Identifying Hepatic Nuclear Factor 1α Mutations in Children and Young Adults With a Clinical Diagnosis of Type 1 Diabetes

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OBJECTIVE — HNF-1α gene mutations (MODY3) present with marked hyperglycemia in lean young adults and may, therefore, be mistaken for type 1 diabetes, with implications for individual treatment and risk of diabetes in other family members. We examined the prevalence of HNF-1α mutations in families with three generations of diabetes identified in a population-based study of childhood diabetes, representing a subpopulation in which misclassification was likely.

RESEARCH DESIGN AND METHODS — In a study population of 1,470 families, 36 families (2.4%) with three affected generations were identified. In the 18 families in whom DNA samples were available, islet autoantibody testing, HLA class II genotyping, and HNF-1α sequencing were performed.

RESULTS — At least one islet autoantibody was found in 13 of 14 probands, and diabetes-associated HLA class II haplotypes were found in 17 of 18. One proband, who had no islet autoantibodies and was homozygous for the protective HLA haplotype DRB1*02-DQB1*0602, had a novel HNF-1α heterozygous nonsense mutation (R54X). This mutation cosegregated with diabetes in the family. The proband, his brother, mother, and maternal grandmother were diagnosed with type 1 diabetes aged 14–18 years and treated with insulin (0.39–0.74 units/kg) from diagnosis. The mother has since been successfully transferred to sulfonylurea treatment.

CONCLUSIONS — Family history alone is of limited value in identification of individuals with HNF-1α mutations, and we propose a stepwise approach that restricts sequencing of the HNF-1α gene to those with a family history of diabetes who also test negative for islet autoantibodies.

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T ype 1 diabetes accounts for the vast majority cases of diabetes in young people, but there are other causes of diabetes in this age-group. Mutations in the hepatocyte nuclear factor (HNF) genes 1α, 1β, and 4α, causing maturity-onset diabetes of the young (MODY), typically present in lean individuals with progressive hyperglycemia and osmotic symptoms in adolescence or early adulthood; therefore, they may be misclassified as type 1 diabetes (1). Mutations in the HNF-1α gene account for 65% of MODY in U.K. populations (2) and make up the majority of cases that might be mistaken for type 1 diabetes. The distinction between MODY and type 1 diabetes is important because patients with HNF-1α mutations are not insulin dependent and are sensitive to sulfonylureas (3). There are also implications for other family members, since the lifetime incidence of type 1 diabetes in the offspring of an affected parent is estimated at 10% (4), whereas risk to the offspring of an affected parent with MODY is 50%.

Type 1 diabetes is characterized by islet autoantibodies that are present at diagnosis in up to 97% of children (5) and by a predisposing HLA genotype. A diagnosis of MODY might therefore be suggested by the absence of islet autoantibodies or the coexistence of class II haplotypes known to confer protection against type 1 diabetes. Of 39 patients identified in a Danish population-based family study of type 1 diabetes who were negative for the highest risk conferring HLA class II alleles HLA-DR3 and -DR4 (6), 4 were found to have HNF-1α mutations. Of 55 unselected Japanese patients with type 1 diabetes (7), HNF-1α mutations were found in 3 and a similar prevalence has been found in series selected for the absence of autoantibodies (8). Japan, however, has a low incidence of type 1 diabetes (9), which might increase the likelihood of finding MODY. In these studies, the majority of "type 1" probands identified with HNF-1α mutations had an autosomal-dominant family history of diabetes. No study has selected European patients with a clinical diagnosis of type 1 diabetes on the basis of their family history.

For clinicians caring for young people with diabetes, information on family history is much more easily available than

Table 1—Comparison of probands from families analyzed in the study and those fulfilling criteria for entry but declining to provide DNA samples

Clinical characteristics	Study sample	Probands not included
n	18	18
Males (%)	44	39
Median age (years) at diagnosis	12.6	10.5
Osmotic symptoms (%)	100	100
Ketosis and weight loss (%)	33	44

There were no statistically significant differences between the two groups.

information about the patient's HLA class II genotype or their autoantibody status. Selecting patients with a clinical diagnosis of type 1 diabetes and an autosomal-dominant family history of diabetes for HNF-1α mutation screening may be an effective approach to identify patients with possible MODY3. We therefore studied islet autoantibodies, HLA genotyping, and HNF-1α sequencing in children from a population-based study with a clinical diagnosis of type 1 diabetes and a three-generation family history of diabetes consistent with autosomal-dominant inheritance. We propose a simple strategy for identification of HNF-1α mutations in children who are considered on clinical grounds to have type 1 diabetes.

RESEARCH DESIGN AND METHODS

Patients
Families were identified from the Bart's Oxford (BOX) study of childhood diabetes. This is a prospective, population-based family study that, since 1985, has recruited >90% of the families of children who have developed type 1 diabetes before the age of 21 years in the former Oxford Health Authority Region, U.K. (10,11). The study population is 95% white European, and the remainder originate mainly from the Indian subcontinent (data from Office of Population Censuses and Surveys for 1991). The classification of type 1 diabetes was based on assignment by the referring clinician and was made on the basis of World Health Organization criteria (12) and a clinical requirement for insulin treatment from diagnosis. Patients with secondary diabetes, known genetic subtypes including MODY, or clinical type 2 diabetes were not included in the study. Referring clinicians are all diabetes specialists and part of the clinical network comprising the BOX study.

Families with three generations of diabetes in a pattern consistent with autosomal-dominant inheritance were selected; affected individuals had to be part of the same genetic line (e.g., daughter, father, paternal grandparent). The probands by definition had a clinical diagnosis of type 1 diabetes, but individuals in other generations could have a clinical diagnosis of type 2 diabetes.

Of 1,470 families recruited to the BOX study by 1 October 2000, 36 families (2.4%) with three affected generations were identified. DNA samples were available on 18 of these probands, of whom 44% were male, with a median age at diagnosis of 12.6 years (range 3.1–16.9). None had features to suggest type 2 diabetes. All presented with osmotic symptoms, and 6 of 18 had documented ketonuria and weight loss at diagnosis. DNA samples were not available on the other 18 families with three affected generations identified from the study. DNA was obtained from either blood samples or mouth swabs. Clinical information on the probands with and without DNA samples was similar (Table 1).

Islet autoantibody assays
Antibodies to GAD and the tyrosine phosphatase (IA-2) were measured in the first available serum sample from the proband. Antibody positivity was defined as a level above the 97.5th centile in 2,860 schoolchildren from the Oxford Region (5).

Antibodies to in vitro–translated $[^{35}S]$-GAD$_{65}$ and $[^{35}S]$-PTP-IA-2$_{ic}$ were measured by immunoassay as previously described (5). The interassay coefficient of variation of the GAD antibody assay was 17% at 1.5 units and 9% at 17 units of antibody. The interassay coefficient of variation of the IA-2 antibody assay was 15% at 1 unit and 21% at 9 units of antibody.

HLA class II genotyping
DNA was extracted from blood (13) and mouthswab samples (14). HLA-DRB1, -DQA1, and -DQB1 typing was performed by PCR using sequence specific primers (PCR-SSP) as previously described (14).

HNF-1α sequencing
All 10 exons and intron/exon boundaries of the HNF-1α gene were amplified by PCR using genomic DNA from the proband and sequence-specific primers as previously described (15,16).

Both strands were sequenced using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's recommendations. Reactions were analyzed on an ABI Prism 377 DNA Sequencer (Applied Biosystems).

Each exon was sequenced in the forward and reverse direction. Exon sequences were compared with published consensus sequences nucleotide sequences accession number AH006664 using the Blast program (http://www.ncbi.nlm.nih.gov) and Sequence Navigator software (Applied Biosystems). Deviations from the consensus sequence were compared with published polymorphisms and mutations.

C-peptide measurement
C-peptide levels were measured in individuals with diabetes in families with HNF-1α mutations. Samples were taken 90 min postprandially, at 20:00 h; the probands had taken no insulin since their morning dose 12 h previously. C-peptide levels were measured by J.D. Teale, Department of Chemical Pathology, Royal Surrey County Hospital, Guildford, U.K.

RESULTS

Islet autoantibodies
Serum samples were available on 14 of 18 probands collected at a median 8 months from diagnosis (range 0–105 months). Of these, 13 were positive for IA-2 antibodies and 8 for GAD antibodies. One proband was negative for GAD and IA-2 antibodies in a sample taken 9 years postdiagnosis. His affected brother was autoantibody negative at diagnosis. The pedigree of this family (family 10) is shown in Fig. 1.

HLA class II genotyping
HLA genotyping of the probands of the 18 families selected revealed that 17 pro-
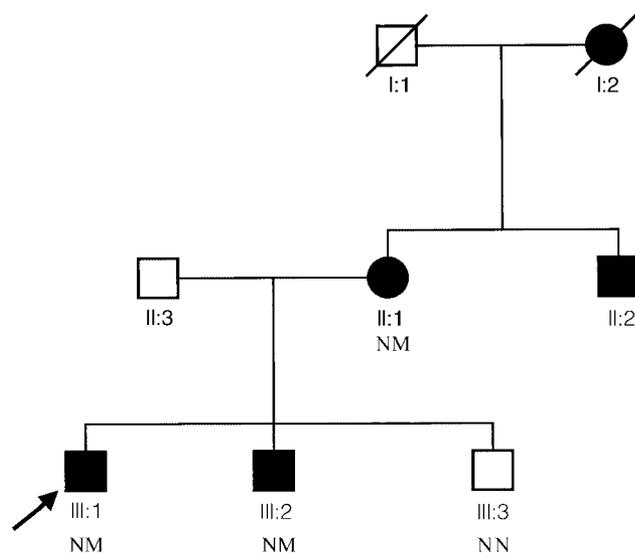
Figure 1—*Pedigree of the study family with MODY3 (family 10). Squares indicate males and circles indicate females. A line across the symbol indicates the individual is deceased. The proband is indicated with an arrow. Filled symbols indicate those family members with diabetes. Mutation status is shown: N, normal allele; M, mutant allele.*

bands had at least one high-risk haplotype. The DRB1*03-DQA1*0501-DQB1*02 haplotype was found in 6 of 17, and the DRB1*04-DQA1*0301-DQB1*0302 haplotype was found in 15 of 17. The highest risk DRB1*03-DQA1*0501-DQB1*02, DRB1*04-DQA1*0301-DQB1*0302 genotype was found in 4 of 17.

One proband was homozygous for the protective DRB1*02-DQB1*0602 haplotype This is the proband of family 10. No other protective HLA class II haplotypes were identified.

HNF-1α sequencing

In 16 of the probands, no variants were found in the HNF-1α gene. In the proband of family 10, with GAD and IA-2 antibodies below the 97.5th centile and a protective HLA class II genotype, a novel mutation R54X (CGA→TGA) was found. This mutation was also present in his mother and sibling who had both been diagnosed with type 1 diabetes; it was not found in an unaffected sibling. The cosegregation of the mutation is shown in Fig. 1. This mutation was not found in 100 normal chromosomes.

In another family, a previously unreported amino acid substitution, H577D, was identified. It did not cosegregate with diabetes in the family, and we conclude that it is a rare polymorphism.

Phenotypic details

The pedigree of family 10 is shown in Fig. 1. The proband (III-1) was diagnosed with type 1 diabetes at 14 years of age and presented with osmotic symptoms of thirst and polydipsia, but no weight loss and with no evidence of ketonuria at diagnosis, nor since. He was treated with insulin from the time of diagnosis, and his current insulin dose is 0.74 units/kg, with an HbA_{1c} of 7.6%. His BMI is 29.7 kg/m^2, and he is complication free. He was IA-2 and GAD antibody negative 9 years postdiagnosis, and he is homozygous for the protective HLA haplotype DRB1*02-DQB1*0602.

His brother (III-2) was diagnosed with type 1 diabetes at age 17 years and presented with osmotic symptoms but no evidence of ketonuria, although he has had one episode of ketonuria since. He was treated with insulin since the time of diagnosis, and his current insulin requirement is 0.47 units/kg with an HbA_{1c} of 11.1%. He has a BMI of 21.6 kg/m^2 and is complication free. He was IA-2 and GAD antibody negative at diagnosis, and his HLA class II genotype is DRB1*02-DQB1*0602, DRB1*0401-DQB1*0301. His mother (II-1) was diagnosed with type 1 diabetes at the age of 18 and presented with necrobiosis lipoidica and osmotic symptoms. At the time of the study, she was being treated with insulin, having

a requirement of 0.4 units/kg and an HbA_{1c} of 8.4%. Her current BMI is 25.2 kg/m^2, and she is complication free, with no recorded episodes of ketonuria. She was negative for IA-2 and GAD antibodies 22 years postdiagnosis, and her HLA class II genotype is DRB1*02-DQB1*0602, DRB1*0401-DQB1*0301. The proband's maternal grandmother (I-2) was diagnosed with diabetes in adolescence and was treated with insulin since the time of diagnosis. His maternal uncle (II-2) was diagnosed with type 2 diabetes at the age of 29 years.

The postprandial C-peptide levels in the proband, his affected sibling, and his mother were 1.70, 0.3, and 0.99 nmol/l, respectively. Individual II-1 has subsequently been taken off insulin and was established on a sulfonylurea. She is currently on gliclazide 80 mg twice daily and has an HbA_{1c} of 7.8%.

CONCLUSIONS — HNF-1α mutations are the most common cause of MODY in the U.K., but they are an uncommon cause of diabetes overall. The clinical features of type 1 diabetes and HNF-1α mutations are similar, and it is useful to identify patients with HNF-1α mutations because of the therapeutic implications for the affected individual and the increased risk of diabetes in their relatives. It is impractical to screen all patients with type 1 diabetes for HNF-1α mutations, and a selective approach to identify individuals for HNF-1α sequencing is required. We used family history as the basis for selection, as multiple affected generations are a cardinal feature of MODY.

This study identified a family with MODY3 (family 10) who had previously been thought on clinical grounds to have familial type 1 diabetes, and all family members were treated with insulin from the time of diagnosis. The novel nonsense mutation R54X described is highly likely to result in diabetes in this family, as it will result in a severely truncated protein. It cosegregates with diabetes in the family and is not present in 100 control chromosomes. The results of autoantibody testing, HLA genotyping, and C-peptide measurement do not support a diagnosis of type 1 diabetes in any family member. In addition, in light of the finding of detectable C-peptide when on insulin treatment, the mother successfully transferred to sulfonylurea therapy after 22 years on

insulin with no deterioration in HbA_{1c}. This demonstrates one of the potential advantages of making a diagnosis of HNF-1α mutation.

Even a three-generation family history did not discriminate well between MODY3 and type 1 diabetes. Of 18 patients with three generations of diabetes tested, only one HNF-1α mutation was identified (5.6%). We are unable to exclude that some of the three-generation families might have a mutation in the other MODY genes, but these are considerably less common than HNF-1α (2). The proband with the mutation differed from the other probands tested by being autoantibody negative and possessing a protective HLA class II genotype. All of the other patients had at least one high-risk HLA class II haplotype, and of 13 tested, all were positive for at least one islet autoantibody.

A comparable study (6), consisting of a population-based sample of North Europeans with a clinical diagnosis of type 1 diabetes, used HLA class II genotype to select individuals who did not possess either of the high-risk type 1 diabetes–associated alleles, DR3 or DR4. From 39 patients, four *HNF-1α* mutations were identified. In three of the families, there were three affected generations, and in the fourth, the father was found to have impaired glucose tolerance in an oral glucose tolerance test. All of the affected individuals were islet autoantibody negative in three of the four families, but in the fourth family, GAD autoantibodies were detected in the proband. The data from Japan support the North European findings with mutations in HNF-1α being described in patients in whom a diagnosis of type 1 diabetes has been made (7). The detection rate of MODY3 is higher in Japanese studies that have selected patients who are pancreatic autoimmune marker negative or have a family history of diabetes (8,12,17)

In view of the poor discriminatory power of family history alone, different strategies are required to identify patients in whom HNF-1α sequencing is likely to detect a mutation. We employed a three-generation history because we felt this would be more closely associated with MODY than type 1 diabetes, though this proved not to be the case. As a consequence, additional selection steps (autoantibody measurement or HLA typing) are needed to increase specificity. Using a

Table 2—*Two strategies for identification of patients with a clinical diagnosis of type 1 diabetes for HNF-1α gene sequencing*

Strategies and screening criteria	Number fulfilling criteria (%)
HLA-based strategy (ref. 6)	
Type 1 diabetes	474
DRB1*03 and *04 negative	26 (5.5)
Family history–based stepped strategy	
Type 1 diabetes	474
Affected parent	67 (14.1)
Affected parent and GAD antibody titer below 97.5th centile	21 (4.4)
Affected parent and GAD and IA-2 antibody titers below 97.5th centile	7 (1.5)

Strategies are applied to 474 patients from the BOX study with full GAD and IA-2 antibody data and full HLA class II genotyping data. There are no significant differences in sex ratio or age at diagnosis between this group and the complete BOX study group. Strategy 1 is based on that used by Moller et al. (6). Strategy 2 is based on pedigree and autoantibody data.

strategy based on a family history in two rather than the three generations may be more sensitive, as 40% of families with MODY give a family history of diabetes extending over only two generations. In Table 2, two approaches are modeled using 474 patients with type 1 diabetes from the BOX study for whom family history, GAD, and IA-2 autoantibody status at diagnosis and full HLA class II genotype data were available. We replicated the Danish strategy based on selection of patients without DRB1*03 or *04 alleles for further tesing. This would result in sequencing of 5.5% of the group, similar to 6.7% in the Danish study (6). In the Danish study, however, only 36% of these patients were positive for either GAD or IA-2 antibodies compared with 77% in our group. An alternative strategy is to supplement pedigree information with autoantibody testing to identify patients for HNF-1α sequencing. Individuals are selected for further investigation in a stepwise manner, beginning with a clinical diagnosis of type 1 diabetes and an affected parent. The next step is antibody testing of this subgroup, followed by HNF-1α sequencing only in those who are negative for both GAD and IA-2 antibodies. This identifies 1.5% of patients if both GAD and IA-2 antibodies are tested and 4.4% if GAD alone is used. Further studies are needed to compare these two potential approaches. We would favor the second approach, as the initial steps are clinically based (family history), with progressively more specialized, restricted, and expensive tests being limited to the

later stages. Neither strategy would detect all cases since the family history of diabetes is not always known, high-risk HLA haplotypes are found in MODY patients, spontaneous HNF-1α mutations occur (18), and patients with HNF-1α mutations and GAD antibodies (6) have been reported.

In summary, MODY due to mutations in the HNF-1α gene is rarely misdiagnosed as type 1 diabetes and was found in only 1 of 18 patients with an autosomal-dominant pattern of diabetes inheritance extending over three generations. Diagnostic yield from genetic testing can be improved by selecting only those individuals negative for GAD and IA-2 antibodies or without high-risk HLA class II haplotypes.

References
1. Fajans SS, Bell GI, Polonsky KS: Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 345:972–980, 2001
2. Hattersley AT: Maturity-onset diabetes of the young: clinical heterogeneity explained by genetic heterogeneity. *Diabet Med* 15:15–24, 1998
3. Pearson ER, Liddell WG, Shepherd M, Corrall RJ, Hattersley AT: Sensitivity to sulphonylureas in patients with hepatocyte nuclear factor-1 alpha gene mutations: evidence for pharmacogenetics in diabetes. *Diabet Med* 17:543–545, 2000
4. Lorenzen T, Pociot F, Hougaard P, Nerup J: Long-term risk of IDDM in first-degree relatives of patients with IDDM. *Diabetologia* 37:321–327, 1994
5. Bingley PJ, Bonifacio E, Williams AJK,

Genovese S, Bottazzo GF, Gale EAM: Prediction of IDDM in the general population. Strategies based on combinations of autoantibody markers. *Diabetes* 46:1701–1710, 1997

6. Moller AM, Dalgaard LT, Pociot F, Nerup J, Hansen T, Pedersen O: Mutations in the hepatocyte nuclear factor-1 alpha gene in Caucasian families originally classified as having type 1 diabetes. *Diabetologia* 41:1528–1531, 1998

7. Yamada S, Nishigori H, Onda H, Utsugi T, Yanagawa T, Maruyama T, Onigata K, Nagashima K, Nagai R, Morikawa A, Takeuchi T, Takeda J: Identification of mutations in the hepatocyte nuclear factor (HNF)-1 alpha gene in Japanese subjects with IDDM. *Diabetes* 46:1643–1647, 1997

8. Kawasaki E, Sera Y, Yamakawa K, Abe T, Ozaki M, Uotani S, Ohtsu N, Takino H, Yamasaki H, Yamaguchi Y, Matsuura N, Eguchi K: Identification and functional analysis of mutations in the hepatocyte nuclear factor-1 alpha gene in anti-islet autoantibody-negative Japanese patients withe type 1 diabetes. *J Clin Endocrinol Metab* 85:331–335, 2000

9. Kida K, Mimura G, Murakami K, Ashkenazi I, Laron Z, The Data Committee for Childhood: Diabetes of the Japan Diabetes Society. Incidence of type 1 diabetes mellitus in children aged 0–14 in Japan, 1986–1990, including an analysis for seasonality of onset and month of birth: JDS study. *Diabet Med* 17:59–63, 2000

10. Bingley PJ, Gale EAM: The incidence of insulin-dependent diabetes in England: a study in the Oxford region 1985–6. *BMJ* 298:558–560, 1989

11. Gardner SG, Bingley PJ, Sawtell PA, Weeks S, Gale EAM, The Bart's-Oxford Study Group: rising incidence of insulin dependent diabetes in children aged under 5 years in the Oxford Region: time trend analysis. *BMJ* 315:713–717, 1997

12. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)

13. Miller SA, Dykes D, Polesky H: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16:1215–1218, 1998

14. Gillespie KM, Valovin SJ, Saunby J, Hunter KM, Savage DA, Middleton D, Todd JA, Bingley PJ, Gale EA: HLA class II typing of whole genome amplified mouth swab DNA. *Tissue Antigens* 56:530–538, 2000

15. Frayling TM, Bulamn MP, Ellard S, Appleton M, Dronsfield MJ, Mackie AD, Baird JD, Kaisaki PJ, Yamagata K, Bell GI, Bain SC, Hattersley AT: Mutations in the hepatocyte nuclear factor-1α gene are a common cause of maturity-onset diabetes of the young in the UK. *Diabetes* 46:720–725, 1997.

16. Ellard S. Bulman MP, Frayling TM, Allen LI, Dronsfield MJ, Tack CJ, Hattersley AT: Allelic dropout in exon 2 of the hepatocyte nuclear factor-1α gene hinders the identification of mutations in three families with maturity-onset diabetes of the young. *Diabetes* 48:921–923, 1999

17. Yoshiuchi I, Yamagata K, Yoshimoto M, Zhu Q, Yang Q, Nammo T, Uenaka R, Kinoshita E, Hanafusa T, Miyagawa Ji, Matsuzawa Y: Analysis of a non-functional HNF-1 alpha (TCF-1) mutation in Japanese subjects with familial type 1 diabetes. *Hum Mutat* 18:345–351, 2001

18. Glucksmann MA, Lehto M, Tayber O, Scotti S, Berkemeier L, Pulido JC, Wu Y, Nir WJ, Fang L, Markel P, Munnelly KD, Goranson J, Orho M, Young BM, Whitacre JL, McMenimen C, Wantman M, Tuomi T, Warram J, Forsblom CM, Carlsson M, Rosenzweig J, Kennedy G, Duyk GM, Thomas JD, et al: Novel mutations and a mutational hotspot in the MODY3 gene. *Diabetes* 46:1081–1086, 1997