITALY)

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INTRODUCTION

The fourth component of complement (C4) is a ß1-globulin (mol.wt. 200 KB) that acts in the classical pathway of complement activation. C4 molecules are encoded by two closely linked loci, C4A and C4B, located with the genes for the steroid 21-OHase in the vicinity to the HLA-DR locus, in the MHC on chromosome 6p. (Campbell et al,1986).

It is characterized by a high polymorphism; the first genetic study to define its pattern dates back to 1969 (Rosenfeld et al.) with agarose gel electrophoresis in plasma or serum. Teisberg et al. in 1976 used similar methods to define one rare and two common structural alleles at a single locus and, in 1977, employed a discontinous buffer to describe the presence of two common and two rare variants and define their allele frequencies.

O'Neill et al. in 1978 observed that the C4 pattern could not be produced by alleles at single locus but C4S and C4F were the product of two different, but closely linked loci. Subsequent electrophoretic methods have been developed (Awdeh and Alper,1980) with the confirm that the C4 system is characterized by a large number of alleles, more than 35, included the null alleles (Mauff et al,1983; Braun et al,1990), encoded by two different loci, with a distinct electrophoretic mobility, chemical reactivity and hemolitic activity.

In literature (Hobart et al,1984; Kemp et al,1987), C4 complement factor has been examined for its association with various diseases, in particular with autoimmune conditions, but it is not commonly investigated in forensic haemogenetics, especially in Italy. The aim of this work is to present a contribution to a better knowledge of the distribution of C4 polymorphism in Italy through the study of a population sample from the Brescia area (Lombardy, Northern Italy).

MATHERIAL AND METHODS

Fresh serum sampleswere obtained from 254 healthy and unrelated blood donors from the Transfusion Centre Of Brescia. Samples were stored at -80°C prior analysis and incubated with Carboxypeptidase and/or Neuraminidase overnight. C4 phenotyping was performed by high voltage agarose gel electrophoresis using Tris/Glycine/Barbital buffer, according to Awdeh and Alper (1980) with minor modifications. Electrophoresis was carried out on 0,45% agarose gel, at the condition of 500V, 100mA and 50W, until a human HbS marker band had migrated 7 cm. After separation, C4 phenotypes were identified by immunofixation. 1ml. of diluited 1:1 anti human C4 antiserum (ATAB) was applied evenly over the gel. The plate was incubated in a humid chamber, pressed with absorbent paper, washed in isotonic saline

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solution, dried and stained with CBB-R250. Some plates were not immunofixed but, in order to distinguish the C4A and the C4B overlapped alleles, they were developed with a functional overlay consisting of 2% sheep erytrocytes sensibilized with rabbit antibody, in 0,1% agarose in barbital buffer and C4 deficient guinea-pig serum.

RESULTS AND DISCUSSION

The distribution of the C4 alleles observed and their frequencies in the population of Brescia are given in Table 1. Good correlation was found between the observed and the expected phenotype distribution, assuming Hardy-Weinberg conditions (Locus A: X^2 = 18,76 for 15 d.f.; $0,2<p<0,3$. Locus B: X^2 = 13,17 for 15 d.f.; $0,5<p<0,7$).

Table 1: C4 alleles and their frequencies

	Alleles observed	Number	Allele Frequencies
Locus A	A3	240	C4*A3 = 0.7652
	A2	26	C4*A2 = 0.0525
	A1	2	C4*A1 = 0.0039
	A13	2	C4*A13 = 0.0039
	A4	4	C4*A4 = 0.0079
	A6	12	C4*A6 = 0.0239
	AQ0	3	C4*AQ0 = 0.1086
Locus B	B1	241	C4*B1 = 0.7737
	B2	44	C4*B2 = 0.0907
	B3	4	C4*B3 = 0.0079
	B5	5	C4*B5 = 0.0098
	B51	2	C4*B51 = 0.0039
	B6	2	C4*B6 = 0.0039
	BQ0	6	C4*BQ0 = 0.1536

No significant differences have been found between our allele frequencies and the others previously published in literature. Particularly, by a comparison between our frequencies and the data obtained in Pavia (De Paoli et al,1987; Abbal et al,1988), a population near geographically but with a different historical past, it results that the values of the commonest alleles are superimposed but, in Brescia, the rare alleles A5, A7 and B4 are not obtained; furthermore a higher value of Q0 is observed, similar to that found in Central Europe population (Kühnl et al,1988) and in accordance with other studies in Caucasians (Awdeh and Alper,1980).

In conclusion, from our observation, it appears C4 is a useful genetic marker, not only for population studies, but, for its apreciable polymorphism and the easy and simply-reproducible method, also for the application to parentage testing.

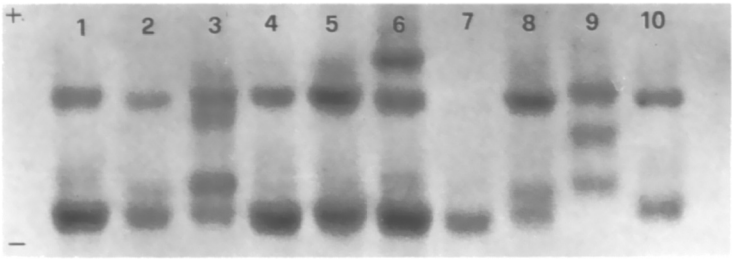

Fig. 1. C4 phenotypes after electrophoresis and immunofixation. From left to right: 1)A3,B1; 2)A3,B1; 3)A3A2,B1B2; 4)A3,B1; 5)A3,B1; 6)A3A6,B1; 7)AQ0,B1; 8)A3,B1B2; 9)A4,B2B5; 10)A3,B1. Anode at the top

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