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Favism: looking for an autosomal gene associated with glucose-6-phosphate dehydrogenase deficiency

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SUMMARY Favism is a severe, acute haemolytic anaemia which occurs in about 20% of G6PD deficient subjects after ingestion of fava beans. Since not all G6PD deficient subjects are sensitive to fava beans, the possibility has been suggested that extra erythrocytic factors may play an important role in the susceptibility to haemolytic favism.

To test the hypothesis that an autosomal enzyme is involved in the pathogenesis of favism, we carried out a β-glucosidase assay in small intestine biopsies from normal subjects and G6PD deficient subjects with or without favism. β-glucosidase might be involved in the absorption and metabolism of fava beans and a quantitative polymorphism could explain the different susceptibility to fava beans of G6PD deficient subjects.

Our observations showed no consistent quantitative polymorphism of β-glucosidase in the subjects examined.

Deficiency of human glucose-6-phosphate dehydrogenase (G6PD) is a widespread X linked disorder which is mainly characterised by susceptibility to haemolytic anaemia after ingestion of certain drugs or toxic substances.[1–3] Favism is a more uncommon aspect of G6PD deficiency occurring after ingestion of fava beans.[4] It is confined largely to certain Mediterranean countries (Sardinia and Greece) although sporadic cases have also been described in other areas of the world.[5] Favism resembles drug induced haemolysis, but differs from it in that fewer than 20% of G6PD deficient subjects appear to be sensitive to fava beans.[6] Subjects sensitive to fava beans are usually carrying the Mediterranean variant of G6PD, but until now no significant biochemical differences in this enzyme have been detected among deficient subjects with or without favism.[1] A study of the incidence of favism in Greece suggests that the haemolytic episodes do not occur randomly in G6PD deficient subjects, but that familial aggregation of cases occurs.[7] These data fit the hypothesis of Mendelian segregation of an autosomal gene which in the heterozygous state enhances the susceptibility to favism of G6PD deficient subjects. Despite this evidence, no definitive conclusions have been reached until now from either the biochemical or the genetic point of view.

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Recently the biochemical role of some substances contained in fava beans has been emphasised. These substances, which may trigger haemolytic crises, are divicine and isouramil, the aglycon moieties of the β-glycosides vicine and convicine present in fava beans.[8] Since β-glucosidase is present in intestinal cells,[9] through the effect of this enzyme the two glycones present and metabolically inactive in fava beans may be cleaved, releasing divicine and isouramil. These substances oxidise in vitro and reduce glutathione (GSH) to glutathione disulphide (GSSG) in G6PD deficient red cells.[8 10] Since not all G6PD Mediterranean subjects are sensitive to fava beans, a different breakdown of these metabolites may occur in relation to a different specific activity of β-glucosidase present in intestinal cells of each subject. Subjects carrying cells with higher specific activity should have a higher rate of breakdown of the two glycones, with larger release of isouramil and divicine.

To test this hypothesis the specific activity of β-glucosidase was measured in biopsy tissue of the small intestine of normal controls and of G6PD deficient subjects with or without a history of favism.

Material and methods

Small intestine biopsies of 1 mm were obtained from

persons who needed a gastroduodenoscopy as part of diagnostic work up. The fragments were frozen, kept at −80°C, and thawed just before the assay. Homogenisation was carried out in ice by 30 strokes of a tight-fitting Dounce glass homogeniser containing 200 µl of distilled water. The homogenate was sonicated in ice by a microsonicator for 30 seconds and immediately assayed.

β-glucosidase activity was tested with the fluorogenic substrate 4-methylumbelliferyl β-D-glucoside (4-MU-β-GLU) (Sigma). The reaction mixture contained 20 µl of the substrate solution (10 mmol/l 4-MU-β-GLU in 0·1 mol/l Na acetate buffer, pH 4·0 and 5·3), 5 and 10 µl of the cell extract, and distilled water to 35 µl. The assay system was incubated for 15 minutes at 37°C and then terminated with 3·0 ml of 0·1 mol/l Tris HCl buffer, pH 10·0. The blank system contained the same reagent but distilled water was substituted for the cell lysate.

The liberated 4-methylumbellipherone (4-MU) was quantified in a fluorometer Farrand 4A. A standard curve was constructed from 4-MU (Sigma). The assay was carried out at pH 4·0 and 5·3 because β-glucosidase has a double pH optimum.[11] One unit of enzymatic activity represented 1 nmol of 4-MU-β-GLU hydrolised per hour at 37°C per mg protein. Each assay was carried out in duplicate. G6PD activity in red cells was performed according to WHO.[12] Protein determination was carried out by the method of Lowry *et al.*[13]

Results

We examined small intestine biopsies from 26 normal males and 19 G6PD deficient males, of whom nine had a past history of favism. Mean levels of β-glucosidase are shown in the table and the figure shows the distribution of each observation.

In agreement with previous results,[11] the activity measured at pH 4·0 is much lower than that measured at pH 5·3. Student's *t* test of the geometric means among the different classes considered (normal, G6PD deficient without favism, and G6PD deficient with favism) did not reveal any significant difference either at pH 4·0 or pH 5·3 ($p>0·8$) (table).

Comparison of the distribution between normal and G6PD deficient subjects (with and without favism) was not significant ($\chi^2>0·07$).

TABLE *β-glucosidase activity from small intestine biopsies.*

Subjects	*No*	*Range nmol/h/mg protein*		*Log mean ± SEM*	
		pH 4·0	*pH 5·3*	*pH 4·0*	*pH 5·3*
Normal subjects	26	8–77	18–312	1·61±0·32	2·12±0·42
G6PD deficient subjects					
Without favism	10	30–118	85–452	1·65±0·52	2·22±0·70
With favism	9	15– 46	52–155	1·52±0·51	2·03±0·68

Note: p values are given in the text.

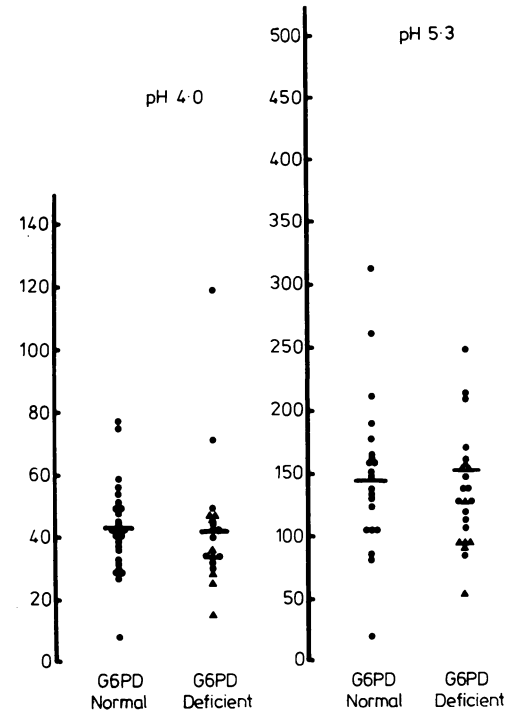

FIGURE *Distribution of β-glucosidase activity from small intestine biopsies (▲ G6PD deficient subjects with favism). Activity is expressed as nmol/h/mg protein.*

Discussion

The basis of haemolytic crises after ingestion of fava beans associated with G6PD deficiency has not yet been elucidated. Previous studies suggested the action of some other genetically determined factor,[14] and the existence of an autosomal gene which favours the haemolytic episodes has been shown.[7] It has been demonstrated that the frequency of carriers of the P^a and P^c alleles of the gene for acid phosphatase in erythrocytes is significantly higher in G6PD deficient males sensitive to fava beans than in the general population.[15] Decreased excretion of D-glucaric acid has been reported to occur in persons who are susceptible to the haemolytic effect of fava beans. It has been postulated that low urinary excretion of D-glucaric acid reflects a genetic abnormality present in favism.

This abnormality is probably located in some enzyme in the metabolic pathway of glucuronic acid.[16] Several authors have presented experimental data suggesting that various GSH oxidising compounds in their inert form may be found in fava beans.[8 10] The main active haemolytic agents in fava beans are divicine and isouramil[8 10] and the different suceptibility to fava beans may depend upon the rate of their production through the action of β-glucosidase. This enzyme is widely distributed in several tissues but it is especially found in the intestinal tract.[9] Assay of the enzyme activity in small intestine biopsy has become a recognised means of diagnosing enzymatic defects such as disaccharidase deficiency.[17] It is known that several enzymes show genetic polymorphisms and the variability of their metabolic activity could be connected with these polymorphisms. A polymorphism of the enzyme which cleaves the inactive metabolites of fava beans could explain the fact that some G6PD deficient subjects are susceptible to favism and others are not.

If our hypothesis were correct, we would expect to find increased specific activity of β-glucosidase in some normal and in some G6PD deficient persons according to the hypothesis of an autosomal gene independent of the X linked gene. In fact the structural locus of the acid form of this enzyme has been recently assigned to chromosome 1.[18] Our results do not show a significant difference in the mean activity of β-glucosidase between normal and G6PD deficient subjects with or without favism. Moreover, the distributions of β-glucosidase activities do not form distinct classes but tend to extend continuously both in normal and in G6PD deficient subjects (figure). Since G6PD deficiency is a necessary condition for the occurrence of haemolytic episodes, but is not by itself a sufficient condition, it will be necessary to test more intra- and extra-erythrocytic factors which could be involved in favism.

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