Insulin Gene Mutations as Cause of Diabetes in Children Negative for Five Type 1 Diabetes Autoantibodies

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OBJECTIVE — Heterozygous, gain-of-function mutations of the insulin gene can cause permanent diabetes with onset ranging from the neonatal period through adulthood. The aim of our study was to screen for the insulin gene in patients who had been clinically classified as type 1 diabetic but who tested negative for type 1 diabetes autoantibodies.

RESEARCH DESIGN AND METHODS — We reviewed the clinical records of 326 patients with the diagnosis of type 1 diabetes and identified seven probands who had diabetes in isolation and were negative for five type 1 diabetes autoantibodies. We sequenced the *INS* gene in these seven patients.

RESULTS — In two patients whose diabetes onset had been at 2 years 10 months of age and at 6 years 8 months of age, respectively, we identified the mutation $G^{B8}S$ and a novel mutation in the preproinsulin signal peptide ($A^{Signal23}S$).

CONCLUSIONS — Insulin gene mutations are rare in absolute terms in patients classified as type 1 diabetic (0.6%) but can be identified after a thorough screening of type 1 diabetes autoantibodies.

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Mutations of the insulin (*INS*) gene associated with neonatal- and infancy-onset diabetes cause sustained stress of the endoplasmic reticulum, which in turn triggers apoptosis of the pancreatic β-cell (1). In patients with insulin mutations with proteotoxic effect, diabetes presents in isolation and the onset of hyperglycemia may occur well outside the neonatal period (1–4). As a consequence, individuals with *INS* gene mutations may be confused with patients having autoimmune type 1 diabetes (1–4).

RESEARCH DESIGN AND METHODS

— We reviewed the clinical records of 326 patients with the diagnosis of diabetes (age range at diagnosis 1–18 years), each consecutively referred to the pediatric diabetes clinic at San Raffaele Hospital during the years 2003–2006. Among these 326, we identified 24 patients who were negative for all the common type 1 diabetes autoantibodies (islet cell antibody [ICA], GAD antibody [GADA], IA-2 antigen [IA-2A], and insulin antibody [IAA]) at the time of diagnosis. The cutoffs (in arbitrary units) were GADA <3, IA-2A <1, and IAA <5; the threshold for positivity in each assay corresponds with 99th percentiles of 200 control subjects with normal glucose tolerance. From the 24 patients negative for autoantibodies, we excluded 4 patients with adolescent type 2 diabetes (Table 1). Among the remaining 20 patients, we selected those with diabetes in isolation, who were tested for the novel type 1 diabetes autoantibody against Zn transporter 8 (ZnT8A; cutoff in arbitrary units <12) (5). Seven patients who were negative for all antibodies were analyzed for insulin gene mutations by DNA direct sequencing, along with four $ZnT8A^+$ patients (control subjects). When appropriate, mutations found were designated according to their position in the mature insulin chains (1).

RESULTS

— In two patients, we detected a heterozygous missense mutation of the *INS* gene: the already described $G^{B8}S$ (or G32S) (2,3) and a novel mutation resulting in a serine for an alanine in the 23rd amino acid of the preproinsulin molecule $A^{Signal23}S$. Both mutations were confirmed by digestion with the appropriate restriction enzyme. DNA sequencing of the *INS* gene of the probands' parents showed a normal sequence (i.e., the mutations arose as spontaneous mutations). No mutation was found in 200 control subjects with normal glucose tolerance or in the $ZnT8A^+$ patients.

The child with the $G^{B8}S$ mutation was born after an uneventful pregnancy (39 weeks of gestation) with a birth weight of 2,770 g (10th centile). At onset of diabetes, he was 2 years 10 months old and lean (BMI 16 kg/m^2, 25th centile for corresponding age) and showed a detectable C-peptide (0.49 ng/ml) that was low, but still measurable 2 years after diagnosis (0.34 ng/ml). Presently, he is 6 years old, his insulin dose is 0.7 units $\cdot$ kg^{-1} $\cdot$ day^{-1}, and his A1C is 8.7% (normal reference <6%).

The individual with the $A^{Signal23}S$ mutation (birth weight 3,350 g, 25–50th centile) presented with typical symptoms of diabetes (polyuria and polydipsia) when he was 6 years 8 months old (A1C 11% at diabetes onset). He was lean (BMI

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Table 1—*Clinical criteria used for classification of new cases of diabetes with onset during childhood or adolescence*

	Type 1 diabetes	Type 2 diabetes	Monogenic diabetes in isolation	HNF1-β (MODY 5)	Wolfram syndrome	Post-CMV infection
n	309	4	7	2	3	1
Type 1 diabetes autoantibodies (ICA, GADA, IA-2A, IAA, ZnT8)*	≥1	0	0	0	0	0
BMI	Not considered	>90th centile	Normal, low	Normal, low	Normal, low	Normal, low
C-peptide (ng/ml)	Low, undetectable	>1.5†	Low, normal, high	<1.5	<1.5	<1.5
Age range at diagnosis of diabetes	1–18 years	12–15 years	2 years 7 months–15 years 4 months	12–14 years	10–14 years	13 years
Features other than diabetes	None	Hypertension, dislipidemia	None	Renal disease, pancreas hypoplasia at ultrasound or NMR, abnormal liver enzymes	Optic atrophy, diabetes insipidus, deafness	Documented perinatal CMV infection, growth retardation, deafness

*Four patients were negative for ICA, GADA, IA-2A, and IAA but positive for ZnT8A. †At least two determinations 6 months apart. CMV, cytomegalovirus; MODY, maturity onset diabetes of the young; NMR, nuclear magnetic resonance.

16.4 kg/m^2, 50th centile). Insulin was started and continued for 6 months; during the following 2 years, the patient went off and on insulin several times (a pattern that may resemble the so-called honeymoon phase of type 1 diabetes). His C-peptide levels, measured 11 and 24 months after onset of hyperglycemia, were 1.32 and 0.7 ng/ml, respectively. He is now 10 years old, his insulin dose is 0.17 units · kg^{-1} · day^{-1}, and his A1C is 6.4%.

CONCLUSIONS — Previously, heterozygous *INS* gene mutations had been detected in adult patients with so-called familial hyperinsulinemia or hyperproinsulinemia who presented with variable phenotypes (mild diabetes or even hypoglycemia) and high serum levels of radioimmunoassayable insulin or proinsulin-like material. More recently, *INS* mutations have been found to be associated with neonatal- and infancy-onset diabetes (1–3). We demonstrated that mutant insulins with proteotoxic effect cannot be secreted when expressed in HEK 293 cell line (1), and it is likely that S^{B8} and $S^{Signal23}$ are also retained in the endoplasmic reticulum. Nevertheless, the patient bearing the mutation in the signal peptide shows a milder clinical course, and we cannot exclude that $S^{Signal23}$ preproinsulin may be partially processed and secreted. Present knowledge indicates that insulin mutations with a proteotoxic effect cause apoptosis of the pancreatic β-cell (1), a process that in most patients takes several months after birth (1–3) or, in some individuals, years (1–4 and this report). Of note, only six patients among those reported in the articles by Støy et al., Edghill et al., Molven et al., and Colombo et al. (1–4) were diagnosed within the first 4 weeks of birth (i.e., the time interval still in use to define the neonatal period); most of them (more than 40) were diagnosed in the first year of life (infancy). Thus, we believe that classifying these patients as having permanent neonatal diabetes is misleading and that this term should be abandoned in favor of the term "monogenic diabetes of infancy," as previously suggested by our group (1). Indeed, at least 12 patients with insulin gene mutations had the diagnosis of diabetes during childhood or adulthood (1–4 and this report), making the neonatal onset an exception.

The Italian proband bearing the $G^{B8}S$ (G32S) mutation had the diagnosis of diabetes at ~3 years of age, ~2 years later

than patients carrying the same mutation as described by Støy et al. (2). Presently, it is not clear why patients with the same *INS* gene mutation, even from the same family, can present with diabetes during infancy, childhood, or adulthood (1–4). It is tempting to speculate that the apoptotic process in some of these patients may be modulated or slowed by the individual's capacity to degrade misfolded insulin (by a process known as endoplasmic reticulum–associated degradation). Another intriguing hypothesis, not mutually exclusive with the previous one, could be that β-cell regeneration may take place in some individuals and not in others. The observation that in these two patients (and in others previously described) (1) insulin secretion was still detectable 2 years after onset of diabetes suggests that either of these mechanisms could be at work.

In conclusion, insulin gene mutations are rare in absolute terms among patients clinically classified as type 1 diabetic (2 of 326 or 0.6%) but can be identified after a thorough screening of type 1 diabetes autoantibodies.

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