Determinants of the Development of Diabetes (Maturity-Onset Diabetes of the Young-3) in Carriers of *HNF-1*α Mutations

Evidence for parent-of-origin effect

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OBJECTIVE — To determine the distribution of the age at onset of diabetes (maturity-onset diabetes of the young-3 [MODY3]) and to identify determinants of the onset of diabetes in carriers of *HNF-1α* mutations.

RESEARCH DESIGN AND METHODS — Extended families (n = 104) with type 2 diabetes inherited in a dominant pattern were recruited and screened for diabetes-causing mutations in *HNF-1α*.

RESULTS — *HNF-1α* mutations cosegregated with diabetes in only 13 families, all with a mean age at onset <35 years. Insulin secretion was diminished or absent in mutation carriers (n = 101), and diabetes developed in 65% by age 25 years and in 100% by age 50 years. If the mutation was inherited from the mother, diabetes onset was very young in those exposed to diabetes in utero; 57 ± 8% were affected by age 15 years as compared with 0.0% in those not exposed ($P < 7 \times 10^{-6}$). By age 25 years, the difference was reduced (85 ± 6 and 55 ± 12%, respectively; $P = 0.02$). If the mutation was inherited from the father, diabetes developed in 52 ± 8% by age 25 years. Age at diagnosis was shown to be highly heritable ($h^2 = 0.47$, $P = 0.003$). When parent of origin was included in the analyses, the magnitude of genetic contribution increased markedly ($h^2 = 0.91$).

CONCLUSIONS — Mutations in *HNF-1α* accounts for diabetes in a small proportion of families with a dominant pattern of inheritance. Age at onset of diabetes in MODY3 families varied widely and was influenced by familial factors (including modifying genes) and parent of origin (whether a mutation carrier was exposed to diabetes in utero).

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Maturity-onset diabetes of the young (MODY) is a subtype of type 2 diabetes defined by an autosomal-dominant mode of inheritance and early age at onset, usually before age 25 years (1,2). However, the upper boundary for the age of onset is arbitrary and not derived from empirical data on penetrance (probability of observing the phenotype given the genotype). Six MODY susceptibility loci have been identified (3–8), and MODY3, which is caused by mutations in the hepatocyte nuclear factor 1α (HNF-1α) gene, accounts for the largest share (9).

The HNF-1α gene (*HNF-1α*) is located on chromosome 12q24 and encodes for a nuclear protein that is expressed in liver, kidney, β-cells of the pancreas, and several other tissues (10–13). HNF-1α is a 631–amino acid protein that forms a homodimer or heterodimer with HNF-1β and binds to regulatory parts of many genes to enhance their expression (14–16). Mutations in *HNF-1α* that result in amino acid substitutions or a truncated protein have been linked in the heterozygous state to the development of early-onset type 2 diabetes (4,9,17–21, and recent update in 22). However, diabetes develops in carriers of *HNF-1α* mutations at different ages and with variable severity, underscoring our ignorance of how the altered protein causes diabetes. The clinical heterogeneity of MODY3 in humans has received little study (23–25). Although inherited as a simple Mendelian trait, manifestation of MODY3 is most likely influenced by environmental as well as additional modifying genes. As with other "simple" Mendelian diseases, its phenotype requires study as a complex trait (26,27).

We screened families with type 2 diabetes segregating as an autosomal-dominant trait for mutations in *HNF-1α*. Families were ascertained without regard to age at diabetes diagnosis, but only those characterized by a young age at diagnosis yielded evidence of mutations. Diabetes manifestation in carriers of *HNF-1α* mutations was affected by exposure to diabetic pregnancy and by other

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Abbreviations: HNF-1α, hepatocyte nuclear factor 1α; IA2, insulinoma-associated antigen 2; MODY, maturity-onset diabetes of the young; OGTT, oral glucose tolerance test; WHO, World Health Organization.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

familial factors, pointing to a complex etiology of MODY3.

RESEARCH DESIGN AND METHODS

Ascertainment and examination of families

Families for the Joslin Study on Genetics of Type 2 Diabetes were ascertained and examined between 1993 and 1999 (8,18,23,28–30). In this family collection, we have already identified two families in which diabetes segregated with mutations in *NEUROD1* (MODY6) (8), two families in which it segregated with mutations in *HNF-4á* (MODY1) (29), and two in which it segregated with mutations in *IPF1* (MODY4) (M.T.M., A.S.K., unpublished data). No families were found in which diabetes segregated with the *GCK* locus (MODY2) (A.D., A. Bektas, unpublished data).

The screening criteria used for the family collection were *1*) a proband and at least one sibling with type 2 diabetes diagnosed between ages 10 and 59 years; 2) three or more generations affected by diabetes; and *3*) unilineal transmission of diabetes. Diabetes in a proband was considered non–insulin dependent if hyperglycemia was managed for at least 2 years without insulin. An additional selection criterion was the availability of a large number of family members (with and without diabetes) willing to participate in the study. A total of 104 multigenerational families with a dominant pattern of inheritance of type 2 diabetes have been identified, and 1,510 family members were examined. The study protocol and informed consent procedures were approved by the committee on human studies of the Joslin Diabetes Center.

Laboratory methods and diagnoses

Fasting blood was drawn for biochemical measurements and DNA extraction. In insulin-treated individuals, fasting blood was used for glucose and C-peptide determination. In diabetic individuals treated with oral agents or diet and in nondiabetic individuals, fasting blood was used for glucose, HbA_{1c}, and serum insulin determinations. Except for insulin-treated individuals, an additional blood sample was obtained in most (86%) 2 h after an oral challenge with 75 g of glucose for blood/plasma glucose and serum insulin determinations.

Glucose was measured in venous samples. Routinely, blood glucose levels were determined using home glucose meters (Onetouch; Lifescan). Whenever possible, plasma glucose values were also measured at the Joslin Clinical Laboratory using a Synchron CX Delta system (Beckman Coulter Instruments, Fullerton, CA) (31). For examinations performed outside of New England, blood or plasma glucose levels were determined at local laboratories, and the methods used were recorded. All glucose measurements were re-expressed in terms of venous plasma as measured at the Joslin Clinical Laboratory. Serum insulin and C-peptide were measured by radioimmunoassay by Linco Research Laboratory (St. Charles, MO). The insulin assay has little cross-reactivity (<0.2%) with human proinsulin. The 2-h insulin and glucose values from the oral glucose tolerance test (OGTT) were combined as an index of insulin sensitivity (defined as $10^4 \times \text{insulin}^{-1} \times \text{glucose}^{-1}$, where insulin is measured in units per milliliter and glucose in millimoles per liter) (32). Serum anti-GAD and anti–insulinoma-associated antigen 2 (IA2) antibody levels were measured by radioligand assays using recombinant GAD and IA2 proteins (33,34). HbA_{1c} was measured in whole-blood specimens using the Tosoh 2.2 Plus glycohemoglobin analyzer (Foster City, CA) in the Joslin Clinical Laboratory. Total cholesterol, triglycerides, and HDL cholesterol were measured using a Synchron CX9 system (Beckman Coulter Instruments), also in the Joslin Clinical Laboratory.

Diabetes was diagnosed if any of the following criteria were met: *1*) treatment with insulin or oral agents and the presence of diabetic hyperglycemia confirmed by the study examination; 2) OGTT blood glucose values meeting World Health Organization (WHO) criteria (fasting levels ≥140 mg/dl or 2 h levels ≥200 mg/dl); or *3*) HbA_{1c} ≥7.0% (normal values <6.1%) in individuals who declined the OGTT or were not fasting when examined (35,36). If glucose values exceeded the WHO criteria for normal glucose tolerance but did not meet any of these criteria, a diagnosis of impaired glucose tolerance was made. A woman with normal glucose values at examination, but a documented history of diabetes during pregnancy, was classified as having gestational diabetes.

Detection of polymorphisms/mutations

DNA was extracted from blood using standard protocols. DNA from 104 probands (individuals with diabetes through whom the families were identified) were used to screen for polymorphisms and mutations in the promoter region and in the 10 exons of *HNF-1α*. PCR primers were similar to those previously reported (9,37). The bidirectional sequencing protocol has been described elsewhere (8). We considered a DNA sequence difference to be a diabetes-related mutation if it resulted in miscoding of the amino acid chain and was present in probands but absent in 72 unrelated nondiabetic individuals (spouses). Subsequently, all available diabetic and nondiabetic relatives were screened for the same mutation. This screening used direct sequencing, antisense oligonucleotide, and restriction fragment–length polymorphism methods.

Age at onset of diabetes

The age at onset of diabetes (also referred to as the age at development of diabetes or hyperglycemia) was defined as the age at diagnosis of diabetes by the subject's physician if diabetic hyperglycemia was confirmed by the study examination. In family 11, the diagnoses of diabetes by the family's physician were the basis for recruiting the family, but diabetes was not confirmed at the study examination. Instead, the family members had impaired glucose tolerance; therefore, no age at diagnosis of diabetes was defined. The age at onset of diabetes within families was characterized in two ways: each pedigree was characterized by the average age at onset of diabetes in the affected members who were examined. In addition, the 13 families with mutations in *HNF-1α* were divided into nuclear families, and each nuclear family was characterized by an age at onset. The carrier status was known for each member of the nuclear families, but not all carriers were affected. Because the mean age at onset could not be calculated, we characterized the sibship's age at onset as the age at which 50% of the mutation carriers in that sibship were affected.

Data analysis

We used three approaches to analyze the data on the 13 families with diabetes segregating with mutations in *HNF-1α*. First,

Table 1—*Characteristics of families and examined family members according to the family's average age at diagnosis of diabetes*

	Young age	Middle age
Families		
*n**	36	68
Examined members	454	1,056
Members with diabetes	227	446
Members with IGT or GDM	31	70
Average number of examined individuals with diabetes per family (range)	6.3 (3–17)	6.6 (3–14)
Examined individuals with diabetes†		
Age (years)	45 ± 18	58 ± 15
Age at diabetes diagnosis (years)	27 ± 16	45 ± 15
IBW (%)	122 ± 24	140 ± 33
Treatment of diabetes (%)		
Insulin	53	45
Oral agents	28	36
Diet	19	19

*There were 10 non-Caucasian families: 4 African-American, 5 Hispanic, and 1 Pacific Islander; †data are means ± SD. Young = 11–35 years, middle = 36–57 years. GDM, gestational diabetes mellitus; IBW, ideal body weight; IGT, impaired glucose tolerance.

the data were examined as whole pedigrees according to mutation type. Whole pedigrees were also used in the variance component analysis to estimate the heritability of the age at onset of MODY3. Second, individual carriers and noncarriers were analyzed as though they were unrelated case and control subjects to compare clinical characteristics of carriers of *HNF-1α* mutations according to the parent-of-origin mutations and exposure to diabetic pregnancy. Finally, the families were divided into nuclear families for analyses by sibship to determine whether the parent of origin of mutations or the type of mutations have an impact on the age at onset of MODY3, independently of the size and complexity of pedigrees.

Variance component analysis. We analyzed the data obtained in the 13 extended MODY3 families using the SOLAR software package (38). SOLAR performs a variance component analysis of family data that decomposes the total variance of the phenotype (age at onset of diabetes) into components that are attributable to additive genetic (polygenic) effects, measured covariates, and unmeasured (random) effects. A series of models were used to estimate the heritability of age at onset, using either no covariates or a series with parent of origin, age, and sex included in a hierarchy. Significance of the heritability (from the null hypothesis of $h^2 = 0$) was determined using a likelihood ratio test. The relative contribution of genetic factors to variation in age at onset is the heritability (h^2), defined by the ratio of the additive genetic variance component to the residual (after removal of covariates) phenotypic variance. The significance of residual h^2 across models was evaluated by the construction of 95% CIs (using the SE of h^2) and determining whether interval overlap occurred. The significance of the change in magnitude of h^2 with respect to a given covariate was determined by computing the −2 log-likelihood difference between a model with all covariates and a model without the covariate in question (distributed as a χ^2 statistic with 1 degree of freedom).

Other statistical methods. Statistical analyses were performed in SAS (*SAS/STAT User's Guide*, version 8e; SAS, Cary, NC). Differences among groups was tested by χ^2 methods (or Fisher's exact test if appropriate) for qualitative variables and ANOVA or regression for quantitative variables. The cumulative incidence (risk) of diabetes according to attained age was estimated by lifetable methods. The significance of differences in cumulative incidence between groups was tested with the log-rank statistic. The significance of differences in cumulative risk at specific ages was tested using their standard errors.

RESULTS

Family collection for Joslin Study on Genetics of Type 2 Diabetes

We identified and examined 104 families (94 Caucasian) with type 2 diabetes that segregated as a highly penetrant autosomal-dominant disorder. In these families, 1,510 individuals were examined (14.5 individuals per family; 722 with diabetes and 106 with impaired glucose tolerance or gestational diabetes). The average age at diagnosis of diabetes in families varied. Three families had a mean age at diagnosis <15 years (the youngest was 11 years), and five families had a mean ≥55 years.

To contrast families by age at onset of diabetes, the families were divided into two classes: 36 young families (average age at onset ≤35 years) and 68 middle-age families (average age at onset >35 years). Although arbitrary, this separation was supported by the mutation findings described below, as well as leaner body weight of the affected members of young families (Table 1).

Detection of polymorphisms/mutations in *HNF-1α*

DNA from one diabetic member (proband) of each of the 104 families was screened by bidirectional sequencing for polymorphisms/mutations in the promoter and in the 10 exons of *HNF-1α*. Altogether, 32 sequence differences were found and examined further in 72 unrelated nondiabetic Caucasians. A total of 17 polymorphisms were found with equal frequency in probands and nondiabetic control subjects (data not shown), and 15 rare mutations were found only in probands. The 15 mutations occurred in 14 probands (2 were in neighboring codons in 1 proband). One mutation that changed Thr to Ala at codon 196 was found in a proband from a minority family in exon 3, but it did not segregate with diabetes in that family. The remaining mutations segregated with diabetes in 13 Caucasian families (Table 2). The oldest of these families had a mean age at onset of 31 years. Therefore, in families that had an autosomal-dominant pattern of diabetes occurrence, mutations in *HNF-1α* were responsible for diabetes in 13 (36%) of the 36 young families but in none of the middle-aged families (Table 1).

A description of *HNF-1α* mutations

Table 2—*Characteristics of mutations in HNF-1α causing diabetes*

Family identity number	Location	Nucleotide		Amino acid		Number of carriers	Diabetes status†	Age (years) at onset	
		Position*	Change	Change	Codon			Mean	Range
Dimerization and DNA binding domains‡									
01	Exon 1	70	GA deletion	Frameshift	24	4	4/–/–	20	14–28
02	Exon 1	130	C deletion	Frameshift	44	4	4/–/–	11	10–13
03	Exon 1	320	T→G	Leu→Arg	107	16	16/–/–	19	9–37
04	Exon 2	21	C→T	Ala→Val	116	9	8/–/1	29	13–48
05	Exon 2	64	C→T	Arg→Trp	131	11	9/1/1	21	11–41
06	Exon 2	110	C→A	Gln→Lys	146	5	5/–/–	15	10–24
		114	A→G	His→Arg	147				
07	Exon 4	5	G→C	Glu→Gln	240	8	4/2/1	25	19–35
08	Exon 4	102	G→A	Arg→His	272	6	6/–/–	22	17–34
Transactivation domain‡									
09	Exon 4	159	C deletion	Frameshift	291	3	3/–/–	13	10–18
10	Exon 4	159	C insertion	Frameshift	291	13	13/–/–	20	8–48
11	Exon 4	159	C insertion	Frameshift	291	5	–/4/1§	—	—§
12	Exon 6	29	CT deletion	Frameshift	379	6	6/–/–	19	13–26
13	Exon 7	19	CA deletion	Frameshift	443	12	9/2/1	31	16–49
Total						101	87/9/5	21	8–49

*Nucleotide number from 5′ end of each exon; †diabetes status (diagnosed diabetes/impaired glucose tolerance at study examination or gestational diabetes/normoglycemic at examination without a previous diagnosis of diabetes or gestational diabetes; ‡*HNF-1α* has three distinct domains *1*) a dimerization domain comprising the first 33 codons, 2) a DNA binding domain defined by codons 100–280, and *3*) a transactivation domain that consists of the remaining 351 codons (10); §two individuals with diabetes diagnosed previously had only impaired glucose tolerance at examination for this study. The age of the five carriers at examination ranged from 16 to 40 years. Mutations in families 03, 05, 08, and 10 were described in a previous publication (18).

cosegregating with diabetes in the 13 pedigrees is shown in Table 2. The mutations were deletions, insertions, or nucleotide substitutions, and they caused either amino acid changes or a frameshift in sequence. Mutations found in the transactivation domains produced only truncated proteins. For each mutation, the number of carriers and their diabetes status as of the time of examination are shown. The diabetes status of carriers varied from family to family, most notably for families 10 and 11, which have the same mutation. All carriers in pedigree 12 had overt diabetes, but this was not so for family 21 (Table 2). The within-family range of ages at diagnosis varied from a minimum of 3 years to a maximum of 40 years (median 16 years) (Table 2).

To examine the risk of developing diabetes for carriers of *HNF-1α* mutations, the cumulative incidence of diabetes according to attained age was estimated by lifetable methods. Diabetes developed in almost all carriers of *HNF-1α* mutations between the ages of 8 and 49 years (and in half of them by age 20 years). Although inclusion of the affected individuals required for the ascertainment of families (two affected family members) can inflate these estimates, the overall results in this case were not appreciably altered by excluding them, with cumulative risk by age 50 years reaching 100% in both cases. Another issue is the inclusion of nine cases of undiagnosed diabetes detected by the screening of families for the study. To assess the impact of including these cases, they were censored as of the date of examination. The cumulative incidence of diabetes by 50 years was reduced from 100 to only 93%. Therefore, all 101 carriers were used in subsequent analyses. Diabetes developed in 65% by age 25 years and in 79% by age 35 years.

Familial clustering of age at onset of diabetes in carriers of *HNF-1α* mutations

To determine the contribution of familial factors to the age at manifestation of diabetes in carriers of HNF-*1α* mutations, genetic variance component analysis was used. In the 13 MODY3 pedigrees, there were 452 pairs of relatives (carriers of *HNF-1α* mutations) available for this analysis. In the model that included no covariates, heritability of age at onset was moderate ($h^2 = 0.47 \pm 0.17$, $P = 0.003$). When parent of origin was included as a covariate, the heritability increased markedly ($h^2 = 0.91 \pm 0.20$, $P = 0.08$ for the increment), although there was overlap in the 95% CIs between the model with no covariates and the model with the parent-of-origin effect. When the significance of the individual covariates was examined, only the parent of origin of the mutation was a significant determinant of the age at manifestation ($\chi^2_1 = 46.9$, $P < 0.0001$). The principal findings, therefore, of the variance components analysis are: *1*) the age at manifestation of diabetes in mutation carriers is highly heritable, and *2*) it differs in some way depending on whether the mutation was inherited from mothers or fathers.

Effect of parent of origin of *HNF-1α* mutations on age at onset of diabetes

To visualize the effect of the parental origin of mutation on the age at diagnosis of diabetes, the cumulative incidence of diabetes in carriers was calculated according to attained age, according to whether the mutation was inherited from the mother or the father (Fig. 1*A*). Among carriers who inherited *HNF-1α* mutations from their mother, diabetes had developed in half of them by age 17 years, whereas among those who inherited mutations from their father, half were af-

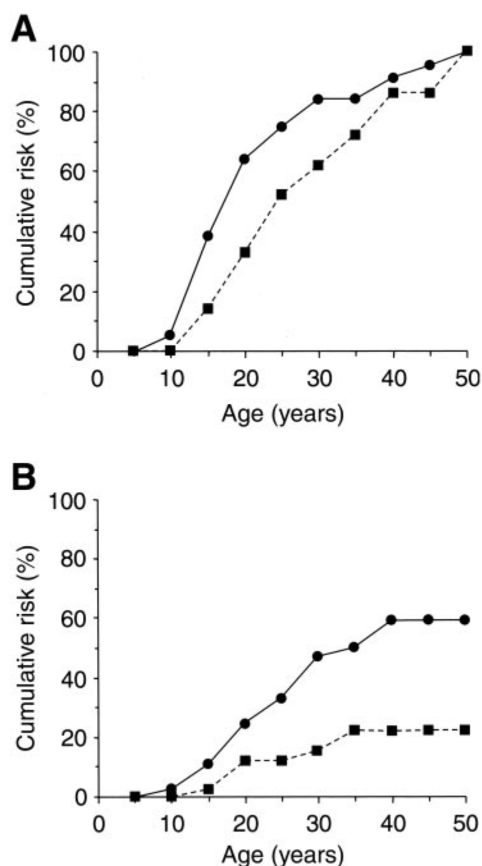

Figure 1—*Cumulative risk of diabetes to age 50 years in carriers of mutations in* HNF1-α *according to the parent of origin of the mutation.* ●, *carriers who inherited mutations from their mother;* ■, *carriers who inherited mutations from their father.* A: *Age at onset of all case subjects that met WHO criteria for diabetes* (P = *0.011 for comparison between carriers who inherited* HNF1-α *mutations from their mother and from their father).* B: *Age at onset of insulin-treated diabetes (diabetes restricted to those case subjects treated with insulin, and onset is date of initiation of insulin treatment).* P = *0.004 for comparison between carriers who inherited* HNF1-α *mutations from their mother and from their father.*

fected by age 23 years, a difference of 6 years ($P = 0.011$).

To see whether this difference was due to close surveillance of the children of diabetic mothers and the diagnosis of mild diabetic hyperglycemia, the diagnosis of diabetes was restricted to insulin-requiring diabetes for calculating the cumulative incidence according to the parent of origin of the mutation (Fig. 1*B*). At age 25 years, for example, insulin-treated diabetes had developed in 33 ± 7% of carriers of maternally inherited mutations but only 12 ± 5% of carriers of paternally inherited mutations ($P = 0.016$). The interval from diagnosis of diabetes to the initiation of insulin treatment also gave evidence against a spurious difference due to maternal surveillance. Within 5 years after the diagnosis, insulin treatment had begun in 33 ± 7% of carriers if the mutation was inherited from mothers but only 16 ± 7% of carriers if inherited from fathers ($P = 0.02$).

Clinical characteristics of carriers also support a conclusion that a more severe, insulin-requiring phenotype was more frequent if the mutation was inherited from the mother (Table 3). Among those treated with insulin, the insulin dose (units/kg) was the same, and the frequencies of individuals without detectable endogenous insulin secretion and of individuals with autoantibodies (GAD or IA2) were similarly low. Curiously, most antibody-positive individuals had a long duration of diabetes (Table 3, legend). Among carriers not treated with insulin, the distributions of other treatments (oral agent, diet, or none) were similar, as were fasting and 2-h postchallenge values of glucose and insulin (Table 3). However, relative to their noncarrier siblings, carriers had slightly lower fasting insulin values, regardless of whether the mutation came from their mother or their father ($P = 0.01$ and $P = 0.006$, respectively), and large deficits in postchallenge insulin levels ($P = 0.0004$ and $P = 0.005$, respectively). Interestingly, the insulin sensitivity index was quite similar in carriers and noncarriers (nondiabetic), regardless of the parent of origin of the mutation. These insulin values are all low relative to young patients with type 2 diabetes who do not have mutations in *HNF-1α* (data not shown).

Carriers were 34 ± 15 years old on average when examined; women and men were affected equally (52% women), and 77% were lean (percent ideal body weight <120). These characteristics did not vary with the parent of origin of the mutation. Serum HDL cholesterol was high in these families, although it was slightly lower in individuals who inherited mutations from their mother (52 ± 12 mg/dl) instead of their father (59 ± 18 mg/dl). Serum triglycerides were low in both groups (103 ± 51 and 113 ± 66 mg/dl, respectively).

Effect of exposure to maternal diabetes in utero on carriers of *HNF-1α* mutations

To evaluate the contribution to younger age at onset of exposure in utero to maternal diabetes in individuals who had maternally inherited *HNF-1α* mutations, we divided them according to the mother's diabetes status during their gestation (Table 4). Those exposed to diabetes in utero had a particularly early onset of diabetes: the cumulative risk by age 15 years was 57 ± 8% as compared with 0.0% in those not exposed ($P < 7 \times 10^{-6}$). By age 25 years, the difference in risks was reduced (85 ± 6 and 55 ± 12%, respectively) but still statistically significant ($P = 0.02$). A higher cumulative incidence of insulin-treated diabetes in the exposed group ($P = 0.04$) accounted for most of this excess occurrence of diabetes by age 25 years. Those exposed to maternal diabetes in utero were slightly heavier at the time of examination, but this difference was not quite significant. In those not treated with insulin, fasting and 2-h values of glucose and insulin were almost identical in the two groups. Of note, the cumulative risk by age 25 years for indi-

Table 3—*Characteristics of carriers of mutations in HNF1α according to parent of origin of the mutation*

	Mutation inherited from		
	Mother	Father	*P*
All carriers			
n	56	45	
Diabetes diagnosed by age 20 years (%)	64	33	0.001
Autoantibodies (%)*	4	10	NS
Insulin-treated diabetes (%)	52	29	0.02
Carriers treated with insulin			
n	26†	10†	
Insulin dose (units/kg)	0.44 ± 0.24	0.39 ± 0.16	NS
Fasting C-peptide <0.2 ng/ml (%)‡	15	10	NS
Remaining carriers			
n	27	32	
Fasting glucose (mg/dl)	116 ± 42	134 ± 45	NS
2-h glucose (mg/dl)	273 ± 102	234 ± 89	NS
Fasting insulin (μU/ml)	6.1 ± 2.1	6.8 ± 2.5	NS§
2-h insulin (μU/ml)	10.3 ± 6.3	13.4 ± 7.6	NS§
2-h insulin sensitivity index	93 ± 73	103 ± 110	NS§
Non carriers			
n	17	27	
Fasting glucose (mg/dl)	89 ± 10‖	85 ± 13‖	NS
2-h glucose (mg/dl)	100 ± 21‖	92 ± 26‖	NS
Fasting insulin (μU/ml)	8.9 ± 4.8‖	12.0 ± 9.0‖	NS§
2-h insulin (μU/ml)	38.6 ± 29.0‖	30.4 ± 38.5‖	NS§
2-h insulin sensitivity index	109 ± 146	148 ± 133	NS§

*Five subjects were GAD^+ and one was $IA2^+$. One was normoglycemic (age 14 years), whereas the remaining five had insulin-treated diabetes (one of the five had undetectable insulin secretion). Diabetes duration was very long (30–50 years), except for one with only 7 years' duration. Antibody results were not available for 13 subjects. †Insulin dose and C-peptide values are unavailable for three subjects in each group. ‡No endogenous insulin secretion. §Significance test performed on log-transformed data, ‖difference between carriers and noncarriers significant ($P < 0.05$).

viduals inheriting a mutation from their mothers but not exposed to diabetes in utero (55 ± 12%) was equivalent to the risk in those who inherited a mutation from their fathers (52 ± 8%).

Determinants of the age at onset of diabetes in nuclear families (sibships)

The preceding analyses were based on individual carriers, and the results were possibly distorted by unequal contributions from large and small sibships. To exclude this possibility, we divided the 13 pedigrees into nuclear families. There were 54 sibships that included at least one affected carrier of a mutation in *HNF-1α*. To characterize the age at onset in sibships with more than one affected carrier, we used the median age at diagnosis. When ranked by age at onset, the sibships fell into three discrete clusters of equal size: 8–15, 17–22, and 26–48 years (Table 5).

The proportion of sibships inheriting mutations from mothers decreased with increasing age at onset in the sibships (χ^2 for linear trend = 5.35, $P = 0.02$). The youngest cluster of sibships consisted almost entirely of sibships that inherited an *HNF-1α* mutation from their mother and were exposed in utero to maternal diabetes. Sibships that inherited a mutation from their father predominated in the oldest age-at-onset cluster. In contrast, the proportion of sibships with mutations in the transactivation domain was constant, regardless of age at onset of diabetes in the sibships (Table 5). The same was true for the frequency of mutations resulting in truncated protein (data not shown).

CONCLUSIONS

— In this study, we examined the role of mutations in *HNF-1α* in the development of diabetes in a large number of extended families in which diabetes was inherited as a highly penetrant autosomal-dominant disorder. In contrast to other reports, this study included large families in which the average age at diabetes diagnosis within families ranged to a high of 57 years. By direct sequencing, we identified *HNF-1α* mutations that in the heterozygous state (dominant effect) caused diabetes in 13 Caucasian families. Diabetes-causing *HNF-1α* mutations were found in 13 of 36 (36%) families with young onsets of diabetes (average age at diagnosis of diabetes within the family ≤35years), and none were found in the remaining 68 families with diabetes diagnosed in middle age. In the 13 families, diabetes typically developed in mutation carriers before age 25 years; however, in 35% of the carriers it did not do so until they reached older ages (up to 49 years). The clinical severity of the diabetes was simi-

Table 4—*Characteristics of carriers of mutations in HNF1-α who inherited the mutation from their mother according to their mother's diabetes status during pregnancy*

	Exposed to maternal diabetes in utero*		
Characteristic	Yes	No	*P*
n	35	20	
Age at examination (years)	34 ± 15	34 ± 16	NS
Percent ideal body weight at exam	115 ± 18	105 ± 19	NS
Diabetes diagnosed by			
Age 15 years (%)	57 ± 8	0 ± 0	0.000007
Age 25 years (%)	85 ± 6	55 ± 12	0.02
Treated with insulin by age 25 years (%)	43 ± 9	17 ± 9	0.04
Carriers not treated with insulin			
Fasting glucose (mg/dl)	119 ± 35	111 ± 53	NS
2-h glucose (mg/dl)	278 ± 109	266 ± 97	NS
Fasting insulin (μU/ml)	6.0 ± 2.5	6.2 ± 1.5	NS†
2-h insulin (μU/ml)	8.7 ± 4.8	11.9 ± 7.4	NS†
2-h insulin sensitivity index	113 ± 94	70 ± 25	NS†

*Mother's diabetes status during pregnancy is unknown for one carrier. †Significance test performed on log-transformed data.

Table 5—*Sibships with at least one carrier of an* HNF1-α *mutation, grouped by median age at diagnosis of diabetes in carrier(s), according to parent of origin of the mutation and type of mutation*

	Median age (years) at diagnosis of diabetes in carrier(s)*			
	8–15	17–22	26–48	Total
Parent of origin†				
Mother	13 (72)	9 (50)	6 (33)	28 (52)
Father	5 (28)	9 (50)	12 (67)	26 (48)
Domain of mutation				
Transactivation	7 (39)	6 (33)	6 (33)	19 (35)
Binding	11 (61)	12 (67)	12 (67)	35 (65)
Total	18 (100)	18 (100)	18 (100)	54 (100)

Data are the number of sibships (%). *There were 58 sibships with at least one mutation carrier (31 with one, 17 with two, 4 with three, and 6 with four). Four were omitted because the single carrier did not have diabetes. Among sibships with two or more affected siblings, there were two sibships in which the pair of affected sibs had diabetes diagnosed in the same calendar year. In three sibships, the diagnoses were 2 years apart, and in four sibships the diagnoses spanned a 3- to 5-year interval. In eight sibships, they spanned 6–10 years; in four they spanned 11–15 years. In three, the span was >15 years (maximum 27 years). †χ^2 for linear trend in parent of origin = 5.35, $P = 0.02$.

larly varied. These findings have implications for diagnostic criteria for MODY (39) and pose a fundamental question: "What are the determinants/mechanisms of such variable, age-dependent expression of the diabetes phenotype in this supposedly monogenic disorder?"

We will now discuss our findings with regard to three sets of determinants that we examined for association with age of manifestation or severity of diabetes in carriers of *HNF-1α* mutations: *1*) spectrum of *HNF-1α* mutations, 2) parent of origin of the mutations, and *3*) other familial factors (environmental and genetic).

The repertoire of the diabetes-causing *HNF-1α* mutations in the present study is similar to that reported previously (22). We found seven mutations in exons that encode for the DNA binding domain. All were single-nucleotide substitutions that resulted in amino acid changes. Two mutations were localized in exon 1 (one in and one outside of the dimerization domain) and resulted in protein truncation caused by a frameshift. The remaining mutations were in exons that encode for the transactivation domain, and these also resulted in truncated proteins caused by frameshifts. Neither the type (truncated versus missense) nor the localization (the three domains) of the mutations had an effect on the clinical course of MODY3. This contrasts with in vitro studies where protein truncations resulting from mutations in the transactivation domain have a dominant-negative effect, whereas a missense mutation localized in other domains results in only partial loss of function (40,41). Our findings are in agreement with recent negative data on genotype/phenotype relationships in MODY3 families from the U.K. (24).

Interestingly, the clinical picture of diabetes was more severe in carriers of *HNF-1α* mutations inherited from the mother rather than the father. If the mutation came from the mother, diabetes was diagnosed at a younger age, and the treatment was more likely to be insulin. The mechanisms underlying this association are not clear. In the general population, data concerning the role of maternal inheritance in the development of type 2 diabetes are conflicting. Data from cross-sectional studies consistently show that probands with type 2 diabetes report diabetes in mothers more frequently than in fathers (42–45). Follow-up studies, which are less susceptible to bias, do not support these findings. The most comprehensive follow-up study, the Framingham Offspring Study, found that maternal and paternal diabetes conferred similar risks of type 2 diabetes (46). Recently, we reported similar findings in families ascertained through patients of the Joslin Clinic (47).

It has been postulated that the metabolic disturbances during diabetic pregnancy, specifically, may affect subsequent risk of diabetes in offspring. For example, a recent study of Pima Indians showed that exposure to diabetes during fetal life conveyed an elevated risk for type 2 diabetes and obesity during adult life (48). In striking contrast, Caucasian offspring exposed in utero to uncontrolled maternal type 1 diabetes do not develop type 1 or type 2 diabetes. Diabetes develops more frequently if the father has type 1 diabetes than if the mother does, and they were not exposed to diabetic pregnancy at all (49). Therefore, the metabolic disturbances of a diabetic pregnancy are not sufficient to modify the risk of diabetes in all exposed offspring. Apparently, the exposure's effect depends on interaction with some genetic susceptibility.

In the present study, we found significant association between exposure to diabetic pregnancy and age at the development of diabetes in carriers of *HNF-1α* mutations. Actually, the diabetic pregnancy effect can account for the difference associated with parent-of-origin mutation. It is important to point out that in contrast to the observation in Pima Indians, in our study the carriers exposed and not exposed to diabetic pregnancy were nonobese and did not differ with regard to insulin secretion or insulin sensitivity. Thus, our findings suggest that exposure to diabetic pregnancy alters pancreatic β-cell mass or function specifically in carriers of *HNF-1α* mutations. The result is the development of diabetes during the second decade of life, whereas nonexposed carriers develop diabetes 5–10 years later. At present, the mechanism of this putative alteration of β-cells is not clear, although various hypotheses may be proposed (50,51).

Using variance component analysis, we found significant heritability (familial clustering) in the age at onset of MODY3 in our families. After accounting for the parent-of-origin effect, the residual heritability of age at onset was very high, suggesting that familial factors have a strong effect on the age of the development of diabetes. These factors may include familial aggregation of environmental factors such as obesity (although carriers as well as noncarriers in our families were not obese) and physical activity, both of which may affect insulin sensitivity and cause an imbalance between insulin secretion and insulin demand. The familial clustering of age at onset was not caused by temporal clustering of screening tests for diabetes within sibships when one of the siblings developed diabetes. Only two sibpairs were diagnosed in the same or adjacent years. In most sibships, the cal-

endar years of diagnosis spanned >5 years (see Table 5).

High heritability for age at onset, however, may also result from genetic factors (in addition to *HNF-1α* mutations) that effect either insulin resistance or insulin secretion. In the preliminary results of a genome scan conducted in these 13 large families, we found suggestive evidence for linkage between age at onset of MODY3 and two chromosomal regions (A.D., S.H. Kim, X. Ma, S.S.R., unpublished data). If correct, these findings would indicate that MODY3 is a complex disorder involving, in addition to *HNF-1α*, other modifying genes as well as exposure to diabetic pregnancy for the full manifestation of the diabetes phenotype early in life. In transgenic mice, complex models have been generated that produce diabetes (52), and in humans the phenotypes of some "simple" Mendelian disorders have been shown to be complex traits (26,27).

Finally, some limitations of our study must be acknowledged. First, the screening for mutations in *HNF-1α* was limited to the proximal promoter, all exons, and the exon/intron boundaries. We have not sequenced the distal 5′ and 3′ ends or the introns of this gene, where unknown regulatory elements may reside. However, none of the remaining young families showed evidence for linkage of diabetes with microsatellite markers located in regions flanking *HNF-1α* (A.D., S.H. Kim, A. Bektas, unpublished data). This makes it unlikely that the negative results of our screening protocols for diabetes-causing mutations in the other families were false negatives.

Second, we ascertained only those families with an autosomal-dominant pattern of inheritance and vertical transmission of diabetes through several generations. Therefore, our estimate of the cumulative risk of diabetes in carriers of *HNF-1α* mutations is most likely higher than that which would be obtained if we had ascertained the families through a single affected carrier. However, the pattern of inheritance may not have a significant effect on parent of origin of *HNF-1α* mutations.

Third, our study has limited precision in determining the age at onset of diabetes. In an unknown number of individuals, the diagnosis of diabetes was delayed until symptoms of hyperglycemia appeared. However, these delays may not have a large impact on the age-dependent penetrance of diabetes in carriers of *HNF-1α* mutations. The nine cases of undiagnosed diabetes detected by the study were equally distributed according to parent of origin of the mutation, so these delays did not confound our major finding of an effect of the parent of origin of the mutation. Moreover, when we analyzed the risk of diabetes in carriers of *HNF1α* mutations according to whether they inherited the mutations from their mother or their father, the difference still existed when we used the age at initiation of insulin treatment as the age of onset MODY3.

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