The Type and the Position of *HNF1A* Mutation Modulate Age at Diagnosis of Diabetes in Patients with Maturity-Onset Diabetes of the Young (MODY)-3

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OBJECTIVE—The clinical expression of maturity-onset diabetes of the young (MODY)-3 is highly variable. This may be due to environmental and/or genetic factors, including molecular characteristics of the hepatocyte nuclear factor 1-α (*HNF1A*) gene mutation.

RESEARCH DESIGN AND METHODS—We analyzed the mutations identified in 356 unrelated MODY3 patients, including 118 novel mutations, and searched for correlations between the genotype and age at diagnosis of diabetes.

RESULTS—Missense mutations prevailed in the dimerization and DNA-binding domains (74%), while truncating mutations were predominant in the transactivation domain (62%). The majority (83%) of the mutations were located in exons 1- 6, thus affecting the three *HNF1A* isoforms. Age at diagnosis of diabetes was lower in patients with truncating mutations than in those with missense mutations (18 vs. 22 years, $P = 0.005$). Missense mutations affecting the dimerization/DNA-binding domains were associated with a lower age at diagnosis than those affecting the transactivation domain (20 vs. 30 years, $P = 10^{-4}$). Patients with missense mutations affecting the three isoforms were younger at diagnosis than those with missense mutations involving one or two isoforms ($P = 0.03$).

CONCLUSIONS—These data show that part of the variability of the clinical expression in MODY3 patients may be explained by the type and the location of *HNF1A* mutations. These findings should be considered in studies for the search of additional modifier genetic factors. ***Diabetes* 57:503–508, 2008**

Heterozygous mutations in the hepatocyte nuclear factor 1-α (*HNF1A*) gene cause maturity-onset diabetes of the young (MODY)-3 (1,2). MODY3 is characterized by a severe insulin secretion defect, a retained sensitivity to sulfonylureas, a decreased renal threshold for glucose reabsorption, and, in rare families, the occurrence of liver adenomatosis (3–6).

The clinical expression of MODY3 is highly variable from one family to another or even within the same family (7). *HNF1A* mutation carriers may be normoglycemic while their siblings may be hyperglycemic at a comparable age (8). Symptoms at diagnosis may be variable. Some patients have metabolic decompensation, while in others diabetes is diagnosed by systematic screening. The severity and the course of insulin secretion defect also vary since approximately one-third of the patients are treated with insulin after 15 years of diabetes duration, whereas others control their diabetes by diet or oral hypoglycemic agents (9).

As in other monogenic diseases, this phenotype variability may be explained by environmental and/or additional genetic factors. Two studies have shown that age at diagnosis of diabetes in offspring carrying a *HNF1A* mutation was lower by 5–10 years when maternal diabetes was diagnosed before pregnancy, suggesting the role of exposure of the fetus to maternal hyperglycemia (10,11). Modifier genetic factors may also modulate the phenotype of the disease. Age at onset of diabetes is partly inheritable within MODY3 families, and putative genetic modifier loci have been mapped but not identified yet (12). In the same vein, it has been recently shown that germ line *CYP1B1*

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HNF1A, hepatocyte nuclear factor 1-α; MODY, maturity-onset diabetes of the young.

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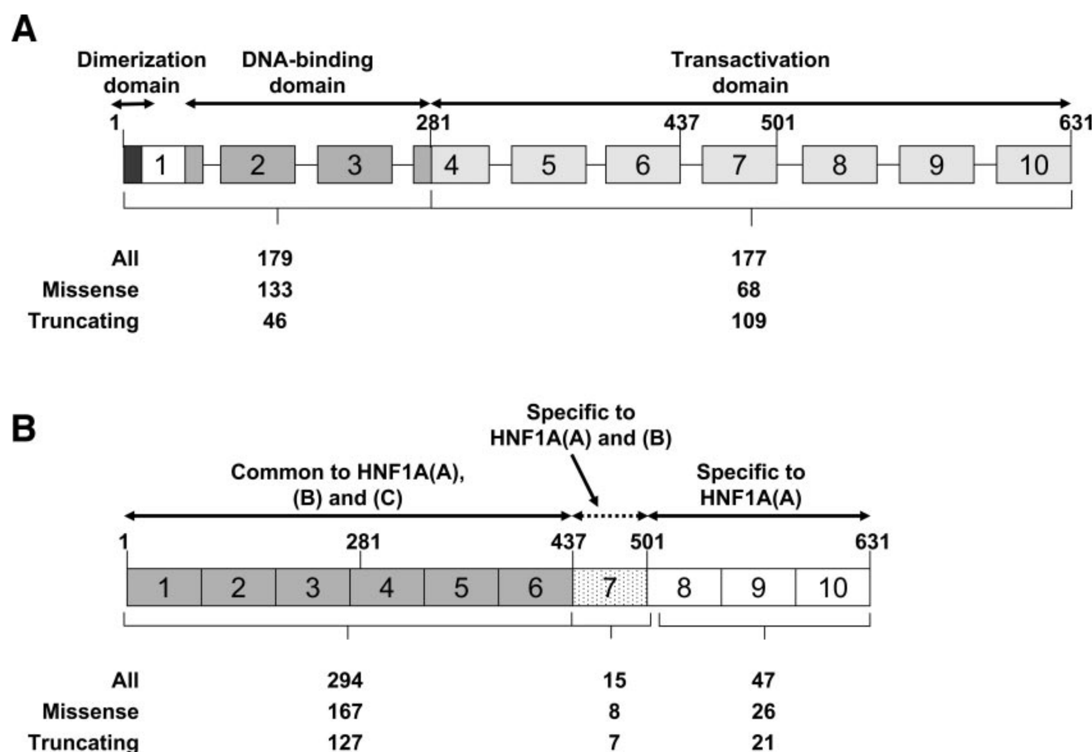

FIG. 1. *HNF1A* mutational spectrum according to the genomic and isoform structures. *A*: The three functional domains are identified on the genomic sequence in dark gray (dimerization domain), mid-gray (DNA binding domain), and light gray (transactivation domain). Each exon is represented by a numbered box. *B*: The exons transcribed in the three *HNF1A* isoforms are indicated and colored according to the number of affected isoforms: the mid-gray boxes correspond to exons common to the three isoforms; the dotted box corresponds to exon 7 specific to the *HNF1A*(A) and (B) isoforms; and the white boxes correspond to exons specific to *HNF1A*(A). The numbers of mutations are indicated under the corresponding affected domain or isoform.

heterozygous mutations, which affect estrogen metabolism, may increase the incidence of hepatocellular adenomas in women with MODY3 (13). The molecular characteristics of the *HNF1A* mutation may also play a role in the severity of the disease. About 200 different mutations have been reported in *HNF1A* (14). HNF1A is composed of three functional domains, and three isoforms are generated by alternative splicing, with different transcriptional properties and tissue expression patterns (15,16). A recent analysis of the *HNF1A* mutation spectrum showed no correlation between the age of onset of diabetes and the type of the mutation (16). An older age of onset was observed in MODY3 patients carrying a *HNF1A* missense mutation affecting specifically the HNF1A(A) isoform, which is highly expressed in the fetal pancreas (16).

Here, we describe the spectrum of *HNF1A* mutations identified in 356 unrelated MODY3 patients, and we show relationships between age at diagnosis of diabetes and the type and position of the mutations.

RESEARCH DESIGN AND METHODS

Patients. This study includes 356 unrelated patients (87% Euro-Caucasians, 60% women) who had been referred for genetic testing from 1998 to 2007 and in whom a *HNF1A* mutation was identified. All patients gave written informed consent.

Mutation analysis. Several criteria were used to ascertain that the novel mutations identified in the present study were pathogenic: nature of the amino acid change, conservation of the residue across species, absence of the mutation in 300 control subjects of Euro-Caucasian origin, and cosegregation of the mutation with young-onset diabetes, when relatives were available.

The molecular spectrum of *HNF1A* mutations was analyzed according to three criteria. First, mutations were classified into two groups according to their predicted functional consequences. One group included missense mutations resulting in amino acid changes, and the second included nonsense, small insertions/deletions, or splicing mutations, predicted to generate premature stop codons (referred to as "truncating mutations"). Second, mutations were analyzed according to the three HNF1A functional domains: NH_2-terminal dimerization domain (amino acids 1–32), DNA-binding domain (amino acids 91–281), and COOH-terminal transactivation domain (282–631) (Fig. 1*A*) (17). Third, mutations were analyzed according to the affected isoforms of *HNF1A*. The *HNF1A*(A) isoform is the full-length transcript comprising the 10 exons, whereas *HNF1A*(B) and *HNF1A*(C) isoforms result from alternative splicing and contain the first seven and first six exons, respectively (Fig. 1*B*). Three groups of mutations were considered: mutations located in exons 1–6, affecting the three isoforms; mutations located in exon 7, affecting isoforms *HNF1A*(A) and (B); mutations located in exons 8–10, involving only the *HNF1A*(A) isoform.

Statistical analysis. Age at diagnosis is reported as median and range. Data were compared with the Mann-Whitney and the Kruskall-Wallis tests. Statistical analyses were performed with GraphPad InStat (GraphPad Software, San Diego, CA).

RESULTS

Characteristics of the *HNF1A* mutational spectrum. Among the 356 unrelated patients, 169 *HNF1A* mutations were identified. Fifty-one have previously been reported, whereas 118 are novel. The 51 known mutations were detected in 200 patients (supplementary Table [available at http://dx.doi.org/10.2337/db07–0859]). By contrast, the 118 novel mutations were mainly private mutations (97 of 118, 82%) (Table 1).

There were 201 (56.5%) missense and 155 (43.5%) truncating mutations (103 small insertion/deletion mutations, 31 nonsense mutations, and 21 splicing defects).

The same numbers of mutations affected the dimerization and DNA-binding domains (the two structurally major domains) and the transactivation domain (179 and 177

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TABLE 1
Description of 118 novel *HNF1A* mutations

Location	Change at the DNA level	Change at the protein level	Mutation type	Occurrence
Exon 1	c.1A>C	p.Met1Leu	Missense	1
Exon 1	c.22C>A	p.Leu8Met	Missense	2
Exon 1	c.41C>T	p.Ala14Val	Missense	2
Exon 1	c.49C>G	p.Leu17Val	Missense	1
Exon 1	c.50T>A	p.Leu17Gln	Missense	1
Exon 1	c.59G>C	p.Gly20Ala	Missense	1
Exon 1	c.77T>C	p.Leu26Pro	Missense	2
Exon 1	c.80T>C	p.Ile27Thr	Missense	1
Exon 1	c.80T>G	p.Ile27Ser	Missense	1
Exon 1	c.82C>T	p.Gln28X	Nonsense	1
Exon 1	c.98C>T	p.Pro33Leu	Missense	1
Exon 1	c.202C>T	p.Arg68Trp	Missense	2
Exon 1	c.206delG	p.Gly69fs	Deletion	1
Exon 1	c.217G>T	p.Glu73X	Nonsense	1
Exon 1	c.225C>A	p.Asp75Glu	Missense	1
Exon 1	c.259A>T	p.Lys87X	Nonsense	1
Exon 1	c.282_283insT	p.Glu95X	Nonsense	1
Exon 1	c.319C>G	p.Leu107Val	Missense	1
Exon 1	c.326delA	p.Gln109fs	Deletion	2
Exon 2	c.346G>A	p.Ala116Thr	Missense	1
Exon 2	c.368T>G	p.Leu123Arg	Missense	1
Exon 2	c.396G>C	p.Glu132Asp	Missense	1
Exon 2	c.397G>T	p.Val133Leu	Missense	1
Exon 2	c.403G>A	p.Asp135Asn	Missense	1
Exon 2	c.410C>G	p.Thr137Ser	Missense	1
Exon 2	c.412G>A	p.Gly138Ser	Missense	1
Exon 2	c.427delC	p.His143fs	Deletion	1
Exon 2	c.436_438dup	p.Gln146dup	Insertion	1
Exon 2	c.436C>T	p.Gln146X	Nonsense	1
Exon 2	c.442C>A	p.Leu148Ile	Missense	1
Exon 2	c.447C>G	p.Asn149Lys	Missense	1
Exon 2	c.460A>G	p.Met154Val	Missense	1
Exon 2	c.461T>C	p.Met154Thr	Missense	1
Exon 2	c.461T>G	p.Met154Arg	Missense	1
Exon 2	c.517G>A	p.Val173Met	Missense	1
Exon 2	c.521C>T	p.Ala174Val	Missense	1
Exon 2	c.523C>T	p.Gln175X	Nonsense	1
Intron 2	c.526 + 1G>C		Splicing Defect	4
Intron 2	c.526 + 5G>A		Splicing Defect	1
Exon 3	c.586A>G	p.Thr196Ala	Missense	1
Exon 3	c.614delA	p.Lys205fs	Deletion	2
Exon 3	c.620_621insG	p.Gly207fs	Insertion	1
Exon 3	c.650C>G	p.Ala217Gly	Missense	1
Exon 3	c.676A>G	p.Lys226Glu	Missense	1
Exon3	c.682_683insG	p.Glu228fs	Insertion	1
Exon 3	c.682G>A	p.Glu228Lys	Missense	1
Exon 3	c.696_697insA	p.Val233fs	Insertion	1
Exon 3	c.704_705insA	p.Cys236fs	Insertion	1
Exon 3	c.711_713+6del	p.Arg238fs	Deletion	1
Intron 3	c.713+1G>C		Splicing Defect	1
Exon 4	c.715G>A	p.Ala239Thr	Missense	1
Exon 4	c.722G>A	p.Cys241Tyr	Missense	1
Exon 4	c.732A>T	p.Arg244Ser	Missense	1
Exon 4	c.732_733delAG	p.Ser247fs	Deletion	2
Exon 4	c.737T>G	p.Val246Gly	Missense	1
Exon 4	c.746_747insC	p.Gln250fs	Insertion	1
Exon 4	c.763G>A	p.Gly255Ser	Missense	1
Exon 4	c.785_786insT	p.Arg263fs	Insertion	1
Exon 4	c.790G>T	p.Val264Phe	Missense	1
Exon 4	c.798C>G	p.Asn266Lys	Missense	1
Exon 4	c.814C>A	p.Arg272Ser	Missense	2
Exon 4	c.827C>G	p.Ala276Gly	Missense	1
Exon 4	c.842T>C	p.Leu281Pro	Missense	2

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TABLE 1
Continued

Location	Change at the DNA level	Change at the protein level	Mutation type	Occurrence
Exon 4	c.865C>T	p.Pro289Ser	Missense	1
Exon 4	c.871C>A	p.Pro291Thr	Missense	1
Exon 4	c.919delC	p.Leu307fs	Deletion	1
Exon 4	c.923C>T	p.Pro308Leu	Missense	1
Intron 4	c.955+2T>C		Splicing Defect	1
Exon 5	c.959_962dupTGCG	p.Tyr322fs	Insertion	1
Exon 5	c.965A>G	p.Tyr322Cys	Missense	2
Exon 5	c.966T>G	p.Tyr322X	Nonsense	1
Exon 5	c.970C>T	p.Gln324X	Nonsense	1
Exon 5	c.984T>G	p.Ser328Arg	Missense	1
Exon 5	c.1017delT	p.Leu341X	Nonsense	1
Exon 5	c.1059_1060insC	p.Thr354fs	Insertion	1
Exon 5	c.1080_1081dupCA	p.Ser361fs	Insertion	1
Exon 6	c.1118C>G	p.Ala373Gly	Missense	2
Exon 6	c.1135C>A	p.Pro379Thr	Missense	5
Exon 6	c.1135C>G	p.Pro379Ala	Missense	8
Exon 6	c.1135C>T	p.Pro379Ser	Missense	2
Exon 6	c.1136delC	p.Pro379fs	Deletion	1
Exon 6	c.1137_1138insT	p.Val380fs	Insertion	1
Exon 6	c.1165T>G	p.Leu389Val	Missense	5
Exon 6	c.1195C>T	p.Gln399X	Nonsense	1
Exon 6	c.1226C>A	p.Pro409His	Missense	1
Exon 6	c.1271C>T	p.Pro424Leu	Missense	1
Intron 7	c.1502-2A>G		Splicing Defect	3
Intron 7	c.1502-2A>T		Splicing Defect	1
Exon 7	c.1369_1383dup	p.Val462fs	Insertion	1
Exon 7	c.1387C>T	p.Gln463X	Nonsense	1
Exon 7	c.1394C>T	p.Ser465Phe	Missense	1
Exon 7	c.1394C>T	p.Ser465Phe	Missense	1
Exon 7	c.1400C>T	p.Pro467Leu	Missense	2
Exon 7	c.1421_1422insA	p.Pro475fs	Insertion	1
Exon 7	c.1444_1445delAG	p.Ser482fs	Deletion	2
Exon 7	c.1465T>G	p.Phe489Val	Missense	1
Exon 7	c.1495C>T	p.Pro499Ser	Missense	1
Exon 7	c.1498C>A	p.His500Asn	Missense	1
Exon 8	c.1509C>A	p.Tyr503X	Nonsense	3
Exon 8	c.1513C>A	p.His505Asn	Missense	3
Exon 8	c.1522G>A	p.Glu508lys	Missense	1
Exon 8	c.1537A>T	p.Thr513Ser	Missense	1
Exon 8	c.1544C>A	p.Thr515Lys	Missense	1
Exon 8	c.1573A>T	p.Thr525Ser	Missense	1
Exon 8	c.1574C>T	p.Thr525Ile	Missense	1
Exon 8	c.1576G>A	p.Asp526Asn	Missense	1
Exon 8	c.1576G>T	p.Asp526Tyr	Missense	1
Exon 8	c.1587_1588insA	p.Asn529fs	Insertion	1
Exon 8	c.1611_1614delGCCC	p.Pro538fs	Deletion	1
Intron 8	c.1623+2T>C		Splicing Defect	1
Exon 9	c.1637A>G	p.Asp546Gly	Missense	1
Exon 9	c.1663C>T	p.Leu555Phe	Missense	1
Exon 9	c.1670_1685dup	p.Thr557_Ala562dup	Insertion	1
Exon 9	c.1673_1674insC	p.Ala559fs	Insertion	1
Exon 9	c.1762C>T	p.Pro588Ser	Missense	1
Exon 10	c.1840_1841delAA	p.Asn614fs	Deletion	1
Exon 10	c.1853_1854delTC	p.Ile618fs	Deletion	1
Exon 10	c.1864_1890dup	p.Ile622_Ser630dup	Insertion	1

HNF1A mutation nomenclature according to accession number NM_000545.3.

cases, respectively). However, missense mutations were much more frequent than truncating ones in the dimerization and DNA-binding domains (74% were missense mutations), while the opposite was noted in the transactivation domain (62% were truncating mutations) (Fig. 1*A*). The distribution was the same when considering only distinct mutations (not shown).

A large majority (83%) of the mutations were located in exons 1–6, thus affecting the three *HNF1A* isoforms; 13% of the mutations, located in exons 8–10, specifically af-

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TABLE 2
Age at diagnosis of diabetes in MODY3 patients according to type and position of *HNF1A* mutations

Position of *HNF1A* mutations	Exons		All mutations		Missense mutations	Truncating mutations	
	1–10		22.5 (3–69)		22 (3–69)	18 (3–49)	
					$P = 0.005$ (missense vs. truncating)		
Relative to the functional domain*					P = NS (missense vs. truncating)		
Dimerization/DNA-binding domain	1–4	P = NS	19 (4–61)	$P = 10^{-4}$	20 (4–61)	18 (6–49)	P = NS
Transactivation domain	4–6		21.5 (3–69)		30 (3–69)	19 (3–45)	
					$P < 10^{-4}$ (missense vs. truncating)		
Relative to the isoform							
HNF1A(A), (B), and (C)	1–6	$P = 0.03$	19 (4–61)	P=0.006	20 (4–61)	18 (4–49)	P = NS
HNF1A(A) and (B)	7		29 (10–47)		33 (11–47)	20 (10–31)	
HNF1A(A)	8–10		24 (3–69)		31 (3–69)	20 (3–44)	

Age at diagnosis indicated as median (range). Comparisons between groups were performed using the Mann-Witney test or Kruskal-Wallis test where appropriate. *Dimerization/DNA-binding domain, amino acids 1–281; transactivation domain, amino acids 282–631.

fected the *HNF1A*(A) isoform; and 4% were located in exon 7, affecting isoforms *HNF1A*(A) and (B). This distribution within the isoforms was very similar when considering either missense or truncating mutations and when considering all or distinct mutations (Fig. 1*B*).

Age at diagnosis of diabetes according to the type and the position of the *HNF1A* mutations. Age at diagnosis of diabetes was available for 352 patients. Median age at diagnosis was lower by 4 years in patients with truncating mutations than in those with missense mutations (18 vs. 22 years respectively, $P = 0.005$).

There was no difference in the age at diagnosis according to the location of the mutation within the dimerization/DNA-binding or transactivation domains (19 and 21.5 years, respectively) (Table 2). However, when both the type of the mutation and its position within the functional domains were considered, marked differences appeared. First, truncating mutations were associated with a lower age at diagnosis than missense ones when they affected the transactivation domain (19 vs. 30 years, $P < 10^{-4}$). By contrast, age at diagnosis was similar for truncating and missense mutations of the dimerization/DNA binding domain (18 and 20 years, respectively). Second, missense mutations affecting the dimerization/DNA-binding domains were associated with a lower age at diagnosis than missense mutations affecting the transactivation domain (20 vs. 30 years, $P = 10^{-4}$).

We then analyzed the age at diagnosis according to the isoforms affected by the mutation. Patients carrying a mutation affecting the three *HNF1A* isoforms had a younger age at onset of diabetes (19 years) than those with a mutation affecting isoforms A and B (29 years) or a mutation affecting only the *HNF1A*(A) isoform (24 years, $P = 0.03$ by ANOVA). No difference in the age at diagnosis was observed in patients with truncating mutations, regardless of the affected isoforms. By contrast, patients with missense mutations affecting the three isoforms were much younger at diagnosis (20 years) than those with missense mutations altering one or two isoforms (31 and 33 years, respectively, $P = 0.006$ by ANOVA).

Because the functional domains and the isoform structure are overlapping within the first six exons (Fig. 1), we compared age at diagnosis associated with missense mutations located in the dimerization/DNA-binding domains (amino acids 1–281) with that associated with missense mutations located in the part of the transactivation domain common to the three isoforms (amino acids 282–437). Age at diagnosis was lower in the former than in the latter (20 vs. 26.5 years, respectively, $P = 0.015$).

DISCUSSION

This large series of *HNF1A* mutations in 356 unrelated MODY3 patients emphasizes the high allelic heterogeneity of *HNF1A*. Among the 169 distinct mutations, 118 were not reported in a recent update (14). The large majority (82%) of the novel mutations were private. The type of mutations differed markedly within functional domains: in the dimerization/DNA-binding domain, 74% of the mutations were missense, whereas in the transactivation domain, truncating mutations were predominant (62%). A similar distribution of *HNF1A* mutations has previously been reported (14). Some missense mutations may have mild functional consequences on the protein, and their clinical expression may depend on the functional importance of the affected domain. Thus, some missense mutations of the transactivation domain may not be associated with overt diabetes or lead to a milder phenotype suggesting type 2 diabetes. In patients with truncating mutations, the mean age at diagnosis of diabetes was 18. This is similar to that previously reported in a large series of MODY3 patients (16) and suggests that truncating mutations have similar functional consequences. Nonsense-mediated decay may be the common mechanism leading to this homogenous phenotype through haplo-insufficiency (18). In patients with missense mutations, diabetes was diagnosed later (by 4 years on average) than in those with truncating mutations. This is in contrast with previous results that did not show relationship between the type of the mutations and age at onset (16). However, we only studied probands, while in the study by Harries et al., 55% of the MODY3 patients were relatives (16). We suggest that analyzing relatives together with the probands may introduce a bias toward the inclusion of young subjects through family

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screening. This would decrease the median age at diagnosis. Moreover, our diagnosis criteria are less restrictive than those often used to raise the diagnosis of MODY3, since we included probands with an age of onset of diabetes above 25 years.

Further analysis combining the type of the mutation and its location within the functional domains revealed striking differences in the age at diagnosis of diabetes. Diabetes was revealed 10 years earlier in patients carrying missense mutations located in the dimerization/DNA-binding domains than in those with a missense mutation in the transactivation domain. We hypothesize that missense mutations affecting the dimerization/DNA-binding domain have more severe functional consequences, such as impaired DNA-binding and protein stability (19).

Recently, it has been shown that the age at onset of diabetes may be influenced by the position of the mutation relative to *HNF1A* isoforms. Missense mutations located in exons eight to 10, that are specific of the *HNF1A*(A) isoform, were associated with an older age of onset (16). The authors suggested that this was due to differences in the expression level of the various isoforms in fetal and adult pancreas. We found that patients harboring missense mutations located in exon 7 or in exons 8–10 were diagnosed more than 10 years later than those with mutations in exons 1–6. However, since exons 1–6 include the dimerization and DNA-binding domains, the observed effect on age at diagnosis could be due either to involvement of the three isoforms or to the position of the mutation within the dimerization/DNA-binding domains (Fig. 1). To distinguish between these two possibilities, we compared mutations affecting the dimerization/DNA-binding domain to that affecting the first part of the transactivation domain, and we observed a younger age at onset in the former than in the latter. Thus, the location of the mutation within a domain crucial for the function of the protein overcomes the fact that the mutation affects the three isoforms of *HNF1A*. This was confirmed by a multivariate analysis (not shown).

The wide variability of MODY3 phenotype has suggested the role of modifier genes. However, such genes have not been identified yet (12). We have shown that truncating mutations, as compared with missense mutations, have an effect on the clinical expression of the disease. Moreover, in patients with missense mutations, which represent more than half of the cases, the position of the mutation relative to the functional domains of HNF1A also plays a role in the severity of the disease. We suggest that these parameters should be considered in the studies aiming at the identification of other factors that may influence the clinical expression of MODY3.

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