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Augsburg-Type Glucosephosphate Isomerase Deficiency

A New Variant Causing Congenital Nonspherocytic Hemolytic Anemia in a German Family

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Glucosephosphat-Isomerase Typ Augsburg

Eine neue Variante als Ursache für kongenitale, nichtsphärocytäre hämolytische Anämie bei einer deutschen Familie

Summary. In a 1-year-old German boy a GPI deficiency was found to be the cause of a chronic nonspherocytic hemolytic anemia with recurrent hemolytic crises. Because of consanguinity of the parents, the patient is true homozygote. The investigation of the biochemical properties of the deficient enzyme revealed an altered electrophoretic behavior, pronounced thermolability, an increased affinity for G6P, an increased affinity for the competitive inhibitor 6-PG, and slightly changed pH optima for both substrates. Electrophoresis after freezing and thawing the hemolysate indicates that the genetic modification of the subunit involves the mechanism of transforming the main band into the secondary bands. The properties of the new deficient GPI indicate a new variant designated GPI Augsburg.

Key words: Hemolytic anemia – Enzyme deficiency – Glucosephosphate isomerase

Zusammenfassung. Bei einem einjährigen deutschen Jungen wurde als Ursache für eine chronische hämolytische Anämie mit rezidivierenden hämolytischen Krisen ein GPI-Defekt der Erythrozyten entdeckt. Bei Konsanguinität der Eltern handelt es sich um einen homozygoten Defektträger. Die Untersuchung der biochemischen Eigenschaften des Defektenzymes ergab ein verändertes elektrophoretisches Muster, eine deutliche Thermolabilität, eine erhöhte Affinität für G6P und für den kompetitiven Inhibitor 6-PG und leicht zum alkalischen verschobene pH-Optima für beide Substrate.

Von der Elektrophorese nach Einfrieren und Auftauen des Hämolysates kann geschlossen werden, daß die genetische Modifikation der GPI-Untereinheit hauptsächlich den Mechanismus betrifft, der beim normalen Enzym die

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Hauptbande in die Sekundärbanden überführt. Die einmaligen Eigenschaften des Defektenzyms sprechen für eine neue Variante, für die nach dem Geburtsort des Patienten der Name GPI Augsburg vorgeschlagen wird.

Schlüsselwörter: Hämolytische Anämie – Enzymdefekt – Glucosephosphat-Isomerase

GPI deficiency is usually associated with a moderate chronic hemolytic anemia and acute hemolytic crises during infections. It is the third most common enzyme defect of erythrocytes after G6PD and PK. Whereas about 1 million G6PD-deficient patients are known, as yet only 34 patients from 30 families with GPI deficiency have been reported [5]. The clinical picture and the course of the disease are relatively uniform but the biochemical findings in consequence of the genetic defect of GPI protein are highly variable so that many different variants of GPI with and without deficiency have been described [5,19,18]. In this communication we report on a German boy with a homozygous GPI deficiency resulting from consanguinity of the parents. His GPI exhibits an abnormal electrophoretic pattern which, interestingly, changes after freezing and thawing of crude hemolysate.

Materials and Methods

The substrates, coenzymes, and auxiliary enzymes for spectrophotometric determinations of enzymes were purchased from C.F. Boehringer and Sons, Mannheim. F-6-P sodium salt was later purchased from Serva, Heidelberg, because of unexplained impurities in the Boehringer preparation. Other chemicals (reagent grade) were obtained from E. Merck AG, Darmstadt, DEAE-Sephadex and Sephadex G-25 and the chromatography columns (K 25/45 and K 50/60) from Pharmacia, Uppsala, Sweden, and the starch for gel electrophoresis from the Electrostarch Company, Madison, Wisconsin.

GPI and other enzyme activities together with the kinetic properties and thermostability of GPI were determined as described previously [2].

Before preparing the hemolysate, platelets and leukocytes were removed by cotton wool filtration [9]; erythrocytes were hemolyzed using digitonin [13]. GPI was purified according to the method published earlier [4] with the exception that the hemolysate was not dialyzed but filtered through a G-25 column equilibrated with 10 mM TRIS buffer containing 1 mM EDTA, pH 8.0. The final preparation was stored in the TRIS buffer and bovine serum albumin was added (5 mg/ml) after the spectrophotometric determination of protein [4].

The starch gel electrophoresis of GPI was performed acco ding to Detter et al. [10].

Case Report[1]

The boy C.P. was born in January 1977 after an uneventful pregnancy. His parents are of German ancestry and their forefathers were related. After birth he was given an exchange transfusion on the assumption that the prolonged jaundice and the anemia resulted from a B/0 constellation. In the following month two further transfusions were necessary. In the 6 th month the anemia improved transiently under application of iron. After a febrile infection of the respiratory tract in January 1978 he developed abdominal pain and pallor and was admitted to hospital (Josefinum,

1 The authors are very grateful to Dr. Freislederer, medical director of Kinderkrankenhaus Josefinum, Augsburg, and Dr. Gisela Jockisch, Augsburg, for providing them with hematological and clinical data

Table 1. Hematologic data of the propositus

	January 1978	June 1978
Hemoglobin (g/dl)	7.0	5.1
Red cell count (10^{12}/l)	2.1	
P.C.V. (%)	21	18.5
Reticolucytes (%)	38.8	13.4
White cell count (10^9/l)	22.4	25.0
Platelet count (10^9/l)	285	450
Bilirubin total (mg/dl)	1.8	2.62
Serum iron (μg/dl)	37	34
Coombstest	negative	–
Osmotic fragility (% NaCl)	0.54–0.40	–

Table 2. Red cell enzyme activities of the propositus

	normals	patient
Glutathione reductase (GR)	4.2 ± 1.2	6.6
Glucose-6-phosphate dehydrogenase (G6PD)	6.3 ± 1.8	15.9
6-phosphogluconic dehydrogenase (6PGD)	3.9 ± 0.7	6.5
Hexokinase (HK)	0.6 ± 0.2	3.03
Phosphoglucomutase (PGLUM)	1.5 ± 0.6	2.64
Glucosephosphate isomerase (GPI)	26 ± 4.5[a]	13.3
Phosphofructokinase (PFK)	4.1 ± 0.7	6.98
Fructosediphosphat aldolase (ALD)	1.6 ± 0.4	4.23
Trisephosphate isomerase (TPI)	1060 ± 205	1890
Glyceraldehyde-3-ph. dehydrogen. (GAPD)	86 ± 16	142
2,3-diphosphoglycerate mutase (DPGM)	3.1 ± 0.4	5.9
3-phosphoglycerate kinase (PGK)	163 ± 29	231
3-phosphoglycerate mutase (PGM)	23 ± 3.9	56.3
Enolase (ENOL)	8.9 ± 1.7	17.9
Pyruvat kinase (PK)	8.6 ± 2.8	29.7
Lactate dehydrogenase (LDH)	92 ± 19	165
Adenylate kinase (ADK)	134 ± 18	194

[a] valid only for a special batch of F6P from Boehringer

Table 3. Red cell GPI and HK activities of the propositus and his siblings

Siblings	Pedigree	NSHA	GPI U/g Hb	%	HK U/g Hb	%
Father, G.P.	III/2	– –	12.7	49	0.51	85
Mother, M.P.	III/3	– –	10.1	39	0.58	97
Propositus, C.P.	IV/1	+ +	5.86	22	1.26	210
Stepbrother, H.P.	IV/2	– –	9.98	37	0.56	94
Normals		– –	26 ± 4.5[a]	100	0.6 ± 0.2	100

[a] valid only for a special batch of F6P from Boehringer

Augsburg). Physical examination revealed severe pallor and moderate jaundice. Liver and spleen were not enlarged. The hematologic data are given in Table 1. Without transfusions the hemoglobin increased within 2 weeks to 11.1 g/100 ml with a reticulocyte peak of 71%. In June 1978 a further hemolytic crisis occurred 1 week after a febrile enterocolitis (hematologic data in Table 1) and a transfusion was given. Four days later the reticulocytes decreased to 0.26%. In later controls the hemoglobin ranged between 8.0 and 12.6 g/100 ml and the leukocytes between 6×10^9 and 11×10^9 cells/l.

Results

The red cell enzyme activities of the propositus during hospitalization in January 1978 are presented in Table 2. At this time GPI activity is about half the normal mean. Many other enzyme activities are high, as explained by the young red cell population with about 40% reticulocytes. Three weeks later when the hemoglobin increased to 11 g/100 ml GPI activity was 5.86 U/g Hb, which is about 20% of the normal mean, and HK activity was 1.26 U/g Hb.

In Table 3 red cell GPI and HK activities of the propositus and his family members are presented. GPI activity in the father's cells is decreased to 50%, in the mother's and the stepbrother's cells to about 40% of the normal mean. The HK activity is elevated only in the propositus' cells, since the family members have no signs of hemolytic anemia.

Figure 1 show the pedigree of the family with the known consanguinity.

Figure 2 presents the results of the thermostability tests of GPI in the hemolysates of the propositus and his parents.

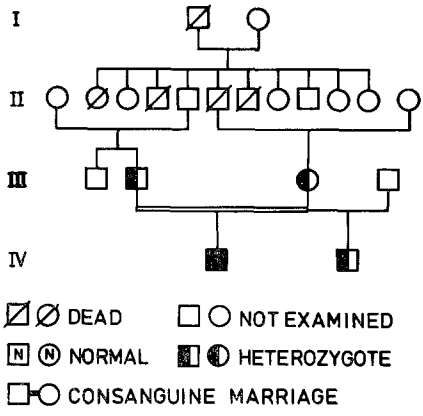

Fig. 1. Pedigree of family P.

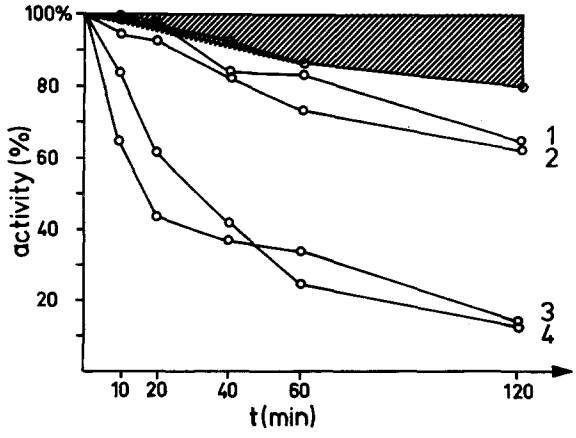

Fig. 2. Thermostability of GPI in the mother's [1], the father's [2], and the proband's [3,4] hemolysate

The propositus' GPI was investigated twice, first in January when the GPI activity was 13.3 U/g Hb and second when GPI activity was 5.86 U/g Hb. Between the two curves representing younger and older GPI molecules there is no significant difference. Compared to normal GPI, the activity decreases continously to 13% residual activity after 120 min. The GPI activity in the father's and the mother's hemolysate decreases only slightly to about 60–65% of the initial activity.

The results of the electrophoretic studies and their interpretation are given in Figs. 3, 4 and 5. Figure 3 presents the GPI pattern after starch gel electrophoresis of the propositus', his father's and mother's hemolysates. Father's and mother's GPI pattern are identical as expected from the consanguinity. Their patterns exhibit five bands. The pattern of the proband consists of three bands. The main band has the same mobility as the first "secondary" band of the normal enzyme. The two weaker bands also have the mobility of the corresponding normal bands.

Figure 4 gives an explanation for the pattern of the homozygote and the heterozygotes in this family. The normal main band consists of molecules with two normal subunits A, the "secondary" bands are, according to Blackburn et al. [7], oxidation products named A′ and A″. The propositus' GPI consists of the genetically changed subunit B. The two additional bands must be secondarily altered molecules, most likely oxidation products as in the case of normal GPI. That the main band consists of the B′B′ molecules may be explained by a higher stability of the B′B′ molecules as compared to the BB molecules. This hypothesis

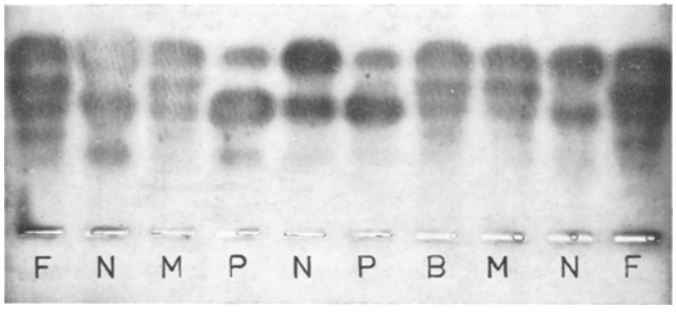

Fig. 3. Starch gel electrophoresis of GPI. N: normal control, M. mother's F: father's, B: stepbrother's and P: proband's hemolysate

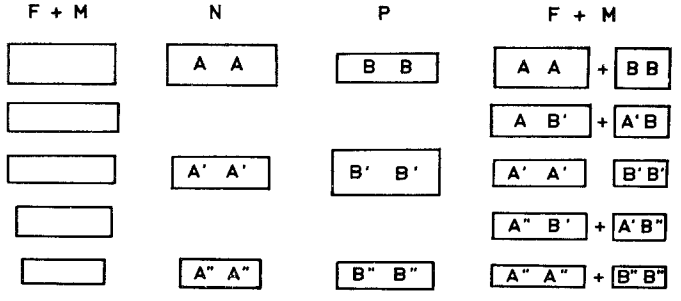

Fig. 4. Interpretation of the GPI electrophoresis in the hemolysates of the family P.

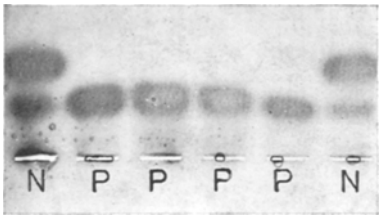

Fig. 5. Starch gel electrophoresis of the proband's hemolysate after freezing and thawing (P), N: normal control

Table 4. Purification of GPI from the patient's red cells

	Volume (ml)	GPI activity (IU/ml)	(IU/Vol)	Yield (%)	Protein (mg/ml)	Spec. activity (IU/mg Protein)
Hemolysate	4.4	0.246	1.08	100	47.1[a]	0.00522
G-25 Eluate	19.5	0.0504	0.982	91	9.6	0.00524
DEAE Eluate	22.5	0.0239	0.54	50	0.0004	60

[a] measured as hemoglobin

Table 5. Kinetic properties of GPI from the patient's red cells

Property	Normal GPI	Patient's GPI
Km (G6P), μM	460. $\pm$ 85	225
Km (F6P), μM	67 $\pm$ 13	74
Vmax(G6P)/Vmax(F6P)	0.96 $\pm$ 0.12	0.714
pH optimum (G6P)	8.0	8.5
pH optimum (F6P)	8.5	9.0
Ki 2,3-DPG (F6P), mM	0.94	1.2
Ki 6-PG (G6P), μM	73	19
Ki 6-PG (F6P), μM	13	10

is supported by the observation that after freezing and thawing the proband's hemolysate the whole activity is found within the main band and the two weaker bands have disappeared, Fig. 5.

The GPI activity before freezing and thawing was 5.86 U/g Hb and after 6.05 U/g Hb. Thus, the weaker bands were not destroyed but rather transformed to GPI molecules which migrate in the main band.

The pattern of the heterozygotes may be explained as a corresponding mixture of the normal and the proband's pattern of GPI molecules. The first band consists of AA molecules and only to a very small extent, if at all, of BB molecules. This is because of the instability of the BB molecules. The second consists of the heterodimers AB′ and A′B, the third band of the oxidation products A′A′ and B′B′ followed by the heterodimers A″B′ and A′B″ and finally by A″A″ and B″B″.

The purification of the propositus' GPI is summarized in Table 4. The final preparation (DEAE eluate) consists of fractions 7 to 12. It was free from any other enzyme activity, especially of PGK, and was stable for 1 week after addition of bovine serum albumin.

The kinetic constants measured with the purified GPI are summarized in Table 5. The Michaelis-Menten constant for G6P is about half that of the normal

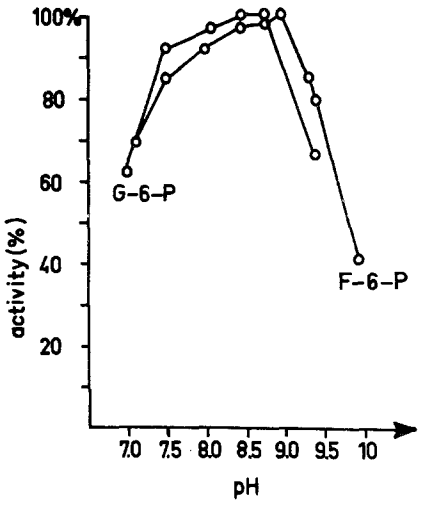

Fig. 6. pH-dependency of GPI activity in the foreward (G6P) and backward (F6P) direction

mean, whereas that for F6P is within the normal range. The ratio of maximal velocity of the forward reaction and backward reaction is slightly decreased. The inhibitor constants of 2,3-DPG and of 6PG for the backward reaction were within normal range, but that of 6 PG for the forward reaction (with G6P as substrate) were decreased to about 25% of the normal mean. Both pH optima were slightly shifted to the alkaline range (Fig. 6).

Discussion

The patient's severe postnatal jaundice and the nonspherocytic hemolytic anemia with recurrent hemolytic crises during infections are explained by GPI deficiency in his red cells. The hematologic data and the course of the disease are typical for GPI deficiency. This is the fifth family of German origin with this deficiency. Because of the known consanguinity of the parents and identical electrophoretic pattern of the parents' GPI the patient is a true homozygote.

The investigation of the biochemical properties of the defective enzymes leads to the designation of different variants [5]. Study of the patient's GPI revealed pronounced abnormalities of the physicochemical as well as of the kinetic properties of the new deficient GPI.

In all cases of GPI deficiency investigated so far the deficient enzyme is more or less thermolabile. From our previous studies it may be concluded that the thermolability in vitro corresponds to a higher instability in vivo, and the instability in vivo is the most important factor in the pathophysiology of the disease [3, 14].

A highly interesting peculiarity of the new deficient GPI is its electrophoretic behavior. As outlined under "Results", the mobility and the position of the three bands is the same as for the normal GPI. However, the fastest band is not the main band as in the normal pattern, but the second band is the main band. After freezing and thawing, only this second band remains and the two weaker bands seemed to be transformed in to the main band. A transformation and not a

simple disappearance may be concluded from the fact that GPI activity after freezing and thawing was not decreased. Thus, we can conclude that in this deficient GPI the genetic mutation concerns not the electrical charge of the changed subunit but rather the mechanism which leads to the so-called secondary bands, which are according to Blackburn et al. oxidation products of GPI molecules [7]. The alteration of this "oxidation" leads to the transformation of the whole GPI activity in the first secondary band. Among the GPI variants described so far this observation is unique. There are three other GPI variants with three bands after starch gel electrophoresis: GPI Narita [15] GPI Los Angeles [8] and GPI Elyria [6]. All three variants differ from the new GPI and from each other.

Concerning the kinetic properties of the patient's GPI a twofold increased affinity for the substrate G6P was observed. Because GPI operates within the cell far below substrate saturation this increased affinity may be favorable for the metabolic situation. An increased affinity for G6P was also observed in GPI Paris [12]. Interestingly, the influence of 6-PG, which inhibits the isomerization of G6P competitively, is different in the two variants. In GPI Paris the Ki of 6-PG for G6P is greatly increased, whereas in the patient's GPI the Ki is decreased four times. Further, both pH optima were shifted to the alkaline range. As yet, only variants with a acidic shift of a pH optimum have been described: GPI Enfants malades [12], and GPI Barcelona [11].

For the new defective GPI firstly the modification of the electrophoretic behavior and secondly the modification of the kinetic properties with increased affinity for G6P, increased affinity for the competitive inhibitor 6-PG and the slightly changed pH optima for both substrates justifies the designation as a new variant, GPI Augsburg in accordance with the birthplace of the patient. The other German GPI variants are GPI Espeln [1], GPI Recklinghausen [2], GPI Nordhorn [4,16] and GPI Paderborn [17]. GPI Paderborn is not sufficiently discriminated from the GPI Espeln described earlier and is therefore probably identical with GPI Espeln.

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