THE GENETICS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY

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Inherited deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) reduces the resistance of the red blood cells to a number of haemolytic agents, notably certain drugs and fava beans. The mode of inheritance of this condition has been studied, and the gene appears most probably to be sex-linked and of intermediate dominance.[1,2] Recent reports of linkage between G6PD deficiency and colour-blindness support this interpretation.[3,4] However, when a White child with favism resulting from G6PD deficiency[5] was found to belong to a large family it was thought to be of interest to examine the available relatives in order to confirm this theory of genetic transmission.

Material and Methods

Blood was collected in acid-citrate-dextrose solution (ACD) and sent by road to the laboratory, where it was tested as soon as possible, and in all cases sooner than 24 hours after collection. It has been established that the tests are valid for as long as several days after collection of blood in ACD.[6] G6PD activity was estimated by the dye decolorization technique of Motulsky and Campbell.[7] The glutathione (GSH) stability test was performed by the method of Beutler[8] as modified by Flanagan *et al.*[9]

Enzyme activity was estimated on all specimens. When deficient activity of G6PD was observed a GSH stability test was performed. The GSH stability of several of the samples showing normal enzyme activity was also tested as a control.

Results

The results are shown in Table I. Blood from male members of the family was either normal or markedly positive to both tests. In some of the females, however, slightly abnormal results were obtained to one or both of the tests. The interpretation of these results cannot be regarded as unequivocal in all cases. Tarlov *et al.*[10] have recently discussed methods of identification of female heterozygotes, and have concluded that none of the *in vitro* techniques available at the present time is capable of detecting more than 80% of the affected females. In their experience the GSH stability test was falsely negative in 30 - 50% of cases, and they quote Allison[11] as having found a high proportion of false negatives with the Motulsky[7] technique. However, Allison[11] regarded as abnormal only those samples not decolorized in 120 minutes; that is to say, he used the same criterion as is applied to males. In our hands the Motulsky[7] test has given very constant results, normal specimens decolorizing the dye within 75 minutes at the outside, and usually within 65 minutes. We have thus regarded decolorization times of longer than 75 minutes as being of significance (in the presence of a normal haematocrit).

Similarly, in normal blood the GSH concentration after incubation is seldom more than a few mg. per 100 ml. less than before, and in our opinion a fall in the GSH level greater than this probably indicates abnormality, particularly when associated with a low initial concentration. We have thus considered these slightly abnormal results to be indicative of the heterozygous state. It is of interest that females whose blood is not abnormal on *in vitro* testing may nevertheless be sensitive to primaquine. Alving *et al.*[2] reported 3 females with normal GSH stability who developed haemolysis on administration of primaquine, and these workers have also observed other sensitive females with normal G6PD activity.

DISCUSSION

The demonstration of G6PD deficiency and GSH stability in 3 or more generations of a family, as in the present study, has been previously reported by Childs *et al.*,[1] and these workers considered the gene to be dominant because of this finding. The marked difference between affected males and females[12,6] gives rise to two possibilities: either the gene is autosomal and sex-modified, or sex-linked (i.e. carried on the X-chromosome). Childs *et al.*[1] favoured sex-linkage because they found a much higher incidence of the condition in the mothers of affected subjects than in the fathers. The recent reports[3,4] of linkage between colour-blindness, a sex-linked condition, and G6PD deficiency establish this mode of inheritance with virtual certainty. Affected males are thus hemizygous for the condition, while most females are heterozygous. The few females manifesting marked enzyme deficiency and GSH instability were originally thought to be homozygous. However, genetic studies have shown that not all markedly affected females are homozygous, since families have been observed in which normal sons have been born to severely defective mothers.[13] It is thus apparent that the penetrance of the gene in females is very variable, and that this simple interpretation is not valid. A study of the chromosomes in one such severely affected but heterozygous female did not reveal any abnormal chromosomal constitution, and established incidentally that primaquine sensitivity is not associated with any detectable morphological abnormality of the X-chromosome.[14]

The pattern of inheritance of sex-linked conditions is well known. An affected male can only have inherited the gene from his mother, since it is carried on the X-chromosome. Similarly, he can only transmit the gene to his daughters, since his sons will receive his Y-chromosome, not his X-chromosome. Affected females, however, may inherit from either parent, and transmit to approximately 50% of their children of both sexes.

In the present family this pattern is well demonstrated, as shown in Fig. 1. Thus the mother of the propositus is affected, not his father; in addition, about half his

TABLE I. DETAILS OF THE FAMILY

Subject	Sex	Age (years)	Relationship to propositus	G6PD activity (maximum normal decolorization 75 minutes)	GSH stability (normally no decrease on incubation) Before (mg./100 ml.)	GSH stability (normally no decrease on incubation) After (mg./100 ml.)	Interpretation
John L.	M	6	Propositus	250 +	34	4	Hemizygous
El.L.	F	13	Sister	70	43	43	Normal
M.L.	F	11	Sister	65	—	—	Normal
Jap.L.	M	10	Brother	250 +	31	12	Hemizygous
Eu.L.	M	7	Brother	60	—	—	Normal
F.L.	M	4	Brother	55	—	—	Normal
A.L.	F	2	Sister	90	54	57	Heterozygous
Jan L.	M	9/12	Brother	250 +	35	11	Hemizygous
F.L.L.	F	34	Mother	120	58	38	Heterozygous
K.L.	M	36	Father	55	56	57	Normal
E.L.F.	M	65	Maternal grandfather	250 +	35	10	Hemizygous
E.J.C.F.	F	64	Maternal grandmother	60	—	—	Normal
E.J.G.F.	F	45	Maternal aunt	90	34	23	Heterozygous
C.E.F.	M	41	Maternal uncle	60	—	—	Normal
L.E.F.	M	39	Maternal uncle	75	51	48	Normal
F.F.	M	10	Son of L.E.F.	75	62	63	Normal
A.F.	M	15	Son of L.E.F.	60	—	—	Normal
E.F.	M	17	Son of L.E.F.	60	—	—	Normal
Y.F.	F	9	Daughter of L.E.F.	60	—	—	Normal
H.E.F.	M	36	Maternal uncle	60	—	—	Normal
Els. F.	F	10	Daughter of H.E.F.	60	—	—	Normal
An. F.	M	6	Son of H.E.F.	60	—	—	Normal
Eli. F.	F	7	Daughter of H.E.F.	60	—	—	Normal
J.E.F.	M	30	Maternal uncle	60	—	—	Normal
J.H.	F	29	Maternal aunt	75	103	36	Heterozygous
R.H.	F	7	Daughter of J.H.	60	33	28	Probably heterozygous
F.H.	M	5	Son of J.H.	60	48	50	Normal
E.J.E.	F	26	Maternal aunt	90	38	31	Heterozygous
A.P.E.	M	21/12	Son of E.J.E.	60	39	37	Normal
J.E.	F	5	Daughter of E.J.E.	60	38	38	Normal
C.F.F.	M	24	Maternal uncle	60	—	—	Normal
A.S.v.d.M.	F	21	Maternal aunt	75	57	44	Probably heterozygous
H.F.	M	66	Great-uncle	360 +	32	3	Hemizygous
H.B.F.	M	35	Son of great-uncle H.F.	60	—	—	Normal
M.F.	F	15	Daughter of H.B.F.	55	—	—	Normal
S.F.	F	12	Daughter of H.B.F.	55	—	—	Normal
D.F.	F	10	Daughter of H.B.F.	60	—	—	Normal
H.F.	M	12	Son of H.B.F.	55	—	—	Normal
L.F.	M	31	Son of great-uncle H.F.	55	—	—	Normal
L.F. jnr.	M	10	Son of L.F.	55	—	—	Normal
K.F.	M	21/12	Son of L.F.	60	—	—	Normal
A.v.R.	F	43	Daughter of great-uncle H.F.	95	36	35	Heterozygous
M.v.R.	M	20	Son of A.v.R.	60	56	58	Normal
J.v.R.	M	15	Son of A.v.R.	65	60	56	Normal
P.v.R.	M	8	Son of A.v.R.	55	44	46	Normal
I.W.	F	21	Daughter of A.v.R.	125	28	20	Heterozygous
A.W.	F	2	Daughter of I.W.	70	42	42	Normal
M.O.	F	42	Daughter of great-uncle H.F.	80	35	30	? Normal
H.O.	M	21	Son of M.O.	65	40	40	Normal
S.O.	F	15	Daughter of M.O.	60	47	50	Normal
O.R.	F	24	Daughter of M.O.	110	35	31	Heterozygous
M.L.	F	23	Daughter of M.O.	100	30	16	Heterozygous
C.L.	M	2	Son of M.L.	360 +	28	2	Hemizygous
G.J.v.R.	F	38	Daughter of great-uncle H.F.	95	38	38	Heterozygous
S.J.v.R.	F	19	Daughter of G.J.v.R.	75	45	45	Normal
G.J.v.R. jnr.	F	18	Daughter of G.J.v.R.	125	34	28	Heterozygous
S.J.v.R.	M	17	Son of G.J.v.R.	60	55	55	Normal
R.J.v.R.	M	15	Son of G.J.v.R.	55	45	47	Normal
J.J.v.R.	M	14	Son of G.J.v.R.	65	38	42	Normal
G.J.v.R.	M	14	Son of G.J.v.R.	60	68	67	Normal
L.J.v.R.	F	10	Daughter of G.J.v.R.	90	35	23	Heterozygous
M.J.v.R.	F	7	Daughter of G.J.v.R.	95	34	17	Heterozygous
La. J.v.R.	F	4	Daughter of G.J.v.R.	55	54	54	Normal
V.F.	F	39	Daughter of great-uncle H.F.	75	36	31	? Normal
P.F.	M	17	Son of V.F.	55	60	64	Normal
M.F.	F	14	Daughter of V.F.	85	45	26	Heterozygous
S.F.	M	8	Son of V.F.	180 +	42	6	Hemizygous
W.O.	F	34	Daughter of great-uncle H.F.	75	30	25	? Normal
C.O.	M	7	Son of W.O.	65	66	64	Normal
H.O.	M	3	Son of W.O.	55	56	56	Normal
F.P.	F	32	Daughter of great-uncle H.F.	110	35	21	Heterozygous
Mar. P.	F	14	Daughter of F.P.	145	28	21	Heterozygous
May. P.	F	11	Daughter of F.P.	85	34	27	Heterozygous
R.P.	M	9	Son of F.P.	55	64	66	Normal
J.P.	M	6	Son of F.P.	360 +	34	3	Hemizygous
W.P.	F	4	Daughter of F.P.	115	30	16	Heterozygous
El.F.	M	62	Great-uncle	360 +	36	0	Hemizygous
E.A.S.	M	58	Son of great-aunt E.S.	55	74	70	Normal
R.S.	M	28	Son of E.A.S.	60	50	50	Normal
L.S.	F	27	Daughter of E.A.S.	55	48	50	Normal
A.v.S.	F	31	Daughter of E.A.S.	60	36	36	Normal
J.v.S.	M	12	Son of A.v.S.	55	46	44	Normal
B.v.S.	M	3	Son of A.v.S.	60	60	58	Normal
S.v.S.	F	9	Daughter of A.v.S.	65	45	40	Normal
E.V.	F	57	Daughter of great-aunt E.S.	75	40	40	Normal
L.V.	F	20	Daughter of E.V.	75	36	40	Normal
M.C.	F	14	Granddaughter of E.V.	110	28	21	Heterozygous

siblings are abnormal, as expected. The mother could have inherited the gene from either parent; in fact, her father, the grandfather of the propositus, is affected. None of his sons, the uncles of the propositus, manifest the condition, while there is evidence that all his daughters are heterozygous.

Both the brothers of the maternal grandfather were found to be enzyme-deficient. Their sister, E.S. (deceased) could not be tested, but since one of her descendants (M.C.) was affected it can be assumed that she, too, carried the gene. Her daughter E.V. must also be a carrier for the same reason, even though on testing her blood neither G6PD deficiency nor GSH instability could be demonstrated. E.V. is thus a heterozygote not detectable by these two techniques.[10]

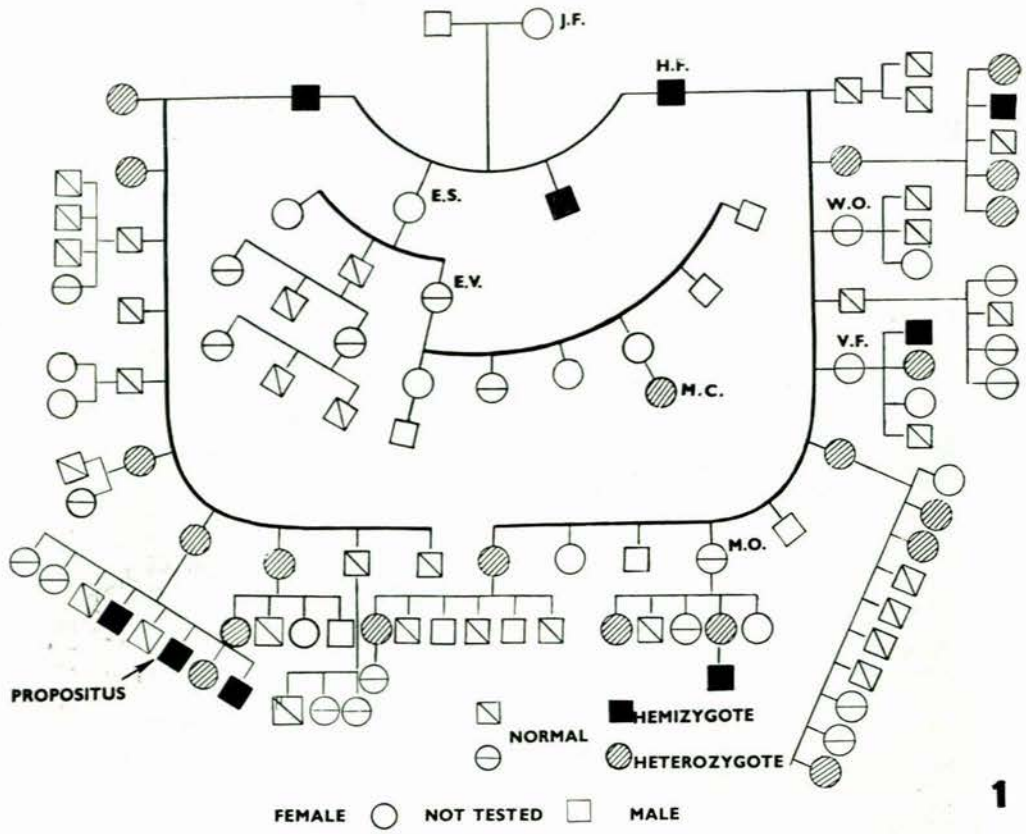

Fig. 1. The family of the propositus.

The family of great-uncle H.F. also manifests the pattern of sex-linkage. Neither of the two available sons is affected, while there is evidence that all the daughters (except one) carry the gene. Although the results of the tests on M.O. and V.F. are not unequivocal, both have children who are definitely abnormal, and therefore they must themselves be heterozygous. The exception is W.O., neither of whose sons is affected. However, it is obviously likely that she, too, is heterozygous, in spite of the lack of definite evidence.

The distribution of primaquine sensitivity in this large family, therefore, is consistent with sex-linkage and intermediate dominance of the gene. This being so, it is possible to infer that the grandfather of the propositus inherited the condition from his mother (J.F.), not his father. She is said to have been of French extraction and to have come to South Africa from Mauritius. The incidence of primaquine sensitivity varies widely in different parts of the world[6] and no reports of its occurrence in Mauritius or in France have been encountered. It is rare in the White population of South Africa.[15]

SUMMARY

The 86 available members of the large family of a child with favism were tested for the associated red-cell defect.

The pattern of distribution of erythrocyte glucose-6-phosphate dehydrogenase deficiency and glutathione instability in the family was found to be consistent with inheritance *via* a sex-linked gene of intermediate dominance.

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