β-Cell Genes and Diabetes

Molecular and Clinical Characterization of Mutations in Transcription Factors

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β-Cell transcription factor genes are important in the pathophysiology of the β-cell, with mutations in hepatocyte nuclear factor (HNF)-1α, HNF-4α, insulin promoter factor (IPF)-1, HNF-1β, and NeuroD1/BETA2, all resulting in early-onset type 2 diabetes. We assessed the relative contribution of these genes to early-onset type 2 diabetes using linkage and sequencing analysis in a cohort of 101 families (95% U.K. Caucasian). The relative distribution of the 90 families fitting maturity-onset diabetes of the young (MODY) criteria was 63% HNF-1α, 2% HNF-4α, 0% IPF-1, 1% HNF-1β, 0% NeuroD1/BETA2, and 20% glucokinase. We report the molecular genetic and clinical characteristics of these patients including 29 new families and 8 novel HNF-1α gene mutations. Mutations in the transactivation domain are more likely to be protein truncating rather than result in amino acid substitutions, suggesting that a relatively severe disruption of this domain is necessary to result in diabetes. Mutations in the different transcription factors result in clinical heterogeneity. IPF-1 mutations are associated with a higher age at diagnosis (42.7 years) than HNF-1α (20.4 years), HNF-1β (24.2 years), or HNF-4α (26.3 years) gene mutations. Subjects with HNF-1β mutations, in contrast to the other transcription factors, frequently present with renal disease. A comparison of age at diagnosis between subjects with different types and locations of HNF-1α mutations did not reveal genotype-phenotype correlations. In conclusion, mutations in transcription factors expressed in the β-cell are the major cause of MODY, and the phenotype clearly varies with the gene that is mutated. There is little evidence to indicate that different mutations within the same gene have different phenotypes.
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HNF, hepatocyte nuclear factor; IPF, insulin promoter factor; MODY, maturity-onset diabetes of the young.

Transcription factor genes play a crucial role in the normal development and function of the β-cell (1). This is highlighted by the identification of mutations in β-cell transcription factors as a cause of early-onset type 2 diabetes—most notably the distinct subtype maturity-onset diabetes of the young (MODY). MODY is characterized by an autosomal dominant mode of inheritance, β-cell dysfunction, and a young age of diagnosis (usually before 25 years) (2). Mutations in the transcription factors hepatocyte nuclear factor (HNF)-1α (3), HNF-4α (4), insulin promoter factor (IPF)-1 (5), HNF-1β (6), and NeuroD1 (7) all cause early-onset diabetes. These genes form crucial links in the cascade of transcription factors that control the appropriate expression of β-cell genes, such as insulin and GLUT2 (1,8,9).

Mutations in different transcription factor genes appear to result in different clinical presentations. HNF-1α mutations are highly penetrant, with 63% of mutation carriers having diabetes by the age of 25 years, 78.6% by 35 years, and 95.5% by 55 years (10). Mutations in HNF-1α result in progressive β-cell dysfunction with increasing treatment requirements and greater risk of complications with age (11,12). Mutations in HNF-4α result in a similar progressive deterioration of β-cell function but appear to be associated with a later age of diagnosis (13–16). The predominant feature of patients with HNF-1β mutations appears to be renal dysfunction, which is often diagnosed before diabetes (6,17–19). Mutations in IPF-1 (PDX-1) are not a common cause of MODY (20–22). Only one MODY family published to date has an IPF-1 mutation that clearly cosegregates with diabetes (5), although the average age at diagnosis in this family (35 years) was somewhat older than that in families with HNF mutations. The mutation in this family (P63fsdelC) had a severe dominant-negative effect in vitro (23). Two recent studies suggest that missense mutations in the coding region of the IPF-1 gene are more likely to represent predisposing alleles in more common forms of type 2 diabetes (24,25) rather than highly penetrant disease-causing alleles. Mutations in the NeuroD1/BETA2 gene have recently been reported as being associated with type 2 diabetes in two families, one of which meets MODY criteria (7). Studies of the HNF-3β (26–28) and NkX2.2 (29) genes—additional

genes involved in the same transcription cascade—have been performed in MODY and young-onset type 2 diabetes families but did not identify any mutations.

The relative contributions of mutations in β-cell transcription factor genes to early-onset diabetes and MODY is not known, although HNF-1α mutations appear to be the most common (30). We describe here the mutations in these factors in a large U.K. cohort of early-onset type 2 diabetes families and their associated clinical characteristics. In addition, we compare the clinical characteristics of subjects with mutations in different functional domains of the HNF-1α gene.

RESEARCH DESIGN AND METHODS

Subjects and mutation identification. We used two approaches to identify mutations in the known MODY genes and NeuroD1. First, a rapid length-polymorphism–based method was used to screen 210 probands (97% U.K.) for insertion/deletion mutations in the poly-C tract of exon 4 of HNF-1α—a known mutation hotspot (31,32). Of these, 160 fulfilled the minimum criteria for MODY: two generations of diabetes with at least one member diagnosed under the age of 25 years. Of the remaining 50 probands, 24 were diagnosed under the age of 25 years but had no apparent family history, and 26 were diagnosed between the ages of 25 and 35 years, with at least one other affected family member.

We have completed a more thorough screening of the MODY genes and NeuroD1 in 101 of the above 210 probands. The details of 53 (13,19,30,33; M.P.B., S.E., A.T.H., unpublished data; C.B., S.E., M.P.B., A.T.H., unpublished data; S.E., A.T.H., unpublished data) of these probands and their families have previously been published as part of smaller cohorts. Ninety of these subjects met the minimum criteria for MODY. In this subset of probands in which the HNF-1α P291fsinsC mutation was not found, we used a combination of linkage analysis and sequencing to identify mutations. Combinations of the following microsatellite markers were used for linkage analysis: D20S170, D20S96, adenosine deaminase (ADA), D20S119, D20S17, D20S197 (HNF-4α), D12S366, D12S321, D12S807, D12S820, D12S342 (HNF-1α), GCK1, GCK2, D7S667, D7S519, D7S2506 (glucokinase), D17S1788, D17S927, D17S800 (HNF-1β), D13S221, D13S1254, and D13S289 (IPF-1). Genotyping was performed on an ABI 377 and by using GENESCAN and GENOTYPER software (ABI-Perkin-Elmer, Foster City, CA). The appropriate gene was sequenced where linkage analysis clearly identified one locus. For families too small for linkage analysis or that had inconclusive linkage results, the HNF-1α gene was sequenced first. In the absence of an HNF-1α mutation cosegregating with diabetes, the remaining genes were screened. We sequenced all exons and intron/exon boundaries of the HNF-1α gene using a previously described method (30) and primers described by Kaisaki et al. (32), except for exon 2, which was amplified using primers described by Ellard et al. (33). The glucokinase, HNF-4α, HNF-1β, IPF-1, and NeuroD1 genes were sequenced using the same method as that for HNF-1α and primers described by Stoffel et al. (34) (glucokinase), Hara et al. (21) (IPF-1), and Malecki et al. (7) (NeuroD1). Details of HNF-1β and HNF-4α primers are available at http://www.diabetes.org/diabetes/appendix.asp. To check for cosegregation with diabetes, we sequenced the appropriate exon in all available family members.

RESULTS

Prevalence of HNF-1α exon 4 poly-C tract mutations. In a total cohort of 210 probands with early-onset type 2 diabetes, 24 (11%) were found to have a 1-bp frameshift mutation at the poly-C tract in exon 4. For 22 of these probands, the mutation was a single-base insertion (P291fsinsC), whereas the remaining two probands had a single-base deletion (P291fsdelC). In all families, the mutation cosegregated with type 2 diabetes. Of 159 families, 22 (14%) fulfilled the minimum criteria of two generations of type 2 diabetes and one member diagnosed under the age of 25 years having a poly-C tract mutation. Two probands were diagnosed under the age of 25 years (6 and 21 years) but had no apparent family history of diabetes.

Relative prevalence of transcription factor mutations. For our cohort of 101 families in which screening of the coding regions of the glucokinase, HNF-1α, HNF-4α, HNF-1β, IPF-1, and NeuroD1 genes is complete, the relative prevalence of the transcription factor genes is shown in Table 1. The inclusion of the 22 MODY families screened for the exon 4 poly-C tract mutations means that the prevalence of HNF-1α mutations is overestimated. However, the prevalence of glucokinase mutations may be overestimated; although most families include a member diagnosed under the age of 25 years, these are often only diagnosed after screening of other family members after the initial identification of a glucokinase mutation (35).

HNF-1α mutations. As shown in Fig. 1, we identified 34 HNF-1α mutations in 59 families including nonsense, missense, frameshift, and splice site mutations. A total of 29 families and 8 mutations have not been described before and are reported in Table 2 with the associated clinical characteristics. The eight novel mutations are W267X, E132K, IVS2nt+1G→A, IVS4nt-2A→G, D135fsdelA, Q474X, IVS8nt+1G→A, and IVS9nt-1G→A. All mutations cosegregated with diabetes in the families and all amino acids altered by missense mutations are conserved across rat, mouse, hamster, chicken, xenopus, and salmon. Mutations are distributed throughout the gene, although there appears to be some clustering in exons 2 and 3 that include the DNA-binding domain. We have not identified any mutations in the dimerization domain.

Of the 34 mutations identified, 14 occur within the DNA-binding domain (codons 150–280), 8 before the DNA-binding domain (codons 1–149), and 12 in the transactivation domain (codons 280–631). The distribution of mutations is different

TABLE 1
Summary of clinical characteristics by gene in total cohort and in those families that had clinical criteria for MODY

Gene	Families (*n*)	Age at diagnosis (years)	Treatment requirements (%)			MODY families
			Diet	Oral hypoglycemic agent	Insulin	
HNF-1α	59	20.4 (12.2–33.5)	27	43	31	57 (63)
Glucokinase	22	20.9 (10.2–41.7)	84	14	2	18 (20)
HNF-4α	2	26.3 (18.5–37.8)	25	75	0	2 (2)
IPF-1	9*	42.7 (27.5–66.1)	13	47	40	0†
HNF-1β	4	24.2 (17.9–32.7)	0	33	67	1 (1)
MODYX	12	24.0 (13.5–42.7)	32	42	26	12 (13)

Data for age at diagnosis are geometric means (range). Data for MODY families are *n* (%). Criteria for MODY are defined as at least two affected family members, with at least one member diagnosed at <25 years of age. *Includes seven families not from the described cohort but in which IPF-1 mutations have been identified by screening for specific mutations (24); †two MODY families with IPF-1 mutations are not included because these mutations do not cosegregate with diabetes.

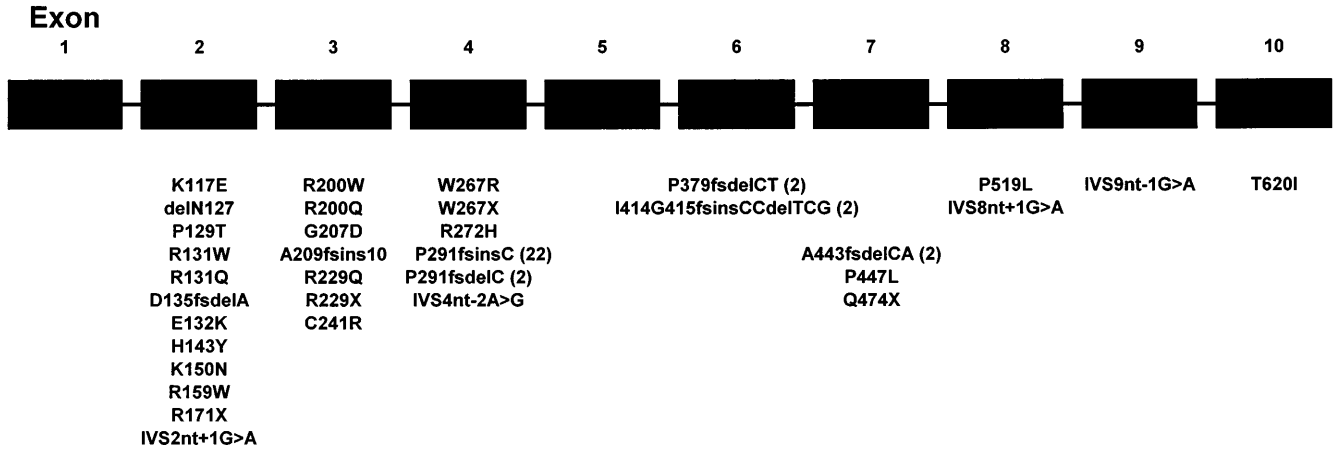

FIG. 1. Distribution of HNF-1α mutations in our cohort of U.K. families.

when considering protein truncating mutations versus amino acid substitutions and different functional domains: of mutations in the transactivation domain, nine are truncating and three result in an amino acid substitution, whereas of the mutations in the DNA-binding domain, five are truncating and nine are amino acid substitutions (χ^2 test for difference: $P = 0.045$). This trend is stronger when comparing mutations in the transactivation domain to all those before this domain (15 missense mutations and 7 truncating mutations before the transactivation domain; χ^2 test for difference: $P = 0.016$).

In addition, we compared the characteristics of patients with mutations in different parts of the HNF-1α gene. Figure

TABLE 2
Characteristics of newly identified families with β-cell transcription factor gene mutations

HNF-1α family	Exon	Codon	Nucleotide change	Coding effect	Mutation designation	Number of diabetic subjects with the mutation	Average age at diagnosis (range)	Treatment			
								Oral hypoglycemic agent and insulin	Diet	Oral hypoglycemic agent	Insulin
DUK 51	4	291	Insertion C	Frameshift	P291fsinsC	5	21.8 (10–27)		1	3	1
DUK 85	4	291	Insertion C	Frameshift	P291fsinsC	2	18.5			2	
DUK 109	4	291	Deletion C	Frameshift	P291fsdelC	1	19			1	
DUK 110	4	267	TGG>TGA	Trp>Stop	W267X	5	21.8 (7–50)		1	4	
DUK 121	2	132	GAG>AAG	Glu>Lys	E132K	3	26.6 (23–33)		1	2	
DUK 128	4	291	Insertion C	Frameshift	P291fsinsC	1	6.0			1	
DUK 145	4	291	Insertion C	Frameshift	P291fsinsC	4	27 (12–45)		1	2	1
DUK 148	4	291	Insertion C	Frameshift	P291fsinsC	5	33.4 (13–74)	1		3	1
DUK 151	4	291	Insertion C	Frameshift	P291fsinsC	3	13.3 (12–14)			1	2
DUK 153	4	291	Insertion C	Frameshift	P291fsinsC	1	17			1	
DUK 164	4	291	Insertion C	Frameshift	P291fsinsC	1	15			1	
DUK 168	Int 2		G>A	Splice site	IVS2nt + 1G>A	3	22 (13–39)			1	2
DUK 176	4	291	Insertion C	Frameshift	P291fsinsC	2	28 (17–39)			2	
DUK 179	Int 4	—	A>G	Splice site	IVS4nt – 2 A>G	5	22.2 (17–29)				5
DUK 196	2	135	Deletion A	Frameshift	D135fsdelA	2	20 (19–21)				2
DUK 213	4	291	Insertion C	Frameshift	P291fsinsC	4	22.5 (11–40)			3	1
DUK 223	4	291	Insertion C	Frameshift	P291fsinsC	3	23.7 (11–45)			3	
DUK 233	4	291	Insertion C	Frameshift	P291fsinsC	5	—	—	—	—	—
DUK 234	4	291	Insertion C	Frameshift	P291fsinsC	2	21.5 (16–27)				2
DUK 243	4	291	Insertion C	Frameshift	P291fsinsC	3	26.3 (20–32)		1	1	1
DUK 247	7	443	Deletion CA	Frameshift	A443fsdelCA	3	—		1	2	
DUK 193	7	474	CAG>TAG	Gln>stop	Q474X	1	29		1		
DUK 169	2	131	CGG>CAG	Arg>Gln	R131Q	2	19.5 (15–24)		1	1	
DUK 43	Int 8	Int 8	G>A	Splice site	IVS8nt + 1G>A	2	36.5 (19–54)		2		
DUK 300	4	291	Ins C	Frameshift	P291fsinsC	1	21		1		
DUK 311	4	291	Ins C	Frameshift	P291fsinsC	2	6.5 (4–9)		1		1
DUK 373	4	291	delC	Frameshift	P291fsdelC	5	18.4 (12–34)			4	1
DUK 358	Int 9	—	G>A	Splice site	IVS9nt – 1G>A	3	20.3 (16–29)			2	1

DUK, Diabetes UK; Int, intron.

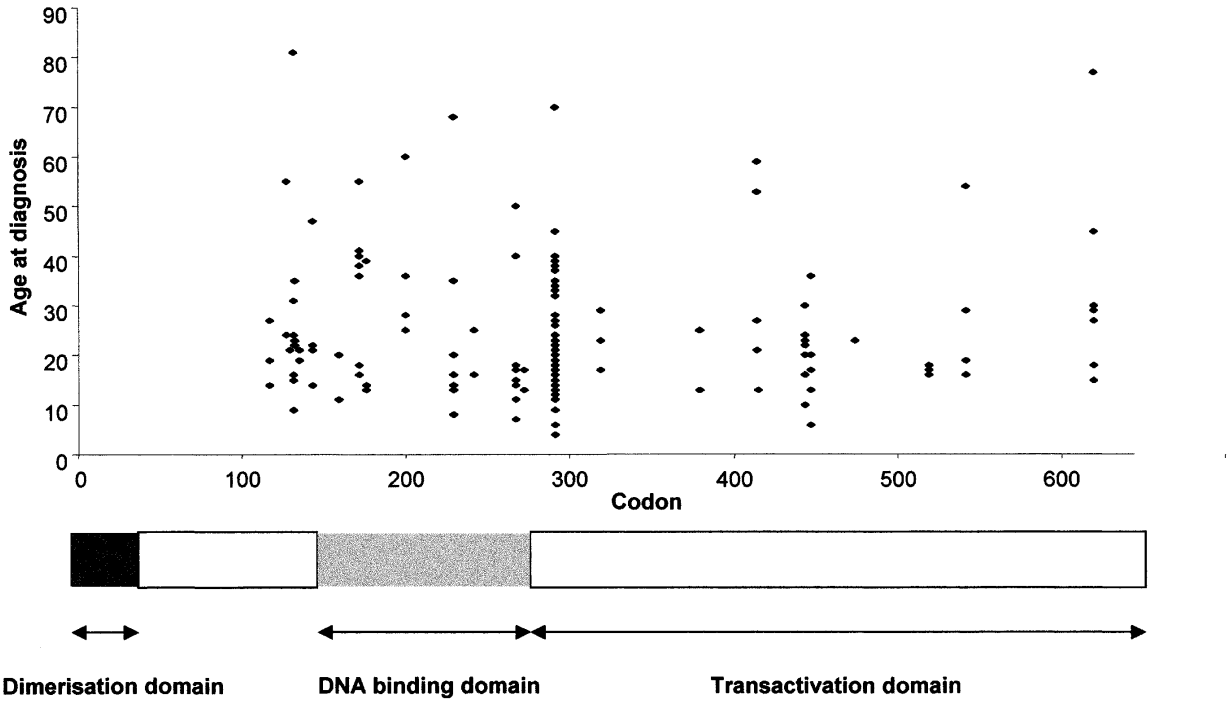

FIG. 2. Scatter plot of age at diagnosis versus codon position in subjects with HNF-1α mutations.

2 shows the distribution of age at diagnosis (average by family) according to position of mutation. We performed comparisons by type and location of HNF-1α mutation. Families with protein-truncating mutations (frameshift, nonsense, and splice-site) were compared with those with non–protein-truncating mutations (missense). There is no evidence that protein-truncating mutations result in a more severe phenotype than missense mutations (mean age of diagnosis 20.3 vs. 20.0 years, $P = 0.91$). Also, families with missense mutations within the DNA-binding domain were compared with those with missense mutations in other parts of the gene. Again, we found no difference in age at diagnosis (20.9 vs. 19.1 years, $P = 0.27$).

HNF-4α mutations. In two of our probands, we identified the HNF-4α missense mutations E276Q (13) and R127W, both of which cosegregated with diabetes in the family. The R127W mutation has previously been described in a Japanese family (14).

IPF-1 mutations. As previously described, we found three IPF-1 missense mutations in nine type 2 diabetes families (24). Only two of these families fulfilled minimum MODY criteria, and in both these families, the mutation did not cosegregate with diabetes. In one family, the proband inherited the mutation from her unaffected father. In the other MODY family, only one affected subject was available for study, and although this person had the mutation, two relatives carrying the mutation were not diabetic after an oral glucose tolerance test.

HNF-1β mutations. To date, we have identified four HNF-1β mutations in four families (19; C.B., S.E., M.P.B., A.T.H., unpublished data). The HNF-1β gene was screened in these families because of a history of renal disease in addition to early-onset diabetes. Only one of these families fitted minimum MODY criteria, but in all four families, the mutation cosegregated with renal disease (19; C.B., unpublished data).

NeuroD1/BETA2. No mutations were identified in the NeuroD1/BETA2 gene. The only change from the published sequence results in a previously described polymorphism, A45T, which was not associated with diabetes in two previous studies (29,36).

MODYX. We identified 12 MODY families in which the coding regions of the six known early-onset type 2 diabetes genes had been screened but no mutations found.

Clinical characteristics. Table 1 gives a summary of the clinical details of all the families in which transcription factor or glucokinase mutations were found but not previously described. Families with glucokinase mutations are not presented in detail because the emphasis of this article is on transcription factors, and the glucokinase families are reported elsewhere (37; G. Spyers, S.E., A.T.H., unpublished data).

Of the transcription factor genes, HNF-1α mutations result in the lowest age at diagnosis (20.4 years). MODYX subjects are diagnosed at an average age of 24.0 years (compared with HNF-1α subjects, $P = 0.052$, Student's t test with log-transformed data). Subjects with IPF-1 mutations are diagnosed at a significantly older age (42.7 years) compared with subjects with mutations in the other transcription factors or with unknown mutations ($P = 0.00002$, 0.01, and 0.0004 compared with HNF-1α, HNF-4α, and MODYX, respectively). Figure 3 shows a Kaplain-Meier plot of survival time without diabetes in years for subjects with mutations in the different transcription factor genes: subjects with a mutation in the HNF-1α gene had a median survival time without diabetes of 21 years compared with 24 years for HNF-4α, 45 years for IPF-1, and 23 years for HNF-1β.

DISCUSSION

In this study, we have shown that heterozygous mutations in β-cell transcription factors are the most common cause of early-onset familial diabetes. This further highlights the crucial role genes in the HNF/IPF-1 β-cell transcription cascade play in normal β-cell function. Whether the primary defect resulting

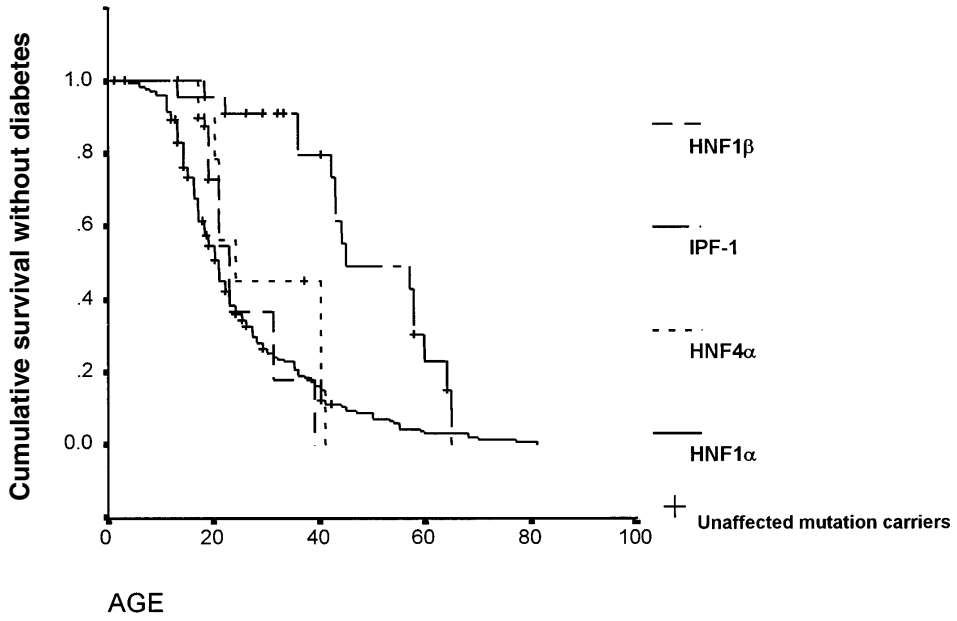

FIG. 3. Kaplan-Meier plot of survival without diabetes for subjects with mutations in the different β-cell transcription factors.

from these mutations is one of β-cell development or function at the fully differentiated stage is unknown. Other genes in this cascade are excellent candidates for all forms of type 2 diabetes.

The single most important gene in our cohort of early-onset families is HNF-1α, with 55% of families (63% of MODY families) having a mutation in this gene. This contrasts with studies of French families where glucokinase mutations appear to be the most common single cause of MODY. A possible reason for this is the more rigorous levels of blood glucose testing performed in children in family studies and in the French health care system (35).

Codon 291 is the most common site for mutations in the HNF-1α gene, and this is almost certainly due to slipped mispairing during DNA replication, causing this region to be a hotspot for mutations, rather than any founder effect (30,32). The type and distribution of HNF-1α mutations across the gene suggest that mutations in the transactivation domain are more likely to be protein-truncating, whereas missense mutations predominate in the DNA-binding domain. This result is consistent with the transactivation domain being more tolerant of minor changes to structure compared with the DNA-binding domain. This suggestion is also supported by the observation that of the three missense mutations in the transactivation domain, two substitute proline, which is the amino acid that creates fixed kinks in polypeptide chains. We can suggest therefore that to result in diabetes, mutations in the transactivation domain need to severely disrupt the HNF-1α protein. Further functional studies of HNF-1α mutant proteins will help clarify this.

We have not shown any genotype-phenotype correlation within the HNF-1α gene, with no relationship between age at diagnosis and location or type of mutation. Hence, protein-truncating mutations result in as severe a phenotype as missense mutations (at least in terms of age at diagnosis), and age at diagnosis is not affected by the location of missense mutations. The P291fsinsC mutation has been reported as showing a dominant-negative effect in vitro (38). However, our subjects with this mutation were not diagnosed at an average age that was significantly different from other subjects (data not shown), as might have been expected if this mutation acts as dominant-negative in vivo but other mutations result in simple gene-dosage effects. Again, further functional studies will clarify this point, but one possibility recently suggested is that protein-truncating mutations may result in an unstable mRNA product that is not translated in sufficient quantity to disrupt the function of the normal product (39).

In some families, HNF-4α mutations are associated with a slightly older age at diagnosis than HNF-1α mutations (13–16). However, when including data from our second family and a further published family (RW) (40), there is no difference between the penetrances: of 66 (46 from the RW pedigree) affected subjects from seven families (14–16,40), 53, 73, and 97% of patients with diabetes were diagnosed by the age of 25, 35, and 55 years, respectively, compared with 63, 78.6, and 95.5% for the same ages for HNF-1α (χ^2 test for difference: $P = 0.72$).

We identified four families with HNF-1β mutations. All these families have some degree of renal disease cosegregating with the mutation and are therefore consistent with the disruption of this gene resulting in renal disease before the early onset of diabetes (6,17–19).

Our study is consistent with previous indications that mutations in IPF-1 (21,22) and NeuroD1/Beta2 are rare causes of early-onset diabetes and MODY (29,36). IPF-1 mutations are more likely to represent predisposing alleles for more typical type 2 diabetes, with an average age at onset of 42 years.

In 12 families, mutations in the coding regions of the known MODY/early-onset diabetes genes do not account for their diabetes. Genes that are part of the β-cell transcription cascade but that have not been screened here will be prime candidates for these families. However, recent studies of French and Japanese subjects suggest that mutations in the Nkx2.2, HNF-3β, and PAX-4 (26–29,36) genes are not the cause of MODY in families unlinked to the known genes.

In conclusion, mutations in transcription factors expressed in the β-cell are the major cause of MODY, and the phenotype varies with the gene that is mutated. There is clear variation between the phenotype seen with mutations in the different genes but little evidence to support different mutations within the same gene having a different phenotype.

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