

Genetic Modifiers of the Age at Diagnosis of Diabetes (MODY3) in Carriers of Hepatocyte Nuclear Factor-1 α Mutations Map to Chromosomes 5p15, 9q22, and 14q24

Sung-Hoon Kim,^{1,2} Xiaowei Ma,^{1,2} Tomasz Klupa,^{1,2} Christine Powers,¹ Marcus Pezzolesi,¹ James H. Warram,¹ Stephen S. Rich,³ Andrzej S. Krolewski,^{1,2} and Alessandro Doria^{1,2}

Mutations in hepatocyte nuclear factor (HNF)-1 α (MODY3) account for the largest proportion of maturity-onset diabetes of the young (MODY) cases in the U.S. This form of diabetes is characterized by impaired insulin secretion in response to glucose, but wide variability exists in the severity of hyperglycemia and in the age at which it becomes clinically manifest. We have previously shown that the age at onset of diabetes in MODY3 families is influenced by familial factors (including modifying genes) and exposure to diabetes in utero. To identify genes influencing the onset of MODY3, we conducted a genome scan in 13 extended MODY families in which diabetes segregates with an HNF-1 α mutation. Linkage with age at onset of diabetes was assessed by genetic variance component analysis using SOLAR. The locus with the strongest evidence of linkage was on chromosome 14q24 (D14S588; logarithm of odds [LOD] = 2.58, $P = 0.0004$). This location overlaps with *IDDM11* and includes *SEL1L*, a negative regulator of the Notch pathway that may control islet development. Linkage evidence also supported loci on 5p15 (D5S817; LOD = 2.44, $P = 0.0004$) and 9q22 (D9S910; LOD = 2.02, $P = 0.0018$). The latter matches a region linked to 2-h insulin levels in Pima Indians. Less strong linkage evidence was observed at three other regions: chromosomes 3p24 (LOD = 1.44), 7q21 (1.20), and 16q23 (1.51). Our data are consistent with the existence of multiple loci that contribute to the expression of the MODY3 phenotype. Identification of these genes will offer new insights into the pathophysiology of MODY that may, in turn, increase our understanding of the cellular events underlying more common forms of diabetes. *Diabetes* 52:2182–2186, 2003

From the ¹Research Division, Joslin Diabetes Center, Boston, Massachusetts; the ²Department of Medicine, Harvard Medical School, Boston, Massachusetts; and the ³Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina.

Address correspondence and reprint requests to Alessandro Doria, MD, PhD, Section on Genetics and Epidemiology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215. E-mail: alessandro.doria@joslin.harvard.edu.

Current affiliation for S.-H.K.: Division of Endocrinology, Samsung Cheil Hospital and Women's Healthcare Center, Sungkyunkwan University School of Medicine, Seoul, Korea.

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HNF, hepatocyte nuclear factor; IGT, impaired glucose tolerance; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; Ngn3, neurogenin 3; OGTT, oral glucose tolerance test; WHO, World Health Organization.

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Maturity-onset diabetes of the young (MODY) is a form of familial diabetes characterized by an early age of onset and an autosomal dominant pattern of inheritance (1). Six MODY genes have been identified to date (2–8). One MODY locus corresponds to the enzyme glucokinase, which is an essential component of the β -cell glucose sensor (2,3). The other five MODY genes are transcription factors (hepatocyte nuclear factor [HNF]-1 α , HNF-4 α , HNF-1 β , insulin promoter factor 1 (IPF1), and NEUROD1) that are involved in islet cell differentiation and metabolism as well as insulin secretion (4–8). Most MODY cases in the U.S. are attributable to mutations in HNF-1 α (9). This form of diabetes, also known as MODY3, is characterized by impaired insulin secretion in response to glucose (10,11). While HNF-1 α mutations are highly penetrant, there is wide variability in the severity of the disease. There is also wide variation in the age at which MODY becomes clinically manifest. Indeed, only 50–60% of HNF-1 α mutation carriers develop diabetes before age 25 years (the traditional criterion for a MODY diagnosis) (9). Such variability does not appear to be related to the type (truncated versus missense) or to the domain localization of the HNF-1 α mutations (9).

In a large panel of HNF-1 α mutation carriers, we found that inheritance of the mutation from the mother and exposure to maternal diabetes in utero are associated with an early onset of MODY (9). In addition, we found the age of diabetes onset to be highly heritable ($h^2 = 0.47$), and when the parent-of-origin of the HNF-1 α mutation was taken into account, the heritability was even greater, with familial (presumably genetic) factors accounting for 91% of the residual variability (9). Thus, the clinical manifestation of MODY3 appears to be more complex than previously thought, being influenced by both modifying genes that are inherited independently of HNF-1 α as well as environmental factors. Similar phenotypic complexity has been proposed for other “simple” Mendelian disorders such as Gaucher disease (12).

To identify loci that influence MODY3 onset, we conducted a genome screen of 13 extended families in which diabetes segregates with an HNF-1 α mutation. The clinical characteristics of these families have been previously

TABLE 1
Characteristics of the *HNF-1 α* mutations segregating with diabetes in the 13 MODY3 families

Family ID no.	Mutation	No. of carriers with diabetes/IGT	Age (years) at onset	
			Mean	Range
01	E24fsdelGA	4/–	20	14–28
02	L44fsdelC	4/–	11	10–13
03	L107R	16/–	19	9–37
04	A116V	8/–	29	13–48
05	R131W	9/1	21	11–41
06	Q146K H147R	5/–	15	10–24
07	E240Q	4/2	25	19–35
08	R272H	6/–	22	17–34
09	P291fsdelC	3/–	13	10–18
10	P291fsinsC	13/–	20	8–48
11	P291fsinsC	–/4	—	—
12	P379fsdelCT	6/–	19	13–26
13	A443fsdelCA	9/2	31	16–49
Total		87/9	21	8–49

described (9). Included in the screen were 87 mutation carriers with diabetes and 9 with impaired glucose tolerance (IGT). The number of individuals per family and the nature of the *HNF-1 α* mutation segregating with diabetes in each family are summarized in Table 1. All 96 study subjects were genotyped by the Mammalian Genotyping Service (Marshfield, WI) for 377 autosomal markers corresponding to a 9.3 cM average spacing. Linkage with age at onset of diabetes was evaluated by variance component analysis using SOLAR (13). Because of its significant effect on MODY onset (9), parent-of-origin of the *HNF-1 α* mutation was included as a covariate in the analysis. Results are reported in Fig. 1 and summarized in Table 2. The locus with the highest multipoint logarithm of odds (LOD) score for age at onset of diabetes was on chromosome 14q24 at 82 cM from p-ter on the Marshfield map (D14S588; LOD = 2.58, empirical $P = 0.0004$) (Table 2). The LOD-1 support interval extends from 68 to 102 cM from p-ter. Two other locations supported linkage, with LOD scores >2. One site occurred on 5p15 (D5S817; LOD = 2.44; $P = 0.0004$) and the other on 9q22 (D9S910; LOD = 2.02; $P = 0.0018$), with the LOD-1 support interval extending from 9 to 41 cM and from 93 to 123 cM for chromosomes 5 and chromosome 9, respectively. Less strong linkage evidence was observed at 3p24 (LOD = 1.44, $P = 0.008$), 7q21 (LOD = 1.20, $P = 0.016$), and 16q23 (LOD = 1.51, $P = 0.007$) (Table 2). To account for the fact that the diagnosis of IGT may precede the diagnosis of diabetes by several years, linkage analysis was repeated after increasing the age at diagnosis of the nine IGT subjects by 5–10 years, as described in RESEARCH DESIGN AND METHODS. The evidence of linkage remained unmodified at 14q24 (LOD = 2.61, $P = 0.0003$), increased at 9q22 (LOD = 2.42, $P = 0.0009$), and decreased at 5p15 (LOD = 1.78, $P = 0.0043$). There were no major changes in the LOD scores at 3p24, 7q21, and 16q23.

In the November 2002 Genome Assembly (www.genome.ucsc.edu), the LOD-1 support interval for the diabetes age-at-onset locus on 14q24 corresponds to 27 Mb (from 57 to 84 Mb). This region partially overlaps with the type 1 diabetes locus (*IDDM11*, 80–84 Mb) that was identified by Field et al. (14) in a subset of families that had no evidence

of linkage to HLA. A type 2 diabetes locus has also recently been described on 14q in an Amish population, but this is placed >50 cM centromeric to our peak (15). According to the latest annotation of the human genome sequence, the 14q24 LOD-1 support interval contains at least 128 genes, many of which could potentially affect glucose metabolism and, in turn, modify the age at onset of MODY. Among these, one interesting candidate is *SEL1L*, a gene that is almost exclusively expressed in the pancreas and islets of Langerhans (16,17). The product of *SEL1L* is a negative regulator of the Notch signaling pathway (17), which controls cell fate decisions in many different settings during development (18). In the pancreas, the Notch pathway moderates islet formation by inhibiting the expression of the *HNF-1 α* -activated pro-endocrine factor neurogenin 3 (*Ngn3*) (19). Thus, a genetic defect of the Notch inhibitor *SEL1L* would result in increased activity of the Notch pathway and worsen the *Ngn3* deficiency caused by *HNF-1 α* mutations. Presumably, the result of this defect would be a more profound deficit of β -cell mass (or function) leading to an earlier development of diabetes. Two nonsynonymous polymorphisms are in the dbSNP database for *SEL1L* (rs2371800 [E182K] and rs1051193 [V714I]). However, after genotyping all 96 *HNF-1 α* mutation carriers for these two single nucleotide polymorphisms, only one heterozygote for rs1051193 (and none for rs2371800) was observed. Both exons and 5' flanking sequence of the gene are being screened to identify additional polymorphisms that may be associated with a young age at onset of MODY in these families.

The peak on chromosome 9q22 corresponds to a locus linked with 2-h oral glucose tolerance test (OGTT) insulin concentrations in Pima Indians (20). The 2-h insulin level is a widely accepted index of insulin resistance in target organs such as skeletal muscle, adipose tissue, and liver. In the presence of insulin resistance alleles at 9q22, carriers of *HNF-1 α* mutations would require more insulin from β -cells to maintain normoglycemia and may therefore develop MODY earlier in life. The LOD-1 support interval at this location spans 27 Mb (from 82.5 to 109.5). Of the 140 genes included in this region, two are known to be directly involved in glucose metabolism and insulin action. One gene codes for fructose-1,6 biphosphatase, a rate limiting enzyme in gluconeogenesis. The other gene is the glycolytic enzyme aldolase B. Deletion and nonsense mutations in this enzyme are a cause of hereditary fructose intolerance (21). Perhaps less severe polymorphisms can influence insulin sensitivity and therefore MODY onset. An effect on insulin sensitivity might also be at play for the locus on 5p15, since this exact location has been linked with circulating levels of the insulin sensitizer adiponectin (22). The LOD-1 support interval spans 27.6 Mb (from 4.1 to 31.7 Mb) and includes 32 known genes, none of which has obvious actions on glucose metabolism.

It must be emphasized that none of the LOD scores obtained in this genome screen meet the criteria for genome-wide significance. In this regard, it must be noted that for effects of the magnitude observed at chromosomes 5, 9, and 14, our family collection provided 75% power to detect linkage with LOD scores >2.5, but only 56% power to detect LOD scores >3.00. The power to detect linkage at genome-wide significance levels may have been further

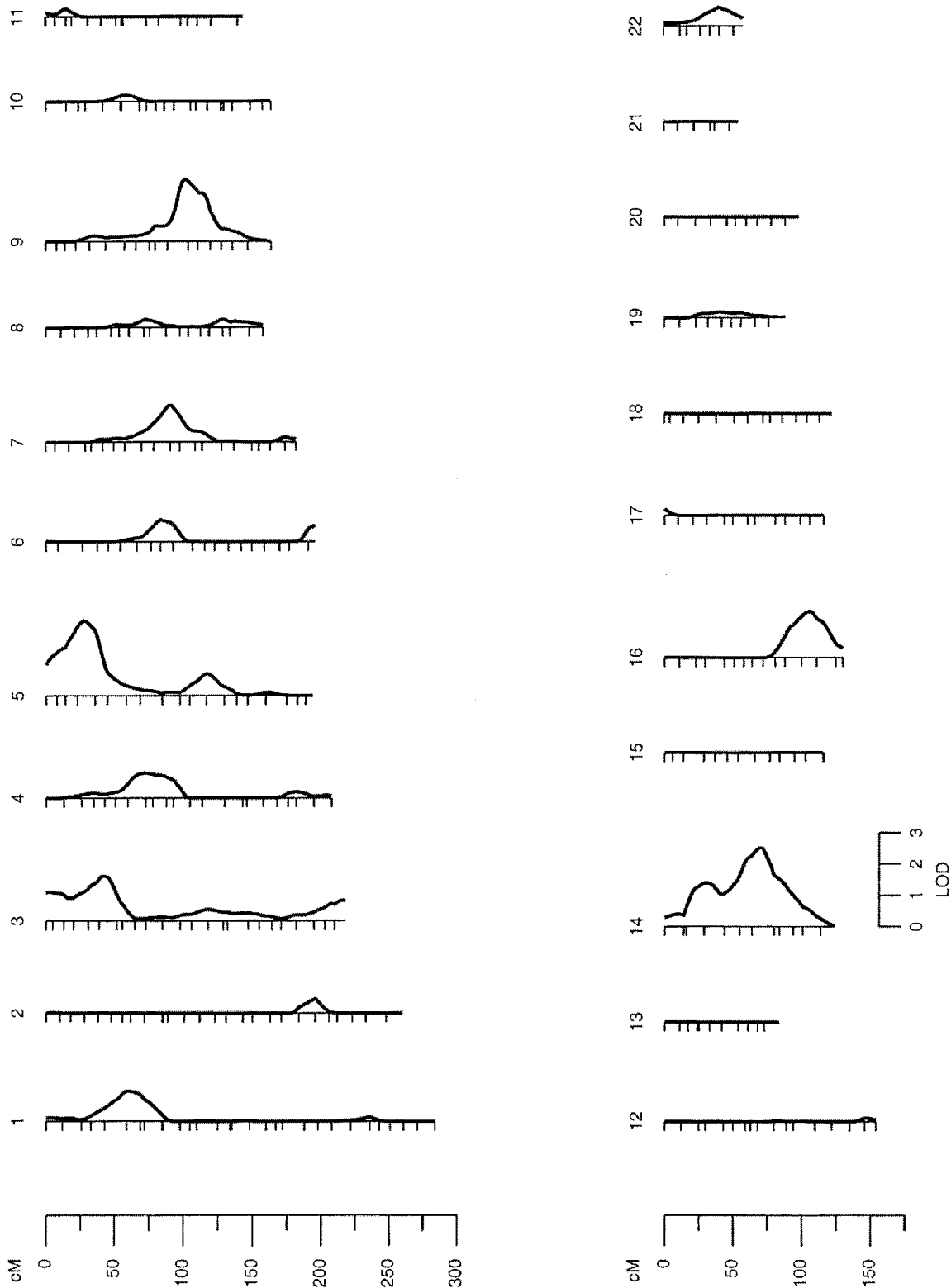


FIG. 1. Results of genome-wide scans for multipoint linkage analysis of age at MODY3 onset. The chromosomal position is represented on the x-axis and the LOD scores on the y-axis. For each chromosome, position 0 corresponds to the most proximal marker in the Marshfield Set 10. Positions in the Marshfield map can be calculated by adding the position of the most proximal marker in that map to the positions in the linkage plots.

reduced by the fact that age at diagnosis is not a perfect surrogate of the age at onset of diabetes.

In summary, these data suggest that multiple loci, inherited independently of MODY, are modifiers of the MODY3

phenotype, although this remains to be substantiated by replication in other studies. Some of these genes may exert their effect at the level of the β -cell, enhancing the insulin secretion deficit caused by HNF-1 α mutations,

TABLE 2
Multipoint LOD scores ≥ 1.0 for linkage with age at diagnosis of MODY3

Chromosome	LOD	<i>P</i> *	Marker†	Position‡
3	1.44	0.008	GATA73D01	47
5	2.44	0.0004	GATA3E10	28
7	1.20	0.016	GATA73D10	90
9	2.02	0.0018	ATA18A07	102
14	2.58	0.0004	GGAA4A12	82
16	1.51	0.007	350VD1	106

*Empiric *P* value from 10,000 replicates; †closest marker in the Marshfield Screening Set 10; ‡position (cM) of the LOD score on the Marshfield map.

while others may influence the onset of MODY3 by affecting the amount of insulin that peripheral tissues require to maintain euglycemia. Identification of these genes will offer new insights to the pathophysiology of MODY that may, in turn, increase our understanding of the cellular events underlying more common forms of diabetes, particularly with regard to the role of gene-gene interactions in the development of hyperglycemia. At the same time, knowledge of these genetic modifiers will provide new tools to evaluate more accurately the prognosis for carriers of HNF-1 α mutations.

RESEARCH DESIGN AND METHODS

Study subjects. Thirteen families segregating HNF-1 α mutations were included in the study. Their characteristics have been previously described (9). Genotyping was performed in mutation carriers who had developed diabetes or IGT and for whom enough DNA was available for a 10-cM genome screen. Of 101 mutation carriers, 96 fulfilled these criteria. 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 ≥ 140 mg/dl or 2 h ≥ 200 mg/dl); or 3) HbA_{1c} $\geq 7.0\%$ (normal values $< 6.1\%$) in individuals who declined the OGTT or were not fasting when examined (23). If glucose values exceeded the WHO criteria for normal glucose tolerance but did not meet any of these criteria, a diagnosis of IGT was made. Age at onset of diabetes or IGT was defined as the age at diagnosis by the subject's physician if abnormal glycemic levels were confirmed by the study examination. For patients with undiagnosed diabetes or IGT that was discovered at the study examination ($n = 18$), the age at onset was defined as the age at examination for the present study.

Genotyping and linkage analysis. A genome scan was performed by the National Heart, Lung, and Blood Institute (NHLBI) Mammalian Genotyping Service at the Marshfield Medical Research Foundation by means of PCR and automated fragment analysis (24). Each individual was genotyped for 377 autosomal microsatellite markers having an average heterozygosity of 0.76 (Marshfield Set 10), resulting in a mean distance between markers of 9.3 cM. The proportion of markers successfully genotyped for each individual ranged from 89.4 to 99.8%, with 84% of the subjects having information on $> 97\%$ of the markers. The overall error rate, as measured on replicate Centre d'Etude du Polymorphisme Humain samples, was 0.5%. After verifying Mendelian inheritance by means of the PEDCHECK software (25) and computing maximum likelihood estimates of allele frequencies, multipoint linkage analysis was performed for the quantitative trait "age at onset" using the SOLAR software package (13). Based on previous analyses (9), we used a model incorporating parent-of-origin of the HNF-1 α mutations and sex as covariates. Empiric *P* values were determined by using the "lodadj" command within SOLAR. This approach samples the null distribution (the distribution of LOD scores obtained under the "no linkage" hypothesis) so that a sorted array of LOD scores is obtained and the location of the observed LOD provides the "empiric" *P* value. A total of 10,000 replicate samples were generated. To account for potential erroneous results from variance components analysis of skewed data, the log transformation was used for the three chromosomes of primary interest (5, 9, and 14). The results of the analyses of transformed data differed little from nontransformed data (slight reduction in maximum LOD score for chromosome 5, slight increase in LOD score for chromosomes 9 and 14, and similar positions [within 5 cM] of the original locations). Thus, results

for nontransformed data are presented. To account for the fact that diabetes is usually diagnosed several years after the diagnosis of IGT, linkage analysis was repeated after adding 5–10 years to the age at diagnosis of the nine IGT subjects. For each of the nine participants a random number (between 0 and 1) was generated (rn) and an increment defined by $\text{INT}[5 + 5 \cdot \text{rn}]$ was added.

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