Mutations in the Insulin Gene Can Cause MODY and Autoantibody-Negative Type 1 Diabetes

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OBJECTIVE—Mutations in the insulin (*INS*) gene can cause neonatal diabetes. We hypothesized that mutations in *INS* could also cause maturity-onset diabetes of the young (MODY) and autoantibody-negative type 1 diabetes.

RESEARCH DESIGN AND METHODS—We screened *INS* in 62 probands with MODY, 30 probands with suspected MODY, and 223 subjects from the Norwegian Childhood Diabetes Registry selected on the basis of autoantibody negativity or family history of diabetes.

RESULTS—Among the MODY patients, we identified the *INS* mutation c.137G>A (R46Q) in a proband, his diabetic father, and a paternal aunt. They were diagnosed with diabetes at 20, 18, and 17 years of age, respectively, and are treated with small doses of insulin or diet only. In type 1 diabetic patients, we found the *INS* mutation c.163C>T (R55C) in a girl who at 10 years of age presented with ketoacidosis and insulin-dependent, GAD, and insulinoma-associated antigen-2 (IA-2) antibody-negative diabetes. Her mother had a de novo R55C mutation and was diagnosed with ketoacidosis and insulin-dependent diabetes at 13 years of age. Both had residual β-cell function. The R46Q substitution changes an invariant arginine residue in position B22, which forms a hydrogen bond with the glutamate at A17, stabilizing the insulin molecule. The R55C substitution involves the first of the two arginine residues localized at the site of proteolytic processing between the B-chain and the C-peptide.

CONCLUSIONS—Our findings extend the phenotype of *INS* mutation carriers and suggest that *INS* screening is warranted not only in neonatal diabetes, but also in MODY and in selected cases of type 1 diabetes. *Diabetes* 57:1131–1135, 2008

M olecular genetic studies of monogenic forms of diabetes such as maturity-onset diabetes of the young (MODY) and neonatal diabetes have provided important insight into the pathophysiology and have led to improved diagnosis and treatment (1–7). In type 1 diabetes, immune-mediated destruction of the pancreatic β-cells plays an important role in the pathogenesis (8). However, some type 1 diabetic children do not present with signs of autoimmunity and are classified as having autoantibody-negative type 1 diabetes, also denoted idiopathic or type 1b diabetes (9–11). Recently, we observed that heterozygous missense mutations in the insulin gene (*INS*) can cause permanent neonatal diabetes (12). The majority of these mutations occurred de novo. Moreover, this phenomenon has been noted in previous studies of *KCNJ11* and *ABCC8* in patients with neonatal diabetes and is in accordance with the sporadic nature of permanent neonatal diabetes.

We hypothesized that *INS* mutations might also cause MODY and could explain some cases of apparent type 1 diabetes. The aim of the present study was therefore to search for *INS* mutations in patients with MODY of unknown etiology as well as in selected patients from the Norwegian Childhood Diabetes Registry.

RESEARCH DESIGN AND METHODS

Physicians refer subjects to the Norwegian MODY Registry based on at least two of the following criteria: first-degree relative with diabetes, onset of diabetes before 25 years of age in at least one family member, insulin level <0.5 units $\cdot$ kg^{-1} $\cdot$ day^{-1}, diabetes diagnosed between age 25 and 40 years of age, or unusual type 1 diabetes (low-dose insulin requirement, no antibodies, or atypical history). The conventional criteria of MODY (13) are therefore not met in all cases. Still, inclusion of subjects based strictly on the conventional criteria would exclude some true MODY patients, e.g., those with de novo mutations, age at diagnosis >25 years, or limited clinical data on the family history of diabetes. We screened DNA samples from 92 probands of the Norwegian MODY Registry for mutations in *INS*; 62 fulfilled conventional MODY criteria, while 30 were categorized as "suspected MODY." None of the probands had mutations in *HNF1A* (14). Moreover, 57 of the probands had a phenotype clinically evaluated as MODY2-like. *GCK* mutations had therefore been excluded in them. Standard oral glucose tolerance testing was performed, and World Health Organization criteria for diabetes were applied.

In addition, we investigated samples from the population-based Norwegian Childhood Diabetes Registry (15). From June 2002 to June 2007, 1,373 subjects were eligible and enrolled. We excluded subjects with mutations in *HNF1A* or *KCNJ11* and one subject with diabetes secondary to pancreatectomy. We then chose to screen two subsets of subjects in the present study. The first consisted of patients who were GAD and insulinoma-associated antigen-2

(IA-2) antibody negative, with or without a family history of diabetes ($n = 124$). The second subset consisted of GAD and/or IA-2 antibody-positive patients with at least one parent with diabetes ($n = 99$). Antibodies were measured the day after diagnosis. We used the following cut offs to define antibody status as negative: GAD <0.08 units and IA-2 <0.1 units. Diabetes in the parents included all types of diabetes. Thus, we sequenced *INS* in a total of 223 subjects regarded as having type 1 diabetes. The reference ranges for fasting C-peptide were 220–1,400 pmol/l for subjects in the MODY family N580 and 400–1,700 pmol/l for subjects in the type 1 diabetes family N781.

We obtained written informed consent from all participants or their parents. The study was approved by the Regional Committee for Research Ethics and the Norwegian Data Inspectorate and performed according to the Helsinki Declaration.

Genotyping. DNA was purified from EDTA blood samples by standard methods. Human *INS* was amplified in two segments using PCR and the primers 5′-CAAGGGCCTTTGCGTCA-3′ together with 5′-GAAGCCAACAC-CGTCCTCA-3′ (exon 2) and 5′-CCCTGACTGTGTCCTCCTGT-3′ together with 5′-AGAGAGCGTGGAGAGAGCTG-3′ (exon 3). The exon and flanking noncoding regions of *INS* were sequenced in both directions using an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). We imported all sequence sample files into the SeqScape Software (Applied Biosystems) and analyzed them for variation in *INS*. Template sequence applied for *INS* was NM_000207 (NCBI database).

RESULTS

INS mutations and MODY. We did not find any pathogenic mutations in the 30 subjects with suspected MODY. We did, however, find a heterozygous mutation in 1 of the 62 families fulfilling conventional MODY criteria (Fig. 1*A* and Table 1). The mutation c.137G>A is predicted to alter arginine to glutamine at residue number 46 (R46Q) of the preproinsulin molecule (Fig. 2). The proband (N580-1) was diagnosed with diabetes at 20 years of age. Initially he was treated with diet only. After 1 year, he needed psychopharmacologic treatment, his BMI increased to 29.6 kg/m², and he required insulin. Subsequently, his psychopharmacologic treatment was changed, he lost weight, and he is now on diet only. N580-1 was GAD and IA-2 antibody negative; his nonfasting C-peptide was undetectable, and his most recent A1C was 5.9% (normal range 4.0–6.0%). His father (N580-3) was diagnosed with diabetes at 18 years of age; he was initially treated with diet only, and after ~20 years a sulfonylurea was introduced. In later years, he has been treated with small doses of insulin. The proband's paternal aunt (N580-4) was diagnosed with diabetes at 17 years of age and has been on a strict diet without need for pharmacological treatment.

INS mutations and type 1 diabetes. Nearly all Norwegian subjects diagnosed with diabetes at age 18 years or under are included in the Norwegian Childhood Diabetes Registry. We first screened *INS* in a group of 124 antibody-negative cases and found a heterozygous mutation in 1 proband. The mutation, c.163C>T, is predicted to cause an arginine to cysteine substitution at residue 55 (R55C) of the preproinsulin molecule (Fig. 2). We subsequently sequenced a further 99 subjects with antibody-positive type 1 diabetes and a parental history of diabetes but identified no further mutations.

The subject N781-1 with the R55C mutation presented with frank diabetes at 10 years of age (Fig. 1*B* and Table 1). She had a blood glucose of 17.6 mmol/l and ketoacidosis, and her A1C was 9.1% (normal range 4.0–6.2%). Autoantibodies against insulin were 5.8 units/ml (normal range <1.0), while fasting C-peptide was detectable (500 pmol/l). She was insulin dependent from the time of diagnosis. Her most recent insulin requirement and A1C were 0.72 units · kg⁻¹ · day⁻¹ and 8.0%, respectively. A recent meal-stimulated C-peptide was detectable (1,050 pmol/l, paired glucose 9.6 mmol/l). Recent autoantibodies

against insulin were positive (6.3 units/ml), while GAD and IA-2 were negative. Her mother (N781-3) had type 1 diabetes diagnosed at 13 years of age (Table 1). She is currently treated with insulin (0.96 units · kg⁻¹ · day⁻¹). Recent meal-stimulated C-peptide was barely detectable (420 pmol/l, paired glucose 11.1 mmol/l). Recent autoantibodies against insulin were positive (5.8 units/ml), while GAD and IA-2 were negative. She also carries the heterozygous mutation. The proband's maternal grandfather (N781-6) had type 2 diabetes diagnosed at 40 years of age. He is treated with insulin (0.47 units · kg⁻¹ · day⁻¹), and his most recent A1C was 6.4%. His current BMI is 42.7 kg/m²,
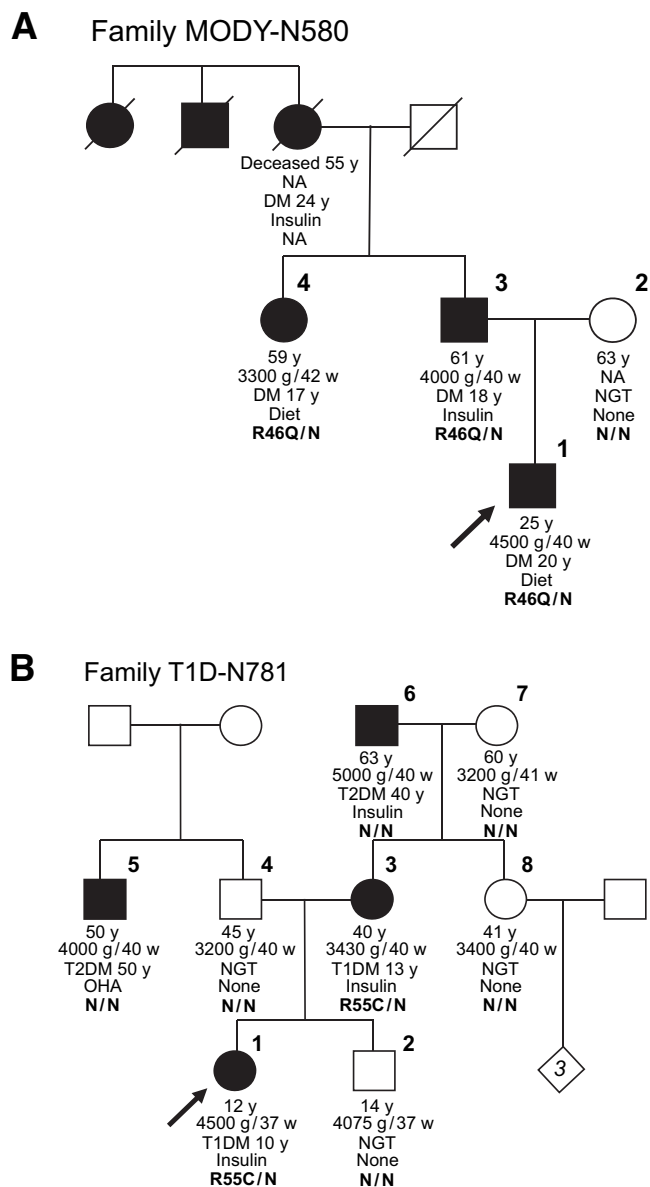
FIG. 1. Mutations in *INS* can cause MODY and type 1 diabetes. *A*: Pedigree of a family with MODY due to the mutation R46Q. The three cases of diabetes in the first generation were unavailable for genetic analysis, but limited clinical information could be obtained for one of them. *B*: Pedigree of a family with antibody-negative type 1 diabetes and the mutation R55C. For both pedigrees, current age, birth weight/gestational age, age of diagnosis, current treatment, and mutation status are listed. Subjects with diabetes are shown in black. Females are represented by circles and males by squares. The probands are marked by arrows. DM, diabetes mellitus; NA, not available; NGT, normal glucose tolerance; OHA, oral hypoglycemic agents; T1DM, type 1 diabetes; T2DM, type 2 diabetes.

TABLE 1
Clinical characteristics of the subjects with the *INS* mutations R46Q and R55C

	Family				
	MODY-N580			T1D-N781	
Subject	N580–1	N580–3	N580–4	N781–1	N781–3
General characteristics					
INS mutation	R46Q	R46Q	R46Q	R55C	R55C
Sex	M	M	F	F	F
Current age (years)	25	61	59	12	40
Centile for birth weight (SD score)	+1.5	+1	−1.5	>+2	+0
Onset of diabetes					
Age (years)	20	18	17	10	13
Clinical manifestation	Hyperglycemia	Glucosuria	Glucosuria	Hyperglycemia, ketoacidosis	Hyperglycemia, ketoacidosis
Recent status					
BMI (kg/m^2)	23.9	25.0	18.1	24.6	37.6
A1C (%)	5.9	6.0	6.0	8.0	8.9
Insulin dose (units · kg^{-1} · day^{-1})	None (diet treated)	0.25	None (diet treated)	0.72	0.96
Other	Bipolar disorder	Neuropathy Hypertension	—	—	—

and he has nephropathy, retinopathy, and neuropathy. Neither he nor the healthy maternal grandmother are mutation carriers. Thus, the proband's mother has a de novo mutation. The paternal uncle (N781-5) was diagnosed with type 2 diabetes at 50 years of age. He is treated with glimepiride (2 mg/day); his most recent A1C was 7.3%, and BMI was 21.4 kg/m^2. He is not carrying the mutation.

The pathogenic role of the INS mutations R46Q and R55C. We did not detect either mutation among 100 healthy blood donors. Neither mutation has been de-scribed previously (12,16). The mutation R46Q alters an invariant arginine at residue 22 of the B-chain. The guanidino group of arginine forms a hydrogen bond with the glutamate at residue 17 of the A-chain and participates in a network of electrostatic interactions with surrounding carbonyl and carboxyl oxygens, which stabilizes the structure of the insulin molecule (Fig. 3). The substitution of arginine B22 by glutamine will disrupt this critical hydrogen bond. The mutation R55C affects the first of the two arginines at the B-chain—C-peptide junction, i.e., the first site of proteolytic processing of proinsulin to insulin. The
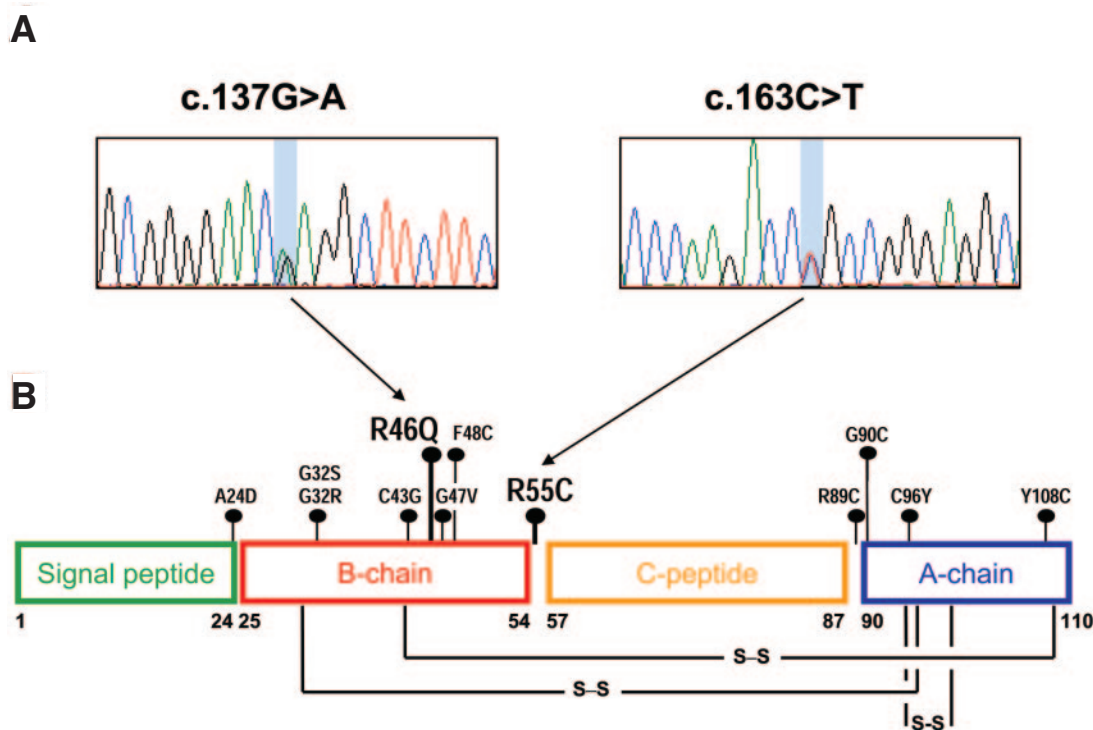

FIG. 2. *A*: DNA sequences of the *INS* mutations c.137G>A (R46Q) and c.163C>T (R55C) found in the Norwegian MODY Registry and the Norwegian Childhood Diabetes Registry, respectively. *B*: Location of the two corresponding amino acid substitutions in the preproinsulin molecule. The 10 mutations identified by Støy et al. (12) are shown in smaller font. Amino acid numbers below the bars show the extension of each peptide fragment in preproinsulin. Note that the amino acids 55/56 and 88/89 form the recognition sites for the proteolytic removal of the C-peptide but are not part of the mature insulin molecule. "S-S" indicates disulfide bridge.
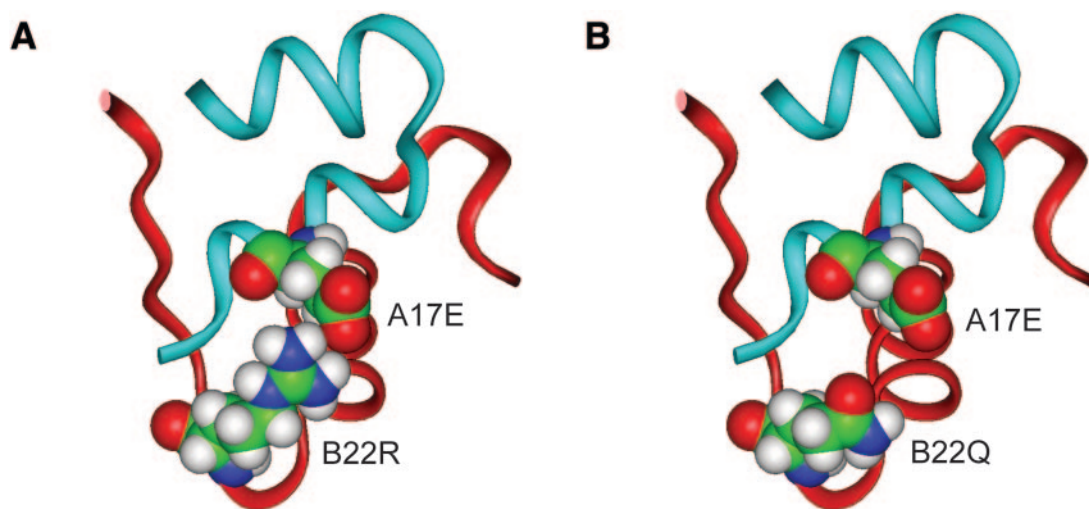
FIG. 3. Predicted effect of the R46Q mutation on structural stability of the insulin molecule. *A*: The native structure of insulin, shown by space-filled image, where the side chain of arginine B22 (B22R) forms a hydrogen bond with the side chain of glutamate A17 (A17E). This hydrogen bond stabilizes the COOH-terminal ends of the A- and B-chains (shown by red and blue ribbons, respectively). *B*: Effect of mutating the arginine to glutamine at B22 (B22Q). Substitution of the long side chain of arginine by the 4.5 Å shorter side chain of glutamine disrupts the formation of a hydrogen bond between residues B22 and A17. B22R is invariant, while A17 tolerates only two stereochemically equivalent amino acid residues, glutamate and glutamine, both of which allow the hydrogen bond between B22 and A17.

substitution of arginine with a neutral residue (in this case cysteine) is not predicted to interfere with the proteolytic processing by proinsulin endoprotease PC1/3. It is thus more likely that the introduction of an unpaired cysteine may affect insulin biosynthesis, as noted for C96Y, the mutant insulin in the Akita mouse, by introducing a defect in folding of the preproinsulin molecule (12,17). Both carriers of the R55C mutation have C-peptide levels in the normal range, thus suggesting that some insulin is being processed and secreted. It is currently not fully understood why these patients, despite evidence of insulin secretion, have severe insulin deficiency, as indicated by ketoacidosis at diagnosis and subsequent requirement for insulin in full replacement doses.

DISCUSSION

We have found that mutations in the gene encoding insulin can cause MODY and antibody-negative type 1 diabetes. Our findings add *INS* to the list of causes of MODY, which currently includes *HNF4A*, *GCK*, *HNF1A*, *IPF1*, *HNF1B*, *NEUROD1*, and *CEL*. The relatively mild phenotype of the three family members with the R46Q mutation suggests that a spectrum of phenotypes may exist in patients with *INS* mutations, ranging from mild diabetes and hyperinsulinemia in patients with the previously described mutations that cause reduced biological activity of the insulin molecule (i.e., B24 Ser, B25 Leu, and A3 Leu) (18,19), to MODY in patients with mutations that are predicted to reduce the structural stability of the insulin molecule (R46Q) and ultimately to neonatal diabetes in patients with mutations that cause severe defects in the biosynthesis of the insulin molecule (for example B8 Ser and B19 Gly) (12).

One could argue that the case with apparent type 1 diabetes (R55C) was MODY that was misclassified. The presentation, however, was like classical type 1 diabetes, with ketoacidosis and frank diabetes. Hence, we believe that most pediatricians on a clinical basis will classify such a patient as having type 1 diabetes. Not all clinics are routinely screening children with newly developed diabetes for antibodies. Although rare, we nevertheless think it

is interesting that patients with a monogenic form of diabetes can be found among those with a diagnosis of type 1 diabetes, an observation that has important implications for diagnosis, genetic counseling, and possibly treatment.

Generally, subjects with neonatal diabetes and *INS* mutations are small for gestational age (12,16). None of our five mutation-positive subjects had low birth weights (Fig. 1). The R46Q mutation of family N580 appears to be functionally mild compared with the *INS* mutations causing neonatal diabetes, as suggested by the much later age of onset, a low A1C, and less-intensive treatment needed. The effect of R46Q on fetal insulin secretion may therefore be negligible, explaining the lack of effect on birth weight. In family N781, the diabetic mother with a de novo mutation had a birth weight in the lower normal range. The relatively high birth weight of her R55C-carrying child can be explained by the mother being diabetic during pregnancy and a near-normal insulin secretion capacity in fetal life. As for R46Q, the age of onset suggests that the phenotype of R55C is milder than that of *INS* mutations causing neonatal diabetes.

Although 80% of the *INS* cases found in patients with neonatal diabetes are de novo, both the probands described here inherited the mutation from a diabetic parent. Thus, our findings as well as those of Edghill et al. (16) indicate that de novo mutations in the *INS* gene are possible when diabetes presents after the neonatal period.

In summary, our results suggest that patients with MODY and autoantibody-negative type 1 diabetes should be screened for mutations in *INS*. The presence of residual β-cell function in the subjects with apparent type 1 diabetes indicates that new approaches for treatment should be considered in such cases with *INS* mutations.

APPENDIX

Other members of the Norwegian Childhood Diabetes Study Group

The following physicians also contributed to the study: Henning Aabech and Sven Simonsen, Fredrikstad; Helge Vogt, Lørenskog; Kolbeinn Gudmundsson, Anne Grethe

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