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Evidence in favour of an association between erythrocyte acid phosphatase phenotype and clinical favism

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Genetica. — *Evidence in favour of an association between erythrocyte acid phosphatase phenotype and clinical favism* (*). Nota di EGIDIO BOTTINI (**), PAOLA LUCARELLI, ROCCO AGOSTINO, RICCIOTTI PALMARINO, LUISA BUSINCO e GIUSEPPINA ANTOGNONI, presentata (***) dal Socio G. MONTALENTI.

RIASSUNTO. — *Dati in favore di una associazione tra favismo e fenotipo delle fosfatasi acide eritrocitarie.*

La frequenza dei fenotipi che portano il gene P^a per le fosfatasi acide eritrocitaria è significativamente più elevata nei bambini maschi con deficit della G–6–PD che hanno avuto crisi emolitiche da favismo, a paragone della popolazione generale.

Questa osservazione sembra indicare che un allele (P^a) di un gene polimorfico in tutte le popolazioni umane influenza la idoneità (fitness) dei fenotipi che lo portano, in speciali condizioni sia genotipiche (deficit di G–6–PD) che ambientali (alimentazione con fave).

It is well known that subjects with red cell G–6–PD deficiency may have a severe hemolytic crisis following the ingestion of 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 (Gd(—)Med) taken at random was found to be less than 30 % [1]. Recently, evidence has been brought to show the existence of an autosomal gene which favours the hemolytic episodes in G–6–PD deficient subjects [2]. The results of our present work suggest an association between some red blood cell acid phosphatase phenotypes and the incidence of clinical favism in male G–6–PD enzymopenic subjects.

A total of 84 male subjects in the pediatric age have been studied: 69 were admitted in the last few years in the Pediatric Clinic of the University of Rome for severe episodes of acute hemolytic anemia due to favism and 15 were taken from 3 villages in the lowlands of Sardinia, they too having been admitted one or more times in the past in the regional Hospitals for severe favism.

In the first group the G–6–PD deficiency was confirmed by a quantitative method, whereas in the Sardinian group by a qualitative (Motulsky) method. The acid phosphatase phenotype was determined according to Hopkinson *et al.* [3]; some hemolysates were treated with 2–mercaptoethanol and the electrophoretic phenotypes resulted identical to those obtained with untreated

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hemolysates. 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 results are indicated in the Table I: 58,33 % of male G–6–PD deficient subjects which had one or more hemolytic episodes due to favism, are carriers of the P^a allele of the red cell acid phosphatases gene either in single or double dose; the frequency of the same phenotypes amongst healthy Italian subjects taken from the Roman and Sardinian areas is 44,59 % (4,5) ($P < 0{,}02$) · P^a gene frequency in the group of children which had episodes of favism is 0,357 and in healthy subjects is 0,258 ($P < 0{,}01$).

TABLE I.

Frequency of RBC acid phosphatase phenotypes in subjects with clinical favism compared to normal subjects.

Phenotype	A	BA	CA	B + CB + C (*)
Subjects with favism:				
observed	11	32	6	35
% frequency	13,09	38,09	7,14	41,66
				(P^a gene frequency 0,357)
Normal subjects:				
observed	74	350	46	584
% frequency	7,02	33,21	4,36	55,41
				(P^a gene frequency 0,258)

(*) Owing to the risk of misclassifying, in these subjects, phenotype B with CB we decided for now to include the two phenotypes, together with phenotype C, in a single class.

Further samples are currently being collected and examined: the presently reported results, although not conclusive, suggest an association between the presence of P^a allele for red cell acid phosphatases and the incidence of clinical favism in male G–6–PD deficient children.

In G–6–PD deficient subjects, erythrocyte reduced glutathione is unstable and its level falls almost to zero under the action of special oxidative drugs and fava beans. We have already shown that oxidized glutathione induces changes in the electrophoretic pattern of red cell acid phosphatases which are associated with a fall of the enzyme activity [6, 7]. We have also observed that the anodic components of the electrophoretic pattern are less stable under the action of oxidized glutathione or acetylphenylhydrazine [8].

Shinoda, in accordance with our results, later showed that A, AB, and B phenotypes are less stable under treatment in vitro with oxidative drugs [9]. Hopkinson *et al.* [10]. Spencer *et al.* [11] and Modiano *et al.* [4] have also shown that amongst the various phenotypes the enzymatic activity of acid phosphatase decreases in this order: $C > CB > CA \geq B > BA > A$.

At the moment the functions of acid phosphatases in 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 hypoteses.

Assuming there is a critical level of acid phosphatase activity for erythrocyte survival, under the action of oxidative drugs this level could be reached more easily in those phenotypes which present a lower activity and which are less stable. One can also hypothesize that some isozymatic combinations (which depend on the acid phosphatase genotype), undergoing a more rapid denaturation as compared to others, form centers of denaturated proteic material which modify the shape of the erythrocyte and the elastic characteristics of the membrane [12] resulting in its destruction.

The observation reported by Modiano *et al.* [4] concerning a slightly lower frequency of gene P^a (although statistically not significant) amongst the lowland Sardinian population as compared to the highland population is compatible with our present results: in fact it is well known that in the former the frequency of the Gd^{Med} gene is much higher respect to the latter and therefore the P^a gene could have undergone a selective negative pressure.

As far as we know the finding presently reported, if confirmed, is the first showing that an allele (P^a) of a gene polymorphic in all human populations, affects the fitness of the involved phenotypes in special genotypic (Gd (—) Med phenotype) as well as not genotypic conditions (ingestion of fava beans). In fact the other similar examples either refer to genetical polymorphisms with limited diffusion (Gd (—), HbS, and so on) or the selection effect involves individuals in the postreproductive age (ABO).

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