# A New Mouse Model of Type 2 Diabetes, Produced by *N*-Ethyl-Nitrosourea Mutagenesis, Is the Result of a Missense Mutation in the Glucokinase Gene

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**Here we report the first cloned *N*-ethyl-nitrosourea (ENU)-derived mouse model of diabetes. GENA348 was identified through free-fed plasma glucose measurement, being more than 2 SDs above the population mean of a cohort of >1,201 male ENU mutant mice. The underlying gene was mapped to the maturity-onset diabetes of the young (MODY2) homology region of mouse chromosome 11 (logarithm of odds 6.0). Positional candidate gene analyses revealed an A to T transversion mutation in exon 9 of the glucokinase gene, resulting in an isoleucine to phenylalanine change at amino acid 366 (I366F). Heterozygous mutants have 67% of the enzyme activity of wild-type littermates ($P < 0.0012$). Homozygous mutants have less enzyme activity (14% of wild-type activity) and are even less glucose tolerant. The GENA348 allele is novel because no mouse or human diabetes studies have described a mutation in the corresponding amino acid position. It is also the first glucokinase missense mutation reported in mice and is homozygous viable, unlike the global knockout mutations. This work demonstrates that ENU mutagenesis screens can be used to generate models of complex phenotypes, such as type 2 diabetes, that are directly relevant to human disease. *Diabetes* 53:1577–1583, 2004**

Type 2 diabetes is a heterogeneous metabolic disorder characterized by hyperglycemia resulting from defects in insulin action and/or insulin secretion. The underlying causes are both genetic and environmental (notably for the latter, obesity [1]).

Several bodies of evidence indicate a genetic component for the disease (lifetime risk of first-degree relatives [2], twin studies [3,4], and genetic admixture studies [5]), and there has been some success in mapping genes to chromosomal regions in human populations (6). Early-onset monogenic forms of diabetes account for ~2–5% of all diabetic patients. This phenomenon is known as maturity-onset diabetes of the young (MODY) and is caused by mutations in a variety of genes, including glucokinase (*Gck*), and the transcription factors hepatocyte nuclear factor 1α (HNF-1α), HNF-4α, HNF-1β, insulin promoter factor-1, and neuroD1 (6). To date, one gene mapped in multigenic type 2 diabetes in Mexican Americans and a Finnish ethnic subpopulation (Botnians) has been cloned, namely the Calpain 10 gene (7). The effect of this gene in European populations is uncertain (see, e.g., 8–12). Polymorphisms in other genes have been identified by association with type 2 diabetes, including, for example, peroxisome proliferator–activated receptor γ and the KCNJ11 component of the ATP-sensitive potassium channel in β-cells (reviewed in 13).

The identification of mouse diabetes genes serves as an adjunct to human studies by exploiting the high statistical power facilitated by large controlled crosses of genetically uniform strains to identify relevant genes even when such gene effects are small (14). A potential source of mice encompassing allelic series across all possible gene determinants of glucose homeostasis is genome-wide *N*-ethyl-nitrosourea (ENU)-induced mutagenesis of the mouse genome (15–17). ENU is an alkylating agent that preferentially induces A/T to T/A transversion mutations at random across the genome in mice and other organisms (15). A specific locus mutation rate of 0.00108 has been determined for the U.K. ENU mouse mutagenesis program, and it has been estimated that dominant mutations will occur at ~25% of this rate (18). These point mutations can create a variety of types of allele, including loss of function, hypomorphs, hypermorphs, and gain of function. This approach allows the discovery of gene function and dissection of protein functional domains in an unbiased manner (15).

Here we report identification of a mouse model of the MODY form of diabetes from a dominant phenotype-driven screen of ENU mutant mice in the Harwell/SB mutagenesis project (16,19) and genetic mapping and positional cloning of the underlying genetic defect. This is the first ENU-derived mouse model of diabetes and specifically of MODY2. It is also a homozygous viable model,

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DHPLC, denaturing high-performance liquid chromatography; ENU, *N*-ethyl-nitrosourea; G-6-PD, glucose-6-phosphate dehydrogenase; Gck, glucokinase; HNF, hepatocyte nuclear factor; IPGTT, intraperitoneal glucose tolerance test; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; OD, optical density.

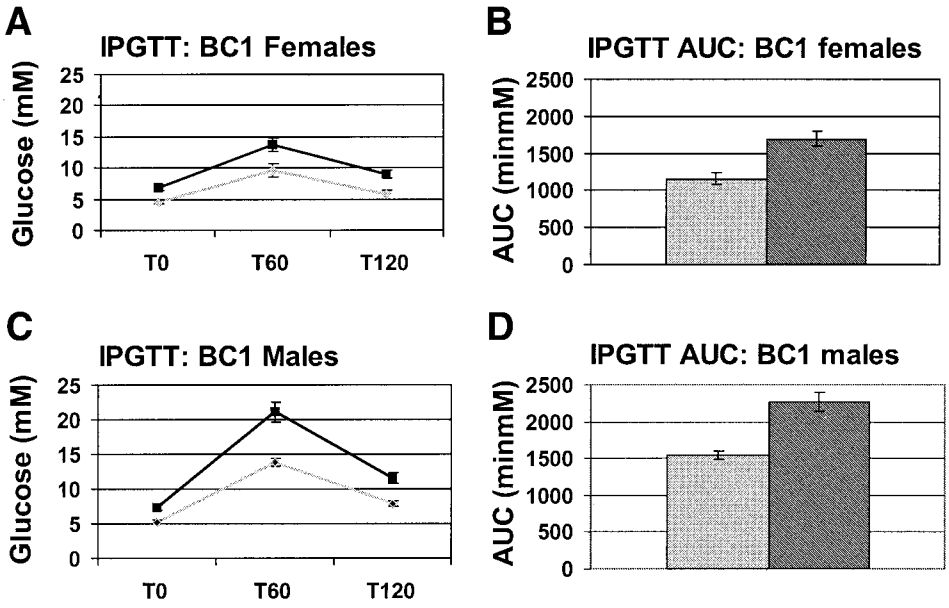


**FIG. 1. IPGTT results for first backcross (BC1) GENA348 mice. All data are from 12-week-old mice. Light gray lines or shaded gray bars are wild-type mice (10 females and 18 males), and shaded black bars or lines are heterozygous (13 females and 17 males) mutants. Data are presented as mean ± SE. *A* and *B*: BC1 female. *C* and *D*: BC1 male. AUC, area under the IPGTT curve. To assess the effect of sex and genotype on glucose tolerance between subjects, we performed repeated-measures ANOVA with between-subjects factors by use of the repeated-measures GLM procedure in SPSS. Type III sums of squares were computed for all terms incorporated in the model. Within-subjects factor "plasma glucose" incorporated correlated measurements IPGTT T0, T60, and T120 plasma glucose. Between-subjects factors were sex and genotype at the *Gck* gene locus. We observed significant effects of sex and genotype ($P < 0.0001$) on plasma glucose but no significant interaction between factors. We stratified the data by sex and genotype, then performed post hoc multiple comparisons between group means by use of Bonferroni multiple comparison tests ($\alpha = 0.05$). We observed male heterozygote (2, 3, and 4), male wild-type homozygote (1), female heterozygote (1 and 4), and female wild-type homozygote (1 and 3), where 1 is *P* value significantly different from male heterozygote, 2 is *P* value significantly different from male wild-type homozygote, 3 is *P* value significantly different from female heterozygote, and 4 is *P* value significantly different from female wild-type homozygote.**

unlike the conventional knockout. These data also provide first proof of the utility of ENU mutagenesis for developing mouse models of diabetes, a disease with complex genetic and environmental components.

## RESEARCH DESIGN AND METHODS

**Animals.** Mice were kept in accordance with U.K. Home Office welfare guidelines and project license restrictions. Mice were maintained under controlled light (12-h light and dark cycle), temperature (21 ± 2°C), and humidity (55 ± 10%). Mice had free access to water (25 ppm chlorine) and were fed ad libitum on a commercial diet (SDS maintenance chow) that contained 2.6% saturated fat.

**ENU mutagenesis.** Procedures used for ENU mutagenesis have been described previously (16,19).

**Identification of mice with blood glucose phenotypes.** Plasma biochemistry screening procedures that were used to identify mice between 8 and 12 weeks of age with glucose phenotypes in a cohort of ENU mutagenized mice have been described previously (19).

**Intraperitoneal glucose tolerance test.** Each mouse was fasted overnight to establish a baseline glucose level "T0" (time zero). Mice were weighed, and a blood sample was collected from the tail vein after administration of local anesthesia (lignocaine cream) using Lithium-Heparin microvette tubes (Sarstedt). The mouse then received an intraperitoneal injection of 2 g glucose/kg body wt (20% glucose in 0.9% NaCl), and T60 and T120 blood samples were taken at 60 and 120 min, respectively, after injection. Plasma was obtained from whole blood by centrifugation (3000 rpm × 10 min). Plasma glucose was measured using a Beckman Glucose analyzer.

**Genotyping.** Genomic DNA was extracted from mouse-tail tissue by use of Qiagen DNeasy tissue kit. Mice were genotyped by use of microsatellite markers. Primer sequences were obtained from published records (20). Microsatellite markers were PCR amplified, the products were resolved using an ABI 377 sequencer and Genescan software and protocols, and the data were processed using ABI Genotyper software.

**Linkage analysis.** Phenotype and genotype data were maintained in Microsoft Excel, SPSS, and MapManager QTX (21) (http://mapmgr.roswellpark.org/mimQTX.html) formats. Genetic maps were constituted using the published map order of the markers (http://www.informatics.jax.org). Linkage between markers and phenotypes was evaluated using the single-marker, interval mapping, and marker interaction mapping features of Map Manager. Raw phenotype data and natural logarithm-transformed data were each subjected to linkage analyses. Thresholds for declaring linkage were as outlined by Lander and Kruglyak (22). Additional statistics, mean, SD, *t* tests, ANOVA, and regression were performed in SPSS (version 10).

**Denaturing high-performance liquid chromatography.** To PCR amplify the *Gck* mutation, primers that flanked exon 9 were designed as follows: forward primer, CCAGGACCCTCAGTGACTTC; reverse primer. AAAAGCCT GGAGTTGAAAGC. PCR amplification of a 358-bp fragment was carried out in a reaction mixture of 15 ng of DNA, 200 μmol/l dNTPs, 2.5 mmol/l $MgCl_2$, 0.2 pmol of primers, 0.45 units of TaqGold polymerase (Perkin Elmer), and 0.025 units of Pfu turbo (Stratagene). Thermal profile conditions were 95°C for 10 min followed by 13 cycles of 94°C for 20 s, 62°C for 60 s with a reduction of 0.5°C per cycle, and 72°C for 60 s, followed then by 19 cycles of 94°C for 20 s, 55°C for 60 s, and 72°C for 60 s, followed finally by 72°C for 5 min and a 15°C hold. After amplification, heteroduplexes were formed using these thermal profile conditions: 95°C for 4 min, followed by 45 cycles of 93.5°C for 1 min with a reduction of 1.5°C per cycle, followed finally by holding at 25°C. Denaturing high-performance liquid chromatography (DHPLC) analysis was carried out using a Transgenomic Wave system operated according to the manufacturer's instructions using a column temperature of 67°C.

**Preparation of protein samples.** Protein samples were prepared from liver samples as described previously (23). Briefly, 0.3-g liver tissue sections obtained from overnight-fasted mice were rinsed in ice-cold PBS buffer, crushed in 5 ml of lysis buffer (containing 50 mmol/l triethanolamine, 100 mmol/l glucose, 100 mmol/l KCl, 5% glycerol, 5 mmol/l EDTA, 5 mmol/l EGTA, 0.02% $NaN_3$, 50 mmol/l PMSF, 1 mmol/l dithiothreitol, and 2.5 mg/ml leupeptin), and then centrifuged at 14,000 rpm for 15 min at 4°C. Supernatants were transferred into fresh microfuge tubes and stored at −70°C until required. Protein concentration was estimated by spectrophotometric quantitation against protein concentration standards using BioRad DC protein reagents and assay protocols.

**Western blot analysis.** Fifty-microgram protein samples were separated on a 4% stacking, 12% resolving SDS-PAGE and electrotransferred onto Hybond ECL nitrocellulose membranes (Amersham Pharmacia) according to the manufacturer's instructions. Gck and actin were detected separately with primary antibodies raised in rabbits (sc-7908 and sc-7210, respectively; Santa

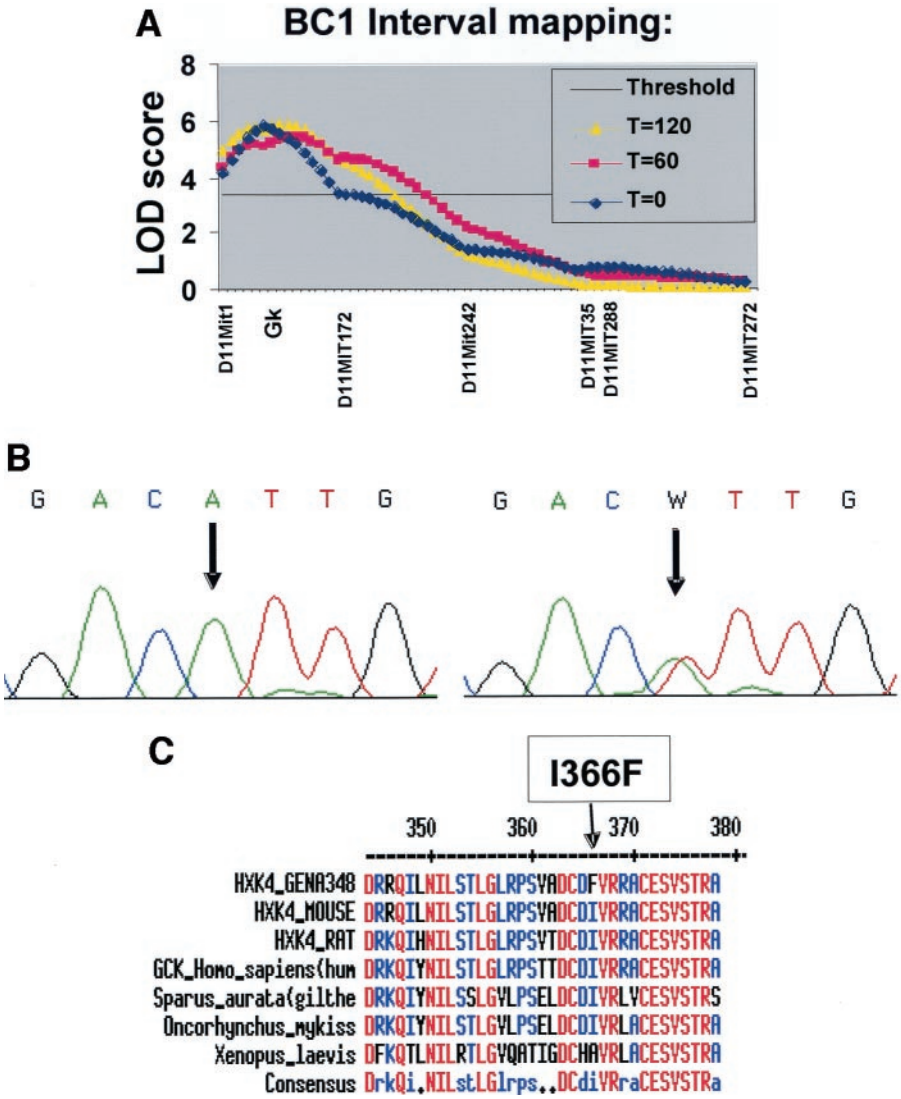


**FIG. 2.** ***A*****: Chromosome 11 interval maps for T0, T60, and T120 plasma glucose showing the LOD scores for each IPGTT subphenotype as a function of map position.** ***B*****:** ***Gck*** **exon 9 mutation: electropherograms of wild-type C3H/BALB/c sequence (left) and GENA348 founder F1 male sequence (right; note heterozygous T/A base), mutated base is arrowed.** ***C*****: Alignment of** ***Gck*** **sequence from GENA348, mouse reference sequence (gi:1708365), rat (gi:123902), human (gi:183227),** ***Sparus aurata*** **(gilthead sea bream; gi:7662681),** ***Oncorhynchus mykiss*** **(rainbow trout; gi:7662683), and** ***Xenopus laevis*** **(African clawed frog; gi:1262840).**

Cruz) using a horseradish peroxidase–conjugated secondary antibody (Bio-Rad) and ECL Plus detection system (Amersham Pharmacia) according to the manufacturers' instructions. Quantification of autoradiographs was carried out by densitometry using AlphaImager (Alpha Innotech) software.

**Gck protein activity assay.** Gck activity was measured by coupling glucose phosphorylation (to glucose-6-phosphate by Gck) to a reporter assay in which glucose-6-phosphate is oxidized to 6-phosphoglucono-δ-lactone with concomitant reduction of NAD+ to NADH as reported previously (24–26). Reaction velocity was estimated from the rate of reduction of NAD+ to NADH in the reporter assay, detected as decreased fluorescence (increased absorbance, optical density [OD]) of the reaction cocktail at 340 nm and 32°C. Each 100-μl reaction contained 5 μl of a 1:10 dilution of the liver protein extract (~0.03 mg wet liver wt/reaction), 50 mmol/l triethanolamine, 100 mmol/l KCl, 20 mmol/l $MgCl_2$, 1 mmol/l NAD+, 10 mmol/l ATP, 0.1% BSA, 1 mmol/l dithiothreitol, either 0.5 mmol/l or 100 mmol/l glucose, and 3 μg/ml glucose-6-phosphate dehydrogenase (G-6-PD; Sigma). Reaction cocktails were prewarmed to 32°C before addition of G-6-PD. Twenty-three OD measurements were taken at 4-min intervals over 88 min. Maximum velocity ($V_{max}$) values were calculated from measurements that fell in the linear portion of the OD time curve. Gck activity was estimated as the difference in glucose phosphorylation rates at 0.5 mmol/l (low $K_m$ hexokinase activity) and 100 mmol/l glucose (total hexokinase activity).

## RESULTS

**The causal basis of GENA348 glucose intolerance maps to chromosome 11.** The free-fed plasma glucose population mean and SD of the cohort of 1,201 male F1 (C3H × BALB/c-ENU–treated) mice was 9.8 ± 1.73 mmol/l. All markedly hyperglycemic mice (initially 15 that were >1 SD above the population mean) were selected for further study (data not shown). The F1 founder of GENA348 had plasma glucose concentrations of 13.4 (>2 SDs) and 12.3 mmol/l (>1 SD) in first and second tests, respectively. The founder was backcrossed (BC) to female C3H mice to test for inheritance of the plasma glucose phenotype and for genetic mapping. For testing glucose tolerance 58 BC1 (35 male and 23 female), 12-week-old mice were given an intraperitoneal glucose tolerance test (IPGTT; Fig. 1).

All mice were individually genotyped with a panel of 69 markers, providing whole genome coverage at an average intermarker distance of 22 cM. A single significant region of linkage was observed on chromosome 11 (Fig. 2*A*) at marker D11Mit172. Additional supplementary markers refined the peak of linkage to the MODY2 homology region around D11Mit1 (cM 1.0, logarithm of odds [LOD] 4.37).

**A mutation in the MODY2 gene,** ***Gck*****, is linked to GENA348 glucose intolerance.** Positional candidate gene sequencing of *CamK2b*, *Grb10*, and the MODY2 gene *Gck* revealed only one mutation of functional consequence, an A/T to T/A transversion mutation at position 1,096 of the *Gck* gene transcript (Fig. 2*B*). We positioned

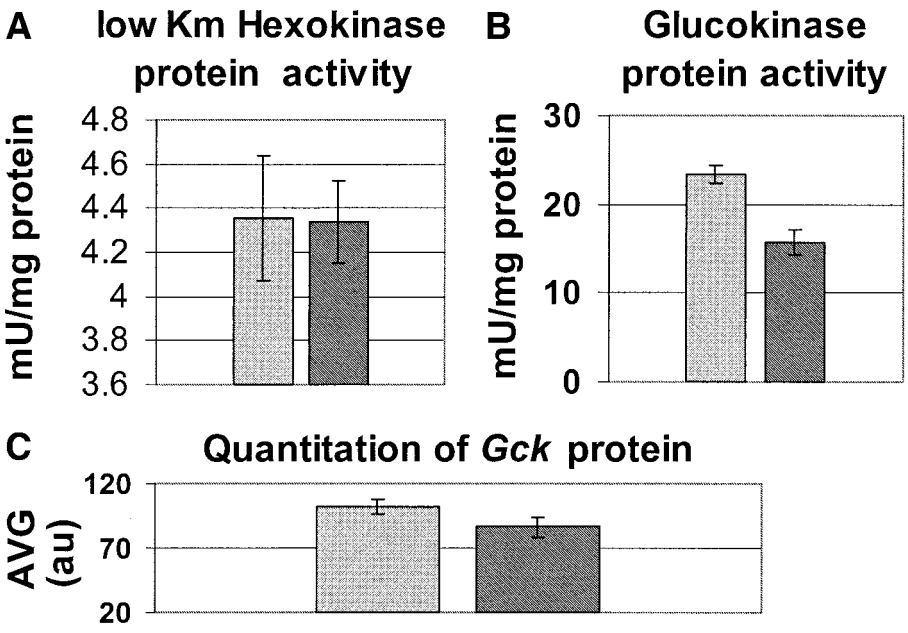


**FIG. 3. The effect of the GENA348 I366F *Gck* gene mutation on Gck enzyme activity. Single-factor ANOVA *P* values comparing wild-type with heterozygotes are given in parentheses. *A*: Low $K_m$ hexokinase activity ($P = 0.96$). *B*: *Gck* activity ($P = 0.001$). *C*: Densitometric quantification of immunodetected *Gck* in the same mice ($P = 0.095$). All data are from 12-week-old BC3 male mice, presented as mean ± SE. The numbers of mice represented for wild-type (light gray bars) and heterozygous mutant (dark gray bars) are 7 and 5, respectively.**

the *Gck* gene in the GENA348 BC1 chromosome 11 linkage map by use of the *Gck* genotypes of BC1 mice (established through DHPLC mutation analysis of a genomic fragment containing the mutation; data not shown). The *Gck* mutation accounts for 37% of fasting plasma glucose (LOD 6.0), 25% of T60 plasma glucose (LOD 5.4), and 33% of T120 plasma glucose (LOD 6.7) in the BC1 generation (Fig. 2*A* and data not shown). There is no significant effect of the mutation on body weight in either sex (data not shown); thus, body weight at 12 weeks of age is not a significant contributor to or consequence of the defective glucose tolerance phenotype of heterozygous mutant mice.

The GENA348 *Gck* gene mutation is located in exon 9 of the gene and results in a missense amino acid change in amino acid 366 from isoleucine to phenylalanine (I366F). Isoleucine has a hydrophobic aliphatic R-group, whereas phenylalanine has a bulky hydrophobic aromatic R-group. Multiple alignment of human, rat, mouse, and fish *Gck* sequences shows that the use of isoleucine at this amino acid position is conserved in evolution, although *Xenopus laevis* uses an alternative hydrophobic aliphatic amino acid (Fig. 2*C*).

***Gck* gene mutation in GENA348 results in impaired glucose phosphorylating activity of Gck protein.** Analysis of the *Gck* gene in BC3 (third backcross to C3H and nominally 94% C3H-derived DNA) mice revealed that the I366F *Gck* gene mutation is significantly associated with reduced glucose tolerance in an IPGTT of mice aged 12 weeks as seen in the BC1 generation (data not shown). A functional analysis assay based on BC3 mouse homogenized liver protein extracts (Fig. 3) also revealed that the *Gck* gene mutation significantly reduced (to 67%) glucose phosphorylating activity of Gck protein in heterozygous mutant mice ($Gck^{GENA348}/+$) when compared with wild-type mice (+/+; Fig. 3*B*). We observed no statistically significant difference in actual Gck protein concentration per unit of total protein (Fig. 3*C*).

**GENA348 *Gck* homozygous mutants are viable but less glucose tolerant than heterozygotes.** We sought to determine whether and to what extent homozygosity for the GENA348 mutation ($Gck^{GENA348}/Gck^{GENA348}$) influences glucose tolerance and specifically glucose phosphorylating activity of the Gck protein. Mice that are homozygous for the GENA348 allele are viable but less glucose tolerant at age 12 weeks than heterozygous or wild-type animals (Fig. 4*A* and *B*); plasma glucose was more than 280% of wild-type at T60 in an IPGTT (all homozygous mutant versus BC3 wild-type mice; data not shown). Body weight was not significantly different in 12-week homozygous animals (data not shown).

We also sought to determine whether and to what extent the glucose concentration–dependent phosphorylating activity of Gck protein was affected by the I366F missense mutation. Homozygous mutant male mice ($Gck^{GENA348}/Gck^{GENA348}$) had ~14% of wild-type enzyme activity in liver extracts at $V_{max}$ (Fig. 4*C*). Glucose phosphorylating activity of the mutant Gck protein from homozygous mutants ($Gck^{GENA348}/Gck^{GENA348}$) was much less responsive to changes in glucose concentration over a range of 0.5–25 mmol/l glucose (Fig. 4*C*, fasting physiological range at ~5 mmol/l). For assessing the effect of the mutation on Gck protein levels, immunoblots of liver and whole pancreas protein extracts were probed with both *Gck* and actin polyclonal antibodies (Fig. 5*A* and *B*, respectively) and quantified using actin as a comparative baseline in samples of different genotype. In the analysis of liver tissue, mutant heterozygotes show a small nominal increase in Gck protein of ~10% and homozygotes show a large 54–66% (two different animals) reduction in Gck protein relative to wild-type mice. In the pancreas, there was a small, nominal decrease of 15% in Gck protein in heterozygotes relative to wild-type littermates and a larger decrease of 22–32% (two different animals) in homozygotes.

## DISCUSSION

Through genetic mapping, we have demonstrated that glucose intolerance in GENA348 is genetically determined by a locus on chromosome 11. Positional candidate gene analysis revealed that an A/T to T/A transversion at posi-

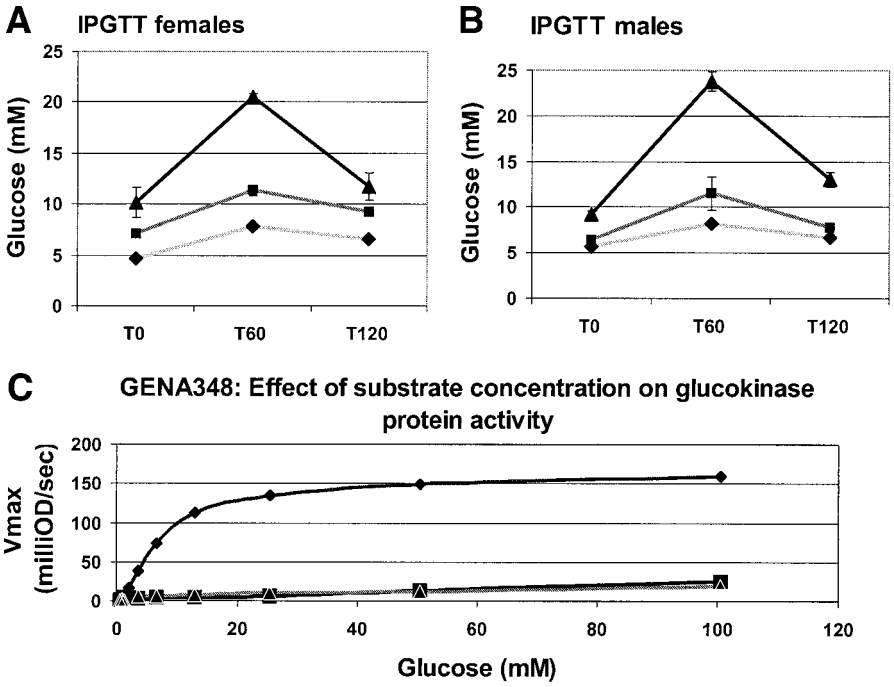


**FIG. 4. Effect of homozygosity of the GENA348 I366F mutation in 12-week-old mice. *A* and *B*: IPGTT at T0, T60, and T120 in female and male mice, respectively. All data are from 12-week-old mice, presented as mean ± SE. Numbers of mice represented are intercross wild-type (light gray lines and ◆, one female and one male), intercross heterozygous mutants (dark gray lines and ■, four females and three males), and intercross homozygous mutants (black lines and ▲, three females and four males). *C*: Effect of substrate concentration on *Gck* activity in wild-type (diamonds) and homozygous mutant (squares and triangles) 12-week male mice, $V_{max}$ *Gck*/mg protein.**

tion 1,096 of the *Gck* gene transcript results in a missense I366F mutation in a conserved residue of the Gck protein. Functional analysis of the *Gck* gene product in hepatic tissue extracts confirmed that the GENA348 mutation reduces glucose phosphorylating activity of the gene product, and this in turn correlates with glucose intolerance in an IPGTT on 12-week-old mice. The pathological consequence of mutations in the *Gck* gene is well studied (27–31). Defective function of the *Gck* gene as a result of dominant mutations results in mild stable fasting hyperglycemia from birth, which deteriorates very slowly with age and is combined with elevated postprandial glucose levels (MODY2) (29,32,33). This results because *Gck* has a key glucose-sensing role (being the rate-limiting step in glycolysis in liver and pancreatic islet β-cells) in the β-cell determining the in vivo glucose and insulin secretion rate dose-response relationship, consequently mutations that impair *Gck* activity impair glucose sensing and consequent insulin secretion (29). Conversely, an activating mutation has been shown to result in a rare heterogeneous clinical condition known as persistent hyperinsulinemic hypoglycemia of infancy, characterized by oversecretion of insulin at low glucose levels (34,35).

The I366F GENA348 allele is novel because no mouse or human diabetes studies have described a mutation in the corresponding amino acid position. In humans, a missense mutation in an adjacent amino acid V367M results in diabetes, indicating along with the GENA348 mutation the functional significance of this gene region in protein function.

Global *Gck* deficiency in homozygous null mice is lethal during the first week after birth as a result of extreme hyperglycemia (26,36). Heterozygous null mice exhibit mild hyperglycemia (26,36). Expression of *Gck* from a transgene specifically expressed only in the β-cell rescues homozygous null mice and in some mice maintains normal or near-normal glucose levels despite a complete absence of *Gck* in the liver (26). This key requirement for β-cell *Gck* is supported by deletion of the β-cell promoter, which creates a β-cell null mutation that is homozygous lethal during the first week of life as a result of severe diabetes (37). Global, β-cell–, and hepatocyte-specific knockouts made using Cre recombinase confirm the critical requirement for β-cell *Gck* to maintain viability, that global or β-cell null heterozygosity results in moderate hyperglycemia and further that liver specific *Gck* null mice exhibit only mild hyperglycemia with defects in glycogen synthesis and glucose turnover (38,39).

Unlike homozygous global and β-cell *Gck* knockout mice, which die perinatally, GENA348 homozygotes are viable but more glucose intolerant than heterozygous mutants. Human patients with homozygous missense GCK gene mutations (M210K and T228M) with resultant neonatal diabetes have also been reported (31). The GENA348 mutation in the homozygous state results in greatly reduced *Gck* activity in the liver and a much reduced $V_{max}$ slope over varying glucose concentrations (including the physiological range), indicating that the mutation directly affects enzyme activity.

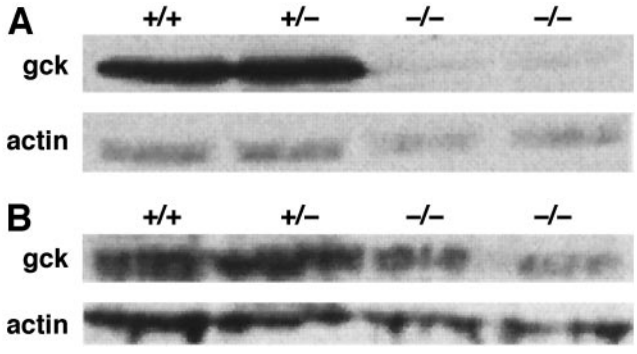


**FIG. 5. Reduction of Gck protein in both the liver and the pancreas of homozygous GENA348 mutants. Immunoblots of intercross offspring of the GENA348 line; Gck and actin protein levels in liver (*A*) and pancreas (*B*). +/+, BC3 wild-type mouse; +/−, BC3 heterozygous mutant mouse; −/−, two homozygous intercross mutant mice.**

In the liver of homozygotes, Gck protein was reduced by 54–66%, and this may be due to several causes. The liver *Gck* gene is under the transcriptional control of insulin (unlike in the pancreas), and the more severe β-cell glucose-sensing defect in homozygotes may result in less circulating insulin and, therefore, less liver *Gck* gene transcription. This could also be compounded by protein instability as a result of the missense mutation. In the human *GCK* gene, it has been shown, for example, that the MODY2 E300K mutation results in a 10-fold reduction in enzyme activity because of protein instability (40). In the mouse, it has also been shown that there is a regulatory locus that cosegregates with the *Gck* gene and that this correlates with hepatic expression with two main haplotypes that are low and high in expression relative to each other (41). In our homozygous mutants, two BALB/c *Gck* alleles are reported (in a different BALB/c substrain) to belong to the low class, unlike C3H/HeJ, which belongs to the high class, although it should be noted that in our heterozygotes (that therefore have both alleles), the Gck protein levels were similar to wild-type C3H/HeH (41). Furthermore, several founders selected for inheritance testing did not yield a significant glucose phenotype in subsequent generations, further indicating that this effect is not a significant factor in our crosses.

In the pancreas, we have found a smaller reduction in *Gck* protein levels than in liver, which may reflect the different regulatory signals for the gene in the β-cell as well as compensatory mechanisms. We propose that this together with residual *Gck* activity is sufficient to ensure the viability of this mutation.

In summary, for GENA348, it is likely that, as in human patients, impaired glucose phosphorylating activity of $Gck^{GENA348}$ Gck protein (through impaired activity compounded by reduced protein levels) results primarily in impaired β-cell glucose sensing and glucose-stimulated insulin release (39) and, consequently, impaired glucose tolerance and diabetes as in MODY2 patients.

The primary screen for mice that exhibit high free-fed blood glucose was carried out between 8 and 12 weeks. This screen may predispose to the identification of early-onset MODY-type mutations, although one other line isolated from this screen does not map to a known MODY gene location (unpublished observation). In subsequent screens, we have used IPGTT assays and have tested at 12 and 24 weeks and identified several models of which two are mapped and do not map to known MODY genes (unpublished observations).

This report demonstrates for the first time the effectiveness of phenotype-driven ENU mutagenesis strategies in the investigation of the genetics of diabetes. It also illustrates the general utility of the ENU approach for generating relevant disease models, especially where the conventional knockout is lethal.

## ACKNOWLEDGMENTS

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