Congenital haemolytic anaemia resulting from glucose phosphate isomerase deficiency: genetics, clinical picture, and prenatal diagnosis

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SUMMARY Glucose phosphate isomerase (GPI) deficiency with severe haemolysis and hydrops fetalis was found in the first child of unrelated, healthy Caucasian parents. The child died at 3 hours. Both parents were found to have 50% of normal red cell GPI activity and qualitative tests on their red cells and white cells showed that each was heterozygous for a different GPI variant allele associated with enzyme deficiency. Tests on the placenta showed that the propositus was a 'compound' heterozygote.

Examination of amniotic cells obtained by amniocentesis on the mother at 28 weeks in her second pregnancy led to the prenatal diagnosis of GPI deficiency. This second child, a 'compound' heterozygote at the GPI locus indistinguishable from the first, was successfully treated by immediate exchange transfusion and subsequent blood transfusions.

Glucose phosphate isomerase (EC: 5.3.1.9) (GPI) is a dimeric enzyme which catalyses the interconversion of fructose-6-phosphate and glucose-6-phosphate. It is determined by an autosomal locus which has been assigned to chromosome 19 (McMorris *et al.*, 1973). Several electrophoretic variants have been identified in population studies on normal healthy subjects in addition to the common phenotype GPI 1 (Detter *et al.*, 1968; Shinoda, 1970; Tariverdian *et al.*, 1970; Welch, 1971; Omoto and Blake, 1972). The variant alleles are mostly rare with the exception of GPI⁸ which reaches polymorphic frequency in some Indian populations (Blake *et al.*, 1971).

Some electrophoretic variants are associated with deficient activity of GPI and may manifest with non-spherocytic haemolytic anaemia. The affected subjects may be homozygous for the variant allele causing GPI deficiency, for example, GPI Espeln, also known as phenotype GPI 9 (Arnold *et al.*, 1973). In other cases, patients have been shown to be heterozygous for two different variant alleles both associated with GPI deficiency, for example, GPI Seattle, also known as phenotype GPI 9-10 (Baughan *et al.*, 1968; Detter *et al.*, 1968). In some other cases,

the product of the variant allele is indistinguishable electrophoretically from normal, and indeed sometimes the alleles appear to be 'silent'.

The various reports of GPI deficiency associated with haemolytic anaemia were reviewed by Paglia and Valentine (1974). Several new cases have been identified since then; for example, GPI Utrecht (van Biervleit, 1975), GPI Nordhorn (Schröter *et al.*, 1974), and GPI Barcelona (Kahn *et al.*, 1977), and the genetic and molecular basis for haemolysis because of GPI deficiency has been reviewed recently (Kahn *et al.*, 1976, 1977; Schröter and Tillmann, 1977). Most of the cases are relatively mild, but there is a report of a family from Kentucky in which 3 of 4 affected children in a family of 8 died, one at the age of 11 days (Hutton and Chilcote, 1974).

A family is described here in which 2 sibs have had severe congenital non-spherocytic haemolytic anaemia because of deficient activity of GPI. The first child died at 3 hours with hydrops fetalis. The second child was diagnosed antenatally by amniocentesis at 28 weeks' gestation, and was successfully treated by exchange transfusion following delivery at 35 weeks and subsequent blood transfusions. Each

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parent was found to be heterozygous for a GPI variant allele associated with enzyme deficiency. Both children were heterozygous for the two different parental variant alleles. This family is of particular interest in view of the severity of the haemolytic disorder and the successful antenatal diagnosis of GPI deficiency.

Materials and methods

PREPARATION OF SAMPLES

Red cells. Whole blood, collected into lithium heparin, was centrifuged at 2000 *g* for 10 minutes and the plasma and white cells removed. Red cells were washed three times in cold 0.9% saline and stored as packed cells in liquid nitrogen.

White cells. 1 ml distilled water was added to the white cell pellet to lyse contaminating red cells and the tube was left to stand for 1 minute; 10 ml 0.9% saline was added and the mixture spun at 2000 *g* for 5 minutes. The supernatant was removed and the above process repeated twice. White cell pellets were stored in liquid nitrogen until required.

Placental extracts. 1:1 w/v homogenates were prepared in distilled water, and cell debris was removed by centrifugation at 15 000 *g* for 20 minutes.

Amniotic cells. The cells were cultured in Eagle's MEM with 10% fetal calf serum. After harvesting, cell pellets were stored in liquid nitrogen.

For enzyme assays haemolysates were lysed by dilutions of approximately 1 in 20 with distilled water. White cells and amniotic cells were sonicated in 1 to 2 drops of distilled water. Before assay, placental extracts and sonicates of white cells and amniotic cells were diluted (about 1:4) with 0.1M Tris buffer pH 8.0. For starch gel electrophoresis, the samples were lysed by freeze-thawing and used without dilution.

Assays. The method for GPI was based on the procedure described by Chapman *et al.* (1962). 2,3-diphosphoglycerate was determined according to the method of Keitt (1971). Other red cell enzymes and glycolytic intermediates were assayed according to methods recommended by the International Committee for Standardisation in Haematology (Beutler *et al.*, 1977).

Starch gel electrophoresis. Horizontal starch gel electrophoresis of GPI was carried out for 18 hours at 4°C. Two buffer systems were used. (1) Bridge buffer 0.3M Tris adjusted to pH 8.6 with HCl; gel buffer 1:15 dilution of the bridge buffer. The potential was set at 5.5 v per cm and the current at 35 mA. (2) Bridge buffer 0.5M Tris and 0.057M citric acid. This was made by adjusting the pH of a 0.057M citric acid solution to pH 8.6 with a 2M

Tris solution. The gel buffer was a 1:25 dilution of the bridge buffer. The potential was 6.0 v per cm and the current 35 to 40 mA.

The stain for GPI was applied as an agar overlay. In a total volume of 40 ml the mixture contained 0.5M Tris pH 8.0, 3.0×10^{-3} M F-6-P, 7.5 mM NADP, 7.0 units G6PD, 7.5 mg MTT, and 5 mM PMS.

HEAT STABILITY STUDIES

Heating of gels. The method described by McAlpin *et al.* (1970) was used at a temperature of 50°C.

Heating of samples. Samples were heated in a water bath at 45°C for periods of from 20 to 60 minutes. Immediately after heating, samples were plunged into ice for 10 minutes. They were then centrifuged at 15 000 *g* for 15 minutes and the supernatants were assayed for GPI activity.

Case report: case 1

CLINICAL FEATURES

This baby (III.1, Fig. 1) was the first child of unrelated, healthy Caucasian parents. The mother (II.2) was aged 35 and the father (II.1) 34 at the time of delivery. The mother had had iron deficiency anaemia 8 to 9 years previously and jaundice at the age of 10 or 11. During pregnancy she worked as a dental nurse. She was mildly hypertensive in the last trimester, but had no other illnesses. During labour which commenced at 35 weeks, there were periods of fetal tachycardia and bradycardia. Delivery was by forceps. The baby was female and had a birth

Fig. 1 Family study of GPI activity of red cells. The children have inherited both parental variant alleles; the maternal and paternal variant alleles are distinguishable by electrophoretic and other qualitative tests.

weight of 2.54 kg. She was pale, oedematous, slightly jaundiced, and was found to have massive enlargement of her liver and spleen. The clinical appearance was that of hydrops fetalis. At 1 minute she had an Apgar score of 1, a heart rate of 30/min, and was apnoeic. She was intubated and intermittent positive pressure ventilation led to an increase in the heart rate, and the baby was breathing spontaneously by 5 minutes. After 15 minutes she had a cardiorespiratory arrest. She was given cardiac massage, further ventilation, and exchange transfusion was started. After 55 ml had been exchanged she had two further cardiac arrests and died 3 hours after birth. Necropsy showed no abnormalities apart from dilation and thickening of the heart and hepatosplenomegaly.

INVESTIGATIONS

Cord blood: group A Rhesus negative; haemoglobin 4.7 g/dl; white cell count $75 \times 10^9/l$; normoblasts 60/100 wbc; platelets $60 \times 10^9/l$; film: gross erythroblastosis and polychromasia, numerous red cell fragments and some spherocytes; direct anti-globulin test negative; plasma methaemalbumin and bilirubin raised; starch gel electrophoresis: normal amounts Hb A and F; brilliant cresyl blue stain for Hb H negative; red cell G6PD and pyruvate kinase (PK) activities normal.

Urine: organic acid content (in $\mu g/\mu g$ creatine) by gas chromatography and mass spectroscopy: raised levels lactic acid (21.1), pyruvic acid (0.3), 3-hydroxybutyric acid (0.7), and malic acid (0.7).

Maternal blood: group O Rhesus positive; Kleihauer test negative; no anti-A haemolysins or atypical antibodies. There was no abnormality in maternal or paternal red cell indices or morphology, and the isopropanol test for unstable haemoglobins was negative. Normal amounts of Hb A and A₂ were shown by electrophoresis, and G6PD and PK activities were normal in both parents. Heinz body stress tests were also normal.

RESULTS OF ENZYME STUDIES

The clinical picture suggested a congenital red cell defect as the cause of haemolytic anaemia. However, the obvious common causes were excluded by the investigations on the cord blood and the parental blood samples. As the parents wished to have children and wanted to have further advice, red cells from both of them were sent to one of the authors (PE) for assay of other enzymes of the glycolytic pathway. The results of these studies showed that both parents had about half the normal level of red cell GPI activity, whereas the activities of the other enzymes tested, and the levels of various glycolytic intermediates including 2, 3 DPG, were normal.

A detailed investigation of the properties and activity of GPI in the parents and the first baby was then carried out using red cells and white cells from the parents and the placenta, which had been stored deep frozen since the delivery. These results are summarised in Table 1. It was confirmed that the parents had about half the normal red cell GPI activity; the placenta had only a quarter of the activity of a control placenta. The father's white cells showed reduced GPI activity compared to the control, but the heat stability of the enzyme in the father's white cells was similar to the control. In contrast, the GPI in the mother's white cells and the placenta was thermolabile compared with the enzyme in appropriate controls. The full heat inactivation profiles are shown in Fig. 2. Michaelis constants and pH optima of the GPI in crude white cell extracts from both parents were within normal range, and so was the K_m F6P for the placental GPI.

On starch gel electrophoresis (Fig. 3a) and isoelectric focusing, the red cell GPI from both parents was indistinguishable from the common isozyme pattern, phenotype GPI 1. The white cell extracts from the father also showed the common GPI 1 isozyme pattern. However, in the mother's white cells a variant component was seen in addition to the usual isozyme. This variant isozyme was more cathodal than the normal isozyme in the Tris/chloride pH 8.6 buffer system, but less cathodal in the Tris/citrate pH 8.6 buffer system. Furthermore, the variant isozyme from the mother's white cells was unstable to heating at 50°, in contrast to the GPI 1 isozyme in the white cells of the mother, the father, and the control (Fig. 3b).

Table 1 GPI activity and properties from parents and placenta of case 1

	Father (II.1)	Mother (II.2)	Case 1 (III.1)	Controls
GPI activity				
Red blood cells				
GPI IU/g Hb	11.6	9.5	—	23.7–26.0
GPI/LDH	7.1	4.9	—	15.0–16.2
White blood cells				
GPI IU/mg protein	2.56	3.84	—	3.39
Placenta				
GPI IU/mg protein	—	—	0.054	0.23*
GPI/LDH	—	—	0.084	0.37*
Thermal stability (% initial activity after 20 mins at 45°C)				
White blood cells	90	65	—	90
Placenta	—	—	57	98
K_m F6P (mM)				
White blood cells	0.70	0.17	—	0.29
Placenta	—	—	0.40	0.36
pH optimum				
White blood cells	8.6	8.6	—	8.6

*Controls were term placentae stored at -20°C for the same duration as the test material in each case.

Starch gel electrophoresis of the placenta showed a heat labile variant isozyme exactly comparable to that seen in the white cells of the mother. There was also a weak isozyme with the same electrophoretic mobility as GPI 1 and of normal heat stability (Fig. 3c).

These results show that the mother was heterozygous for the common *GPI*¹ allele and a variant allele which determines a heat labile, electrophoretically-distinct form of GPI. The father is probably also heterozygous for *GPI*¹ and a variant allele. However, in his case, the variant allele appears to determine a

Fig. 2 Heat stability profiles of GPI in various tissues from the parents II.1 and II.2, and their offspring III.1 and III.2.

Fig. 3 Photographs of starch gels. (a) GPI isozymes in red cells from parents II.1 and II.2 compared with the GPI 1 control. Electrophoresis in Tris/citrate at pH 8.6. (b) GPI isozymes in white cells from (i) mother, (ii) GPI 1 control, after heating the isozymes in situ in the gel at 50° for 0, 15, and 30 min. Electrophoresis in Tris/chloride at pH 8.6. (c) GPI isozymes in placentae from (i) control (extract diluted), (ii) case 1 (extract undiluted), and (iii) case 1 (extract diluted) after heating the isozymes in situ in the gel at 50° for 0, 5, and 10 min. Electrophoresis in Tris/citrate at pH 8.6.

form of GPI with low specific activity, which is indistinguishable by electrophoresis, heat stability, and kinetics from the common GPI 1 isozyme. The results obtained from the placenta of case 1 confirmed that this child inherited both parental variant GPI alleles.

Case report: case 2

PREGNANCY

The mother became pregnant again while the above investigations were being carried out. She remained normotensive throughout this pregnancy, did not work as a dental nurse, and rested more than in her first pregnancy. Since we had no previous experience of GPI assay in cultured cells from amniotic fluid and did not know what the prognosis for another affected child would be, it was agreed with the parents not to attempt early prenatal diagnosis with a view to termination if the fetus was thought to be affected. However, it was decided that it would be worth monitoring the last trimester and inducing early if the fetus appeared to be severely affected.

AMNIOCENTESIS

This was performed at 28 weeks. The liquor was stained and showed an optical density of 0.22 at 450 nm, confirming a raised bilirubin level. Amniotic fluid cells were cultured and the GPI was characterised (Table 2). The activity was about 37% of the mean of 4 controls and the enzyme was found to be heat labile. After heating, the cells retained only 1% of the initial GPI activity, compared with 94% in the control cells. Electrophoretic studies revealed an isozyme pattern identical to that seen in the placenta of the first baby. It was concluded that this second child was, like the first, heterozygous for the 2 different variant GPI alleles, and was at risk for haemolytic anaemia.

Table 2 GPI activity and properties of cultured amniotic fluid cells, red cells, and placenta from case 2 (III.2)

Cells	GPI activity	GPI/LDH activity	Thermal stability (% initial activity after 20 mins at 45°C)	Km F6P (mM)
Amniotic cells	0.27	—	1.1	—
Controls (IU/mg protein)	0.73*	—	94	—
Red cells	9.15	2.8	—	—
Controls (IU/g Hb)	23.7–26.0	15.0–16.2	—	—
Placenta	0.139	0.067	53	0.39
Controls (IU/mg protein)	0.36*	0.22	98	0.36

*Controls were term placentae stored at -20°C for the same period as the test material in each case.
*Mean of four controls.

CLINICAL FEATURES

The labour started spontaneously at 35 weeks' gestation, but the fetus developed type II cardiac decelerations. A lower segment caesarian section was performed with delivery of a male infant of birth-weight 2.61 kg and head circumference 34 cm. He was pale and had gross enlargement of the liver, but was not hydropic. At 1 minute he had an Apgar score of 4. He was intubated at 2 minutes and given intermittent positive pressure ventilation with oxygen, with an immediate improvement in heart rate, and he breathed spontaneously at 4 minutes. He had regular adequate respirations by 9 minutes.

The cord blood haemoglobin was 6.8 g/dl, white cell count $12.3 \times 10^9/l$, normoblasts 78/100 wbc, and reticulocytes 80%. The red cell morphology was identical to that seen in case 1. Heinz bodies were not seen. Blood group was A Rhesus negative and the direct antiglobulin test was negative. The cord blood bilirubin was raised at 110 µmol/l.

Exchange transfusion was carried out at 3 hours with 200 ml A Rhesus negative packed red cells. The baby had rapid shallow respiration, but did not require high concentrations of oxygen. An x-ray showed cardiac enlargement and confirmed gross hepatic enlargement. Over the next 5 hours the abdomen became soft, the liver slightly smaller, and it became possible to feel an enlarged spleen.

Plasma unconjugated bilirubin rose to a maximum of 200 µmol/l, but after the first 24 hours the bilirubin was predominantly conjugated. After exchange transfusion the baby's haemoglobin level rose to 11.5 g/dl and the reticulocyte count fell to 2 to 3%, but rose again to 29% at one month. His haemoglobin initially remained high, but at just over 1 week dropped again to 8 g/dl and subsequently continued to fall slowly. Urine samples collected on the second and third day showed a relatively normal pattern of organic acid excretion, though gluconic and glucaric acid were somewhat raised.

He was discharged home at 1 month at a weight of 2.68 kg and a haemoglobin level of about 6 g/dl. He was feeding on his own satisfactorily. He was followed twice weekly thereafter. By 6½ weeks his haemoglobin had fallen further to 4.9 g/dl, despite iron, folate, and vitamin supplements. At this time he had begun to tire on feeding, so he was given a blood transfusion to a Hb level of 15 g/dl. By 11 weeks his Hb had again dropped to 5.4 g/dl and he received a further transfusion. His growth and development are otherwise normal.

GPI ASSAYS

The results obtained on the cord blood and the placenta from case 2 confirmed the findings on the amniotic cells (Table 2). There was severe deficiency

of GPI and the isozymes were indistinguishable by electrophoresis and heat stability from those seen in the placenta of case 1, and in the amniotic cells from case 2.

MERCURY ASSAYS

Because the mother had worked as a dental nurse during her first pregnancy, it was suspected that she might have been exposed to mercury vapour from the amalgam used for tooth fillings. Such intoxication, if it occurred, might exacerbate the effects of GPI deficiency and contribute to the more severe degree of haemolysis seen in the first baby. Accordingly, placental samples from both pregnancies were kindly assayed for mercury by Professor D. G. Wibberley of the University of Aston. His reported findings are shown in Table 3.

FAMILY STUDIES

Results of GPI assays on red cells from various members of the family are given in Fig. 1. In addition to the parents, levels in the heterozygote range were observed in maternal sibs II.3 and II.4 and in one subject in the earlier generation I.3.

Discussion

The severity of non-spherocytic haemolytic anaemia has varied in reported cases of GPI deficiency. Most cases have been diagnosed during childhood and those patients who have had a splenectomy have shown reduced haemolysis and dependence on transfusion, with the sole exception of GPI Utrecht in which there is also a unique susceptibility to the effect of drugs and infections (Helleman and van Biervliet, 1976). Less common are the more severe cases which present with neonatal jaundice (Paglia *et al.*, 1969; Miwa *et al.*, 1973a, b), which may even require exchange transfusion, and in one case (Hutton and Chilcote, 1974) GPI deficiency has resulted in neonatal death. As far as we can ascertain, of the two affected sibs presented here, case 1 is the first instance in which haemolysis has been so severe as to result in hydrops fetalis.

In this family both parents appear to be heterozygous for a different GPI variant allele associated with deficiency of the enzyme. The variant allele, when in heterozygous combination with the common (*GPI*¹) allele, does not result in any clinical abnormality. The sibs inherited both the variant parental alleles and were thus heterozygous for two different

deficient alleles. Double or compound heterozygosity such as this has been reported in other patients with congenital GPI deficiency (Baughan *et al.*, 1968; Detter *et al.*, 1968; Blume *et al.*, 1972; Engelhardt *et al.*, 1973; Schröter *et al.*, 1974; van Biervliet *et al.*, 1975; Kahn *et al.*, 1977). In this case, there was no parental consanguinity as has been seen in several families with apparent homozygosity of affected subjects (Paglia *et al.*, 1969, 1975; Arnold *et al.*, 1973, 1977; Nakashima *et al.*, 1973; Beutler *et al.*, 1974; Hutton and Chilcote, 1974; Kahn *et al.*, 1978).

Both sibs in this family were heterozygous carriers of the same two variant alleles at the GPI locus, and it is difficult to be sure why the first child was relatively more severely affected than the second. One possibility which has been explored is mercury intoxication of the mother during the first pregnancy, when she was working as a dental nurse. The presence of mercury could have exacerbated the severity of the haemolytic disorder in the first baby. However results obtained from placental mercury estimations (Table 3) suggest that this was not the case.

The organic aciduria detected in the first affected child was probably secondary to this infant's moribund state at the time of birth. The relatively normal urinary organic pattern in the second child excludes any abnormality characteristic of this disorder.

The maternal variant GPI showed normal Michaelis constant and pH optimum, but marked heat lability and altered electrophoretic mobility on starch gels in the buffer systems used. Since previously reported electrophoretic variants with abnormal heat stability have not been examined in these two buffer systems, it is not possible to say whether they are identical with this particular heat labile variant (Hutton and Chilcote, 1974; Schröter *et al.*, 1974; Paglia *et al.*, 1975; Kahn *et al.*, 1976, 1978). The father's GPI showed normal Michaelis constant, pH optimum, heat stability, and electrophoretic mobility. His low GPI activity was probably the result of a variant allele which determines a form of GPI with low specific activity, which is indistinguishable from the usual GPI 1 isozyme by the tests used. Studies are in progress to characterise further the paternal variant enzyme.

Heterozygotes for a dimeric enzyme such as GPI would usually be expected to show 3 major isozymes resulting from the random association of 2 sub-units. The middle band (heterodimer) of the triplet would be the most intense and the 2 homodimers would be expected to be equally active. In the mother's white cells, 2 isozymes of approximately equal intensity were seen, one with the same mobility as the common GPI 1 isozyme. It seems likely that the latter isozyme has the sub-unit structure $\alpha^1\alpha^1$, where α^1 is the

Table 3 Placental mercury in $\mu\text{g}/100\text{ g}$ fresh weight

Sample	Mercury level
Placenta of III.1	1.06
Placenta of III.2	0.68
Control of placenta (i)	0.20
Control placenta (ii)	1.47

product of the GPI^1 allele, and the variant isozyme corresponds to the heterodimer $\alpha^1\alpha^M$, where α^M is the product of the maternal variant allele. The absence of the variant homodimer $\alpha^M\alpha^M$ is probably the result of its being particularly unstable. An alternative, but less likely, explanation is that structural features of the variant (α^M) enzyme sub-units prevent their dimerisation with each other but not with the normal (α^1) sub-units. In red cells which cannot synthesise new protein, the effects of the instability are more pronounced so that only the normal homodimeric $\alpha^1\alpha^1$ was seen in the mother's red cells.

Two-banded isozyme patterns were also seen in the placentae of the sibs. One isozyme had normal mobility, but was very weak compared with the usual GPI 1 isozyme. It is proposed that this isozyme is the variant homodimer $\alpha^P\alpha^P$, where α^P is the product of the paternal variant allele. The other isozyme, with mobility identical to the variant isozyme seen in the mother, is probably the variant heterodimer $\alpha^M\alpha^P$. As was the case with the mother, the variant homodimer $\alpha^M\alpha^M$ was not seen. According to this interpretation, the variant isozymes seen in the mother and children are different in their sub-unit structures, $\alpha^1\alpha^M$ and $\alpha^M\alpha^P$, respectively, but are both unstable since each contains the α^M sub-unit.

The father exhibited a single GPI isozyme. It seems likely that this consists of three components $\alpha^1\alpha^1$, $\alpha^1\alpha^P$, and $\alpha^P\alpha^P$, all with the same electrophoretic mobility.

The distinctive features of the variant forms of GPI in this family made it possible to predict the outcome of the second pregnancy. The 4 possible genotypes of the offspring and the distinctive biochemical characteristics of the GPI in each case are shown in Table 4.

Antenatal diagnosis of GPI deficiency early in pregnancy has not previously been reported for the obvious reason that, on the basis of cases reported so far, when properly treated it is only rarely fatal and has a good prognosis. The neonatal death of the first child in this family suggested that the outlook might be less favourable than usual and that antenatal diagnosis should be considered. Since we were uncertain of the prognosis for another affected child, it was decided not to attempt early antenatal diagnosis with a view to termination, but to do an amniocentesis in the third trimester. Thus, if the fetus appeared to be severely affected appropriate measures, such as early induction and exchange transfusion, could be undertaken. In the event, this turned out to be the right decision and the value of late antenatal diagnosis has been shown. It is proposed to increase the amount of blood administered at each transfusion in order to extend the time interval between transfusions without allowing the haemoglobin

Table 4 The 4 possible genotypes expected among the offspring of parents of the index case and their biochemical characteristics. There is an equal probability (1:4) for the occurrence of each genotype

Genotype	Phenotype	Characteristics of GPI		
		Activity (expressed as % of control)	Heat stability (expressed as % of control after heating at 45°C for 20 mins)	Isozymes (after starch gel electrophoresis)
$GPI^1 GPI^1$	Normal	normal (100%)	stable (100%)	normal (GPI 1)
$GPI^1 GPI^M$	Heterozygote	low ($\approx 50\%$)	unstable ($\approx 60\%$)	normal (GPI 1) + variant (GPI ^M)
$GPI^1 GPI^P$	Heterozygote	low ($\approx 50\%$)	stable ($\approx 100\%$)	'normal' (GPI ¹ + GPI ^P)
$GPI^M GPI^P$	Double heterozygote	very low ($<40\%$)	unstable (60% or less)	variant (GPI ^M) + 'normal' (GPI ^P)

GPI^1 represents the common allele; GPI^M represents the maternal variant allele which determines a heat labile electrophoretically distinct form of GPI; GPI^P represents the paternal variant allele which determines a GPI with low specific activity which is indistinguishable by heat stability and electrophoresis from GPI^1 .

concentration to fall below a level that will permit normal growth and development. Hopefully, after splenectomy long term transfusion will not be necessary.

It is of interest that though GPI studies on the amniotic cells showed the second child to be affected, this was also strongly suggested by the yellow colour of the amniotic fluid as it was withdrawn and the confirmation that this was because of raised bilirubin. The above procedure is to be recommended in the management of pregnancy where there is a risk of a child with GPI deficiency. Until the frequency of GPI deficiency as a cause of hydrops fetalis has been determined, it ought to be looked for in all cases of unexplained hydrops fetalis with severe haemolysis.

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