Favism: Association with Erythrocyte Acid Phosphatase Phenotype

Abstract. *The frequency of carriers of the* P^a *and* P^c *alleles of the gene for acid phosphatase in the erythrocyte is significantly higher in male subjects deficient in glucose-6-phosphate dehydrogenase and having hemolytic clinical favism than it is in the general population. This observation seems to indicate that alleles* (P^a *and* P^c) *of a gene polymorphic in all human populations affect the fitness of the involved phenotypes in special genotypic and nongenotypic conditions.*

Subjects with erythrocytic deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) may have a severe hemolytic crisis after ingesting fava beans. The G-6-PD deficiency is the necessary condition for the occurrence of hemolytic episodes but by itself is not sufficient [the incidence of clinical favism in a group of enzymopenic subjects taken at random was less than 30 percent (*1*)]. An action of some other genetically determined factor has been suggested mainly by Sartori (*2*), and recent evidence shows the existence of an autosomal gene which favors the hemolytic episodes in subjects deficient in G-6-PD (*3*). Our results indicate an association between some erythrocyte acid phosphatase phenotypes (*4, 5*) and the incidence of clinical favism in male subjects deficient in G-6-PD (*6–8a*).

Two hundred twenty-one subjects with a positive history of hemolytic favism have been studied. Seventy-one (69 boys and 2 girls) were admitted in the last few years to the Pediatric Clinic of the University of Rome for severe episodes of acute hemolytic anemia due to favism. Of 150 (104 males and 46 females) subjects from the Oristano area (Sardinia) who were referred by the local doctors, only a certain number of them have a positive history of hospital admission and transfusions. All subjects were confirmed to be deficient in G-6-PD.

We used as a control for the group admitted to our Pediatric Clinic the data obtained by Modiano *et al.* (*9*) on 417 normal adult Roman subjects; they were mainly blood donors, and more than 90 percent were males. As a control for the Sardinian group, 264 subjects (105 males and 159 females) were collected at random in the area of Oristano. Their ages were between 7 and 14 years. The frequency of the Gd^{Med} gene (*10*) in the group was 0.23 [based on the frequency of Gd(−),-Mediterranean phenotype for males]. Eleven subjects had a positive history for hemolytic favism. No difference between males and females has been observed for the frequency of the acid phosphatase alleles.

The acid phosphatase phenotype was determined according to the method of Hopkinson *et al.* (*5*); some hemolyzates were treated with 2-mercaptoethanol, but the electrophoretic phenotypes resulting were identical to those obtained with untreated hemolyzates. In a few cases the parents were also examined, and the acid phosphatase phenotype of the children was found compatible with that of their parents.

The frequencies of phenotypes and genes for erythrocyte acid phosphatase in the various groups of subjects examined are shown in Tables 1 and 2. The frequencies of the P^b gene are compared in Table 3. The groups of males differ significantly from the control groups, showing a marked deficiency in P^b corresponding to an increase in the numbers of P^a and P^c alleles. The group of females does not differ significantly from the control group.

In Table 4 are shown the frequencies of P^b in Sardinian males with the following phenotypes: (i) Gd(+),B; (ii) Gd(−),Mediterranean without a history of favism; and (iii) Gd(−),Mediterranean with a history of favism. The first two groups include the Oristano male controls and 77 other male subjects of the same age group collected according to the same criteria in the zone of Tortolì, which, like that of Oristano, is a lowland Sardinian area with a high frequency of Gd^{Med} gene. The frequencies of acid phosphatase alleles in the two control groups of Oristano and Tortolì were practically identical. The frequency of P^b in Gd(−),Mediterranean subjects without a history of favism is significantly higher than that in Gd(−),Mediterranean subjects with favism ($P < .05$) and slightly higher than that in normal males. The latter finding was also to be expected because of the P^b deficiency observed in Gd(−),Mediterranean subjects with favism; however, it is difficult to give an estimate of the expected frequency of P^b in Gd(−),Mediterranean subjects without favism because the actual incidence of favism in such subjects is not known (in fact, 30 percent is to be considered only as the upper limit). In 63 male subjects who had had favism, quantitative assay of acid phosphatase activity of the various groups of phenotypes indicated that the relative activity was very similar to that found in normal controls (Table 5).

The possibility that the observed excess of some phenotypes in subjects with favism would be the consequence

Table 1. Frequencies of erythrocyte acid phosphatase phenotypes in subjects deficient in G-6-PD who had had hemolytic favism (*17*). In parentheses are reported the expected absolute frequencies calculated on the basis of frequencies in the respective control group. For calculation of χ^2, phenotype C has been associated with CB. N.S., not significant.

Subjects	Mean age at first hemolytic episode	Phenotype frequencies						Comparison with the respective control group	
		A	CA	BA	B	CB	C	$\chi^2_{4d.f.}$	P
Normal Romans		36	14	132	183	51	1		
Roman males with favism	4	8 (5.96)	6 (2.32)	27 (21.84)	19 (30.28)	9 (8.44)	(0.16)	9.5059	$< .05$
Sardinian control group		14	7	76	141	26			
Sardinian males with favism	17	12 (5.51)	7 (2.76)	27 (29.94)	42 (55.54)	15 (10.24)	1	12.1296	$< .02$
Sardinian females with favism	29	4 (2.44)	1 (1.22)	9 (13.24)	26 (24.57)	6 (4.53)		2.0692 ($\chi^2_{3d.f.}$)	N.S.

of a misclassification due to the formation of artifacts induced by GSSG (oxidized glutathione) can be excluded for several reasons: (i) the phenotype of the child was always compatible with that of the parents; (ii) 2-mercaptoethanol did not modify the electrophoretic pattern of the considered phenotypes, even though this substance can reverse the effect of GSSG (*8*); (iii) typical artifacts observed during in vitro incubation with GSSG were consistently absent; (iv) gene frequencies of acid phosphatase in G-6-PD deficient subjects who did not have favism were different from those observed in G-6-PD deficient subjects who had had favism (Table 4); and (v) in the various groups of phenotypes, acid phosphatase activity in male subjects who had had favism was very similar to that in normal controls.

Table 2. Gene frequencies of erythrocyte acid phosphatase in subjects deficient in G-6-PD who had had hemolytic favism.

Subjects	Gene frequencies			Total
	P^a	P^b	P^c	
Normal Romans	0.261	0.658	0.080	0.999
Roman males with favism	.355	.536	.109	1.000
Sardinian control group	.210	.727	.063	1.000
Sardinian males with favism	.279	.606	.115	1.000
Sardinian females with favism	.196	.728	.076	1.000

Table 3. Frequency of P^b allele of erythrocyte acid phosphatase in subjects deficient in G-6-PD who had had hemolytic favism. In parentheses are reported the expected absolute frequencies calculated on the basis of frequencies in the respective control group. N.S., not significant.

Subjects	Alleles (No.)		Comparison with the respective control group		Comparison with Sardinian females with favism	
	$P^a + P^c$	P^b	$\chi^2_{1d.f.}$	P	$\chi^2_{1d.f.}$	P
Normal Romans	285	549				
Roman males with favism	64 (47.16)	74 (90.84)	7.6622	$<.01$		
Sardinian control group	144	384				
Sardinian males with favism	82 (56.73)	126 (151.27)	10.3530	$<.01$	4.1705	$<.05$
Sardinian females with favism	25 (25.09)	67 (66.91)	0.0002	N.S.		

Table 4. Frequency of P^b allele of erythrocyte acid phosphatase in Sardinian male subjects deficient in G-6-PD with or without a positive history for hemolytic favism. In parentheses are reported the allele frequencies in percentages.

Subjects	Alleles (No.)		Comparison with Gd(−),-Med subjects without favism	
	$P^a + P^c$	P^b	$\chi^2_{1d.f.}$	P
Gd(+),B	89 (29.9)	209 (70.1)		
Gd(−),Mediterranean without favism	14 (24.1)	44 (75.9)		
Gd(−),Mediterranean with favism	82 (39.4)	126 (60.6)	4.5933	$<.05$

Table 5. Erythrocyte acid phosphatase activity (expressed as micromoles of para-nitrophenol liberated in 30 minutes per gram of hemoglobin at 37°C) in normal subjects and in male subjects deficient in G-6-PD who had had hemolytic favism (*18*). S.D., standard deviation.

Acid phosphatase phenotype	Subjects deficient in G-6-PD			Normal subjects		
	No.	Mean activity	S.D.	No.	Mean activity	S.D.
A	7	130.3	24.7	16	123.3	20.7
BA	16	147.9	31.5	48	142.6	20.3
B	25	166.9	23.4	75	162.7	20.0
CA	5	171.2	36.6	5	174.6	9.8
CB	9	192.8	32.2	22	192.2	29.8
C	1	220.0		1	270.0	

We therefore think that these results prove satisfactorily that male subjects with different phosphatase phenotypes have different susceptibilities to the hemolytic effect of *Vicia faba* (*11*). The group of females is too small and is not clinically comparable (only seven Sardinian females have a positive history for admission to a hospital and transfusion in pediatric age) with the male group examined (all the Roman and about half the Sardinian males have been admitted to the hospital and transfused in pediatric age) for us to draw any definitive conclusion. Further data confirming the observed association between acid phosphatase phenotype and clinical favism would come from the analysis of the distribution of favism among siblings segregating for G-6-PD deficiency and P^a or P^c alleles.

In G-6-PD deficient subjects, erythrocytic reduced glutathione is unstable, and its concentration falls almost to zero under the action of special oxidative drugs and fava beans. Oxidized glutathione induces changes in the electrophoretic pattern of erythrocytic acid phosphatase which are associated with a decrease in the enzyme activity (*7*). In addition, the anodic components of the electrophoretic patterns of acid phosphatase are less stable than the cathodic ones under the action of oxidized glutathione or acetylphenylhydrazine (*8*). Shinoda, in accordance with our results, showed that A, BA, and B phenotypes of acid phosphatase when treated in vitro with oxidative drugs are less stable than the other phenotypes (*12*). Hopkinson *et al.* (*13*), Spencer *et al.* (*14*), and Modiano *et al.* (*9*) have also shown that among the various phenotypes the enzymatic activity of acid phosphatase decreases in this order: C>CB>CA≥B>BA>A.

The functions of acid phosphatase in the red blood cell are not well known, and it is therefore difficult to give a satisfactory explanation of the observed association. Nevertheless, on the basis of the above evidence, we feel justified in advancing some hypotheses.

If it is assumed that there is a critical amount of acid phosphatase activity necessary for survival of the erythrocyte, then under the action of oxidative drugs this amount could be reached more easily in those phenotypes which present a lower activity and which are less stable. Moreover, one can hypothesize that some isozymatic combinations, which depend on the acid phosphatase genotype, undergoing a more rapid denaturation than others, might form centers of denatured proteic mate-

rial which would modify the shape of the erythrocyte and the elastic characteristics of the membrane, resulting in its destruction (*15*).

The observation that there is a slightly lower frequency of gene P^a (although statistically not significant) among the lowland Sardinian population than among the highland population (*9*) is confirmed by our data on individuals of the Oristano and Tortolì areas (our Sardinian control groups) and is in line with association between favism and acid phosphatase phenotypes. In fact, in Sardinian lowlanders the frequency of the Gd^{Med} gene is higher than it is in the highlanders; therefore the P^a gene could have undergone a selective negative pressure in the lowland areas.

As far as we know, ours is the first report showing that alleles of a gene coding for an enzyme polymorphic in all human populations affect the fitness of the involved phenotypes in special genotypic [Gd(−),Med phenotype] and nongenotypic conditions (ingestion of fava beans). Other similar examples refer to some genetic polymorphisms with limited diffusion [Gd(−), HbS, and so on] and to the association between blood groups and infectious diseases (*16*). On the contrary, the association of blood groups with internal diseases, because the selective effect involves mainly individuals past the reproductive age, is not likely to influence gene frequencies.

EGIDIO BOTTINI
PAOLA LUCARELLI
ROCCO AGOSTINO
RICCIOTTI PALMARINO
LUISA BUSINCO
GIUSEPPINA ANTOGNONI

Centro di Genetica Evoluzionistica del CNR (Istituto di Genetica della Facoltà di Scienze) and *Clinica Pediatrica dell'Università di Roma, Rome, Italy*

References and Notes

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4. Red cell acid phosphatase is an enzyme dependent on sulfhydryl groups, with an electrophoretic polymorphism determined by the occurrence of three common alleles: P^a, P^b, and P^c at an autosomal locus; correspondingly there are six electrophoretic phenotypes: A, B, C, BA, CA, and CB (*5*).
5. D. A. Hopkinson, N. Spencer, H. Harris, *Nature* **199**, 969 (1963).
6. A search for such association was strongly prompted by previous in vitro studies (*7, 8*) in which we demonstrated that red blood cell acid phosphatase is highly susceptible to the inactivating action of oxidized glutathione and acetylphenylhydrazine and that the isoenzymes of acid phosphatase show a different resistance toward these substances. As a conclusion to the last study in the series, we stated, "From a more general standpoint one wonders whether the differential liability of isoenzyme fractions towards toxic agents could result in a differential fitness in favour of the genotypes bearing the most stable combination of isoenzymes." Under these circumstances the a priori probability of an association between hemolytic event and phosphatasic phenotype appeared to be definitely not negligible, in contrast with the situation of most of the similar associations reported in the literature (*8a*).
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10. According to the "Nomenclature of Glucose-6-Phosphate Dehydrogenase in Man" (*ibid.*, p. 545), the male phenotype with the normal enzyme is designated by Gd(+),B. The male phenotype with a deficient enzyme having an electrophoretic mobility similar to B is designated by Gd(−),Mediterranean, and the corresponding allele is designated by $Gd^{Mediterranean}$.
11. Prof. Harry Harris has told us that Dr. Hopkinson, who has studied 29 Greek subjects with favism, obtained data showing an excess of P^a gene. Although not significant, this result, obtained independently from ours in a third population, can be regarded as a confirmation of the association between hemolytic favism and acid phosphatase phenotype.
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17. The two girls admitted in our clinic for favism are not included: both showed B phenotype for acid phosphatase. Fourteen females from Sardinia showed an intermediate G-6-PD deficiency.
18. Subjects deficient in G-6-PD were not tested for thalassemia. Values for normal subjects are those reported by Modiano *et al.* (*9*).
19. We thank Prof. Harry Harris for helpful discussion. This work was supported by the Italian National Research Council.

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Electrical Activity of the Hypothalamus: Effects of Intraventricular Catecholamines

Abstract. *The injection of epinephrine into the third ventricle of the rat brain causes a biphasic elevation and depression in the integrated multiple-unit electrical activity of the median eminence. Activity in the arcuate nucleus decreases after the injection of the catecholamines. These changes in the integrated multiple-unit electrical activity may be related to the secretion of hormones by the anterior pituitary gland.*

The hypothesis that brain catecholamines play an important role in the regulation of the secretion of hormones from the anterior pituitary is strongly supported (*1*). Catecholamines have been administered intraventricularly to bypass the limited permeability of the blood-brain barrier to the amines. The injection of epinephrine into the third ventricle of the estrous rabbit or the pentobarbital-blocked proestrous rat induces ovulation (*2*). Intraventricular injection of dopamine causes the release of follicle-stimulating hormone and luteinizing hormone in the male rat (*3*). Norepinephrine inhibits stress-induced release of adrenocorticotropin when injected into the third ventricle of the dog (*4*). Norepinephrine increases the secretion of growth-hor-

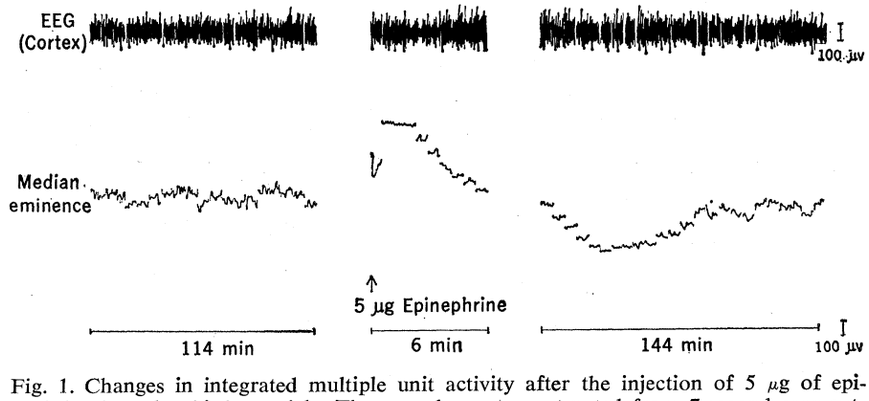

Fig. 1. Changes in integrated multiple unit activity after the injection of 5 µg of epinephrine into the third ventricle. The record was reconstructed from 7-second segments extracted from a continuous record at successive 6-minute intervals. For the first 6 minutes after the injection of the catecholamine, however, 7-second segments were extracted every 30 seconds.