

Allelic Drop-Out in Exon 2 of the Hepatocyte Nuclear Factor-1 α Gene Hinders the Identification of Mutations in Three Families With Maturity-Onset Diabetes of the Young

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Maturity-onset diabetes of the young (MODY) is a monogenic form of type 2 diabetes characterized by autosomal dominant inheritance, an early age of onset (usually <25 years of age) and β -cell dysfunction (1). Genetic heterogeneity has been demonstrated by the identification of five genes to date. The most common cause of MODY in most populations is a mutation in the hepatocyte nuclear factor (HNF)-1 α gene (MODY3) (2–10). Mutations in the glucokinase gene are associated with MODY2 (11,12), and a small number of families have been reported that have mutations in the HNF-4 α (13–15), insulin promoter factor-1 (16), or HNF-1 β (17,18) genes. At least one additional gene has not yet been localized.

Prior to the identification of the MODY3 gene as HNF-1 α , we performed linkage analysis of this region on chromosome 12q in one Dutch and 10 U.K. MODY families. Analysis of D12S86 using standard criteria (19) demonstrated that eight families showed evidence for linkage, with logarithm of odds (LOD) scores 0.6–2.9 (20). Mutations were detected in five of these families (BDA01, BDA02, BDA04, BDA06, BDA19) (Ref. 3 and T.M.F., M.P.B., S.E., A.T.H., unpublished observations) by direct sequencing of the 10 exons and flanking introns using the primers originally described by Yamagata et al. (2). In the other three linked families (BDA 05, BDA 12, and BDA 13), which had LOD scores of 2.9, 0.6, and 0.8, repeated sequencing in both directions of the coding regions, exon/intron boundaries, and the promoter did not detect any mutations. On one occasion, a nonsense mutation, R171X

(CGA TGA), in exon 2 was identified in the proband of the Dutch family (BDA05), but when the remaining members of this family were investigated, we were unable to detect this mutation in any of the 10 affected subjects, and repeat polymerase chain reaction (PCR) amplifications of the proband DNA failed to confirm the presence of the mutation.

We hypothesized that the failure to detect the mutation in exon 2 might be due to nonamplification of the allele containing the mutation (allelic drop-out). To investigate this, we designed primers with binding sites internal to the original primers described by Yamagata et al. (2). The sequences of the internal primers are as follows: INT2F 5'-CAGGAC CGCAGCCCCACCTATG-3' and INT2R 5'-GGTAGGGTCAT TACTTACGCT-3'. Sequencing of the purified products revealed the presence of the R171X mutation in all 11 affected subjects from family BDA05 (Table 1).

The exon 2 internal primers were then used for PCR amplification and sequencing of the probands from families BDA12 and BDA13. Two further missense mutations were then identified that fully segregated with the diabetic phenotype within the pedigrees: K117E (AAG GAG) in family BDA12 and H143Y (CAC TAC) in family BDA13 (Table 1).

To investigate whether the allelic drop-out had occurred as a result of a polymorphism in one of the original primer binding sites, we designed primers complementary to regions further upstream from the original forward primer and downstream from the original reverse primer (external primer set). The sequences of these primers are as follows: EXT2F 5'-CAAGTCTCTGTCCCTCATGACC-3' and EXT2R 5'-TCC CACTGACTTCCTTTCC-3'. Sequencing of these PCR products revealed a G C base substitution at nt+66 of intron 2 in all 21 affected subjects from the three families (4 homozygotes and 17 heterozygotes), with the C allele present on the same chromosome as the mutations. This polymorphism is located nine bases from the 5' end of the original reverse primer binding site (Fig. 1).

The G C nt+66 polymorphism was present in 7 of 22 (32%) nondiabetic control subjects (allele frequency 16%) and 12 of 33 (36%) MODY probands (allele frequency 18%). The allele frequencies did not differ significantly between control subjects and probands ($P = 0.72$).

Inhibition of the amplification of one allele such as we have observed is most likely to occur when a polymor-

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HNF, hepatocyte nuclear factor; LOD, logarithm of odds; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction.

TABLE 1
HNF-1 α gene mutations in MODY3 families

Family	Exon	Codon	Nucleotide change		Coding effect		Mutation designation	Number of diabetic subjects with the mutation	Average age at diagnosis (range)	Treatment		
										Diet	OHA	Insulin
BDA05	2	171	CGA	TGA	Arg	Stop	R171X	11	33.1 (16-60)	6	5	—
BDA12	2	117	AAG	GAG	Lys	Glu	K117E	4	18.5 (14-27)	2	2	—
BDA13	2	143	CAC	TAC	His	Tyr	H143Y	6	27 (14-47)	1	4	1

OHA, oral hypoglycemic agents.

phism is located at or near the 5' end of the primer binding site (i.e., the 3' end of the primer). The G C polymorphism at nt+66 of intron 2 is situated approximately in the middle of the original reverse primer binding site and might, therefore, be predicted to have a less critical effect that may be affected by factors including the choice of annealing temperature.

In our three families, mutations in exon 2 of the HNF-1 α gene were not detected because of allelic drop-out of the

mutation-containing allele due to a common polymorphism within the 3' primer binding site. Other causes of preferential amplification of one allele may include 1) differential denaturation caused by differences in melting temperature of two alleles (e.g., HLA locus), 2) length differences between alleles when *Taq* polymerase is limiting (e.g., variable number of tandem repeats analysis), and 3) stochastic fluctuation in the number of copies of each allele when the initial number of templates is very small (21).

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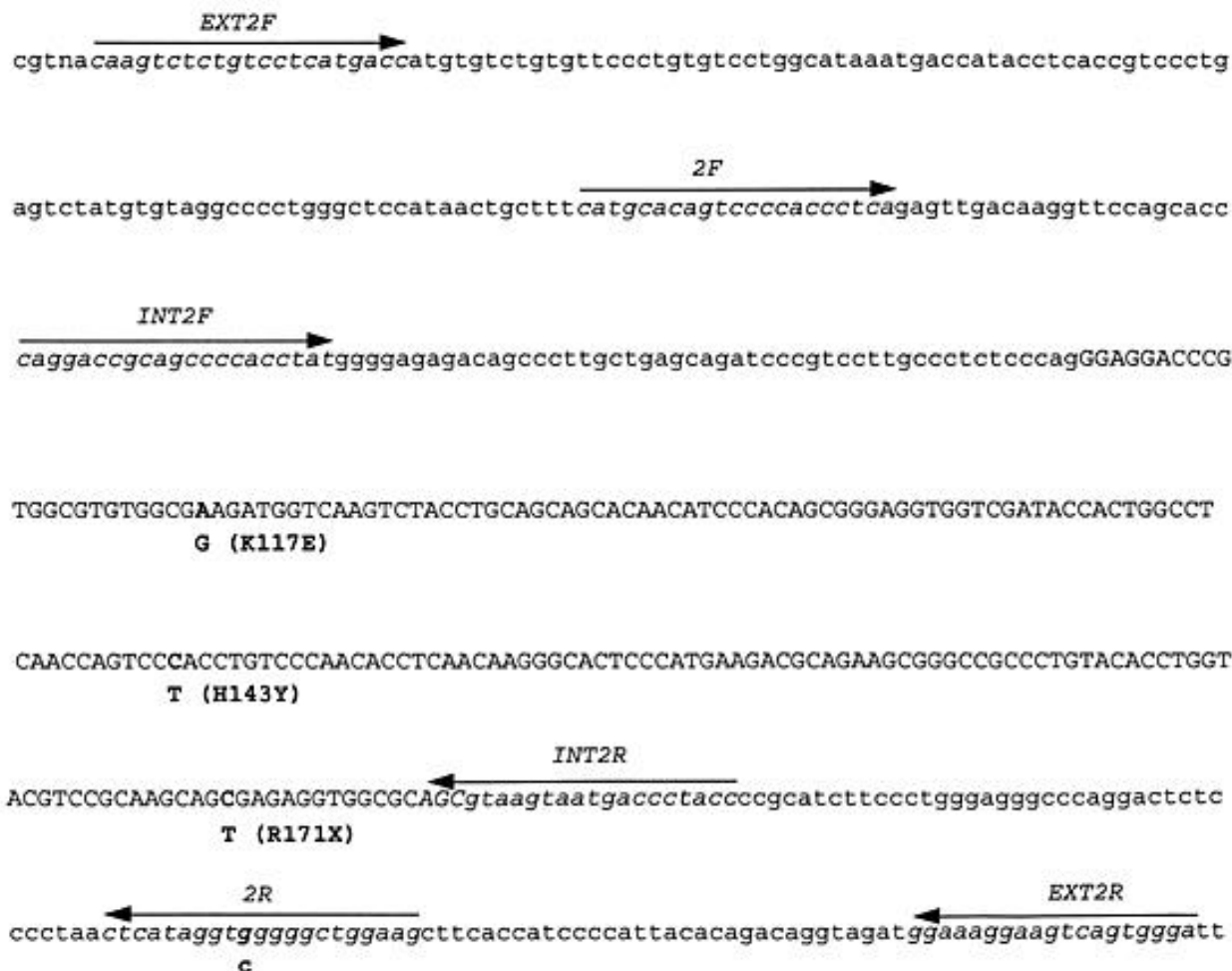


FIG. 1. Partial sequence of HNF-1 α gene. Exon and intron sequences are shown in upper- and lowercase, respectively. The polymorphism and mutations identified in this study are shown in bold, with the designation of the mutations noted. The primer binding sites of the three sets of primers, original primers described by Yamagata et al. (2) (2F and 2R), internal primers (INT2F and INT2R), and external primers (EXT2F and EXT2R) are shown in italics and with arrows.

The K117E is a novel missense mutation, but the R171X and H143Y mutations have been previously reported in a French (5) and a Danish (6) family, respectively. K117E is highly likely to be a mutation rather than a polymorphism, since 1) it results in the replacement of glutamic acid (an acidic charged polar amino acid) for lysine (a basic charged polar amino acid); 2) lysine at codon 117 is conserved in rat, mouse, hamster, chicken, salmon, and *Xenopus*; 3) it cosegregates with diabetes in the family; and 4) it was not seen in 100 control chromosomes.

This is, to our knowledge, the first report of allelic drop-out preventing the detection of a mutation in a gene that causes diabetes. To date, we have identified 30 different HNF-1 α mutations in U.K. MODY families (3) and (S.E., M.P.B., T.M.F., L.I.S.A., A.T.H., unpublished observations). Of these mutations, 12 (40%) are located within exon 2. Data from other groups support our finding that this exon is a frequent site for HNF-1 α mutations (2–10). Substitution of the external primers above for the original exon 2 primers described by Yamagata et al. (2) may reveal HNF-1 α mutations in MODY families with hitherto unidentified mutations.

This finding is also important for mutation analysis of other genes in patients with diabetes. If no mutation in the coding region, splice sites, or promoter is found in a gene showing strong linkage to diabetes, then the possibility of allelic drop-out should be considered.

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