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Guido Modiano, Rosaria Scozzari, Franca Gigliani, Giorgio Filippi, Bachisio Latte

Studies on red cell phosphoglucomutase and adenylatekinase polymorphisms in Sardinia

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Genetica. — *Studies on red cell phosphoglucomutase and adenylate-kinase polymorphisms in Sardinia* (*). Nota di GUIDO MODIANO (**), ROSARIA SCOZZARI (**), FRANCA GIGLIANI (**), GIORGIO FILIPPI (**) e BACHISIO LATTE (***), presentata (****) dal Socio G. MONTALENTI.

RIASSUNTO. — Sono state determinate le frequenze geniche per la PGM e per le AK eritrocitarie in località della Sardegna con incidenza della endemia malarica pregressa variante tra zero fino a 100%.

Non è stata trovata indicazione di eterogeneità per nessuno di questi due polimorfismi.

Si può quindi concludere che la malaria non ha influenzato le frequenze gèniche della fosfoglucomutasi né della adenilatochinasi.

Date le sfavorevoli frequenze geniche relative a quest'ultimo sistema in Sardegna, la esclusione di un effetto della malaria su questo polimorfismo è basata però su un test scarsamente sensibile.

L'allele AK^2 è risultato in Sardegna molto meno frequente che in Italia (provincia di Roma).

The reports of a positive correlation in the distribution of Thalassaemia and that of malignant malaria brought Haldane [1], 1949, to formulate the hypothesis that malaria might have been one of the major ecological factors responsible for the maintenance of this clearcut example of balanced polymorphism. Namely the lethality of the gene in homozygous condition would be compensated by an higher biological fitness of the heterozygous carrier in a malarial environment.

Later on it was realized that such hypothesis could also very well explain the distribution of the genes for sickle cell and other haemoglobin variants as well as that of the sex linked gene responsible for the so-called G6Pd–deficiency. Up to now substantial evidence supporting this hypothesis has been collected and critically reviewed [2, 3].

Since all the above mentioned examples of genetical polymorphisms involve alterations of the red cell biochemical make up, it seems reasonable to assume that the mechanisms through which the corresponding genes afford protection to their carriers may be the " unusual ", red cell biochemical machinery for which they are directly or indirectly responsible and which may be less suitable than the " normal " one for a successful intraglobular growth and multiplication of the malarial parasite.

This effect could directly depend upon the presence of the " abnormal " primary gene products in the red cell (say an abnormal haemoglobin or enzyme)

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(**) Department of Genetics, Faculty of Science, University of Rome, Italy.

(***) Town Hospital, Nuoro, Italy.

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or it could—more likely—be the result of a complex interaction at the cellular level between gene products of " abnormal " as well as of " normal " genes.

In the first instance the exposure of a population to malarial endemia for several generations should simply result in an increase in the gene frequency of one or more of the adaptive genes up to equilibrium levels depending upon the fitnesses of the different genotypes and genotype combinations.

In the second instance it could happen that in the long run, the whole genetical structure of populations continuously exposed to malaria, becomes sharply different from that of populations free from it, at least in so far as the genes controlling red cell biochemical characters are concerned and independently of the degree of their ethnical similarity.

It appears therefore reasonable to hypothize that a prolonged action of one or both these mechanisms of selection could very well bring about the appearance or disappearance of certain alleles or even of certain red cell biochemical polymorphisms directly influenced by malaria, but it could also lead to the modification of the gene frequencies of certain other red cell polymorphisms, whose distribution is ubiquitary and clearly independent of malaria.

The combined distribution of thalassaemia, G6Pd-deficiency, sickle and other haemoglobin variants in malarial areas is a clear example of the first type of selective mechanism. On the whole the frequencies of the genes responsible for these conditions are positively correlated with one another but when their distribution is carefully studied within given areas or villages, an interesting picture emerges:

the positive correlation holds true in fact only between G6Pd-deficiency and thalassaemia or sickle cell trait (or other Hb variants), while the last two conditions tend instead to be negatively correlated.

Thus, in Sardinia, where some of the highest frequencies of G6Pd-deficiency and thalassaemia have been reported, sickle cell trait is virtually absent, unlike what happens in Greece and other mediterranean populations whose prolonged contacts with Sardinians are well known. In Greece itself anyhow an inverse correlation between the frequencies of thalassaemia and sickle cell trait has been noticed when the distribution of these two traits has been studied per villages and the same can be said for the distribution of haemoglobins S and C in Africa [2].

The search of interaction of the second type has hardly started to date, namely for the very fact that it is only a few years since three new examples of red cell biochemical polymorphisms—whose maintenance is known to be independent of malaria—were discovered (Table I).

Among these, the A.P. polymorphism is the only one which has been thoroughly studied from this standpoint, with the conclusion that malaria does not appear to have influenced its distribution in Sardinia (Modiano et al., 1967 [4]).

In the present paper the results of similar investigations performed in Sardinia for the PGM and the AK polymorphisms are reported.

As already stated elsewhere (3 and 5) Sardinia is an ideal place for this kind of study because of the availability within the island of numerous and highly inbred human isolates which, though ethnically homogenous, have been living in ecological environments which were extremely diverse in so far as the distribution of *Plasmodium falciparum* was concerned, with a well documented (Fermi, 1938 [8]) differential malarial morbidity ranging from 0 % in the mountains to 100 % down in the plains.

The samples.

The subjects on which the present investigations were carried out, as well as their parents, were born in Sardinia.

They were unrelated adult individuals of both sexes randomly selected through several hospitals, scattered throughout the island of Sardinia, or through the private practice of village doctors.

They were affected by various minor incidental diseases.

Methods.

The bloods were collected by vein puncture with heparin vacutainers (Becton, Dickinson and Company-Columbus, Nebraska) and kept cool (4° C to 10° C) until used.

For the PGM determination the hemolysates were obtained by a duplicated cycle of freezing and thawing of the packed and twice washed red cells. A mixture of acetone and dry ice was used for the freezing and a 37° C waterbath for the thawing.

For the AK determination the hemolysates were obtained by the addition of approximately 1.5 volumes of distilled water to the packed and twice washed red cells.

The PGM phenotypes were determined according to Spencer et al., 1964 [6], the AK phenotypes according to Fildes et al., 1966 [7].

Results and discussion.

The results will be discussed under two headings, *a* and *b*.

a) *Frequencies of* PGM_1 *and* AK *genes versus malaria.*

In order to look for a possible correlation between past malarial morbidity and the gene frequencies of a particular polymorphism, each individual observation must be classified for the phenotypes of the given polymorphism and for malarial morbidity.

Thus, the gene frequencies were calculated within groups of individuals whose birth place had the same malarial morbidity or the same altitude. The altitude is, as everybody knows, inversely correlated with *Anopheles* diffusion and consequently with malarial endemicity. This second criterion

of classification was considered a useful complement because at the time when the rates of malarial morbidity were estimated by Fermi (1938 [8]), the disease was already more or less completely eliminated from a number of areas of the island. Therefore the figures of malarial morbidity given by Fermi at the time were in many areas much lower than those which had been actually operating before (for discussion see Siniscalco et al., 1966 [3].

PGM$_1$ polymorphism.

Fig. 1 shows the comparisons between the gene frequencies for the PGM$_1$ locus observed in four groups of Sardinian subjects subdivided according to the past malarial morbidity of their birth place.

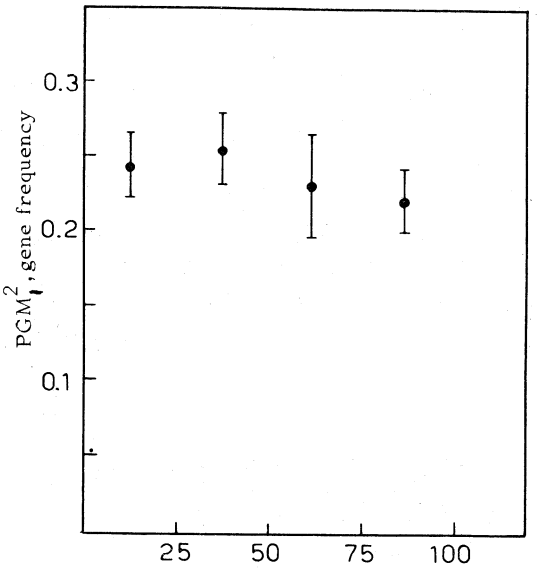

Fig. 1.

The first group includes all the subjects which were born in areas with past malarial morbidity from zero up to 25 %; the range for the second group goes from 26 to 50 % and so on. The sizes of these subgroups and the agreement with the Hardy-Weinberg equilibrium were the following:

Group	Number of individuals	χ^2 for H.W.	P
1	195	2.96	> 0.05
2	167	0.55	> 0.30
3	74	0.35	> 0.50
4	193	0.07	> 0.70

TABLE I.

Formal genetics and racial distribution of some polymorphisms involving red cell enzymes.

ENZYME	Locus	Genetic determination	Codominant alleles observed in			References
			Caucasian	Negro	Mongoloid	
Glucose–6–phosphate–dehydrogenase (G6Pd)	Gd	X–linked	Gd^A, $Gd^{Med\ (*)}$ and rare variants	Gd^A, Gd^{A-}, Gd^B and rare variants	Gd^B, $Gd^{Med\ (*)}$	WHO report (1967)
Acid phosphatase (A.P.) .	P	Autosomic	P^a, P^b, P^c	P^a, P^b, and rare variants	P^a, P^b	Lisker et al. (1967)
Phosphoglucomutase (PGM)	PGM_1	Autosomic	PGM_1^1, PGM_1^2 and rare variants	PGM_1^1, PGM_1^2 and rare variants	PGM_1^1, PGM_1^2	Hopkinson et al. (1966)
	PGM_2	Autosomic	PGM_2^1 and rare variants	PGM_2^1, some other common alleles and rare variants	PGM_2^1	Hopkinson et al. (1966) Cavalli–Sforza (1967) Arends et al. (1967)
Adenylatekinase (AK) . .	AK	Autosomic	AK^1, AK^2 and a rare variant AK^3	AK^1 and a rare variants AK^3		Fildes et al. (1966) Bowman et al. (1967)
6–phospho–gluconate–dehydrogenase (6–PGd)	PGD	Autosomic	PGD^A, PGD^B and rare variants			Fildes et al. (1963)

(*) The $Gd^{Mediterranean}$ allele attains high frequencies only in malarial or past malarial areas.

The good agreement between observed and expected figures for equilibrium in all of these four samples make them suitable for the problem of looking for a possible correlation between past malarial endemia and PGM_1 gene frequencies. It is clear that no indication of heterogeneity between these subgroups has been found.

Absence of heterogeneity was also found when the data were subdivided in two groups according to the altitude of the birth place of the subjects involved (see Table II). The χ^2 (1 d.f.) of the comparison between the PGM_1 gene frequencies of these two groups was 1.604 with $P > 0.20$.

TABLE II.

Distribution of red cell phosphoglucomutase phenotypes in lowland and highland areas of Sardinia.

SOURCE	No. of tested individuals	PHENOTYPES (observed numbers)			Chi-Square for H.W. equilibrium (1 d.f.)	P
		1-1	2-1	2-2		
Lowland (below 400 metres)	310	175	113	22	0.406	> 0.50
Highland (over 400 metres)	323	196	110	17	0.093	> 0.90

		LOWLAND	HIGHLAND
Estimates of gene frequencies	PGM_1^1	0.747	0.777
	PGM_1^2	0.253	0.223
		1.000	1.000

AK polymorphism.

In Table III the AK phenotypic and gene frequency distributions observed among 997 individuals are compared with the past malarial morbidity of their birth place ranging from 0 to 33%, 34 to 66%, 67 to 100% respectively.

TABLE III.

Distribution of red cell adenylate kinase genes and phenotypes in areas of Sardinia with different past malarial morbidity.

Malarial morbidity	0-33				34-66				67-100			
Phenotypes	1-1	2-1	2-2	Totals	1-1	2-1	2-2	Totals	1-1	2-1	2-2	Totals
	466	18	0	484	236	3	0	239	268	5	1	274
Estimates of gene frequencies . .	$AK^1 = 0.981$				$AK^1 = 0.994$				$AK^1 = 0.987$			
	$AK^2 = 0.019$				$AK^2 = 0.006$				$AK^2 = 0.013$			
	1.000				1.000				1.000			

The unfavourable gene frequencies of this polymorphism make the H.W. equilibrium not testable (because of the existence of only two phenotypes at an appreciable frequency). Also the possible correlation with past malarial endemia is very poorly testable, though we are certainly in the position to exclude a gross influence of this ecological factor on the AK polymorphism.

The same conclusion is reached when the same data are plotted against the altitudes of the birth place of the propositi (see Table IV).

TABLE IV.

Comparison between gene frequencies distributions observed in lowland and highland areas of Sardinia.

SOURCE	No. of tested individuals	AK^1	AK^2
Lowland (below 400 metres)	428	842	14
Highland (over 400 metres)	569	1124	14

Chi-Square (1 d.f.) = 0.580, P > 0.30.

In conclusion it seems fair to state that, in Sardinia, the distribution of these additional examples of red cell biochemical polymorphisms have not been affected by malaria.

b) *Frequencies of* PGM_1 *and* AK *genes in Sardinia as a whole.*

Since the Sardinian populations, so far studied, are homogeneous in their distribution of PGM_1 and AK types, the data have been pooled together in order to get better estimates of gene frequencies for these two polymorphic traits (see Table V and VI).

TABLE V.

Distribution of red cell phosphoglucomutase phenotypes in a sample of 633 Sardinians.

PHENOTYPES	Incidence	Absolute frequencies	
		Observed	Expected
1–1	58.6	371	367.8
2–1	35.2	223	229.4
2–2	6.2	39	35.8
Totals . . .	100.0	633	633.0

Chi-Square (1 d.f.) = 0.546 (for deviation from Hardy-Weinberg equilibrium) P > 0.30.

Estimates of gene frequencies . . . . . $PGM_1^1 = 0.76$, $PGM_1^2 = 0.24$, 1.00

TABLE VI.

Distribution of red cell adenylate kinase phenotypes in a sample of 1033 Sardinians.

PHENOTYPES	Frequencies	
	Relative	Absolute
1–1	97.1	1004
2–1	2.8	28
2–2	0.1	1 (*)
Totals . . .	100.0	1033

Estimates of gene frequencies $AK^1 = 0.985$, $AK^2 = 0.015$, 1.000

(*) The AK phenotype of his two parents and of his two children is 2–1. His wife is 1–1.

The gene frequencies for the PGM_1 has turned out to be the same as those found in the Italian mainland (Modiano et al., unpublished data [9]) and in other Caucasian populations (see Table VII).

TABLE VII.

Gene frequencies for red cell phosphoglucomutase: A comparison between a group of 633 Sardinians and several other populations.

POPULATION	Totals	Gene frequencies (*)		References
		PGM_1^1	PGM_1^2	
Sardinia	633	76.2	23.8	
Caucasian				
England	2115	76.4	23.5	Hopkinson et al. (1966)
San Francisco	271	77.7	22.3	Lie-Injo Luan Eng (1966)
U.S.A.	282	77	23	Giblett (in press)
Greek	88	69.3	30.7	Hopkinson et al. (1966)
Iraqi Jews	69	67.4	32.6	
Turkish Cypriot	243	69.8	30.0	
Icelanders	129	81.8	18.2	Mourant et al. (1967)
Habbanites Jews	222	43.0	57.0	
Mongoloid				
U.S.A. (mostly Japanese)	84	74	26	Giblett (in press)
Japan (Ainus)	182	90	10	
Eskimos	299	82.4	17.6	Scott et al. (1966)
Athabaskans	127	89.4	10.6	
Aleuts	53	85.8	14.2	
MEXICO				
Huasteco	233	80.5	19.5	Lisker et al. (1967)
Cora	100	89.0	11.0	
Huichol	72	83.3	16.7	
SOUTHERN VENEZUELA				
Yanomama Indians	338	93.8	(**)	Arends et al. (1967)
Negro				
Yoruba (Nigeria)	153	75.8	23.9	Hopkinson et al. (1966)
Bantù (South Africa)	99	79.2	20.2	
Living in England	103	78.6	21.4	
Living in S. Francisco	284	80.5	19.5	Lie-Injo Luan Eng (1966)
Living in U.S.A.	202	84.2	15.8	Brewer et al. (1967)
Living in U.S.A.	336	80	20	Giblett (in press)

(*) Only PGM_1^1 and PGM_1^2 genes have been reported.
(**) Only PGM_1 gene frequency has been reported.

The gene frequencies for the AK locus were found, instead, to be quite different from those found in Rome ($\chi^2_{1\,d.f.} = 15.4$, $P < 0.001$) and even more different from those found in England (see Table VIII).

TABLE VIII.

Gene frequencies for red cell adenylatekinase: a comparison between a group of 1033 Sardinians and other groups.

SOURCE	Totals	Gene frequencies		References
		AK^1	AK^2	
Sardinia	1033	98.5	1.5	
Caucasian				
Rome	738	96.5	3.5	Modiano et al. (1967b)
England	960	94.9	5.1	Fildes et al. (1966)
U.S.A.	254	97.2	2.8	Brewer et al. (1967)
U.S.A.	1315	95.2	4.7	Bowman et al. (1967)
Negro				
Ghana & Nigeria	800	100.0	0.0	quoted by Bowman et al. (1967)
Babynga Pygmies	± 300	100.0	0.0	Cavalli-Sforza (1967)
Mongoloid				
Yanomama Indians	(*)	100.0	0.0	Arends et al. (1967)
Mixed				
U.S.A. Negroes	1063	99.3	0.6	Bowman et al. (1967)
U.S.A. Negroes	139	98.6	1.4	Brewer et al. (1967)

(*) Not indicated.

Since this finding of a different gene frequency distribution for the AK locus is apparently constant throughout the island of Sardinia, it may be considered as an additional example of a peculiar " Sardinian " gene frequency as already observed for the MN and Rh systems (3 and 5).

This adds further strength to the assumption that the Sardinian isolates are ethnically homogeneous, an argument which is a *sine qua non* condition for interpreting the finding of the positive correlation between malaria, thalassaemia and G6Pd–deficiency as evidence in favour of the hypothesis that

Plasmodium falciparum has indeed been the ecological factor responsible for the maintenance of these three red cell polymorphisms in the island of Sardinia.

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