J. Biol. Res. 2009 n. 1 - Vol. LXXXII - Rubbettino - Soveria Mannelli

Use of electrophoresis for the determination of protein polymorphisms: application to the study of isolated human populations

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KEY WORD: electrophoresis, seroproteins, mountain populations

Abstract

Third factor of complement (C3), group specific component (Gc), properdin B factor (Bf), haptoglobin (Hp) and transferrin (Tf) polymorphisms were studied in a mountain communities samples from the Italian-French Alps.
Electrophoresis was used to determine the seroprotein systems, since both traditional and high-resolution methods have proved to be valid tools in anthropogenetic investigations.
Principal components analysis and maximum likelihood estimation were used to analyse the genetic relationships among populations.

Introduction

Plasma contains numerous proteins, each of which has specific functions. The key role of plasma proteins and their relatively easy analysis by electrophoresis make their determination a valid diagnostic tool.
Different individuals present different molecular characteristics of the same protein, attributable to the underlying genetic systems. These polymorphisms are of anthropological interest when the variants reach frequencies of at least 1% (Facchini, 1988). In fact, seroproteins (above all haplotypes) have proved to be valid markers for calculating genetic distances and for assessing relationships between populations. Previous studies have shown the utility of classic seroprotein markers in reconstructing the biological history of populations, particularly in regard to the relationships with their environment and the historically confirmed contacts with other populations.
The aim of our research is to study the genetic characteristics of populations living in mountain communities in order to identify the microevolutionary processes underlying adaptation to this ecosystem and the socio-cultural transformations that have affected the genetic structure of the populations.
We performed a genetic characterization of these communities using five plasma polymorphisms that are very important markers in human genetics research: third factor of complement (C3), group specific component (Gc), properdin B factor (Bf), haptoglobin (Hp) and transferrin (Tf).
Electrophoresis was used to determine the seroprotein systems, since both traditional and high-resolution methods have proved to be valid tools in anthropogenetic investigations: electrophoresis is an effective method for the identification of different polymorphisms since it provides good resolution of the protein components (Spedini, 1997).

Materials and Methods

The sample consisted of 542 adults of both sexes, native to the communities for at least three generations: 60 from Giaglione, 145 from mountain communities of Valle d'Aosta (La Thuile e Valgrisenche), 150 from Vallouise, 80 from Postua and 107 from Biella.
The Gc, C3 and Bf systems were determined by cellulose acetate electrophoresis and subsequent immunofixation with specific anti-Gc, anti-C3 and anti-Bf antisera (Alper et al., 1972; Germenis et al., 1982); the Hp and Tf systems were determined by agarose electrophoresis and subsequent immunofixation with specific anti-Hp and anti-Tf antisera (Smithies, 1955; Smithies, 1957).
Electrophoresis: a technique used to separate the molecules of a mixture by the application of an electric field; the dissolved molecules migrate at a speed determined by their charge/mass ratio. Proteins usually have a clear positive or negative position according to the mixture of charged amino acids they contain.
Electrophoretic migration occurs on a porous solid support, such as cellulose acetate, or on amide gel soaked with an electrolytic solution (buffer) to allow passage of the current (Alberts, et alt. 2004). The sample is applied to a point of the support and moistened with the buffer solution. The ends of the strip are immersed in separate containers containing buffer and the electrodes are placed in these containers. When a continuous current is applied,

the ions in the sample migrate toward the electrode with opposite charge at a characteristic speed, forming separate uniform bands. Proteins are separated using alkaline buffers, which negatively charge them and cause them to migrate toward the positively charged anode. When the electrophotogram is complete, the various components of the sample are marked with the specific antibody for the particular protein under study and the position of the marked proteins is subsequently identified by Coomassie Blue staining.

Statistical procedures

The genetic relationships between populations were analysed by *maximum likelihood* (Cavalli Sforza et al., 1967) and multivariate techniques (principal components and *cluster analysis*). The general statistical programs SPSS, Phylip v. 3,6c (Felsenstein, 1989) were used.

Results and discussion

Table 1 reports the allele frequencies of the mountain populations for each seroprotein system.

Table 1. Allele frequencies

Giaglione, Postua and particularly Vallouise and Biella have the lowest Gc1 allele frequencies among European populations, much lower than the lowest values recorded thus far in Europe: Germans 0,68 and Polish 0,66 (Spedini, 1966). The frequencies of the Gc2 alleles (0,46 for Biella, 0,41 for Vallouise, 0,38 for Postua and 0,34 for Giaglione) are exceptionally high among European populations (mean 0,20), although high frequencies have also been observed in Sweden, Finland and a region on the eastern side of the Pyrenees (Constans et al., 1978). Italians and Europeans in general have maximum values of the Gc1 allele between 0,70 and 0,80 and Gc2 allele frequencies between 0,20 and 0,30.

The C3 polymorphism frequencies in all the communities differ significantly from the mean values of European populations, in which the incidence of the C3S allele is about 80% and that of C3F is around 20% (Scacchi, 1987). Therefore, Postua and Vallouise represent a limit value for Italian and European populations.

The values of the BfS and BfF variants of properdin also differ from the mean European values and the difference for the latter is significant for all the communities.

The examined sample falls within the European type, although it is different from the other French populations, whose reported frequencies are around 0,81 (Hauptman, 1977) and 0,70 (North, 1981).

In contrast, none of the communities differ from the mean European values for haptoglobin (north-western Italy Hp1 0,35 and Hp2 0,65 and Liguria Hp1 0,30 and Hp2 0,70) (De Stefano et al., 1987).

The allele frequencies recorded in our samples generally agree with those reported for Italy and Europe, showing a higher frequency of Hp2 than Hp1 (around 0,4), with a slight tendency toward lower frequencies in the Alpine communities. The geographical distribution of Hp1 seems to confirm the hypothesis of an increasing east-west and north-south gradient. De Stefano et al. (1987) noted that the distribution of the Hp alleles in Italy is heterogeneous, with high values of Hp1 in regions with a malarial past, confirming the hypothesis of a selective advantage of the Hp1 and Hp2-1 phenotypes in malarial areas due to the higher Hb-binding capacity of the Hp1 allele product.

The gene frequency of Tf C in our samples is similar to those of other Italian populations (non-significant differences).

Comparison with other populations

We compared the results for our communities with those previously reported for the Italian population to determine the biological distances.

Figure 1 shows the results of the principal components analysis of the allele frequencies of the various polymorphisms.

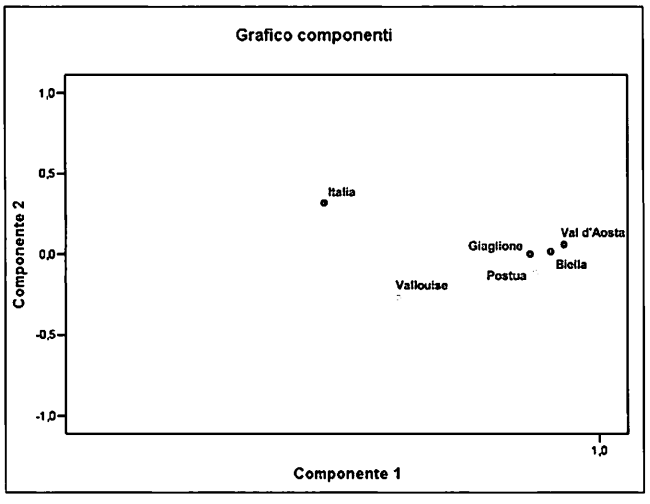

Fig. 1 - *Principal components analysis*

The examined communities are distant from both the general Italian and Vallouise populations, which are also separate from each other. Although together in a fairly tight cluster, the communities still show their own peculiar biological characteristic Fig. 2 - shows the dendrogram obtained by cluster analysis.

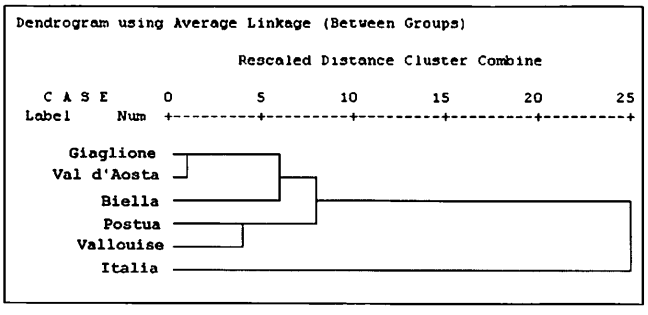

Fig. 2 - *Cluster analysis.*

Our four study populations (Vallouise, Giaglione, Val d'Aosta mountain communities and Postua) generally have very similar frequencies; in fact, some populations are

identical for several alleles. Separate consideration of the individual systems was not informative. The picture obtained by pooling the five systems reveals higher similarity among Vallouise, Postua and Biella on the one hand and Giaglione and the Val d'Aosta mountain communities on the other.
The maximum likelihood dendrogram used to evaluate the relationships among the populations highlights the isolation of Vallouise, Giaglione and Postua and their distance from the Italian mean (figure 3).

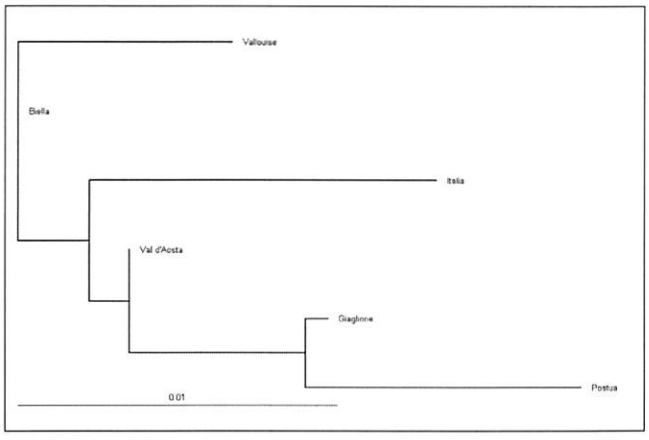

Fig. 3 - *Dendrogram based on the maximum likelihood.*

The genetic distances between our communities and the other Mediterranean and transalpine populations are high, especially for Vallouise (0,05741) and Postua (0,072), indicating that these small communities can be considered genetic isolates.

Conclusions

The electrophoresis is an effective method for the identification of different polymorphisms since it provides good resolution of the protein components.
The genetic analysis confirms the results of biodemographic studies suggesting isolation of the populations of Vallouise, Giaglione and the Val d'Aosta mountain communities, probably due to a founder effect and low gene flow, as found in previous studies for Postua.
The seroprotein pattern revealed very low variability: the study populations generally have very similar frequencies (for some alleles, they are identical).
Separate examination of the individual systems showed that the two populations that differ the most for C3 are Vallouise and the Val d'Aosta mountain communities, while for Gc and Bf the most different populations are Biella and Giaglione, in agreement with the geographical position and distance.
When the five systems are considered together, the relationship between genetic distance and geographical position is confirmed.
The present study demonstrated that the distribution of the allele frequencies of these polymorphisms can adequately discriminate the transalpine populations from the Mediterranean ones or from small communities with peculiar characteristics like Vallouise and Postua.

		Postua N=80	Biella N=107	Vallouise N=150	Giaglione N=60	Valle d'Aosta mountain communities N=145	Mean European values
C3	S	0,52	0,60	0,48	0,62	0,66	0,80 (Scacchi & al., 1987)
	F	0,48	0,40	0,52	0,38	0,34	0,20 (Scacchi & al., 1987)
Gc	1	O,62	0,54	0,59	0,66	0,62	0,73 (Spedini., 1966)
	2	0,38	0,46	0,41	034	0,38	0,27 (Spedini, 1966)
Bf	S	0,60	0,66	0,58	0,53	0,56	0,69-0,8 (Davrinche et al., 1981; Kuhn et al.. 1978) in Spedini.1966
	F	0,40	0.34	0,42	0,47	0,44	0.18 (Mauff et al. 1975) in Spedini, 1966
Hp	1	0,33	0.37	0,41	0,42	0,37	0.40 (Facchini, 1988)
	2	0,67	0.63	0,59	0,58	0,63	0,60 (Facchini et al., 1988)
Tf	C	0,94	1,00	0,98	0,97	1,00	0,90- 0,98 (Fuciarelli et al., 1997)
	B	0,03	/	0,02	0,03	/	0,02 (Fuciarelli et al., 1997;)
	D	0,03	/		/	/	0,02 (Fuciarelli et al., 1997)

Table 1 - *Allele frequencies*

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