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Liver glycogenosis due to phosphorylase kinase deficiency: *PHKG2* gene structure and mutations associated with cirrhosis

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Mutations in three different genes of phosphorylase kinase (Phk) subunits, *PHKA2*, *PHKB* and *PHKG2*, can give rise to glycogen storage disease of the liver. The autosomal-recessive, liver-specific variant of Phk deficiency is caused by mutations in the gene encoding the testis/liver isoform of the catalytic γ subunit, *PHKG2*. To facilitate mutation detection and to improve our understanding of the molecular evolution of Phk subunit isoforms, we have determined the structure of the human *PHKG2* gene. The gene extends over 9.5 kilonucleotides and is divided into 10 exons; positions of introns are highly conserved between *PHKG2* and the gene of the muscle isoform of the γ subunit, *PHKG1*. The beginning of intron 2 harbors a highly informative GGT/GT microsatellite repeat, the first polymorphic marker in the *PHKG2* gene at human chromosome 16p11.2–p12.1. Employing the gene sequence, we have identified homozygous translation-terminating mutations, 277delC and Arg44ter, in the two published cases of liver Phk deficiency who developed cirrhosis in childhood. As liver Phk deficiency is generally a benign condition and progression to cirrhosis is very rare, this finding suggests that *PHKG2* mutations are associated with an increased cirrhosis risk.

INTRODUCTION

Glycogen storage disease of the liver due to phosphorylase kinase (Phk) deficiency can be caused by mutations in three genes coding for Phk subunits and isoforms: the liver isoform of the α subunit, α_L (gene symbol, *PHKA2*), the β subunit (*PHKB*) or the testis/liver isoform of the γ subunit, γ_{TL} (*PHKG2*). These three subtypes of liver Phk deficiency differ in mode of inheritance and biochemical tissue involvement. Mutations in the *PHKA2* gene cause X-linked recessive liver-specific Phk deficiency (1–5), *PHKB* mutations cause autosomal-recessive combined Phk deficiency of liver and muscle (6,7) and *PHKG2* mutations cause autosomal-recessive liver-specific Phk deficiency (8). As they affect an X-linked gene, *PHKA2* mutations account for the large majority of liver Phk deficiency cases. Mutations in the *PHKB* gene have been identified in six patients; four of them are compound-heterozygotes without parental consanguinity, indicating that mutant *PHKB* alleles are also relatively abundant in the population. Mutations in *PHKG2* seem to be the rarest variant, with only three patients described to date, all of them homozygous offspring of consanguineous parents.

The clinical appearance of liver Phk deficiency with mutations in these three genes is similar. Most patients present as infants with a protuberant abdomen because of hepatomegaly, and with growth retardation, delayed motor development and elevation of triglycerides, cholesterol and transaminases. Fasting hypoglycemia and a characteristic rounded face (‘dollface’) are observed in a minority of patients. Muscle weakness is often observed also in the liver-specific deficiencies with *PHKA2* and *PHKG2* mutations, presumably due to secondary mechanisms, whereas, conversely, muscle symptoms may be lacking even in subjects with liver and muscle Phk deficiency due to *PHKB* mutations. The presence or absence of clinical muscle involvement is therefore inconclusive. The prognosis is normally good. Typically, patients improve with age and become asymptomatic as adults, reaching normal height at about the age of 20 years. The phenotype of *PHKB* mutations seems to be particularly mild, both biochemically and clinically (6,7).

The development of liver fibrosis and even cirrhosis in Phk deficiency has been reported but seems to be very rare. Hers *et al.* (9) mention two cases of liver cirrhosis with esophageal varices and three deaths from liver adenomas and malignant tumors in a series of 205 patients with deficiency of the phosphorylase system, most of them presumably with Phk deficiency. Two cases of cirrhosis developing in childhood in association with documented liver Phk deficiency were later published (10,11). In contrast, liver cirrhosis develops almost invariably in infants with type IV glycogenosis (branching enzyme deficiency); in type III glycogenosis (debranching enzyme deficiency), liver fibrosis is characteristic but rarely progresses to cirrhosis (9,12).

In this study, we have determined the structure of the human *PHKG2* gene and, based on these data, performed a molecular-

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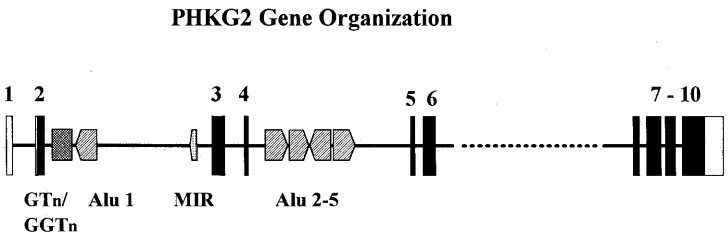

Figure 1. Organization of the human *PHKG2* gene. Exons are indicated by boxes (black, coding sequences; white, untranslated sequences). *Alu* elements are symbolized by hatched arrows, an MIR element by a stippled arrow and the $(GT)_n/(GGT)_n$ microsatellite repeat by a cross-hatched box. The region of intron 6 that was not sequenced is represented by a dotted line. The total length of the gene as shown is 9.5 knt.

genetic analysis of the two published cases of liver Phk deficiency with cirrhosis (10,11). In both, we found translation-terminating mutations in the *PHKG2* gene. Together with a case of severe fibrosis among the first three *PHKG2* cases reported (8), and given the otherwise rare occurrence of *PHKG2* mutations in the patient population with liver Phk deficiency, this finding suggests that in liver Phk deficiency, *PHKG2* mutations are associated with an increased risk of fibrosis and cirrhosis.

RESULTS AND DISCUSSION

Structure of the human *PHKG2* gene

Previously, the gene of the muscle isoform of the Phk γ subunit, *PHKG1*, was characterized from mouse and rat and shown to be organized into 10 exons extending over 15 kilonucleotides (knt) of genomic DNA (13,14). Assuming that the *PHKG2* gene may be of similar organization and size as the gene of its isoform, we aimed to amplify it directly from genomic DNA by analogously dividing the published human *PHKG2* cDNA sequence (15) into 10 putative exons and designing pairs of PCR primers corresponding to the distal ends of neighboring exons. With these primers, we could indeed amplify the complete *PHKG2* gene, between the extreme ends of the cDNA, in 10 pieces overlapping at their ends within the exon sequences. The overall organization of *PHKG2* is given in Figure 1 and its sequence in Figure 2.

Our assumption of a similar exon organization of *PHKG1* and *PHKG2* proved to be correct. Both genes have, in the course of evolution, diverged down to 59% identity of predicted amino acid sequence and have been translocated to different chromosomes (*PHKG1*, human chromosome 7; *PHKG2*, human chromosome 16). However, the positions of introns within the coding sequences have been conserved perfectly, being inserted in identical fashion into codons of directly corresponding amino acids (Table 1). Even the position of intron 1 is similar although it lies within the 5′-untranslated sequence (18 nt upstream from the start codon in human *PHKG2* as opposed to 33/34 nt in rat/mouse *PHKG1*) (Fig. 2). In contrast, the lengths of corresponding introns vary considerably between *PHKG2* and *PHKG1* whereas they are well conserved between rat and mouse *PHKG1* (Table 1). This is in agreement with previous observations that intron positions are better conserved than intron lengths in the course of evolution (16,17). *PHKG2* introns 5, 7, 8 and 9 are strikingly short (Table 1), close to or even below the value of 80 nt that was determined to be necessary for efficient and accurate splicing (18).

The overview (Fig. 1) shows that the 10 exons are grouped into four blocks, so that the complete *PHKG2* coding sequence and the flanking intron sequences can be amplified conveniently in only four pieces for mutation analysis (see below). Sequence analysis identified one *Alu* and one MIR (19) repetitive element in intron 2, and a block of four closely successive *Alu* elements in different orientations in intron 4. The two pairs of inverted repeats would allow the formation of cruciform DNA. All five *Alu* elements belong to the Sx subfamily, with 94–95% sequence identity, which would enhance their potential for recombination with each other.

Table 1. Organization of the human *PHKG2* gene and comparison with the rat (r) and mouse (m) *Phkg1* genes

Exon	Size (nt) *PHKG2*	cDNA position	Amino acid interrupted *PHKG2*	*Phkg1* (r + m)	Intron	Size (nt) *PHKG2*	*Phkg1* (r)	*Phkg1* (m)
1	>75	1–75	5′-UTR	5′-UTR	1	312	3300	4700
2	113	76–188	Ar/g	Ar/g	2	2151	412	376
3	176	189–364	I/le	I/le	3	267	390	398
4	55	365–419	Le/u	Le/u	4	2082	4200	3800
5	66	420–485	Ar/g	Ar/g	5	96	2050	2200
6	164	486–649	G/lu	G/lu	6	2600	860	570
7	91	650–740	Le/u	Me/t	7	94	144	137
8	154	741–894	Leu/Ile	Leu/Val	8	69	860	817
9	126	895–1020	Arg/Val	Lys/Val	9	88	291	286
10	>551	1021–1571						

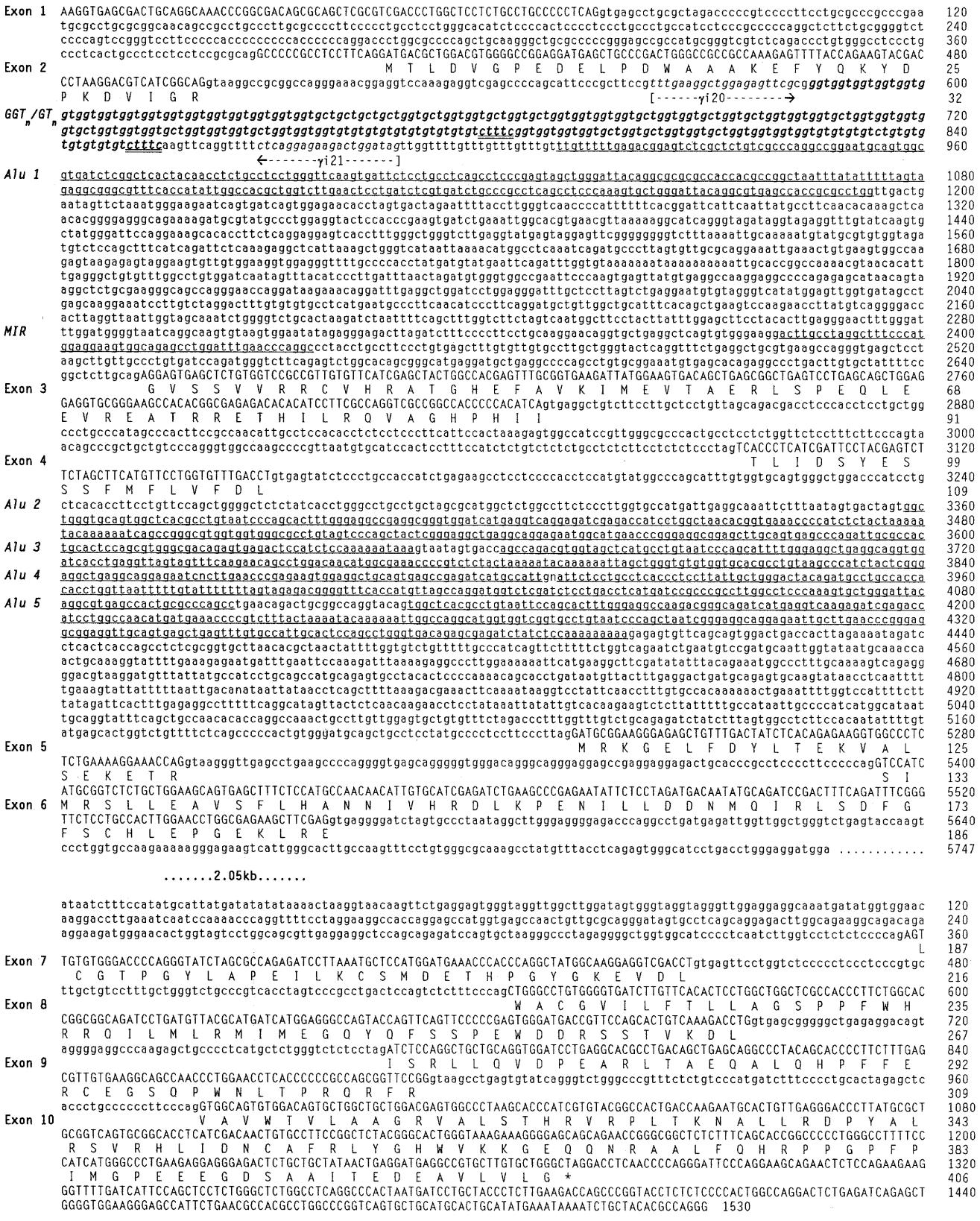

Figure 2. Sequence of the human *PHKG2* gene. The sequence is given in two contigs, with a gap of ~2050 nt in intron 6. Exons are given in uppercase letters and introns in lowercase letters. *Alu* and MIR sequences are underlined. Bold italic print highlights the microsatellite repeat, with the CTTTC sequence that marks the end of its internal duplication doubly underlined. The primers γi20 and γi21 employed for amplification of the repeat are indicated.

The published chromosomal assignment of *PHKG2* to 16p11.2–16p12.1 is based on an incompletely processed heterogeneous nuclear (hn) cDNA clone mapped by Southern blot hybridization to a high-resolution somatic cell panel (20). Comparison of this hn cDNA sequence with the *PHKG2* gene shows that it corresponds to the end of exon 2, exon 3 (but without intron 2), intron 3, exon 4 and the beginning of intron 4 including half of *Alu* element #2. However, there are a considerable number of mismatches (3.2%) between the hn cDNA sequence and ours. As our own sequences determined from two unrelated individuals are completely identical and the mismatches concentrate in the central region of the 893 nt long hn cDNA, we suspect that the discrepancy does not reflect polymorphisms or the existence of two distinct though very closely related genes, but is probably due to errors in the hn cDNA sequence that was apparently obtained by single-pass terminal sequencing.

Polymorphic microsatellite repeat in intron 2

There is a complex microsatellite repeat at the beginning of intron 2. A series of GGT (interspersed with GCT) and GT repeats ends on CTTTC (doubly underlined in Fig. 2) and is partially duplicated. The sequence of this region given in Figure 2 was obtained from a homozygous individual and comprises 72 trinucleotide and 22 dinucleotide units. To investigate whether this region can serve as a polymorphic marker within the *PHKG2* gene, we amplified it with primers γi20 and γi21 from 10 unrelated individuals. High allelic heterogeneity was observed, with sizes of PCR products ranging between 310 and 360 nt. Already when resolved only on a simple 1.5% agarose gel, different alleles within one sample and different band patterns of different samples could be discriminated in eight of 10 cases each. Two samples giving closely spaced doublets on an agarose gel were analyzed by radioactive labeling and electrophoresis on a denaturing gel, yielding sizes of 312/318 and 339/351 nt, respectively. The sequence in Figure 2, obtained from one of the two samples giving a single sharp band in agarose gel electrophoresis, yields a size of 324 nt for this interval. Moreover, sequencing of another sample (the 312/318 doublet) showed that in addition to length polymorphism there is sequence heterogeneity due to GGT↔GCT replacements (data not shown). Though we have not performed a quantitative analysis of polymorphism information content, these observations indicate a very high degree of allelic heterogeneity.

PHKG2 mutations in two patients with liver Phk deficiency and early cirrhosis

Two patients with liver Phk deficiency and cirrhosis, both daughters of consanguineous parents, were analyzed for mutations in the *PHKG2* gene. Each of them was found to be homozygous for a single sequence abnormality. Patient A had a deletion of a cytosine residue in codon 93 (exon 4; Fig. 3A), leading to a frameshift after 23% of the coding sequence and translation termination after 17 additional codons. Patient B had a C→T transition in codon 44 (exon 3) resulting in an Arg44ter nonsense mutation (Fig. 3B). In the earlier biochemical analysis of her family, her father and two siblings had Phk activities in the heterozygote range whereas her mother had normal Phk activity in repeated tests, so that a new maternal mutation was suspected in spite of her parents' consanguinity (11). However, when we analyzed her parents' DNA, we found them both heterozygotic for

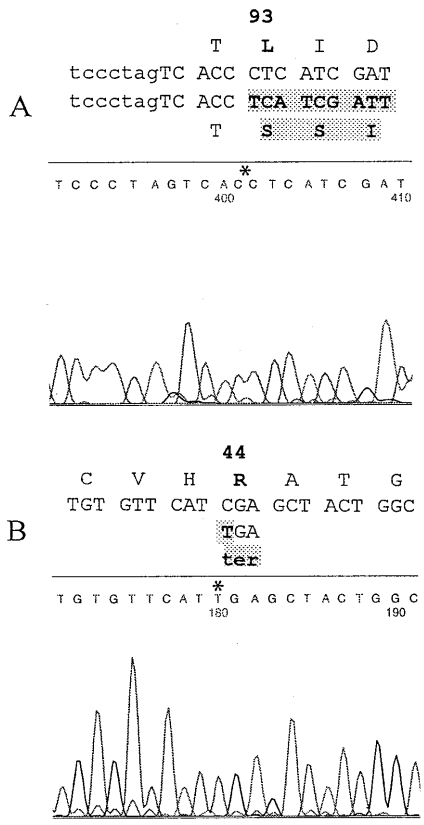

Figure 3. Homozygous translation-terminating mutations in the *PHKG2* genes of two patients with liver Phk deficiency and cirrhosis. Mutated positions are marked by asterisks in the sequencing electropherograms.

the nonsense mutation. This indicates that the mother's normal Phk activity had been misleading and could not rule out that she is actually a carrier of the mutation.

In a previous study in which we identified the first three human *PHKG2* mutations (8), also in female patients of consanguineous parentage, we noted that Phk deficiency due to mutations in this gene might tend to result in a more severe clinical and biochemical phenotype. Residual enzymatic activity was very low, and lipids and transaminases particularly high. Although patient 1 of that investigation has taken a benign clinical course, with developmental remission of symptoms, patient 2 has had persistent hypoglycemia and acidosis (similar to patient B of the present study) as well as mild fibrosis, and patient 3 developed severe fibrosis before the age of 2 years. In the present study, we have therefore analyzed both published cases of Phk deficiency with liver cirrhosis for mutations in the *PHKG2* gene, and indeed found them to be homozygous for one frameshift and one nonsense mutation, respectively. The severe phenotype of these patients is corroborated by the observations of hepatocellular adenoma in patient A, of lactic acidosis in patient B and of an abnormal glucagon response in both (see case reports in Materials and Methods), findings that are unusual in Phk deficiency (6,9,21).

These results add to our knowledge of genotype–phenotype correlations in the genetically complex Phk system. Although the number of *PHKG2* patients identified to date is still low (five), the available data strongly suggest that Phk deficiency with mutations in *PHKG2* tends to be associated with a more severe phenotype than Phk deficiency with mutations in *PHKA2* or *PHKB*, and in particular

to carry an increased risk of developing liver cirrhosis at an early age. Our findings suggest that among patients with liver Phk deficiency, those with *PHKG2* mutations have a worse prognosis, so that they warrant special diagnostic and therapeutic attention.

MATERIALS AND METHODS

Gene amplification and sequencing

PCR was performed in 50 µl volumes containing 200–300 ng of genomic DNA, 300 µM of each dNTP and 150 ng of each primer. Primer sequences are available upon request. To bridge the long introns, we employed 2 U of expand long template DNA polymerase (Boehringer Mannheim) in the manufacturer's buffer 1, and for other PCRs 0.5 U of Goldstar DNA polymerase (Eurogentec) in 20 mM ammonium sulfate, 0.01% Tween-20, 1.5 mM $MgCl_2$, 75 mM Tris–HCl (pH 9.2). Amplification was performed in 45 cycles following a step-down protocol. After denaturation for 40 s at 96°C, annealing (40 s) was carried out at a temperature 2°C below the calculated primer melting temperatures in the first five cycles, 4°C below in the next 10 cycles and 6°C below in the final 30 cycles. Elongation was performed at 68°C for 3 min in the first five cycles, for 3.5 min in the next 10 cycles and for 4 min in the final 30 cycles. Control reactions with only one primer of each pair were performed to identify unspecific side products. After electrophoresis on low-melting-point agarose gels, PCR products were sequenced directly from gel slices using the DyeDeoxy Cycle Sequencing kit from ABI essentially as described (5). The exons, the short introns, and ~50 nt at the ends of the long introns 2, 4 and 6 were sequenced in both directions, whereas the main parts of introns 2 and 4 were sequenced in one direction by primer walking. Moreover, most of the gene sequence was obtained from DNA of two or more unrelated individuals, except the central regions of intron 2 (nt 629–1320) and intron 4 (nt 3700–4900) which were sequenced from one person only.

Microsatellite repeat analysis

The repeat region was amplified with the primers γi20 [5′-(nt 563)-TTTGAAGGCTGGAGAGTTCG-3′] and γi21 [5′-(nt 887)-CTATCCAGTCTTCTCCTGAG-3′] (Fig. 2). PCR without radioactive label was performed in 50 µl containing 20–50 ng of genomic DNA, 200 µM of each dNTP and 300 ng of each primer, employing Goldstar DNA polymerase (Eurogentec) and buffer as described above. Initial denaturation was at 96°C for 60 s, followed by 10 cycles of 96°C for 45 s, 58°C for 40 s, 72°C for 60 s, and finally by 35 cycles of 96°C for 45 s, 56°C for 40 s, 72°C for 60 s. Products were visualized on a 1.5% agarose gel. Amplification with radioactive label was carried out under the same conditions, except the following parameters: 10 µl volumes containing 20–50 ng of genomic DNA, 5% dimethylsulfoxide, 60 ng of each primer and 1 µCi of [α-^{32}P]dCTP (in addition to 200 µM cold dNTPs), and an annealing temperature of 56°C in all 45 cycles. Products were resolved on a 6% polyacrylamide sequencing gel with an M13 sequencing ladder standard and visualized by overnight autoradiography of the dried gel at room temperature.

Case reports and mutation analysis

Detailed descriptions of both patients have been published. Patient A (EO.O; ref. 10) is the daughter of healthy Japanese parents who are first cousins. She was found to have adenomatous hyperplasia and cirrhosis of the liver at the age of 15 years. She had short stature and abdominal swelling as a young child but eventually grew to almost normal height. Histological examination of a liver biopsy showed glycogen storage. Phk activity in erythrocytes was 10% the value of a healthy subject. The change in blood glucose with glucagon stimulation was blunted after a meal, and absent when the patient was fasting. Today, with the patient aged 26 years, the liver tumor has not changed in size, but the cirrhosis has progressed, with the development of esophageal varices and ascites.

Patient B (BA.R; ref. 11) is the daughter of healthy consanguineous Saudi-Arabian parents (first cousins); one brother and one sister are healthy. She came to medical attention at 2 and 4 months of age with early-morning seizures and abdominal extension. At 11 months, she was admitted in a semi-comatose condition after a seizure, and presented with massive hepatomegaly, growth retardation (weight and length at the 5th percentile), low blood glucose (1.8 mM) and high serum lactate, transaminases and triglycerides. Liver glycogen was markedly elevated (20%; normal, 2–6%). Phk activity was undetectable in liver and erythrocytes but normal in muscle. The phosphorylase a/a + b ratio in liver was low (0.1; normal, 0.4–0.5). During follow-up until age 5.5 years, recurrent seizures, hypoglycemia, transaminases fluctuating between five and 25 times the normal values, lactic acidosis and a flat glucagon response were observed. Sequential liver biopsies showed progression to cirrhosis. Now, at the age of 8 years, she has not recently had hypoglycemic symptoms or convulsions, but continues to be shorter than normal (below the 5th percentile).

Table 2. PCR primers (A–D) and additional sequencing primers (S) for mutation analysis of the *PHKG2* gene

PCR/Seq.		Primer	Position/direction		Sequence
A		γTL F1	–89[a]	>	5′-TGAGCGACTGCAGGCAAAC
A		γi 21	Ex2 +387	<	5′-CTATCCAGTCTTCTCCTGAG
	S	γTL AR	Ex2 +66	<	5′-CAAACGGAAGCGGGAATGC
B		γTL BF	Ex3 -56	>	5′-TGTGCGGAAATGTGAGCAC
B		γTL BR	Ex4 +67	<	5′-CACAAATGCTGGGCCATAC
C		γTL CF	Ex5 -67	>	5′-GCACTGGTCTGTTTTCTCAG
C		γTL CR	Ex6 +66	<	5′-AGCCAACCAATCTCATCAGG
D		γTL FD	Ex7 -71	>	5′-AGCAGAGATCCAGTGCTAAG
D		γTL R3	1293[a]	<	5′-CAGAGGAGCTGGAATGATCA
	S	γTL H2	808[a]	>	5′-AGGCTGCTGCAGGTGGATC
	S	γTL R2	875[a]	<	5′-TCAAAGAAGGGGTGCTGTAG

[a]These primers lie within exons. Nucleotide positions therefore refer to the cDNA sequence.

Genomic DNA was isolated from whole blood, and four regions of the *PHKG2* gene that encompass the complete coding sequence (exon 2, exons 3 and 4, exons 5 and 6, and exons 7–10) were amplified and analyzed by direct sequencing essentially as described previously (5). Primers for PCR and sequencing are given in Table 2.

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REFERENCES

1. Hendrickx, J., Coucke, P., Dams, E., Lee, P., Odievre, M., Corbeel, L., Fernandes, J.F. and Willems, P.J. (1995) Mutations in the phosphorylase kinase gene *PHKA2* are responsible for X-linked liver glycogen storage disease. *Hum. Mol. Genet*., **4**, 77–83.
2. Van den Berg, I.E.T., Van Beurden, E.A.C.M., Malingre, H.E.M., Ploos van Amstel, H.E.K., Poll-The, B.T., Smeitink, J.A.M., Lamers, W.H. and Berger, R. (1995) X-linked liver phosphorylase kinase deficiency is associated with mutations in the human liver phosphorylase kinase α subunit. *Am. J. Hum. Genet*., **56**, 381–387.
3. Hirono, H., Hayasaka, K., Sato, W., Takahashi, T. and Takada, G. (1995) Isolation of cDNA encoding the human liver phosphorylase kinase alpha subunit (*PHKA2*) and identification of a missense mutation of the *PHKA2* gene in a family with liver phosphorylase kinase deficiency. *Biochem. Mol. Biol. Int*., **36**, 505–511.
4. Hendrickx, J., Dams, E., Coucke, P., Lee, P., Fernandes, J.F. and Willems, P.J. (1996) X-linked liver glycogenosis type II (XLG II) is caused by mutations in *PHKA2*, the gene encoding the liver alpha subunit of phosphorylase kinase. *Hum. Mol. Genet*., **5**, 649–652.
5. Burwinkel, B., Shin, Y.S., Bakker, H.D., Deutsch, J., Lozano, M.J., Maire, I. and Kilimann, M.W. (1996) Mutation hotspots in the *PHKA2* gene in X-linked liver glycogenosis due to phosphorylase kinase deficiency with atypical activity in blood cells (XLG2). *Hum. Mol. Genet.*, **5,** 653–658.
6. Burwinkel, B., Maichele, A.J., Aagenaes, Ö., Bakker, H.D., Lerner, A., Shin, Y.S. and Kilimann, M.W. (1997) Autosomal glycogenosis of liver and muscle due to phosphorylase kinase deficiency caused by mutations in the phosphorylase kinase β subunit (*PHKB*). *Hum. Mol. Genet*., **6**, 1109–1115.
7. Burwinkel, B., Moses, S.W. and Kilimann (1997) Phosphorylase kinase deficient liver glycogenosis with an unusual biochemical phenotype in blood cells associated with a missense mutation in the β subunit gene (*PHKB*). *Hum. Genet*., in press
8. Maichele, A.J., Burwinkel, B., Maire, I., Sövik, O. and Kilimann, M.W. (1996) Mutations in the testis/liver isoform of the phosphorylase kinase γ subunit (*PHKG2*) cause autosomal liver glycogenosis in the *gsd* rat and in humans. *Nature Genet.*, **14,** 337–340.
9. Hers, H.-G., van Hoof, F. and de Barsy, T. (1989) Glycogen storage diseases. In Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D. (eds), *The Metabolic Basis of Inherited Disease*. 6th edn. McGraw-Hill, New York, pp. 425–452.
10. Shiomi, S., Saeki, Y., Kim, K., Nishiguchi, S., Seki, S., Kuroki, T., Kobayashi, K., Harihara, S. and Owada, M. (1989) A female case of type VIII glycogenosis who developed cirrhosis of the liver and hepatocellular tumor. *Gastroenterol. Jap*., **24**, 711–714.
11. Kagalwalla, A.F., Kagalwalla, Y.A., Al Ajaji S., Gorka W. and Ali M.A. (1995) Phosphorylase b kinase deficiency glycogenosis with cirrhosis of the liver. *J. Pediatr.*, **127**, 602–605.
12. Chen, Y.T. and Burchell, A. (1995) Glycogen storage diseases. In Scriver, C.R., Beaudet, A.L., Sly, W.S. and Valle, D. (eds), *The Metabolic and Molecular Bases of Inherited Disease*. McGraw-Hill, New York, 7th edn, Vol. I, pp. 935–965.
13. Cawley, K.C., Akita, C.G., Angelos, K.L. and Walsh, D.A. (1993) Characterisation of the gene for rat phosphorylase kinase catalytic subunit. *J. Biol. Chem.*, **268,** 1194–1200.
14. Maichele, A.J., Farwell, N.J. and Chamberlain, J.S. (1993) A B2 repeat insertion generates alternate structures of the mouse muscle γ-phosphorylase kinase gene. *Genomics*, **16,** 139–149.
15. Hanks, S.K. (1989) Messenger ribonucleic acid encoding an apparent isoform of phosphorylase kinase catalytic subunit is abundant in the adult testis. *Mol. Endocrinol.*, **3**, 110–116.
16. Aperlo, C., Boulukos, K. E., Sage, J., Cuzin, F. and Pognonec, P. (1996) Complete sequencing of the murine USF gene and comparison of ist genomic organization to that of mFIP/USF2. *Genomics*, **37**, 337–344.
17. Kuhen, K.L., Shen, X., Carlisle, E.R., Richardson, A.L., Weier, H.-U.G., Tanaka, H. and Samuel, C.E. (1996) Structural organization of the human gene (PKR) encoding an interferon-inducible RNA-dependent protein kinase (PKR) and differences from its mouse homolog. *Genomics*, **36**, 197–201.
18. Wieringa, B., Hofer, E. and Weissmann, C. (1984) A minimal intron length but no specific internal sequence is required for splicing the large rabbit β-globin intron. *Cell*, **7**, 915–925.
19. Smit, A.F.A. and Riggs, A.D. (1995) MIRs are classic, tRNA-derived SINEs that amplified before the mammalian radiation. *Nucleic Acids Res.*, **23**, 98–102.
20. Whitmore, S.A., Apostolou, S., Lane, S., Nancarrow, J. K., Phillips, H. A., Richards, R. I., Sutherland, G. R. and Callen, D.F. (1994) Isolation and characterisation of transcribed sequences from a chromosome 16 hn-cDNA library and physical mapping of genes and transcribed sequences using a high-resolution somatic cell panel of human chromosome 16. *Genomics*, **20**, 169–175.
21. Willems, P.J., Gerver, W.J.M., Berger, R. and Fernandes, J. (1990) The natural history of liver glycogenosis due to phosphorylase kinase deficiency: a longitudinal study of 41 patients. *Eur. J. Pediatr.*, **149**, 2682–2671.