decreased attentional functioning is the central cognitive problem, observed as a slower functioning on tests of simple and choice reaction time and the digit symbol task and decreased performance on the forward digit span (4–6). To test the hypothesis that recurrent severe hypoglycemia results in decreased attentional functioning, we examined cognitive functioning in nine adult male type 1 diabetic patients with a history of recurrent (>5 episodes) severe hypoglycemia (HH), nine adult male type 1 diabetic patients with no history of severe hypoglycemia (NH), and nine matched healthy control subjects. Patients were recruited from the diabetes outpatient clinic of our hospital (mean age 36.9 ± 6.7 years). To rule out potential effects of age-related intellectual decline, patients older than 50 years were not included. Likewise, to avoid confounding effects of hypoglycemia on the developing brain, patients who were included had diabetes onset after the age of 17 years. In addition, we ensured that none of the patients included suffered from secondary complications, nor from previous head injury, epilepsy, cerebrovascular disease, alcohol abuse, or psychiatric illness. History of severe hypoglycemia was obtained by self-report, asking patients how often they had suffered severe hypoglycemia, which was defined as "not having been able to recover from the hypoglycemia without the assistance of others." The three groups were comparable for mean age, educational level, intelligence (7), and depression scores (Well-Being Questionnaire) (8). The NH and HH groups were comparable for mean diabetes duration (14 and 13.4 years, respectively), age at onset, and glycemic control (HbA_{1c} 7.8 and 7.4%, respectively). Between-group comparison revealed no significant differences in mean blood glucose levels at testing (all in the nonhypoglycemic range). Patients and control subjects were tested neuropsychologically using two measures of attention. First, they were tested with the Bourdon Inhibition Test (BIT), a two-choice reaction-time vigilance test (9) that allows a subject's reaction time performance to be described in terms of speed (slowness) of processing, concentration, and errors. Second, a modified version of the Wechsler Adult Intelligence Scale (WAIS) digit span subtest was administered, where three (instead of two) series of the same length were read aloud to the subjects, thereby minimizing floor effects and allowing for separate interpretation of the forward and backward condition (10). Between-group analyses were performed using *t* tests for independent samples and one-way analysis of variance.

No significant differences ($P < 0.05$) were found between the two patient groups on any of the outcome measures, nor did we find any significant differences in cognitive performance between diabetic patients as a group and the healthy control subjects. Although the data were derived from a relatively small sample, they strongly suggest that a history of severe hypoglycemia is not inevitably associated with attentional dysfunction, at least not in otherwise healthy male patients with late-onset type 1 diabetes. We should, of course, bear in mind that attention is a highly complex process, incorporating elements of focused, sustained, selective, and divided attention across visual and auditory/verbal modalities. We cannot exclude the possibility that other cognitive tests may have shown different results. However, we did examine different aspects of attention (i.e., vigilance, speed, concentration) and related functions (short-term and working memory), and mean scores for the groups were strikingly comparable. To substantiate our findings, replication in a larger prospective study is warranted, with careful documentation of both frequency and intensity of severe hypoglycemic episodes.

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A Novel Missense Mutation in the Homeodomain of the Hepatocyte Nuclear Factor-1α/ Maturity-Onset Diabetes of the Young 3 in a Japanese Early-Onset Type 2 Diabetic Patient and Time-Course of Glucose-Stimulated Insulin Secretion

Recently, mutations in the gene encoding the hepatocyte nuclear factor (HNF)-1α, a homeodomain-containing transcription factor, were shown to be a cause of maturity-onset diabetes of the

Table 1—*OGTT profiles of the proband and the father*

Subject	Age Years	Age Months	BMI (kg/m²)	Glucose (mmol/l) 0	30	60	90	120	180	Insulin (pmol/l) 0	30	60	90	120	180	ΣIRI (pmol/l)
Proband	14	6	18.9	6.6	13.8	15.7	15.2	12.3	7.3	60	198	222	210	162	66	918
	15	3	19.3	8.4	17.6	21.9	22.7	24.6	17.7	54	210	174	186	198	156	978
	15	8	19.7	6.3	13.7	18.2	17.1	15.7	12.4	36	192	234	204	216	156	1,038
	17	4	21.1	4.9	9.4	10.6	9.6	10.7	7.8	42	180	222	222	234	114	1,014
	20	3	22.8	7.8	18.4	22.7	26.3	27.8	20.4	48	144	132	144	132	102	702
	22	2	19.5	8.7	16.2	23.6	26.0	23.9	22.6	48	84	90	84	60	54	420
Father	47	4	22.3	9.7	25.3	24.8	19.4	15.2	12.0	0*	36	54	36	18	0*	144

Standard OGTTs were performed; 100 g glucose was loaded in each OGTT except for 50 g glucose in the first OGTT of the proband. Measured plasma glucose and serum insulin concentrations are shown with age, BMI, and sum of insulin values (ΣIRI). *Below the sensitivity level of the assay (<6 pmol/l).

young (MODY) (1,2). Furthermore, mutations in the HNF-1α gene were frequently found in early-onset type 2 diabetic patients (2,3) and also in Japanese type 1 diabetic patients (4). We screened the HNF-1α gene in early-onset (<35 years) Japanese diabetic subjects with positive family histories of diabetes in first-degree relatives. The 10 exons, with flanking introns, and the promoter region were amplified by polymerase chain reaction (PCR), and then the PCR products were directly sequenced as described (2). Among 12 type 1 and 12 type 2 diabetic patients, one novel missense mutation (Arg-203-His; R203H) was identified heterozygously in a type 2 diabetic patient. This mutation was absent in 74 unrelated healthy subjects (148 chromosomes) and in an additional 77 type 2 diabetic patients (154 chromosomes).

The patient was a 33-year-old woman who was not obese; BMI at the diagnosis and maximal BMI were 18.9 and 22.8 kg/m², respectively. Glycosuria was first detected by an annual school health examination at age 14 years, and the patient's diabetes was diagnosed soon by oral glucose tolerance test (OGTT). Her insulin secretion was substantially decreased, but islet cell antibody and GAD antibody were both negative. The patient had been treated with diet alone for 1 year and with oral hypoglycemic agents (glibenclamide, 1.25–3.75 mg/day) for 15 years and then with insulin for 3 years because of the patient's plan for pregnancy.

Arginine-203 of HNF-1α, 5th residue in the DNA binding homeodomain, contacts the DNA in the minor groove, and the corresponding residue is extremely (~97%) conserved among the >300 members of the homeodomain family (5). Thus, the R203H mutation could affect DNA recognition and/or binding, and presumably a major cause of diabetes in the patient. Clinical features of the patient, such as normal weight, impaired insulin secretion, and absence of GAD antibody, were in accord with the representative diabetes phenotype in MODY with HNF-1α mutations (MODY3) (6). In the present study, in addition, we could observe the time-course of glucose tolerance and insulin secretion by OGTT for about 8 years from the diagnosis (Table 1). The insulin secretion capacity was low at the diagnosis, was retained for 3 years, and then gradually decreased. The glucagon-stimulated C-peptide value was 0.66 nmol/ml at the 17th year from the diagnosis. Therefore, the speed of decline of the proband's insulin secretion from onset, roughly, appears to be slower than that in diabetes because of the mitochondrial 3243 mutation (7,8) but faster than that in diabetes with glucokinase mutations (MODY2) (9).

Both of the patient's parents were diabetic. Her father's diabetes was noticed at age 40 years by a health examination in his company. He had undergone a gastric resection at age 32 years because of a gastric ulcer, and died of esophageal cancer at age 60 years. The patient's mother's diabetes was diagnosed by OGTT at age 47 years. The glucose tolerance of two elder sisters was assessed by OGTT, revealing that the eldest sister had normal glucose tolerance at age 22 years and the second eldest sister showed impaired glucose tolerance at age 18 years. By direct sequencing of exon 3 of HNF-1α, the mother and two sisters all represented a normal genotype. Although we could not analyze her father's DNA, the father's insulin secretion capacity at 47 years of age was markedly reduced (Table 1), suggesting that the mutant allele was transmitted from the father.

Marked within-family heterogeneity of clinical phenotype was reported in MODY3 patients (6). In the present study, the onset age of the father, probably carrying the mutant allele, was much higher than that of the proband. The proband may have inherited an additional diabetes-susceptibility gene from the mother, who also showed overt diabetes without the HNF-1α mutant allele. In addition, the father's habit of light eating because of the past gastric ulcer and subsequent gastrectomy might have delayed the development of his diabetes.

In conclusion, we found a novel mutation in the HNF-1α gene with small-scale screening of early-onset type 2 diabetic patients with family histories of diabetes, supporting that HNF-1α mutations are an important cause of early-onset type 2 diabetes or MODY.

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An Epitaph for Sulfated Insulin

Immunologic profile of the last patients as they are switched from sulfated beef to human insulin

Immunologic reaction to exogenous insulin can lead to several clinical manifestations, ranging from local cutaneous reaction at the injection site to severe anaphylaxis (1). High levels of anti–insulin immunoglobulin (Ig) G antibodies can also bind to exogenous insulin and cause immune insulin resistance (IIR), which could lead to frequent ketoacidosis (2,3). Sulfated insulin (SI) was developed in 1964 by adding two to eight sulfate groups to beef insulin. It was commercialized mainly for the treatment of patients suffering from IIR and also from insulin allergy. The use of SI has been shown to decrease insulin requirement in IIR, with improvement of insulin peak action kinetics in these SI-treated patients (4). It may also induce a specific T-cell suppressor response to insulin (3).

Novo Nordisk has been the only pharmaceutical company to produce this form of insulin in recent years. According to the company's most recent survey, performed in 1993, SI was used by only 26 patients worldwide, all of whom resided in Canada. Of the 26 patients originally identified, 11 were no longer using SI for various reasons at study commencement in 1996. The present study was undertaken to monitor the clinical condition and immunologic status of these patients while switching from sulfated beef insulin to recombinant human insulin (HI). Every attempt was made to contact the patients directly and through their physicians, and a standardized questionnaire was sent to each patient's primary care physician to complete in conjunction with the patient. The attending physicians of patients willing to participate in the study were advised to initially decrease the daily insulin dose by approximately 25% when switching from SI to HI (Novolin ge insulin, Human Biosynthetic; Novo Nordisk) and, subsequently, to titrate the insulin dose upward to achieve good glycemic control. Diabetes management was left to their discretion. All patients participating in this study gave their informed written consent, and the study protocol was approved by the human ethics committee of the Toronto Hospital, University of Toronto.

After the insulin was switched, the body weight, daily insulin dose required, type of insulin, frequency of injections, fasting glucose level, and frequency of mild and severe hypoglycemia were recorded at each visit. Blood was drawn at baseline, at 2–4 weeks, at 8–12 weeks, and at or after 25 weeks to monitor HbA_{1c} (normal range, 4.3–6.1%) and to measure specific anti-insulin antibodies and insulin-specific T-cell proliferation. Insulin-specific antibodies were quantitated by enzyme-linked immunosorbent assay using horseradish peroxidase (HRP)-conjugated polyclonal goat anti–human IgM, IgG, IgA, and IgE (Caltag, San Francisco, CA), or HRP-conjugated anti-human IgG1, IgG2, IgG3, and IgG4 monoclonal antibody (PharMingen, San Diego, CA) as developing antibodies. Assays were performed in triplicate, and the results expressed in optical density (OD) units. Results from individual assays were standardized using sera from individuals with high-titer insulin-specific antibodies. For measurement of insulin-specific proliferation to various concentrations of HI, pork insulin, beef insulin, or SI (0, 50, 100, and 500 μg/ml), peripheral blood lymphocytes were isolated by Ficoll-Hypaque density centrifugation, and proliferation was measured by addition of tritiated thymidine (1 μCi per well) for the final 18 h of incubation. All assays were performed in triplicate, and results were expressed as counts per minute above background proliferation in the absence of antigen.

Of the 15 patients identified as using SI in 1996, 13 agreed to participate in the study. SI was used by eight patients for IIR, by four for insulin allergy, and by one for severe lipoatrophy. The mean duration of SI use was 16.2 ± 9.1 years (mean ± SEM). One patient withdrew from the study because of a suicide attempt. Another patient died during follow-up from causes unrelated to diabetes. After the switch from SI to HI, there was no significant change in HbA_{1c} (9.2 ± 1.7% at baseline vs. 9.5 ± 1.7% 12 weeks after the switch), although there was a statistically significant increase in daily insulin dose required after 12 weeks (73 ± 41 U/day at baseline [$n = 9$] vs. 86 ± 53 U/day at >12 weeks [$n = 6$], $P < 0.05$). This small but significant increase in daily insulin requirement at 12 weeks, which was not associated with a reduction in HbA_{1c}, could be interpreted as suggesting that SI may have some beneficial effect