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Discovery of a Genetic Metabolic Cause for Mauriac Syndrome in Type 1 Diabetes

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A mechanistic cause for Mauriac syndrome, a syndrome of growth failure and delayed puberty associated with massive liver enlargement from glycogen deposition in children with poorly controlled type 1 diabetes, is unknown. We discovered a mutation in the catalytic subunit of liver glycogen phosphorylase kinase in a patient with Mauriac syndrome whose liver extended into his pelvis. Glycogen phosphorylase kinase activates glycogen phosphorylase, the enzyme that catalyzes the first step in glycogen breakdown. We show that the mutant subunit acts in a dominant manner to completely inhibit glycogen phosphorylase kinase enzyme activity and that this interferes with glycogenolysis causing increased levels of glycogen in human liver cells. It is known that even normal blood glucose levels physiologically inhibit glycogen phosphorylase to diminish glucose release from the liver when glycogenolysis is not needed. The patient's mother possessed the same mutant glycogen phosphorylase kinase subunit, but did not have diabetes or hepatomegaly. His father had childhood type 1 diabetes in poor glycemic control, but lacked the mutation and had neither hepatomegaly nor growth failure. This case proves that the effect of a mutant enzyme of glycogen metabolism can combine with hyperglycemia to directly hyperinhibit glycogen phosphorylase, in turn blocking glycogenolysis causing the massive liver in Mauriac disease.

Although it has been 85 years since Pierre Mauriac (1) described the well-known syndrome of growth failure and delayed puberty in children with poorly controlled type 1 diabetes that bears his name, the mechanistic cause of the syndrome remains poorly understood. The hallmark of the syndrome is extreme liver enlargement from massive deposition of glycogen. Although the prevalence of poor glycemic control from noncompliance with insulin administration among patients with type 1 diabetes is common, chronic severe hepatomegaly and growth failure in type 1 diabetes are rare in children whose type 1 diabetes is poorly controlled. This suggests that other factors, such as a mutation in an enzyme of glycogen metabolism, combined with persistent hyperglycemia cause the hepatomegaly. However, even though a genetic cause of the syndrome has been previously suggested, there has never been a report of a genetic enzyme defect as a cause of Mauriac syndrome. Here we report for the first time a genetic mechanism as a cause for Mauriac syndrome in a Caucasian male child who presented with typical islet cell antibody–positive type 1 diabetes at age 30 months, and who, after years of hyperglycemia, developed growth failure and massive hepatomegaly caused by glycogen deposition during adolescence.

We identified a mutation in an enzyme of glycogen metabolism and show that the mutation completely inhibits the activity of the enzyme and increases glycogen deposition in human liver cells. Homozygous or compound heterozygous mutations in numerous enzymes involved in the pathway of glycogen metabolism cause glycogen storage disease with hepatomegaly, growth failure, and hypoglycemia that appear in infancy. Because the onset of Mauriac syndrome typically occurs in adolescence, this argues against a homozygous mutation in a gene encoding an enzyme of glycogen metabolism as a cause of the syndrome. We hypothesized that a heterozygous mutation in a gene that encodes an enzyme of glycogen metabolism combined with hyperglycemia cause the syndrome. We found support for this hypothesis by

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identifying a heterozygous mutation at a highly conserved amino acid position in the catalytic subunit of the liver glycogen phosphorylase kinase (PhK) enzyme complex in the patient with Mauriac syndrome. This large enzyme complex activates glycogen phosphorylase, which is the enzyme that catalyzes the first step in glycogen breakdown in the liver. We expressed the mutant enzyme subunit in human liver cells and show that it acts in a dominant-negative manner to inhibit the catalytic activity of the PhK enzyme complex to cause excess glycogen accumulation in the human liver cells. The patient's mother possessed the same mutant enzyme, but did not have diabetes or hepatomegaly, and his father had childhood type 1 diabetes with very poor glycemic control, but lacked the mutation and had neither hepatomegaly nor growth failure. This patient's case proves that, although neither hyperglycemia alone nor a mutated enzyme alone are sufficient to cause Mauriac syndrome, a mutant enzyme of glycogen metabolism combined with chronic hyperglycemia can cause the syndrome.

RESEARCH DESIGN AND METHODS

Subjects

Blood samples were collected from subjects under a protocol approved by the Institutional Review Board of the University of Wisconsin School of Medicine and Public Health. Written informed consent was obtained from each subject and/or the subject's legal guardian.

Sequencing Genomic DNA

DNA was purified from the peripheral blood buffy coat of the patient with Mauriac syndrome and the other patients with the Qiagen Flexigene DNA kit according to the directions of the manufacturer. Exons and adjacent introns of relevant genes were sequenced in both directions using a standard protocol (2). PCR amplification of DNA was performed in a volume of 25 μL/well in a mixture containing 100 ng of genomic DNA, 0.2 μmol/L PCR primers, and 1× EconoTaq Plus 2× Master Mix (product 30035–1; Lucigen). For amplification, touchdown PCR was used as follows: 95°C for 5 min, followed by 14 cycles of 94°C for 20 s for melting, 72°C for 1 min for elongation, and a starting temperature of 63°C for 1 min for annealing. During these 14 cycles, the annealing temperature was decreased by 0.5°C with each cycle, and then 20 more cycles were performed with the annealing temperature maintained at 56°C and with the same temperatures as cited above for melting and annealing. After the final cycle, the temperature was maintained at 72°C for 5 min and then at 4°C. PCR reaction products were cleaned with Diffinity RapidTips (Diffinity Genomics, West Henrietta, NY) and used in the sequencing reaction. PCR amplification of DNA was performed in strip tubes in a volume of 20 μL/well in a mixture containing 5 μL of DNA, 1 μL of DMSO, 1 μL of primer, 3 μL of buffer, and 2 μL of BigDye Terminator version 3.1 (BigDye and buffer; Applied Biosystems, Palo Alto, CA). The sequencing reaction conditions were as follows: 95°C for 5 min, followed by 50 cycles of 96°C for 10 s for melting, 50°C for 10 s for elongation, and 58°C for 4 min for annealing. After the final cycle, the temperature was maintained at 72°C for 7 min and then at 4°C until the DNA sequencing of both strands was performed on the PCR-amplified genomic DNA using the same forward and reverse primers that were used for PCR amplification of the genomic DNA. Cleanup of the sequencing reaction was performed with magnetic beads (Agencourt CleanSEQ, catalog #A29151; Beckman Coulter) and plate, according to the directions of the manufacturer. DNA fragments were sequenced on a 3730xl DNA Analyzer (Applied Biosystems) using a 50-cm array and POP-7 polymer (2). The PHKG2 G → A mutation in exon 9 of the patient with Mauriac syndrome was confirmed by sequencing the same region in three additional separate samples of his DNA sequenced in both directions. Sequences of all primers used are available upon request.

Quantitative PCR and Immunoblotting

Quantitative PCR (3) and immunoblotting (4–6) were performed as previously described.

Quantifying the PHKG2 Mutation in mRNA Transcripts of the Patient

RNA was isolated from the peripheral white blood cell buffy coat, and the reverse transcription reaction was performed with a PHKG2 gene–specific reverse primer. The PCR was performed using PHKG2 gene–specific forward and reverse primers, and the resulting fragments were cloned into the pGEM-T Easy Vector System (Promega). The plasmid DNA was isolated from recombinant clones, and the identity of PHKG2 wild-type and mutant clones were assessed by DNA sequencing.

Plasmid-Mediated Expression of Mutant and Wild-Type PHKG2 in Human Liver Huh-7.5 Cells

The full-length PHKG2 wild-type (CGG → Arg at amino acid position 309) and PHKG2 mutant (CAG → Gln at amino acid position 309) gene sequences were synthesized by GenScript (Piscataway, NJ). The gene sequences were PCR amplified and cloned directionally into the pIRES2-GFP vector (Clontech) using *Nhe*I and *Xho*I restriction enzymes. The Huh-7.5 cells (7) were transfected with empty vector, pIRES2-PHKG2-wild-type, or pIRES2-PHKG2 (G → A) mutant plasmid DNA using Lipofectamine 2000 (Invitrogen). The transfected cells were selected with growth medium containing 800 μg/mL G418. After 2 weeks, the selected cells were used for further analysis. For measuring glycogen PhK enzyme activity and immunoblot analysis, the G418 selected cells were first grown in DMEM cell culture (contains 25 mmol/L glucose) and 10% FBS, then switched to RPMI-1640 cell culture medium (contains 11.1 mmol/L glucose) and 10% FBS and maintained for 1 week. At all times, the G418 drug selection was maintained. The reduction of the glucose concentration was intended to relieve any possible suppression of the

activity of any enzyme of glycogen metabolism, including the enzyme complex PhK and PHKG2, by the very high concentration of glucose in the DMEM.

Lentivirus-Mediated Expression of Mutant and Wild-Type PHKG2 in Human Liver Huh-7.5 Cells

To confirm the results of the plasma-mediated expression of the PHKG2 proteins in the liver cells, lentivirus was also used to express the PHKG2 protein. The PCR-amplified synthetic gene described above was cloned into the pLJM1-GFP vector (Addgene) using *Age*I and *Eco*RI restriction enzymes. We removed the GFP coding sequence from this vector and fused an HA tag at the COOH-terminal of the PHKG2 gene. Similarly, the original vector was manipulated to contain an HA tag at the COOH-terminal of the GFP coding region to serve as a control. To rescue lentiviruses, the plasmid DNA along with packaging mix from Lenti-X lentivirus expression systems (Clontech) was transfected into 293-T cells as per the manufacturer protocol, and after 48 h the supernatant was collected, clarified, and stored at −80°C in small aliquots. To generate the Huh-7.5 cell lines expressing PHKG2, the cell monolayer was infected with virus supernatant. The next day, the selection drug puromycin was added to growth media (final concentration 2 μg/mL), and further selection was performed for 2 weeks with the selection media changed every 4 days. Cells were maintained in RPMI-1640 cell culture medium for 1 week and were examined for the expression of PHKG2 protein by immunoblotting and for PhK enzyme activity.

PhK Enzyme Activity

The cells were homogenized in 220 mmol/L mannitol, 70 mmol/L sucrose, and 5 mmol/L potassium HEPES buffer, pH 7.5, containing protease inhibitor and 1 mmol/L dithiothreitol. The homogenate was centrifuged at 20,000*g* for 10 min at 4°C, and the supernatant fraction was removed and diluted to a concentration of 1 μg protein/μL. Ten microliters of the supernatant fraction was incubated

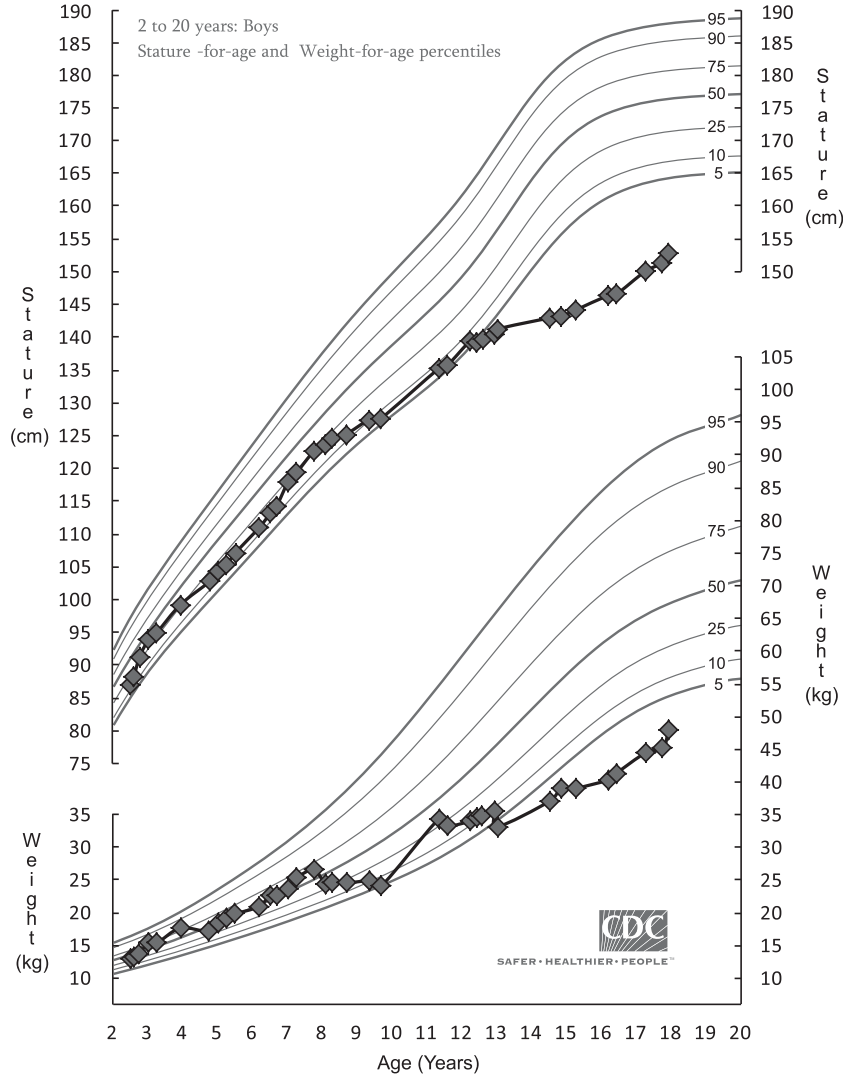

Figure 1—CDC growth chart showing the onset of growth delay during adolescence in the patient with Mauriac syndrome.

in an enzyme reaction mixture of 50 μL of 20 mmol/L $MgCl_2$, 0.1 mmol/L dithiothreitol, 1 μmol/L ATP, and 40 mmol/L Tris buffer, pH 8.0, with or without 1 μmol/L glycogen phosphorylase b (catalog #P-6635; Sigma-Aldrich) at 30°C for 10 min. Ten-microliter aliquots of the kinase reaction mixture were transferred to wells of a white 96-well plate to measure the enzyme activity using luminescence with the ADP-GLO Assay Kit (catalog #9101; Promega) according to the manufacturer instructions. Each condition was measured in quadruplicate wells. Luminescence was measured for 1–2 min on a Tecan GENios Microplate Reader using Kinetic software. The slope of the reaction was calculated with Magellan software. The rate without phosphorylase b was subtracted from the rate with phosphorylase b to give the enzyme activity attributable to PhK. Enzyme activity was expressed as relative light units per minute per microgram of protein.

Glycogen Content of Cells

The human liver Huh-7.5 cell lines, containing lentivirus-overexpressed mutant PHKG2 or overexpressed wild-type PHKG2, were maintained in DMEM cell culture medium containing 10% FBS. Control cell lines were the parent Huh-7.5 cell line or the Huh-7.5 cell line containing scrambled short hairpin RNA. Cells were maintained in the presence of insulin (350 mU insulin/mL) for up to 24 h to stimulate glycogen synthesis. Cells were also maintained in DMEM medium modified to contain 1.5 mmol/L glucose and no glutamine for up to 8 h to stimulate glycogen breakdown. To harvest the cells for glycogen analysis, cells maintained as monolayers on 10-cm tissue culture plates were washed twice with PBS and scraped from plates into 1.5-mL microfuge test tubes. A sample of the cell suspension was saved for protein analysis, and cells were immediately boiled. The boiled sample was centrifuged to remove the precipitated cell pellet, and glycogen in the supernatant fraction was measured by a standard procedure (8,9).

RESULTS

The Patient With Mauriac Syndrome

Severe hepatomegaly and growth failure (Fig. 1) were present in the patient at 13 years of age after having type 1

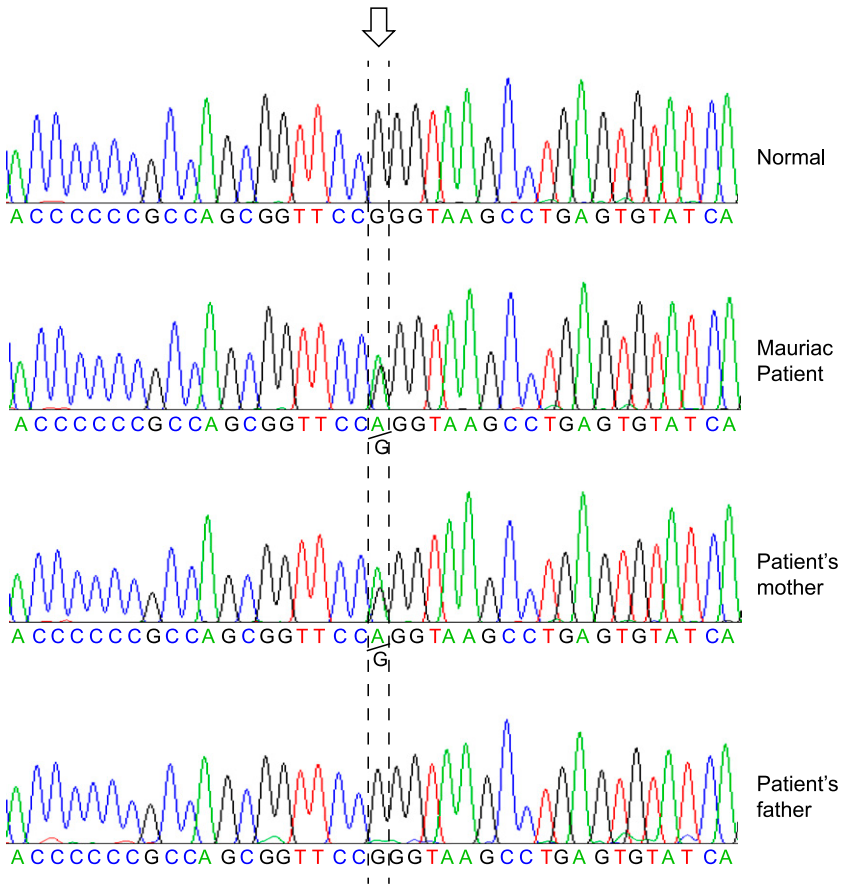

Figure 2—The G → A heterozygous mutation in PHKG2 in the patient with Mauriac syndrome and his mother. The figure shows the tracing of the nucleotide sequence surrounding the PHKG2 G → A mutation in DNA samples from the Mauriac patient and his mother, as well as a tracing of the normal sequence from his father's DNA and a tracing from 1 of 231 other Caucasian patients with type 1 diabetes whose glycemic control ranged from extremely poor to excellent and did not have an enlarged liver, and who all had normal PHKG2 exon sequences.

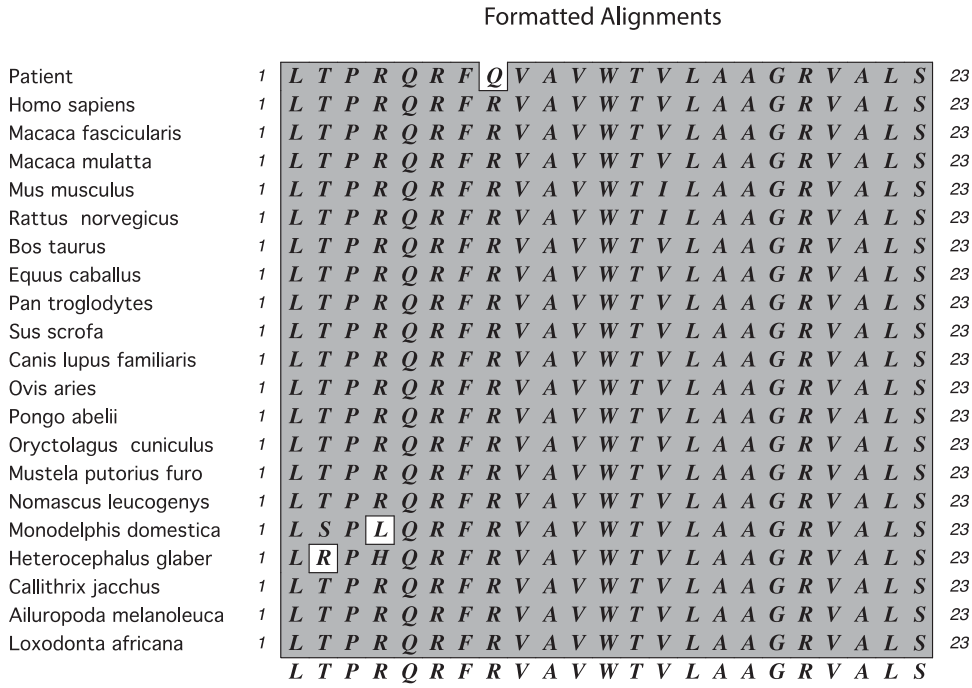

Figure 3— Conservation of arginine at amino acid position 309 of the human PHKG2 protein, and in PHKG2 of other mammals and genera. The figure shows the mutation to glutamine at position 309 in the patient and the conservation of arginine at residue 309 in all other PHKG2 proteins shown in the figure. Protein sequences were from the National Center for Biotechnology Information website.

diabetes for 11 years. The patient's hyperglycemia had worsened over the previous 3 years as severe hepatomegaly developed with his liver extending into his pelvis. His hemoglobin A_{1c} value was 10.2%, reflective of an average blood glucose value of 250–325 mg glucose/dL over the previous months. He was hospitalized briefly for respiratory distress attributed to his liver impinging on his diaphragm. An abdominal ultrasound examination was consistent with homogeneous glycogen deposition throughout an extremely enlarged liver. A liver biopsy sample showed cells swollen with glycogen, no inflammation, and no steatosis. He was also neutropenic (blood neutrophil count 510 cells/μL blood, normal 1,700–7,500 neutrophils/μL blood). When the glycemic control improved slightly, the respiratory distress and neutropenia disappeared, but his liver continued to extend to well below his umbilicus. Thirty months later, his liver again enlarged into his pelvis and the neutropenia recurred. He stated that a fear of hypoglycemia caused him to give himself lower than prescribed amounts of insulin. (See the detailed case history in the Supplementary Data.)

Causes of Liver Glycogen Storage Disease

Homozygous or compound heterozygous mutations in enzymes of glycogenolysis, including liver glycogen phosphorylase (10,11), glycogen debranching enzyme (12,13), glucose-6-phosphatase-α, and the glucose-6-phosphate transporter (14), cause hepatomegaly and growth failure as well as hypoglycemia due to failure of the liver to rapidly release glucose into the bloodstream sufficient to maintain a normal blood glucose level. Although glucose-6-phosphatase-α is expressed primarily in gluconeogenic tissues, such as liver and kidney, the glucose-6-phosphate transporter is expressed ubiquitously. A second glucose-6-phosphatase, glucose-6-phosphatase-β, is encoded by the gene G6PC3 and exhibits a ubiquitous pattern of distribution similar to the glucose-6-phosphate transporter. Glucose-6-phosphatase-β and the glucose-6-phosphate transporter contribute to the maintenance of neutrophil homeostasis and function, and mutations in either of these two enzymes are known causes of congenital neutropenia (14–16). Another clue that our patient could have a mutation in an enzyme of glycogen metabolism was that twice when his liver was at its largest he also had transient neutropenia. However, no pathogenic variations in the above-mentioned five enzymes were found (Supplementary Data).

Discovery of a Mutation in the PHKG2 Subunit of PhK

Glycogen phosphorylase catalyzes the first step in glycogen breakdown, and mutations in the glycogen phosphorylates gene PYGL can cause glycogen storage disease type VI (10). We found no pathogenic variations in the glycogen phosphorylase gene (Supplementary Data). The activity of glycogen phosphorylase is upregulated by PhK, which is a large 1.3×10^6 molecular weight hexadecameric enzyme complex that contains four copies of each of four subunits (α, β, γ, δ). PhK regulates glycogenolysis by converting the less active *b* form of glycogen phosphorylase to the active *a* form to activate glycogen breakdown. The α-subunit of PhK exists as skeletal muscle and liver

isoforms, encoded by different genes, PHKA1 and PHKA2, respectively. The β-subunit is the same in liver and muscle, and is encoded by one gene (PHKB). The δ-subunit is calmodulin, which is found in many body organs. The γ-subunit also exists in muscle and liver isoforms, encoded by the PHKG1 and PHKG2 genes, respectively. Homozygous or compound heterozygous mutations in the genes that encode the α-, β-, and γ-subunits of liver PhK can also give rise to glycogen storage disease of the liver (types IXa, IXb, and IXc, respectively) (17–21) with generally milder hypoglycemia and more variable phenotypes than those seen in patients with the severe forms of glycogen storage disease. No pathogenic variations in PHKA2 were observed (Supplementary Data).

PHKG2 is the catalytic subunit of the liver PhK complex. We found a heterozygous G → A mutation in exon 9 of the PHKG2 gene in the patient with Mauriac syndrome that was not present in 231 other Caucasian children with type 1 diabetes without hepatomegaly and who showed the whole spectrum of glycemic control (Fig. 2). The patient's mother possessed the same heterozygous mutation, and the patient's father did not possess the mutation (Fig. 2). The mutation causes an arginine-to-glutamine (R309Q) amino acid change within domain N of PHKG2. Domain N spans amino acid residues 302–326 of the protein and contains the autoinhibiting pseudosubstrate domain that binds calmodulin. The arginine at position 309 of PHKG2 is conserved in >20 species (Fig. 3) and the Polymorphism Phenotyping version 2 program (22) interpreted the mutation as damaging with a very high probability of 0.992. In contrast, the program predicted a change from an arginine to a glutamine at position 307 of the protein, two residues in front of R309, as not damaging, with a benign score of 0.128. The expressions of normal and G → A PHKG2 mRNA transcripts in the patient's peripheral white blood cells were about equal (12 wild-type and 10 G → A cDNAs of 22 clones), which is consistent with the expected hemizygous expression of the mutation.

Expression of the Mutant PHKG2 in Human Liver Cells Inhibits PhK Enzyme Activity and Inhibits Glycogen Degradation

We showed that minimal overexpression of the R309Q PHKG2 in human liver cells can completely inhibit the activity of the entire PhK enzyme complex. The hemizygous presence of the R309Q PHKG2 subunit predicts that it would disrupt the normal interaction of the subunits of the PhK enzyme complex which would severely inhibit its catalytic activity. This is because the calmodulin binding region of PHKG2 where the mutation is located interacts not only with calmodulin, but also with the C terminus of the α-subunit and the N terminus of the β-subunit of the PhK enzyme complex (23,24). We generated two sets of cell lines derived from the human liver cell line Huh-7.5 to give about twice the normal levels of PHKG2 with overexpression of either the R309Q PHKG2 or the wild-type PHKG2 compared with a control cell line—one set by stable transfection with a plasmid and another set by infection with lentivirus (Fig. 4). The liver cell lines with the overexpressed wild-type subunit should contain 100% normal PHKG2 subunit. The liver cell lines with overexpressed mutant PHKG2 should contain 50% mutant PHKG2 subunit and 50% normal PHKG2 subunit; the same as in the patient where the expression of the mutant PHKG2 is hemizygous. The 50% presence of the R309Q PHKG2 mutant subunit caused complete inhibition of PhK enzyme activity, whereas

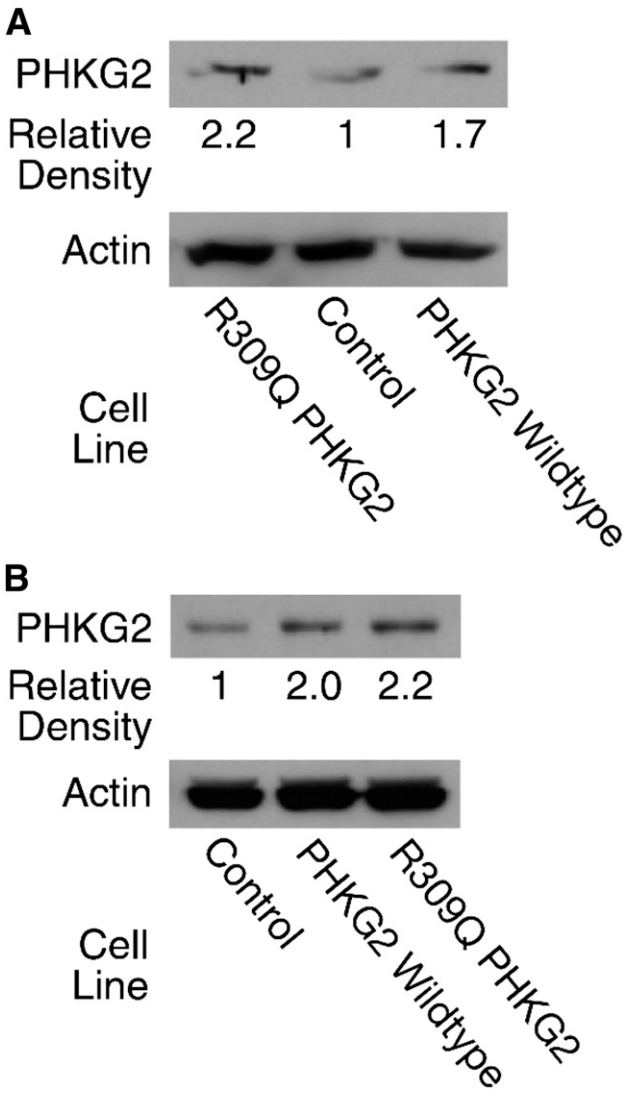

Figure 4—Immunoblot showing approximately twofold relative overexpression of the PHKG2 wild-type and R309Q PHKG2 mutant proteins in human liver Huh-7.5–derived cell lines. Vectors containing wild-type and the mutant PHKG2 gene sequences and a scrambled DNA sequence (Control) were used to generate stable cell lines with overexpressed wild-type PHKG2 or the mutant R309Q PHKG2 proteins. Cell lines shown in panel *A* were generated with a plasmid, and cell lines shown in panel *B* were generated with lentivirus, as described in the RESEARCH DESIGN AND METHODS section. The relative densities of the PHKG2 bands are shown. The PHKG2 immunoblot was stripped of antibody and reprobed with antibody to β-actin to demonstrate equal loading of cell protein across the lanes.

the liver cells containing only wild-type PHKG2 had PhK enzyme activity equal to that of the control liver cell lines containing a scrambled DNA sequence (Fig. 5*A*).

We also showed that the presence of the R309Q PHKG2 subunit in the human liver cells increases cell glycogen levels. The cell line possessing the mutant R309Q PHKG2 contained 33–70% higher glycogen levels than the wild-type or the control cells when the cells were maintained for short time periods either in the presence of low glucose to stimulate glycogen breakdown or in the presence of insulin to stimulate glycogen formation (Fig. 5*B* and *C*). This supports the idea that the hemizygous R309Q mutation was pathogenic in a dominant-negative manner and blocked the ability of PhK to activate glycogen phosphorylase, and that this inhibited glycogenolysis.

DISCUSSION

Glucose Directly Inhibits Glycogen Phosphorylase

The R309Q mutation in PHKG2 acting in a dominant manner to inhibit PhK and glycogenolysis by itself cannot entirely explain the patient's hepatomegaly because glycogen phosphorylase possesses a low level of activity in the absence of activation by PhK and also because hepatomegaly was not present in the patient's mother who possessed the same heterozygous mutation (Fig. 2) and did not have diabetes. A second factor contributing to this patient's severe hepatomegaly was the very high blood glucose level itself. Hyperglycemia alone can occasionally cause mild or moderate glycogen deposition in the liver in diabetes (glycogen hepatopathy), but it does not cause the chronic massive deposition of liver glycogen and associated growth failure seen in Mauriac syndrome, and this mild glycogen deposition is usually easily ameliorated with short-term improvement in glycemic control (25–29). The patient's father had type 1 diabetes, which had been diagnosed at 6 years of age. It was very poorly controlled, and he had neither hepatomegaly nor growth failure. The concentration of glucose in the blood and liver is the same, and glucose uptake and output by the liver and its incorporation into glycogen is influenced by the prevailing blood glucose concentration (10,12,30–32). Glucose, at normal concentrations found in the blood (3.9–5 mmol/L), inhibits glycogen breakdown by directly binding to

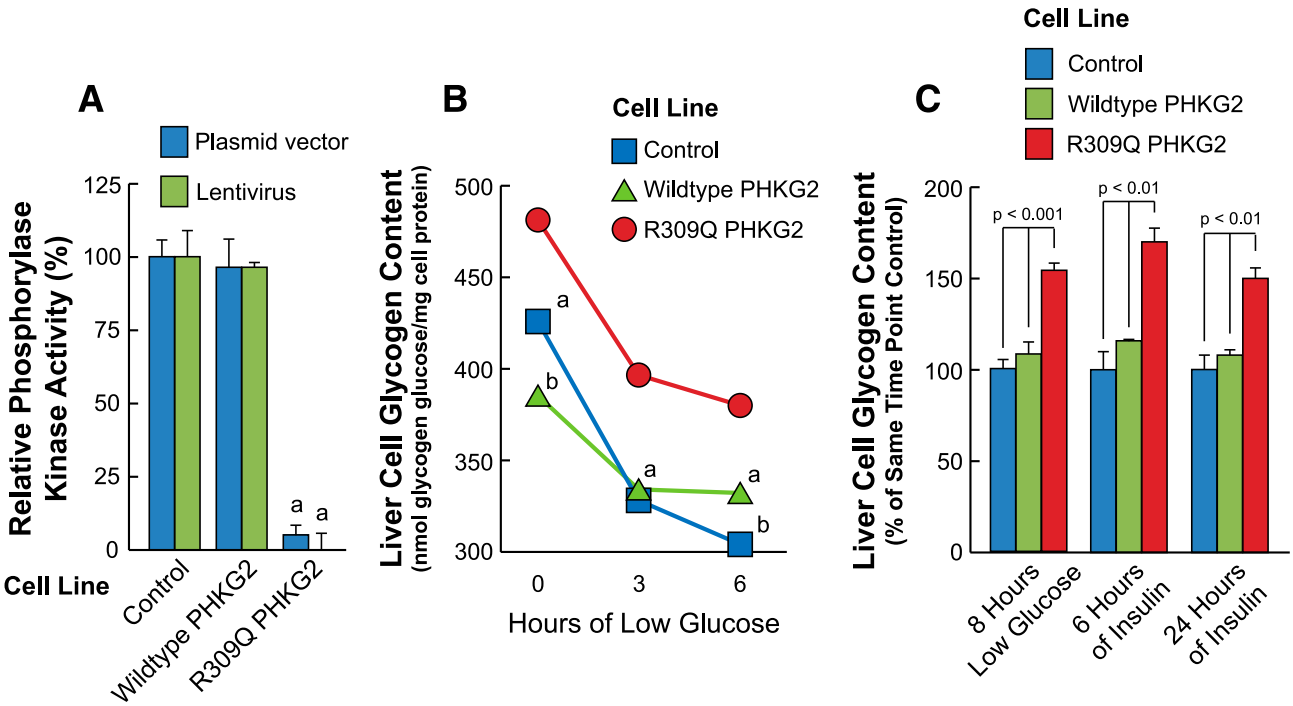

Figure 5—Expression of the R309Q PHKG2 mutant enzyme in human liver cells completely inhibits the enzyme activity of the PhK enzyme complex and inhibits glycogen breakdown in the presence of either low glucose, to stimulate glycogen breakdown, or insulin, to stimulate glycogen formation. *A*: PhK enzyme activity. Plasmid vectors and lentiviruses containing either the wild-type PHKG2, R309Q mutant PHKG2 or a scrambled nucleotide sequence (Control) were overexpressed in the Huh-7.5 human liver cell line to generate two sets of stable cell lines overexpressing approximately twofold the normal PHKG2 levels in the case of the wild-type or approximately onefold R309Q plus approximately onefold normal PHKG2 proteins compared with the control, as shown in Fig. 4. Enzyme activities are the mean ± SE of three preparations for each cell line. [a]$P < 0.001$ vs. control or wild type. *B*: Glycogen content. A scrambled DNA (Control) and the wild-type and mutant PHKG2-expressing lentivirus cell lines were incubated for 3 and 6 h in the presence of low (1.5 mmol/L) glucose to stimulate glycogen breakdown (mean ± SE, SE values are within symbols, $N = 4–6$ replicates for each cell line). [a]$P < 0.01$ or [b]$P < 0.001$ vs. R309Q PHKG2. *C*: Glycogen content. The parent human liver cell line (Control), the wild-type PHKG2 cell line, or the R309Q mutant PHKG2 lentivirus cell line were incubated in the presence of low glucose for 8 h to stimulate glycogen breakdown or in the presence of insulin for 6 or 24 h to stimulate glycogen formation (mean ± SE, $N = 4–6$ for each cell line).

the active site of glycogen phosphorylase *a*, competitively inhibiting its phosphorylase activity with a K_i of 1.7 mmol/L glucose (30–33) and also making the phosphorylase more susceptible to inactivation by dephosphorylation by a phosphatase (12,30–33). This inactivates the phosphorylase when the blood glucose level is normal and glycogenolysis is not required to maintain the blood glucose level (10,30–32). Extremely high blood glucose levels (e.g., as reflected from the patient's hemoglobin A_{1c} values, a typical average 2- to 3-month blood glucose level of 14–18 mmol/L, which is 8- to 11-fold the K_i of glucose for the enzyme) should inhibit glycogen degradation even more than normal.

Conclusion

The case of the patient with Mauriac syndrome provides conclusive proof that the mutation in PHKG2 (Fig. 2) combined with the extreme hyperglycemia acted together to inhibit glycogenolysis, and caused the severe hepatomegaly and possibly hypoglycemia and transient neutropenia in our patient. Our patient's hemoglobin A_{1c} values were not worse than numerous other patients with type 1 diabetes with poor glycemic control who do not have an enlarged liver and extremely poor growth. The family history is even more convincing. Each parent had one causal factor for Mauriac syndrome without having the syndrome. If the patient was indeed prone to hypoglycemia, as he reported, the inability to rapidly release glucose from liver glycogen could explain an increased susceptibility to hypoglycemia. Although mutations in PHKG2 or other subunits of the PhK complex have not been reported to cause neutropenia, mutations in either of two other enzymes of glycogen metabolism (glucose-6-phosphatase-β and the glucose-6-phosphate transporter) cause congenital neutropenia (14–16). PhK is expressed ubiquitously, including in leukocytes (14–21), and the mutant PHKG2 likely explains the transient neutropenia that occurred twice when the patient's glycemic control was extremely poor. The hemizygous R309Q mutation in PHKG2 destabilizes the PhK enzyme complex and inhibits the activity of the entire enzyme complex in a dominant-negative mechanism (Fig. 5*A*). This block in the ability of PhK to activate the liver glycogen phosphorylase plus hyperglycemia directly hyperinhibiting glycogen phosphorylase strongly inhibits the ability of glycogen phosphorylase to degrade glycogen (Fig. 5*B* and *C*). The results predict that a heterozygous mutation might be found in a subunit of the PhK enzyme complex or another enzyme of glycogen metabolism in other cases of Mauriac syndrome that undergo complete genetic screening of glycogen metabolism enzyme genes.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. M.J.M. designed the experiments, researched the data, and wrote the manuscript. N.M.H. and I.H.A. designed and executed the experiments, researched the data, and contributed to writing the manuscript. M.J.L. contributed to the design of and executed the experiments. M.A.K. and S.W.S. executed the experiments. M.J.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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