

# Heterozygous Missense Mutations in the Insulin Gene Are Linked to Permanent Diabetes Appearing in the Neonatal Period or in Early Infancy

## A Report From the French ND (Neonatal Diabetes) Study Group

Michel Polak,<sup>1,2,3</sup> Aurélie Dechaume,<sup>4,5</sup> H el ene Cav e,<sup>6</sup> Revital Nimri,<sup>7</sup> H el ene Crosnier,<sup>8</sup> V eronique Sulmont,<sup>9</sup> Marc de Kerdanet,<sup>10</sup> Raphael Scharfmann,<sup>1,2</sup> Yael Lebenthal,<sup>7</sup> Philippe Froguel,<sup>4,5,11</sup> and Martine Vaxillaire<sup>4,5</sup>

**OBJECTIVE**—Permanent neonatal diabetes (PND) is defined by chronic hyperglycemia due to severe nonautoimmune insulin deficiency diagnosed in the first months of life. Several genes, including *KCNJ11* and *ABCC8*, which encode the two subunits of the ATP-sensitive K<sup>+</sup> channel (K<sub>ATP</sub> channel) can cause PND. Mutations in the insulin (*INS*) gene have been recently described in families with neonatal diabetes. Our study aimed to investigate the genetic anomalies and clinical heterogeneity in PND patients who are negative for a K<sub>ATP</sub> channel mutation.

**RESEARCH DESIGN AND METHODS**—We screened the *INS* gene by direct sequencing in 38 PND patients and in one child with nonautoimmune early-infancy diabetes, where no mutation in *GCK*, *KCNJ11*, and *ABCC8* was identified. A detailed clinical phenotyping of the patients was carried out to specify the diabetes features in those found with an *INS* mutation.

**RESULTS**—We identified three missense mutations in the *INS* gene in four probands. Two of four mutations were inherited in a dominant manner, and the familial description evidenced a marked variability in age of diagnosis and disease progression. In our cohort, the *INS* mutations may represent ~10% of all permanent neonatal diabetes cases, having a later presentation of diabetes and no associated symptoms compared with cases with K<sub>ATP</sub> channel mutations.

From the <sup>1</sup>Faculty of Medicine, Ren e Descartes Paris 5 University, Paris, France; <sup>2</sup>Inserm U845, Necker Enfants Malades Hospital, Paris, France; the <sup>3</sup>Department of Pediatric Endocrinology, Necker Enfants Malades Hospital, Paris, France; <sup>4</sup>CNRS 8090, Institute of Biology, Pasteur Institute, Lille, France; the <sup>5</sup>University of Lille 2, Lille, France; <sup>6</sup>Genetic Biochemistry, Robert Debr e Hospital, Paris, France; <sup>7</sup>Pediatric Endocrinology and Diabetes, Schneider Medical Center, Petah Tikva, Israel; the <sup>8</sup>Department of Pediatrics, Saint-Germain en Laye Hospital, Saint-Germain en Laye, France; <sup>9</sup>Pediatric Endocrinology, Franco-American Hospital, Reims, France; <sup>10</sup>Pediatric Endocrinology, H opital Sud, Rennes, France; and <sup>11</sup>Genomic Medicine, Hamner-Smith Hospital, Imperial College, London, U.K.

Address correspondence and reprint requests to Dr. Martine Vaxillaire, CNRS UMR 8090 & Institut de Biologie, Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP 245, 59019 Lille, France. E-mail: martine.vaxillaire@good.ibl.fr.

Received for publication 24 September 2007 and accepted in revised form 22 December 2007.

Published ahead of print at <http://diabetes.diabetesjournals.org> on 28 December 2007. DOI: 10.2337/db07-1358.

Additional information for this article can be found in an online appendix at <http://dx.doi.org/10.2337/db07-1358>.

ISPAD, International Society for Paediatric and Adolescent Diabetes; K<sub>ATP</sub> channel, ATP-sensitive K<sup>+</sup> channel; PND, permanent neonatal diabetes; TND, transient neonatal diabetes.

  2008 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**CONCLUSIONS**—Heterozygous *INS* gene mutations can cause isolated permanent early-infancy diabetes and should be assessed in neonatal as well as in childhood diabetes appearing like type 1, when autoimmune markers are absent. New pharmacogenomic strategies may be applicable, since residual  $\beta$ -cell function is still present in some patients. *Diabetes* 57:1115–1119, 2008

**D** diabetes in childhood, including so-called "neonatal diabetes," is unrelated to autoimmune destruction of the pancreatic  $\beta$ -cells in the vast majority of cases (1). Neonatal diabetes is a rare (~1:300,000 newborns) but potentially devastating metabolic disorder characterized by mild to severe hyperglycemia with low levels of circulating insulin within the first months of life (1). The presentation of the disease can be transient (transient neonatal diabetes [TND]) or permanent (permanent neonatal diabetes [PND]), which differ in the duration of insulin dependence early in the first months/years of life and in the molecular mechanisms responsible for the severe insulin secretion defect. More than half of TND cases are associated with abnormalities of an imprinted region on chromosome 6q24 (2), whereas mutations in the two subunits (Kir6.2 and SUR1) of the ATP-sensitive K<sup>+</sup> channel (K<sub>ATP</sub> channel) of the pancreatic  $\beta$ -cell have been characterized as a common cause of both TND and PND (3–5). In addition, a few cases of PND were attributed to mutations in the genes encoding the glycolytic enzyme glucokinase (*GCK*) (6) and insulin promoter factor-1 (*IPF-1*) (7), and syndromic cases including neonatal diabetes are caused by rare mutations in *PTF1A*, *FOXP3*, *GLIS3*, *TCF2*, and *EIF2AK3* (1).

Our previous studies from the French ND (neonatal diabetes) case series, and others from the International Society for Paediatric and Adolescent Diabetes (ISPAD) cohort, have demonstrated that dominant mutations in both subunits of the K<sub>ATP</sub> channels expressed in the neuroendocrine cells lead to a range in the severity of the disease. These phenotype specificities depend on the mutation in Kir6.2 or SUR1: from mild transient hyperglycemia to PND for SUR1 mutations (4,8,9) and from PND to more severe phenotypes associated with developmental delay and epilepsy usually for Kir6.2 and for some SUR1 mutations (10,11). In the patients referred to the French ND Study Group, over 50 cases presenting with very-early-

onset diabetes are not yet defined for their molecular origin (8). This suggests that other gene defects are involved in key mechanisms regulating insulin processing and secretion and/or the survival process of insulin-producing  $\beta$ -cells.

Stoy et al. (12), using a positional cloning approach in a pedigree including four diabetic members (age at diagnosis from 13 to 52 weeks), identified the insulin (*INS*) gene as responsible for diabetes in this family and replicated their findings in unrelated neonatal diabetic patients and in the ISPAD cohort, showing 10 heterozygous missense mutations in 16 probands.

In the present study, 38 PND patients from the French ND cohort and 1 child with nonautoimmune early-infancy diabetes, who are negative for a mutation in *GCK*, *KCNJ11*, and *ABCC8* (4,8,10), were screened for the *INS* gene by direct sequencing. The H family (Table 1) was referred to us to sequence *ABCC8*, as mutations in this gene may be present in patients with diabetes from the neonatal period to young adulthood (4,8). As *GCK* (*MODY2*) and *HNF1A* (*MODY3*) sequences were also found to be normal in this family, the proband HA was included in the current study, despite diabetes appearing like nonautoimmune early-onset type 1 rather than bona fide neonatal diabetes.

The three exons of *INS* were amplified in two fragments using PCR (supplementary Table 1 [available in an online appendix at <http://dx.doi.org/10.2337/db07-1358>], and both strands were sequenced by using a standard protocol (4) and 3730xl DNA analyzer (Applied Biosystems, Foster City, CA). Three missense mutations—A24D (c.71 C>A in exon 2), R89C, and C96Y (c.265 C>T and c.287 G>A in exon 3)—were identified in four probands diagnosed with a permanent form of diabetes. A detailed description of these patients is shown in Table 1. The same three mutations were found in PND patients from the original report (12). We also investigated whether the identified mutations were inherited from a parent or arose de novo by sequencing a DNA sample of the parents when available. In two families (H and B), the R89C mutation was inherited in a dominant manner from the mother. The sister of proband HA who presented with early-onset diabetes was also found with the R89C mutation (Table 1). The A24D and C96Y mutations were not inherited from a parent in families F and G. Such family relationships were confirmed by genotyping a panel of six microsatellite markers as previously used (4). Eleven patients with TND from the French cohort, in which chromosome 6 anomalies had been excluded (1), were also screened for the *INS* gene, but no mutation was identified in these patients.

All three mutations are located in critical regions of the preproinsulin molecule and may affect the proteolytic processing of insulin precursors or disrupt insulin biosynthesis and induce endoplasmic reticulum stress, as proposed in the previous studies (12,13). The two amino acid changes at the signal peptide and A-chain C-peptide cleavage sites (A24D and R89C) are present in several unrelated neonatal diabetic patients (in three and four patients, respectively, when considering the current and previously reported cases [12]); this likely reflects the potential deleterious effect of these preproinsulin cleavage mutants on the folding and secretion of the normal insulin peptide. Importantly, the C96Y mutation found in two unrelated patients (this study and the previous report [12]) was also shown to act in a dominant manner in the Akita diabetic

mouse and consequently to activate the unfolded protein response (13).

The clinical features of four proband children, of one relative child (sister of proband HA), and of the two affected mothers (except for data at birth and at presentation, which were not available for the two mothers) are shown in Table 1. Diabetes was diagnosed in the children at a median age of 8.5 months (range 25 days to 4.25 years). One child presented with mild hyperglycemia (7.2 mmol/l), whereas the four others had marked hyperglycemia (mean plasma glucose at diagnosis 27.3 mmol/l, range 7.2–49.5). One case (FZ-A24D) presented with severe ketoacidosis, one (HA-R89C) presented with hyperglycemia and ketoaciduria, and three (HL-R89C, BG-R89C, and GI-C96Y) were diagnosed based on polyuria and polydipsia. The age at diagnosis in family H was consistent with familial type 1 diabetes. However, autoantibodies associated with type 1 diabetes (islet cell antibody, GAD65, and insulinoma-associated protein 2 antigen) were all negative in the two siblings, as well as in the other three younger patients. Pancreas ultrasonography was normal when performed (in four of five of the children cases). Two of five of the young cases were small for gestational age (<3rd centile), including one of the offspring of a mother with diabetes (BG-R89C), suggesting a certain degree of insulin deficiency in utero. Initial insulin was required for those five patients and could never be stopped. At the last follow-up examination (age range 22 months to 9.8 years), insulin dose ranged from 0.42 to 0.70 units  $\cdot$  kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup> (median 0.60) with good metabolic control in each of the patients. These are relatively low insulin requirements, especially for the children and mother in family H, compared with what is required in type 1 diabetic patients in the same time frame (usually  $\sim$ 1 unit  $\cdot$  kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup>). Consistent with a partially preserved  $\beta$ -cell secretory function, C-peptide levels in those two children were detectable, and a normal response to a glucagon test was observed when they were 7.5 and 3.4 years of age, i.e., 3 and 1 years, respectively, after diabetes onset (Table 1). Interestingly, in patient HL, before the start of insulin therapy (at 4 years and 3 months), 2-h postprandial glucose level was moderately high (9 mmol/l) and insulinemia was high (126 mU/l), with a C-peptide level of 4.4 ng/ml, which proves an endogenous insulin secretion in this affected child at the age of 3 years and 5 months, an unusual situation in type 1 autoimmune diabetes.

Three patients (among whom a brother and a sister) have inherited the R89C mutation from their diabetic mother. In family H, the mother (HB) was discovered to have diabetes at 3 years of age and has a good metabolic control at age 38 years with a low dose of insulin ( $\sim$ 0.35 units  $\cdot$  kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup>). In contrast, the mother in family B, who was discovered at 3 months of age to have diabetes, had a poor metabolic control over the years and developed severe retinopathy, neuropathy, and macroangiopathy; at present (aged 35 years), she had to undergo amputation of both feet.

Clinically, two main differences were found by comparing in our cohort the children with an *INS* mutation and those with early-onset diabetes due to  $K_{ATP}$  channel mutations: the disease starts later with a median age of 8.5 months compared with 57 and 33 days in the cases with a mutation of *KCNJ11* or *ABCC8*, respectively (Table 2); no neuropsychological or neuromotor dysfunctions are present in children with an *INS* mutation in contrast to the children with a  $K_{ATP}$  channel mutation, where 6 of 17

TABLE 1  
Clinical characteristics of patients with permanent early-infancy diabetes who are carriers of an *INS* mutation

	Family F:		Family H			Family B		Family G:	
	Proband FZ	Proband HA	Sister HL	Mother	Proband BG	Mother	Proband GI		
Sex	F	M	F	F	F	F	F	M	
Ethnicity	European	European	European	European	European	European	European	Middle Eastern	
Mutation	A24D	R89C	R89C	R89C	R89C	R89C	R89C	C96Y	
At birth									
Weight (g/percentile)	2,080/<3	2,920/25	3,650/80	38	NA	NA	2,090/<3	3,000/10	
Gestation week	39	38.5	38	NA	NA	38	NA	41.5	
At presentation									
Age (months and years)	0.8 months	4 years, 3 months	2 years, 4 months	4 years	8.5 months	3 months	4.8 months		
Presentation	Ketoacidosis	Ketoaciduria	Polyuria/polydipsia	NA	Polyuria/polydipsia	NA	Polyuria/ polydipsia		
Glucose (mmol/l)	38.8	18.4	7.2	NA	49.5	NA	22.5		
Autoantibodies	0	0	0	NA	0	NA	0		
Pancreas ultrasonography	N	N	N	NA	N	NA	NA		
Neuropsychological assessment	N	N	N	N	N	N	N		
C-peptide (ng/ml)									
Basal	Undetectable	0.5	0.5	Undetectable	Undetectable	Undetectable	Undetectable	NA	
Under glucagon (peak level)*	NA	3.3	2.6	Undetectable	NA	NA	NA		
Current status									
Age (months and years)	22 months	9 years, 10 months	6 years, 1 month	38 years	6 years, 6 months	35 years	4 years, 10 months		
Weight (Kg)	12.3	25.5	19	51	23	54	17		
Type of insulin therapy	Pump	Injection	Injection	Injection	Pump	Injection	Pump		
Insulin dose (units · kg <sup>-1</sup> · day <sup>-1</sup> )	0.60	0.56	0.42	0.32	0.70	0.75	0.70		
A1C (%)†	7.3	6.5	6.7	7	7.5	7.1	NA		

\*Normal response: >150% over baseline. †Upper limit of normal values for A1C: 5.9%. N, normal; NA, not available.

TABLE 2  
Comparison of clinical characteristics of patients with diabetes caused by a mutation in *INS*, *KCNJ11*, or *ABCC8*

	Molecular genetic etiology			<i>P</i> *
	<i>INS</i>	<i>KCNJ11</i>	<i>ABCC8</i>	
<i>n</i>	5	18†	17‡	
Age at diagnosis (days, months, years)	8.5 months (25 days to 4.25 years)	57 days (1–127 days)	33 days (1–125 days)	<0.001
Gestational age (weeks)	38 (38–41.5)	39 (38–41)	40 (34–41)	NS
Birth weight (g)	2,920 (2,080–3,650)	2,710 (2,110–3,260)	3,040 (1,660–3,350)	NS
<3rd centile ( <i>n</i> )§	2	4	5	NS

Data are medians (range). \*Differences between groups calculated using Mann-Whitney and Fisher's exact tests. †The *KCNJ11* group includes 16 cases with PND and 2 presenting with TND. ‡The *ABCC8* group includes 2 cases with PND and 15 presenting with TND. §Number of patients.

patients with an *ABCC8*/*SUR1* mutation and 4 of 18 patients with a *KCNJ11*/*Kir6.2* mutation in our cohort do have such developmental anomalies. From our findings, diabetes caused by an *INS* mutation was found to be isolated in contrast to what has been reported in some patients previously described (12). Indeed, none of the patients from our cohort had acanthosis nigricans, a finding suggestive of insulin resistance, whereas four patients in the previous study have such a skin lesion.

In the current study, the three mutations identified were present in five children and two mothers with childhood-onset diabetes and not found in the transient form in accordance with the original report (12). In our cohort, the *INS* mutations account for 13% of neonatal diabetes cases where the genetic etiology was unknown and for ~10% of all permanent neonatal diabetes cases, compared with 4% for *ABCC8* and 35% for *KCNJ11* mutations (4,10; A.D., H.C., M.V., unpublished data). A higher prevalence of PND due to *INS* mutations was reported in the ISPAD cohort (~20%), which could be explained by more strict inclusion criteria in the French cohort, particularly age of diagnosis and presentation at diagnosis for the patients who were mostly referred as newborns. The exception of family H is of great importance, as children with an *INS* mutation can present clinically as type 1 diabetic patients. This is of special relevance, as the age of diagnosis of type 1 diabetes has decreased over the years with many children diagnosed before or around 4 years of age (14), and highlights the need of knowing the status for autoimmune markers in those children; if negative, then a monogenic form of diabetes should be searched for. Another biological feature that may help distinguish diabetes linked to *INS* mutations from autoimmune type 1 diabetes is a higher insulin level at presentation.

Highly variable penetrance and clinical presentation of diabetes, both within and between families, is indeed documented from our study cohort for the patients carrying an *INS* mutation. Furthermore, previous works have reported familial hyperproinsulinemia with normal to mild glucose intolerance in adults caused by *INS* mutations at residues R89P/H (15,16) and H34D (17). Cellular biology experiments are needed to further understand these contrasting disease phenotypes. A clinical variability in the age at onset of diabetes in early infancy was also reported in patients with known Wolcott-Rallison syndrome, another disease linked to endoplasmic reticulum stress (18). This severe condition is due to mutations in the *EIF2AK3* gene that encodes the pancreatic eukaryotic initiation factor 2 $\alpha$  kinase or PERK. Severe defects in fetal/neonatal  $\beta$ -cell proliferation and differentiation were observed in the PERK-deficient mice, which results in low  $\beta$ -cell mass,

defects in proinsulin trafficking, and abrogation of insulin secretion and culminate in PND (19).

A better understanding of the mechanism(s) leading to  $\beta$ -cell dysfunction in the patients with diabetes caused by an *INS* mutation will be crucial to define new treatments. As in the Akita mouse model, it may relate to the general concept of protein toxicity (20). The protein toxicity and overload in the endoplasmic reticulum are molecular defects already known to be involved in congenital diabetes insipidus, where the disease begins after the neonatal period. Indeed, this delay represents the time to destroy vasopressin-secreting neurons (21). The *INS* mutations associated with permanent early-infancy diabetes extend this concept demonstrated in diabetes insipidus to diabetes. Strategies to increase insulin secretion by upregulating the normal allele of the *INS* gene may be applicable, as long-term residual  $\beta$ -cell function may exist in some patients with diabetes due to an *INS* mutation.

In conclusion, heterozygous *INS* gene mutations are a cause of isolated permanent early-onset diabetes and should be searched for in neonatal as well as in childhood diabetes appearing like type 1, when autoimmune markers are absent, and when there is a strong autosomal dominant pattern of inheritance.

#### ACKNOWLEDGMENTS

This study was supported by the European Union (Integrated Project EuroDia LSHM-CT-2006-518153 in the Framework Programme 6 [FP6] of the European Community, to P.F.). We also acknowledge partial support from the French nonprofit associations Aide aux Jeunes Diabétiques (to M.P.) and Association Française des Diabétiques (to R.S.).

We thank all of the families for their participation in the study. We thank Julie Støy, Louis H. Philipson, and Graeme Bell for providing information before their publication; Sabrina Pereira and Marion Marchand for participation in the genetic and molecular studies; Dr. Chantal Metz who initially took care of the newborn ZF; Dr. Marie-Laure Anciaux who provided care to the mother in family H; and Prof. Sophie Christin-Maitre for providing updated information on the mother of proband B. We are very grateful to Prof. Paul Czernichow for his continuous support on the projects dedicated to neonatal diabetes and to Prof. Moshe Phillip for the cooperation between his group and the French ND Study Group.

#### REFERENCES

- Polak M, Cave H: Neonatal diabetes mellitus: a disease linked to multiple mechanisms. *Orphanet J Rare Dis* 2:12, 2007

2. Gardner RJ, Mackay DJ, Mungall AJ, Polychronakos C, Siebert R, Shield JP, Temple IK, Robinson DO: An imprinted locus associated with transient neonatal diabetes mellitus. *Hum Mol Genet* 9:589–596, 2000
3. Hattersley AT, Pearson ER: Pharmacogenetics and beyond: the interaction of therapeutic response, beta-cell physiology, and genetics in diabetes. *Endocrinology* 147:2657–2663, 2006
4. Babenko AP, Polak M, Cavé H, Busiah K, Czernichow P, Scharfmann R, Bryan J, Aguilar-Bryan L, Vaxillaire M, Froguel P: Activating mutations in the *ABCC8* gene in neonatal diabetes mellitus. *N Engl J Med* 355:456–466, 2006
5. Flechtner I, Vaxillaire M, Cavé H, Scharfmann R, Froguel P, Polak M: Diabetes in very young children and mutations in the insulin secreting cell potassium channel genes: therapeutic consequences. In *ESPE Developmental Series*. Karger, Basel, Switzerland, 2007, p. 86–98
6. Njolstad PR, Sovik O, Cuesta-Munoz A, Bjorkhaug L, Massa O, Barbetti F, Undlien DE, Shiota C, Magnuson MA, Molven A, Matschinsky FM, Bell GI: Neonatal diabetes mellitus due to complete glucokinase deficiency. *N Engl J Med* 344:1588–1592, 2001
7. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF: Pancreatic agenesis attributable to a single nucleotide deletion in the human *IPF1* gene coding sequence. *Nat Genet* 15:106–110, 1997
8. Vaxillaire M, Dechaume A, Busiah K, Cavé H, Pereira S, Scharfmann R, Perez de Nanclares G, Castano L, Froguel P, Polak M: New *ABCC8* mutations in relapsing neonatal diabetes and clinical features. *Diabetes* 56:1737–1741, 2007
9. Flanagan SE, Patch AM, Mackay DJ, Edghill EL, Gloyn AL, Robinson D, Shield JP, Temple K, Ellard S, Hattersley AT: Mutations in ATP-sensitive K<sup>+</sup> channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. *Diabetes* 56:1930–1937, 2007
10. Vaxillaire M, Populaire C, Busiah K, Cavé H, Gloyn AL, Hattersley AT, Czernichow P, Froguel P, Polak M: Kir6.2 mutations are a common cause of permanent neonatal diabetes in a large cohort of French patients. *Diabetes* 53:2719–2722, 2004
11. Flanagan SE, Edghill EL, Gloyn AL, Ellard S, Hattersley AT: Mutations in *KCNJ11*, which encodes Kir6.2, are a common cause of diabetes diagnosed in the first 6 months of life, with the phenotype determined by genotype. *Diabetologia* 49:1190–1197, 2006
12. Stoy J, Edghill EL, Flanagan SE, Ye H, Paz VP, Pluzhnikov A, Below JE, Hayes MG, Cox NJ, Lipkind GM, Lipton RB, Greeley SA, Patch AM, Ellard S, Steiner DF, Hattersley AT, Philipson LH, Bell GI, for the Neonatal Diabetes International Collaborative Group: Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci U S A* 104:15040–15044, 2007
13. Izumi T, Yokota-Hashimoto H, Zhao S, Wang J, Halban PA, Takeuchi T: Dominant negative pathogenesis by mutant proinsulin in the Akita diabetic mouse. *Diabetes* 52:409–416, 2003
14. Knerr I, Wolf J, Reinehr T, Stachow R, Grabert M, Schober E, Rascher W, Holl RW: The accelerator hypothesis: relationship between weight, height, body mass index and age at diagnosis in a large cohort of 9248 German and Austrian children with type 1 diabetes mellitus. *Diabetologia* 48:2501–2504, 2005
15. Warren-Perry MG, Manley SE, Ostrega D, Polonsky K, Mussett S, Brown P, Turner RC: A novel point mutation in the insulin gene giving rise to hyperproinsulinemia. *J Clin Endocrinol Metab* 82:1629–1631, 1997
16. Collinet M, Berthelon M, Bénit P, Laborde K, Desbuquois B, Munnich A, Robert JJ: Familial hyperproinsulinaemia due to a mutation substituting histidine for arginine at position 65 in proinsulin: identification of the mutation by restriction enzyme mapping. *Eur J Pediatr* 157:456–460, 1998
17. Chan SJ, Seino S, Gruppuso PA, Schwartz R, Steiner DF: A mutation in the B chain coding region is associated with impaired proinsulin conversion in a family with hyperproinsulinemia. *Proc Natl Acad Sci U S A* 84:2194–2197, 1987
18. Senev V, Vattem KM, Delepine M, Rainbow LA, Haton C, Lecoq A, Shaw NJ, Robert JJ, Rooman R, Diatloff-Zito C, Michaud JL, Bin-Abbas B, Taha D, Zabel B, Franceschini P, Topaloglu AK, Lathrop GM, Barrett TG, Nicolino M, Wek RC, Julier C: Wolcott-Rallison Syndrome: clinical, genetic, and functional study of EIF2AK3 mutations and suggestion of genetic heterogeneity. *Diabetes* 53:1876–1883, 2004
19. Zhang W, Feng D, Li Y, Iida K, McGrath B, Cavener DR: PERK EIF2AK3 control of pancreatic beta cell differentiation and proliferation is required for postnatal glucose homeostasis. *Cell Metab* 4:491–497, 2006
20. Ron D: Proteotoxicity in the endoplasmic reticulum: lessons from the Akita diabetic mouse. *J Clin Invest* 109:443–445, 2002
21. Russell TA, Ito M, Ito M, Yu RN, Martinson FA, Weiss J, Jameson JL: A murine model of autosomal dominant neurohypophyseal diabetes insipidus reveals progressive loss of vasopressin-producing neurons. *J Clin Invest* 112:1697–1706, 2003