# REVIEW

# Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF)1 and HNF4 families: functional and pathological consequences

**G U Ryffel**

Universitätsklinikum Essen, Institut für Zellbiologie (Tumorforschung), Hufelandstraße 55, D-45122 Essen, Germany

(Requests for offprints should be addressed to G U Ryffel; Email: gerhart.ryffel@uni-essen.de)

**ABSTRACT**

Mutations in the human genes encoding the tissue-specific transcription factors hepatocyte nuclear factor (HNF)1α, HNF1β and HNF4α are responsible for maturity onset diabetes of the young (MODY), a monogenic dominant inherited form of diabetes mellitus characterized by defective insulin secretion of the pancreatic β-cells. In addition, the mutated HNF1β gene causes defective development of the kidney and genital malformation. This review summarizes the main features of these transcription factors and discusses potential events leading to the specific disease phenotypes.
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## INTRODUCTION

Transcription factors are regulatory proteins composed typically of a DNA binding domain that specifically recognizes a short DNA sequence of about 10 base pairs and of an activation or repression domain that influences gene transcription. A classical approach for the isolation of transcription factors is based on the use of a short DNA sequence as affinity resin to purify transcription factors as DNA binding proteins from nuclear extracts. Using this approach the hepatocyte transcription factors HNF1α, HNF3α, β, γ, HNF4α (reviewed in Cereghini 1996) and HNF6 (Lemaigre *et al*. 1996) have been isolated from rat liver using promoter elements of genes specifically expressed in hepatocytes. Upon cloning of the corresponding cDNAs and using these nucleic acid probes in hybridization experiments, it turned out that these liver transcription factors are not restricted to hepatocytes and thus their names are misleading. Furthermore, the similar names of the HNFs describe transcription factors of most distinct structure and therefore reflect only their common origin of purification. Recently, in humans, mutations in some of these HNFs have been identified that cause an early onset of type II diabetes referred to as MODY (maturity onset diabetes of the young) and/or severe renal defects. These diseases associated with 'hepatic' transcription factors document most convincingly the essential function of these transcription factors in non-hepatic cell types.

In this review, I will focus on HNF1α, HNF1β and HNF4α, as up to now mutated forms of these three 'hepatic' transcription factors have been identified to cause human diseases. It has also been proposed that the HNF3β gene is involved in late-onset type II diabetes (Zhu *et al*. 2000), but the evidence is not yet clear as the two missense mutations found could represent polymorphisms, and other groups have questioned the relevance of HNF3β mutations in humans (Abderrahmani *et al*. 2000).

## THE HNF1 GENE FAMILY ENCODING TWO RELATED TISSUE-SPECIFIC TRANSCRIPTION FACTORS IN VERTEBRATES

HNF1α and HNF1β (vHNF1), that has been isolated due to its sequence homology to the α-protein, are two related tissue-specific transcription factors


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of the homeodomain family (Cereghini 1996). They are unique members of this gene family as they contain an extra 21 amino acid stretch in the homeobox that is part of the DNA binding domain. In addition, both transcription factors contain a POU A related domain as a second element involved in DNA binding (Fig. 1). A further feature unique to HNF1 proteins is the N-terminal dimerization domain that allows homo- and heterodimerization of the HNF1 proteins. The C-terminal part contains the transactivation domain that is less conserved between the two proteins (Fig. 1). Both these genes have been highly conserved in vertebrate evolution with homologs in the frog, *Xenopus* (Bartkowski *et al*. 1993, Demartis *et al*. 1994) and an HNF1 protein has even been found in salmon (Deryckere *et al*. 1995). In humans, HNF1α and HNF1β are located on chromosomes 12 and 17 respectively and both consist of 10 exons. This genomic structure and also the temporal and spatial expression pattern of HNF1α and HNF1β are conserved in vertebrate species, as in all species analyzed the expression of HNF1β precedes activation of the HNF1α gene during embryogenesis. Consistent with this differential temporal expression pattern, inactivation of the HNF1β gene in the mouse leads to early embryonic lethality at day 7·5 due to an abnormal extraembryonic region, poorly organized ectoderm as well as defective differentiation of the parietal and visceral endoderm (Barbacci *et al*. 1999, Coffinier *et al*. 1999). The early embryonic lethality of mice with homologous inactivation of the HNF1β gene has precluded any analysis of HNF1β function in later stages of development.

In contrast, the absence of HNF1α gene functions is only manifested after birth with hepatomegaly, phenylketonuria and a Fanconi syndrome characterized by severe urinary loss of glucose, phosphate and amino acids in one report (Pontoglio *et al*. 1996). A more detailed analysis revealed defective insulin secretion in the pancreatic β-cells of HNF1α-deficient mice, but no impairment was found in heterozygous animals (Dukes *et al*. 1998, Pontoglio *et al*. 1998, Pontoglio 2000). In another mouse lineage with a homozygous inactivation of the HNF1α gene, dwarfism as well as non-insulin dependent diabetes were seen to predominate (Lee *et al*. 1998). A careful analysis of these HNF1α-deficient mice revealed alterations in fatty acid homeostasis leading to the enlarged fatty livers typical of HNF1α-deficient mice (Akiyama *et al*. 2000). Furthermore, in these mice hepatic glucose-6 phosphate transporter (G6PT) mRNA levels are significantly reduced and the glucose-6-phosphatase system is disturbed (Hiraiwa *et al*. 2000) explaining growth retardation, hepatomegaly, hyperlipidemia and renal dysfunction.

## HNF1 TARGET GENES

The target genes of the HNF1α and HNF1β transcription factors can either be deduced from the presence of binding sites in promoters and enhancers of genes (see Tronche *et al*. 1997 for an extensive list) or, more convincingly, by the analysis of gene expression upon artificial introduction or inactivation of HNF1 in specific cell types. The binding site for the HNF1 proteins is a palindromic sequence of 13 base pairs with GTTAATNATT ANC as the consensus sequence. However, an HNF1 target site is not sufficient to direct HNF1 dependent transcription, but rather the cooperation within a complex network of various transcription factors defines gene expression.

Using homologous inactivation of the HNF1α gene in the mouse has shown that transcription of most hepatic genes initially thought to be under strict HNF1α control is only weakly affected by the absence of HNF1α (Pontoglio *et al*. 1996). This may reflect the fact that HNF1β present at a low level in hepatocytes takes over the function of the α-protein, but may also be due to the fact that other transcription factors interacting with the regulatory elements of the HNF1 target genes can compensate for loss of HNF1α function. A notable exception to this general rule is that the gene encoding phenylalanine hydroxylase (PAH) is totally silent in the absence of HNF1α and thus leads to the observed phenylketonuria in HNF1α-deficient animals. Quite surprisingly, this inactivation of the PAH gene involves an inactive chromatin structure illustrating that HNF1α also plays a role in chromatin remodeling (Pontoglio *et al*. 1997). Another HNF1α-dependent target gene in hepatocytes is the glucose-6-phosphatase transporter gene that contains an HNF1 binding site in the promoter and is downregulated in the HNF1α-deficient mice (Hiraiwa *et al*. 2000). Concerning target genes in the kidney, it has been shown that the expression of the sodium dependent glucose cotransporter 2 (SGLT2) in tubular cells is reduced and that the SGLT2 promoter contains a functional HNF1α binding site (Pontoglio *et al*. 2001). Thus the inactivation of the SGLT2 gene seems to play a role in urinary glucose loss in the renal Fanconi syndrome of HNF1α-deficient mice.

Due to the early embryonic lethality of HNF1β-deficient mice (see above), potential HNF1β target genes could only be traced by the formation of embryoid bodies that are composed of an outer endoderm layer similar to the visceral endoderm surrounding the inner ectodermal layer of the egg cylinder. These multicellular structures can be formed when embryonic stem (ES) cells are cultured

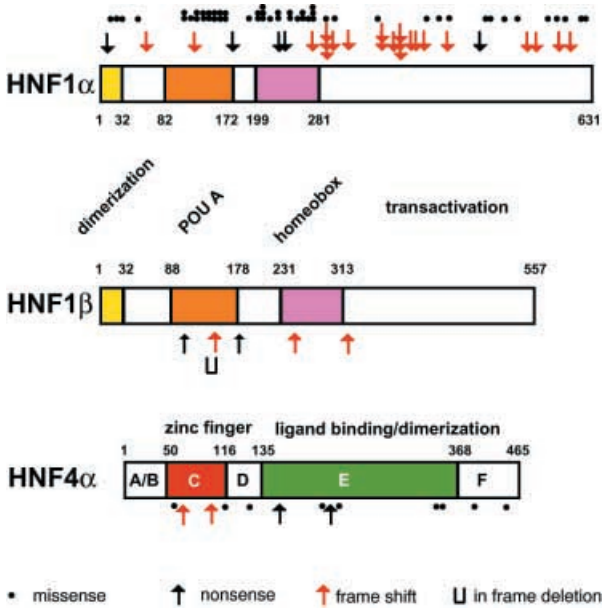



FIGURE 1. Structure and mutations of the HNF1α, HNF1β and HNF4α proteins. A schematic drawing of the human HNF1α and HNF1β protein is shown with the dimerization domain (yellow) and the two domains involved in DNA binding (homeodomain in orange, POU A domain in pink). The structure of the human HNF4α2, the splice variant containing the full-length exon 8 (Sladek & Seidel 2001), is drawn with the DNA-binding domain (zinc finger in red) and the potential ligand binding domain used also for dimerization (green). The locations of frame shift and nonsense mutations generating truncated proteins are indicated with red and black arrows respectively. The in-frame deletion is marked with a bracket and the missense mutations are indicated with a dot. For HNF1α the mutations given include the ones listed recently (Ellard 2000) as well as the novel frame shift mutations listed in Table 1 and all the missense mutants given in Fig. 2. The mutations found in the HNF1β gene are those according to Fig. 4. In HNF1β, two missense mutations A241T and G492S have recently been reported that seem to represent variants and are not involved in any disease (Weng *et al*. 2000). The mutations in HNF4α are given in detail in Fig. 3.

in suspension. Using ES cells with homozygous inactivation of the HNF1β gene, embryoid body formation is severely impaired as no external visceral endoderm cell layer is formed and the bodies remain small without the typical cysts. In these defective structures the expression of the genes encoding the HNF4α1 splice variant, HNF1α and HNF3γ is reduced compared with normal embryoid bodies, whereas the activity of the genes encoding the transcription factors GATA4 and GATA6 is induced to normal levels (Barbacci *et al*. 1999, Coffinier *et al*. 1999). This implies that HNF1β plays a major role in establishing the expression of various transcription factors in the visceral endoderm of the embryo, but it is not yet resolved whether these are direct target genes of HNF1β.

As an alternative way to look for HNF1 target genes the transcription factors have been introduced into cells lacking these transcription factors. Using dedifferentiated hepatoma cell lines lacking HNF1α and HNF4α proteins the expression of transfected HNF1α may lead to hepatic differentiation including the activation of the HNF4α gene and even of the endogenous HNF1α gene (Bailly *et al*. 1998). However, the outcome of such experiments is quite variable depending on the recipient cell type used and on the presence of other factors referred to as activators or extinguishers. The role of such factors can also be deduced from somatic cell hybrids between hepatoma cells and fibroblasts (Bulla 1999). Using such cell hybrids the role of HNF1α and HNF4α in the remodeling of the human chromatin domain containing the α1-antitrypsin and the corticosteroid-binding globulin gene has been established (Rollini & Fournier 1999). In an opposite approach, the inactivation of HNF1α has been performed by the introduction of a dominant negative mutant of the HNF1α gene into the β-cell derived insulinoma cell line, INS-1 (Wang *et al*. 1998, 2000*a*). From such experiments, the genes encoding insulin, glucose transporter-2, L-pyruvate kinase, aldolase B, 3-hydroxy-3-methylglutaryl coenzyme A reductase and mitochondrial 2-oxoglutarate dehydrogenase (OGDH) have been identified as HNF1α target genes in pancreatic β-cells. All these genes encode functions essential for metabolism and secretion of pancreatic β-cells and are known to contain HNF1 binding sites in their promoter or enhancers. In conclusion, these experiments show that HNF1α contributes to cell identity, but clearly its role is limited as the effect will depend on the cell type and/or the presence of other transcription factors. In general, the introduction of HNF1α does not lead to the establishment of a new cell type and this may distinguish HNF1α from transcription factors such as MyoD that acts as a master regulator and leads quite frequently to myogenic gene expression program (Rawls & Olson 1997).

## THE TISSUE-SPECIFIC TRANSCRIPTION FACTOR HNF4α

HNF4α is a member of the nuclear receptor superfamily with two zinc fingers as the DNA

binding domain and thus represents a transcription factor very distinct from the HNF1 proteins (Sladek & Seidel 2001). HNF4α belongs to the factor subfamily 2A and is designated as NR2A1 (Nuclear Receptors Nomenclature Committee 1999). This subfamily includes HNF4β, so far only identified in the frog, *Xenopus* (Holewa *et al*. 1997), and HNF4γ present in humans (Drewes *et al*. 1996) and mice (Taraviras *et al*. 2000). The functional domains of HNF4α consist of an N-terminal activation function (AF-1, also referred to as A/B domain), two zinc fingers responsible for DNA binding (C domain), a potential ligand binding domain (domain E) and the F domain (Fig. 1). The C-terminal part contains a region involved in homodimerization, the activation function AF-2, as well as a short repressor region. Based on the three dimensional structure of the ligand binding domain of the related progesterone receptor, 12 helix bundles and 4 β-sheets can also be located in the ligand binding domain of HNF4α (Bogan *et al*. 2000). A series of coactivators have been identified that interact with defined regions of the HNF4α protein (listed in Sladek & Seidel 2001). No definitive ligand for HNF4α has been identified as the recent claim that fatty acylcoenzyme A thioesters might be ligands (Hertz *et al*. 1998) has been challenged by the finding that these compounds exert only a minor effect and have a limited specificity (Peiler *et al*. 2000, Sladek & Seidel 2001). Thus HNF4 remains an orphan receptor, but the identification of potential ligands is still an important issue as such compounds might be important in influencing HNF4 activity in the organism. Recently, a low molecular compound has been identified in embryonic extracts of *Xenopus* that inhibits quite specifically the DNA binding properties of HNF4 (Peiler *et al*. 2000). However, this inhibitor is distinct from the usual ligands of the nuclear hormone receptor superfamily, as it does not require the ligand binding domain of HNF4α to exert its inhibitory function. The biological significance of this compound and its potential use to interfere with HNF4 action are unclear and will remain so as long as the chemical identity of the HNF4 inhibitor is not solved.

In humans, the gene encoding HNF4α is located on chromosome 20 and the open reading frame is located on 10 exons. As two additional exons (exon 1C and 1B) have been identified between the original exon 1 and 2, and exon 8 can be differentially spliced to exon 9, many splice variants exist whose functional relevance is not yet clear (Sladek & Seidel 2001).

The HNF4 transcription factor family has been extensively conserved with members found even in insects (Sladek & Seidel 2001). Observations in the fly, *Drosophila* (Zhong *et al*. 1993) and the frog, *Xenopus* (Holewa *et al*. 1996, 1997) suggest that HNF4 plays a role early in embryonic development, since this transcription factor is a maternal component of the egg. An early function in embryogenesis seems also to occur in mammals, as HNF4 expression has been reported in murine embryonic stem cells (Nakhei *et al*. 1998) and the knock-out of the gene in the mouse leads to defective gastrulation with embryonic lethality at day 9 (Chen *et al*. 1994). This critical phase of early development in the mouse primarily depends on the requirement for HNF4α for the proper function of the extraembryonic tissue at day 6·5, as the supply of wild-type visceral endoderm rescues the development of the murine embryo through midgestation (Duncan *et al*. 1997). In these rescued embryos, specification and early development of the liver occurs, but the fetal liver fails to express many genes typically expressed in hepatocytes, including genes encoding apolipoproteins, metabolic proteins and serum factors (Li *et al*. 2000), thus establishing the importance of HNF4α for proper terminal differentiation of hepatocytes. In these rescued mice embryos no data are available for the extent of differentiation in other organs expressing HNF4α such as the kidney, the intestine and the pancreas. In all species analyzed, HNF4α expression precedes the expression of HNF1α and HNF1β, implying that HNF4α is a transcription factor higher up in the hierarchy of regulatory proteins. Consistent with this potential dominance of HNF4α, a functional binding site for HNF4 has been identified in the promoter of the HNF1α gene (Tian & Schibler 1991, Kuo *et al*. 1992, Zapp *et al*. 1993) and overexpression of HNF4α in *Xenopus* embryos induces ectopic activation of the HNF1α gene (Holewa *et al*. 1996, Nastos *et al*. 1998). However, an HNF1 binding site has also been identified in the HNF4 promoter suggesting a reverse regulation as well (Zhong *et al*. 1994). Furthermore, the analysis of embroid bodies with inactivated HNF3α or HNF3β genes revealed that both these transcription factors affect HNF4α and HNF1α expression (Duncan *et al*. 1998). Whereas HNF3α seems to act as a repressor, HNF3β supports the expression of HNF4α substantially. Clearly, the activities of the HNF4α and HNF1α genes are regulated by a complex transcriptional regulatory network, whose main features are just being identified.

## HNF4α TARGET GENES

HNF4α regulates genes at the transcriptional level by interacting with the HNF4 binding site in the

promoter or enhancer. These potential target genes with an HNF4 consensus sequence are predominantly expressed in hepatocytes (Sladek & Seidel 2001). However, the presence of a target site within the promoter/enhancer does not mean that the corresponding gene is exclusively under the control of HNF4α, as HNF4β and HNF4γ and even unrelated transcription factors such as chicken ovalbumin upstream promoter-transcription factor (COUP-TF), retinoic acid receptor/retinoid X receptor or peroxisome proliferator-activated receptor (PPAR) can interact with these binding sites (Sladek & Seidel 2001). The recent analysis of knock-out mice whose early development has been rescued (see above) proves that the genes encoding the transcription factor HNF1α and the pregnane-X-receptor as well as the genes of apoAI, apoAII, apoB, apoCII, apoAIII, aldolase B, phenylalanine hydroxylase, L-fatty acid-binding protein (LFAP), transferrin, retinol-binding protein and erythropoietin are target genes in hepatocytes (Li *et al*. 2000). A similar list of HNF4α target genes has been identified in embryoid bodies containing visceral endoderm, an embryonic tissue with many hepatic functions (Shih *et al*. 2000). Recently, the function of HNF4α in pancreatic β-cells has been analyzed in insulin secreting INS-1 cells by conditional expression of either wild-type HNF4α or a dominant negative mutant of HNF4α that efficiently suppresses the endogenous HNF4α transcription factor (Wang *et al*. 2000*b*). In these experiments the genes encoding insulin, glucose transporter 2, L-pyruvate kinase, aldolase B, 2-oxoglutarate dehydrogenase E1 subunit and mitochondrial uncoupling protein-2 were affected in the manipulated β-cells. As the spectrum of genes affected is strikingly similar to that of its downstream transcription factor HNF1α, that is also influenced in these cell clones, the effect is probably mediated via the simultanous decrease in HNF1α expression. These results establish that HNF4α regulates many activities crucial for proper glucose metabolism and insulin secretion in pancreatic β-cells.

Based on all these data it is clear that HNF4α plays an important role in many distinct functions of the organism. Most notable are the apolipoproteins whose dysfunction may lead to artherosclerosis, and the coagulation factors whose lack of expression results in hemophilia. An extensive list of potential target genes and their physiological functions has recently been published (Sladek & Seidel 2001). So far, three human genes have been identified containing naturally occurring mutations in the HNF4 binding site that lead to a disease. Two genes encode factors involved in blood coagulation (factor VII and factor IX) and thus a mutated HNF4 binding site in the promoter of these genes is responsible for hemophilia (Sladek & Seidel 2001, see also Carew *et al*. 2000). The third gene is the transcription factor HNF1α where a mutation in the HNF4 binding site causes MODY3 (Gragnoli *et al*. 1997, see below).

## MATURITY ONSET DIABETES OF THE YOUNG (MODY) CAUSED BY MUTATED HNF1α, HNF1β AND HNF4α TRANSCRIPTION FACTORS

Maturity onset diabetes of the young (MODY) is an autosomal dominant inherited disease in humans that is characterized by an early onset of non-insulin dependent diabetes mellitus (NIDDM) (recent reviews: Hattersley 1998, Froguel & Velho 1999, Winter *et al*. 1999, Winter & Silverstein 2000). Clinically this diabetes is manifested by defective insulin secretion of the β-cells of the pancreas and is defined by the occurrence of diabetes in at least two generations with at least one member affected under the age of 25 years. So far, heterozygous mutations in 5 genes that lead to this disease have been identified in humans. One gene, referred to as MODY2, encodes the enzyme glucokinase expressed in the β-cells and senses the glucose level in the blood. A defective enzyme due to a mutated gene will not measure the glucose level properly and thus failure in appropriate insulin secretion occurs. All the other MODY genes identified encode cell-specific transcription factors that are all expressed in pancreatic β-cells. MODY4 represents mutations in the gene encoding the transcription factor IPF-1 (insulin promoter factor-1) also referred to as PDX-1 (pancreatic duodenal homeobox-1), IDX-1 (islet duodenum homeobox-1) or STF-1 (somatostatin transcription factor-1) that has been initially identified as a transcription factor for pancreas development and islet peptide hormone expression (Habener & Stoffers 1998). In contrast, MODY1, MODY3 and MODY5 are the genes for HNF4α, HNF1α and HNF1β respectively, three transcription factors that were primarily not considered to be regulators in the endocrine pancreas (Huang & Tsai 2000). The data available suggest that people carrying one mutated allele are born with completely normal physiological and biochemical functions of the pancreatic β-cells and that diabetes will only occur at some stage during adolescence. The penetrance of diabetes in patients with mutations in one of these three genes is quite high, with more than 95% by the age of 55 years (Frayling *et al*. 2001). Overall, MODY3 representing a mutated HNF1α gene is the most prominent

TABLE 1. Mutations in the HNF genes

| | Type of mutations | | | |
|---|---|---|---|---|
| | Missense | Nonsense | Frameshift | In frame deletion/ insertion |
| **Gene** | | | | |
| HNF4α | 7 | 2 | 2 | 1 |
| HNF1α | 52 | 5 | 20 | 0 |
| HNF1β | 0 | 1 | 4 | 1 |

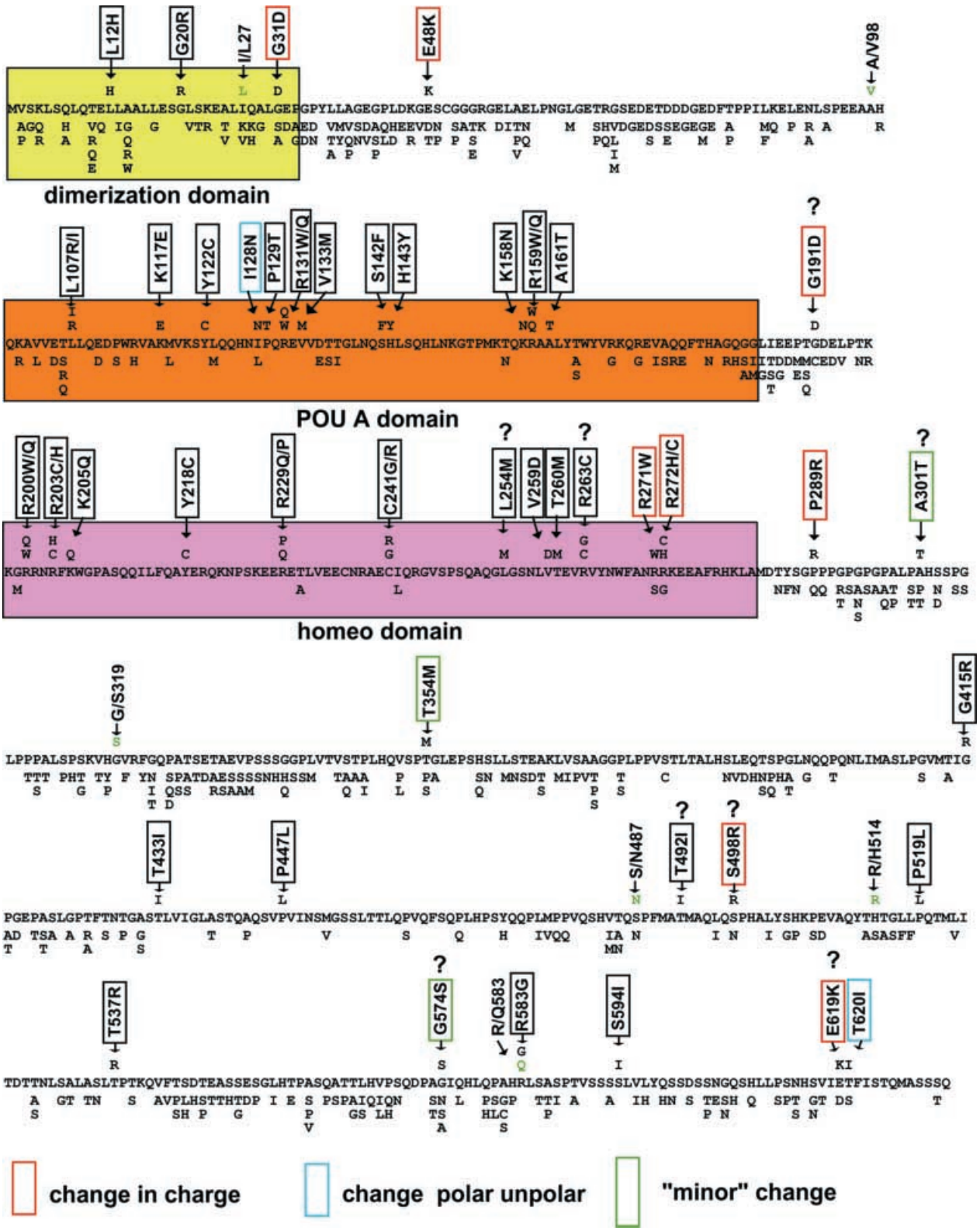
The mutations for HNF4α and HNF1β are listed in Figs 3 and 4 respectively. The mutations for HNF1α are the ones listed in Ellard (2000) supplemented by additional missense mutations given in Fig. 2 and the novel frameshift mutations S121fsdelC (Costa *et al*. 2000), R272fsdelGC (Lindner *et al*. 1999*b*) and S315fsinsA (Lehto *et al*. 1999*b*).

form, amounting to about 60% of cases, whereas MODY1 and MODY5 representing HNF4α and HNF1β mutations are rarely seen (1–2%). The remaining patients are MODY2 (mutated glucokinase gene), MODY4 (IPF-1) and MODYX (cases where the mutated gene defect has not been identified yet) representing some 20%, ~1% and 15% of the MODY families respectively. It seems that there is a genuine difference in the frequency of these various forms of MODY depending on the racial groups investigated.

## COMPARISON OF THE TYPES OF MUTANTS FOUND IN THE HNF1α, HNF1β AND HNF4α GENES OF MODY PATIENTS

The main structural and functional features of HNF1 and HNF4 proteins have been identified quite extensively (see above). Thus, when the first mutations were found in these genes several groups analyzed the features that were destroyed by the mutants, suspecting that a unique property might be identified that would explain the disease. Based on the fact that HNF1α is the most abundant gene mutated in MODY patients, more than 75 distinct mutants have been identified in this gene (for the most up-to-date list see Ellard 2000). In Table 1 the mutants are classified into missense mutants encoding a change in amino acid sequence, nonsense and frameshift mutants leading to truncated proteins, and in-frame deletions or insertions generating proteins with internal deletions or insertions respectively. About 2/3 of the HNF1α mutants are missense mutants and most other mutants generate proteins truncated at the C-terminus either by a point mutation leading a stop codon (nonsense mutation) or, more frequently, a nucleotide deletion/insertion resulting in a frameshift encoding an altered amino acid sequence downstream of the mutational event. This spectrum of mutations shown in Fig. 1 seems to represent mutations occurring at random. A similar distribution is found for the HNF4α gene, although in this case only 12 distinct mutants have been identified so far (Table 1 and Fig. 1). In contrast, the six mutations of the HNF1β gene all generate truncated proteins due either to nonsense mutations (2 cases) or to frameshifts (4 cases) obtained by deletions or insertions. This distinction is most compelling as HNF1α and HNF1β are encoded by two closely related genes (Fig. 1). This graphic illustrates that the truncations in the HNF1α gene are predominantly in the C-terminus representing the activation domain, whereas the same types of mutants seem to be more randomly distributed in

FIGURE 2. Missense mutations in the human HNF1α protein. The amino acid sequence of the human HNF1α protein (Bach *et al*. 1990) is given and the domains for dimerization (yellow) and for DNA binding (POU A in orange and homeodomain in pink) are boxed. Below the human amino acid sequence amino acids are listed that are different to those at the corresponding position in the proteins of the rat (Frain *et al*. 1989), mouse (Blumenfeld *et al*. 1991), hamster (Emens *et al*. 1992), chicken (Grajer *et al*. 1993) and *Xenopus* a and b (Bartkowski *et al*. 1993) proteins. Diabetes-associated missense mutations (boxed) and polymorphisms not associated with any human disease (without boxes) are given. Black boxes refer to missense mutations changing an amino acid which is fully conserved in all HNF1α proteins, whereas the colored boxes indicate mutations occurring at non-conserved positions. The red, blue or green boxes indicate changes in charge, changes from an unpolar to a polar amino acid (or vice versa) or a 'minor' change respectively. The missense mutations include the ones listed in Ellard (2000) and are complemented by the mutations G20R (Hua *et al*. 2000, Rose *et al*. 2000*b*), V133M (Boutin *et al*. 1997, Costa *et al*. 2000), R203H (Awata *et al*. 1998), Y218C (Jap *et al*. 2000), L254M (Yamada *et al*. 1997), V259D (Boutin *et al*. 1997, Costa *et al*. 2000), A301T (Cox *et al*. 1999), G/S319 (Hegele *et al*. 2000), S/N487 (Urhammer *et al*. 1997, Yamada *et al*. 1997, Behn *et al*. 1998, Lehto *et al*. 1999*b*, Rissanen *et al*. 2000), T492I (Cox *et al*. 1999), S498R (Cox *et al*. 1999), R/H514 (Behn *et al*. 1998), G574S (Boutin *et al*. 1999), R/Q583 (Urhammer *et al*. 1997) and S594I (Boutin *et al*. 1997). Missense mutations marked with a question mark refer to data that seem to exclude a MODY mutation but rather indicate a correlation to early or late onset diabetes II. In these cases a polymorphism not associated with any disease is not excluded. Amino acids given above the human sequence refer to the changes found and polymorphisms are in green.



the HNF1β gene. The missense mutations found in the HNF1α gene are spread over the entire gene, but are clearly concentrated in the DNA binding domains (POU A and homeobox) of the N-terminal part (Fig. 1). The relatively high incidence of mutations in the HNF1α gene reveals that there is a hotspot at codon P291, as insertion of a C in the polyC tract of exon 4 leading to frame shift is seen in at least 22 distinct families. A frequent instability is also found at P379 (7 families) involving insertion or deletion of pyrimidines (Ellard 2000). Mutations at Arg codons (R131, R159, R171, R200, R229, R272) are also found in independent families (Ellard 2000), most likely due to the well known deamination of methylcytosin in CpG dinucleotides. In conclusion, it appears that the mutations found in the HNF1α and the HNF4α genes reflect the various mutagenic events known to occur and there seems to be no selection for specific types. In contrast, the MODY5 mutations, all leading to truncated HNF1β proteins, clearly represent a selection mechanism.

## TYPE OF MUTANTS FOUND IN THE HNF1α GENE OF MODY PATIENTS

The high incidence of mutations in the HNF1α gene allows an evaluation as to whether missense mutations occur at random in the HNF1α protein or are restricted to amino acid positions conserved throughout vertebrate evolution. In Fig. 2, the amino acid sequence of the human HNF1α protein is presented and the changes in amino acids as found in the rat, mouse, hamster, chicken and *Xenopus* proteins are listed under the human protein sequence. Out of 52 missense mutations identified in the HNF1α gene, 44 distinct amino acid positions are involved and 31 (70%) are located at fully conserved amino acid positions, predominantly in the conserved domains for dimerisation and DNA binding (POU A and homeodomain). The mutations at non-conserved positions of the HNF1α protein involve 8 position alterations that lead to a charge change (red boxes) not present in any of the HNF1α proteins, and thus may profoundly affect the structure and function of the protein. At two positions, I128N and T620I (blue boxes in Fig. 2), a change from a non-polar to a polar amino acid and vice versa is seen, respectively, that might have functional implications. However, there are also 'minor' changes at 3 positions (A301T, T354M and G574S) that are quite variable between the HNF1α proteins of the different species. However, in two of these cases (A301T and G574S) the clinical data do not support the identification of MODY, but rather document an early or even late onset of type II diabetes. As this type of diabetes is quite frequent, such single cases do not allow a clear cut conclusion. Furthermore, in some cases the data available do not as yet prove that the corresponding change in amino acid really is responsible for MODY, but rather they may document a variant factor apparently associated with the disease. It could be that the amino acid change marks the responsible allele, but the critical mutation has not yet been identified and may affect, for instance, elements essential for gene regulation. This notion is supported by the few cases where mutations in the promoter of the HNF1α gene have been identified as critical parameters (see below). In summary, Fig. 2 illustrates that missense mutations found in MODY3 patients are predominantly located at evolutionary conserved amino acid positions.

A wealth of functional data have been collected over the last few years to identify a common and specific defect in the various MODY3 genes that triggers the pathological process in the pancreatic β-cells. In general, a multitude of defective functions has been described using *in vitro* and *in vivo* assays. Therefore, a recent classification is quite useful to get an overview (Vaxillaire *et al*. 1999), although the application of this classification to the experiments made in different laboratories using distinct cell lines and reporter systems is subject to interpretation. Table 2 summarizes the functional data on 19 mutants that have been analyzed. Ten were found to have a reduced transactivation activity (class I), whereas nine showed a complete loss-of-function. These loss-of-function mutants include most frameshift or nonsense mutants that generate truncated proteins. Four of these loss-of-function mutants were reported to have dominant negative properties. This property may not be too surprising, as HNF1α acts as a dimer, but the significance of this dominant negative effect is not yet clear. If a mutation such as K158N reduces the transactivation of HNF1α (Yamada *et al*. 1999), it is expected that in a mixture of wild-type and mutated factors an intermediate activity will be observed that depends on the ratio of the two factors and thus can readily be seen by adding a large excess of the mutant. This may represent an artificial situation as in the heterozygous MODY patients an equal amount of wild-type and mutated factor is expected. Of course it may be more significant if a mutated factor has no activity on its own and in addition inhibits the wild-type protein present in equimolar amounts. Such a situation may apply for the missense mutant P447L (Vaxillaire *et al*. 1999) and the two

frameshift mutants P291 fsinsC (Yamagata *et al*. 1998, Vaxillaire *et al*. 1999) and T547E548 fsdelTG (Vaxillaire *et al*. 1999). But, in general, a dominant negative effect cannot represent the cause of MODY3 and even if it could explain a few cases, there is no evidence that these patients have a more serious disease. Based on the fact that the structural and functional features of HNF1α are fairly well understood, the mutational defect can be explained in many mutants. For instance, the mutants L12S and G20R in the dimerization domain disrupt the ability to form HNF1α dimers as well as the interaction with the coactivator DCoH to form the tetrameric DCoH-HNF1α complex (Hua *et al*. 2000, Rose *et al*. 2000*a*). On the other hand, many MODY3 mutants pinpoint essential features that were not known previously allowing the deduction of some novel functional anatomy of the HNF1α protein (Vaxillaire *et al*. 1999).

TABLE 2. Functional properties of the HNF1α mutations

| | Effect | Missense | Truncations |
|---|---|---|---|
| **Class** | | | |
| I | Decreased | LH12[1,2,3,4,5]<br>Y122C[6]<br>R131Q[6]<br>R159Q[2,6]<br>G191D[1]<br>R203C[2]<br>K205Q[6]<br>R263C[1,3]<br>R272H[6] | P379fsdelCT[1,3] |
| IIa | Loss | G20R[4,5]<br>S142F[6] | R171X[6]<br>R229X[2]<br>L584S585fsinsTC[1,3] |
| IIb | Loss and dominant negative | K158N[2]<br>P447L[6] | R291fsinsC[6,7]<br>T547E548fsdelTG[6] |

The functional data are taken from [1]Yang *et al*. (1999), [2]Yamada *et al*. (1999), [3]Okita *et al*. (1999), [4]Rose *et al*. (2000*b*), [5]Hua *et al*. (2000), [6]Vaxillaire *et al*. (1999) and [7]Yamagata *et al*. (1998).

## REGULATORY MUTANTS IN THE PROMOTER AREA OF MODY3 PATIENTS

The analysis of MODY3 patients has revealed a few cases where the mutational event seems to be outside of the protein coding region (Table 3). The most notable case has been the mutation of the HNF4 binding site of the HNF1α promoter referred to as –283A-G mutation, where a single nucleotide exchange from A to G in the potential HNF4 binding site was reported (Gragnoli *et al*. 1997). Indeed, it was verified that this nucleotide exchange dramatically reduces binding of HNF4α to the HNF1α promoter and that in transfection assays a –283A-G mutant promoter was much less activated by the transcription factor HNF4α (Lausen *et al*. 2000). The importance of the HNF4 binding site within the HNF1α promoter is further supported by the presence of this site in the *Xenopus* promoter, where it plays a crucial role in the expression of the HNF1α gene in transgenic animals (Ryffel & Lingott 2000). As HNF4α is also a gene involved in MODY (see below) and the data of the –283A-G mutation is supported by a three-generation family tree (Gragnoli *et al*. 1997), this mutation defines a *bona fide* MODY3 disease. A less clear situation may be the –119Gdel mutation (Godart *et al*. 2000) that is supported by a three-generation pedigree, but no functional data are available for the performance of this mutated promoter, and the potential transcription factor binding to this site is unknown. Further unresolved cases involving early onset diabetes patients with no established family history are listed in Table 3. However, in some mutations potential binding sites are involved and/or changes of the activity of promoter constructs in transfection assays have been reported. As the HNF1α promoter is extensively conserved between *Xenopus* and higher vertebrates (Zapp *et al*. 1993), this comparison may constitute another feature to evaluate the significance of changes in the promoter area. Clearly, more data and experiments are needed to establish whether these are variants or functionally relevant nucleotide changes. The fact that MODY mutations occur in the promoter area implies that loss-of-function of one HNF1α allele is a crucial event in the genesis of MODY. It may also indicate that other regions that regulate the activity of the HNF1α gene are potential regions in which to look for MODY causing mutations. These could potentially include far upstream sequences such as locus control regions (LCR) that are, for example, known to be mutated in some β-thalassemia patients (Li *et al*. 1999).

## TYPE OF MUTANTS FOUND IN THE HNF4A GENE OF MODY PATIENTS

The mutations of the HNF4α gene representing the MODY1 gene are summarized in Fig. 3. The amino acid sequence of the human HNF4α gene represents the sequence encoded by the HNF4α2 splice variant (Furuta *et al*. 1997) and the numbering starts at the methionine initially thought to mark the translation start site. However, the cloning of HNF4α from *Xenopus* (Holewa *et al*. 1996) and other species

TABLE 3. MODY3 mutants/variants in the HNF1α promoter

| | Conserved position | Binding site | Function | Family history (# patients) | Family tree | Reference |
|---|---|---|---|---|---|---|
| **Mutant** | | | | | | |
| −562G-A | Rat | | ND | No | | Cox *et al*. (1999) |
| −527C-T | | | ND | No | | Lehto *et al*. (1999*b*) |
| −283A-G | Rat, *Xenopus* | HNF4 | Minus 80% | Yes (9) | 3 gen. | Gragnoli *et al*. (1997) |
| del-233-234 | Rat, *Xenopus* | C/EBP? | ND | No | | Cox *et al*. (1999) |
| −218T-C | Rat, *Xenopus* | NF-Y? | Minus 20% | Yes (2) | | Godart *et al*. (2000) |
| 207,206ins27 | Rat | HNF3? | ND | Yes (1) | | Godart *et al*. (2000) |
| −187C-T | Rat | AP-1? | ND | Yes (1) | | Godart *et al*. (2000) |
| −154-153 insTGGGGGT | Rat | | ND | Polymorphism | | Lehto *et al*. (1999*b*) |
| −124G-C | Rat | | Plus 100% | Yes (1) | | Yoshiuchi *et al*. (1999) |
| −119G-A | Rat | | ND | Yes (2) | | Godart *et al*. (2000) |
| −119Gdel | Rat | | ND | Yes (4) | 3 gen. | Godart *et al*. (2000) |
| −97T-G | | | ND | Yes (2) | | Godart *et al*. (2000) |
| −62C-G | | Myc/Max? | ND | Yes (1) | | Godart *et al*. (2000) |

The reverse numbering starts at the ATG translation start codon of the open reading frame. Using this numbering the transcription start site is located at −225. Cases where the nucleotide change involves a part conserved in the rat or *Xenopus* promoter is indicated. Potential binding sites are given and the question marks indicate that the binding has not been verified experimentally. The function refers to the activity of the mutated promoter compared with the wild-type promoter in transfection assays. The family history is given and the number in brackets refers to the number of mutant carriers identified. The number of generations (gen.) in a family tree is given.
ND=not determined.

(Sladek & Seidel 2001) suggested that there might be an alternative start methionine nine amino acids upstream. Below the human sequence the amino acids that differ in the corresponding vertebrate proteins are listed and these highlight positions that can be considered not crucial for function. The seven missense mutations found in the HNF4α gene in association with diabetes all change positions which are fully conserved in vertebrate HNF4α proteins and therefore give a strong indication of functional significance (Fig. 3). The in-frame insertion mutant, V328ins, is located in helix 10 of the ligand binding domain and is expected to alter this highly conserved structural element of nuclear receptors. The frameshift mutants, F75fsdel and K99fsdel, generate truncated proteins lacking part of the zinc finger domain essential for DNA binding, whereas the nonsense mutants, R154X and Q268X, retain the DNA binding part but lack a substantial portion of the potential ligand binding domain. Thus all these truncated proteins have lost essential domains of HNF4α.

The functional performance of several HNF4α mutants have been analyzed in *in vitro* and *in vivo* systems. The nonsense mutant, R154X, retained some reduced DNA binding activity (Lausen *et al*. 2000, Peiler *et al*. 2000) and showed some residual transactivation potential in transfection experiments depending on the cell type used (Laine *et al*. 2000, Lausen *et al*. 2000). One report found an inhibitory effect of the mutated HNF4α protein as assayed in transfection experiments by adding increasing amounts of the R154X mutant to wild-type protein. However, this effect was significantly lower as compared with an artificial HNF4α deletion construct (amino acids 1 to 358) lacking the AF-2 module (Laine *et al*. 2000). As R154X is known to bind to DNA albeit less efficiently (Lausen *et al*. 2000, Peiler *et al*. 2000), the reduction of the activity of the cotransfected wild-type protein probably reflects some dilution of the strong wild-type activity and should not be considered as a dominant negative effect. Most similar data were obtained for the nonsense mutant, Q268X, by showing that this mutant lacks DNA binding, has some altered localization in the cell compartment and has lost its transactivation potential. More importantly, a dominant negative action on the wild-type factor has been excluded by experiments in several laboratories (Stoffel & Duncan 1997, Sladek *et al*. 1998, Lausen *et al*. 2000). Thus, the MODY mutants R154X and Q268X encode transcription factors whose activities are more or less destroyed by the mutation and it is most likely that the frameshift mutants F75fsdelT and K99fsdelAA that have not yet been functionally characterized are similar loss-of-function mutations. A significant loss of activity has also been reported for the R127W missense mutant (Lausen *et al*. 2000, Yang *et al*. 2000). A substantial decrease in the activity was also found for the E276Q mutant in two reports (Suaud *et al*. 1999, Lausen *et al*. 2000), and a third report

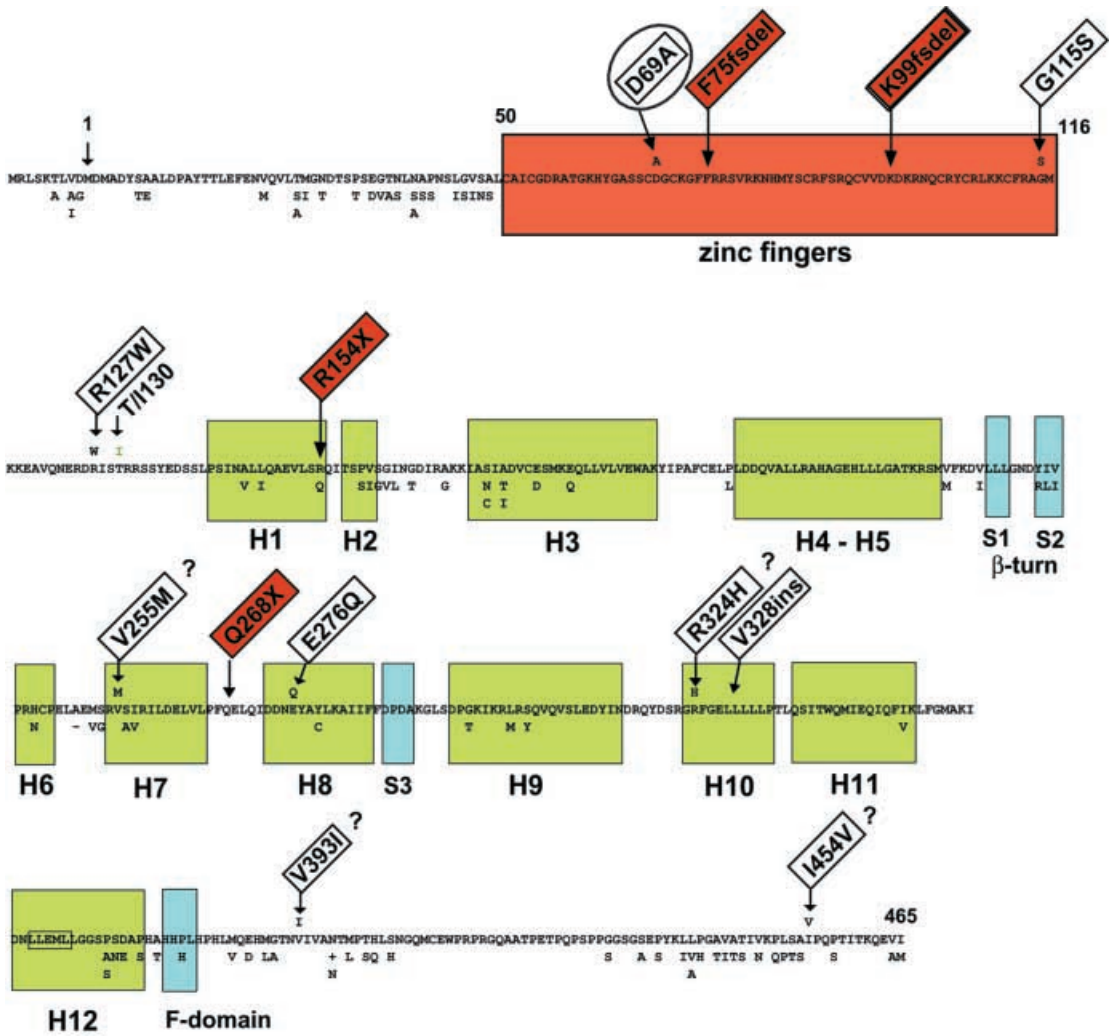



FIGURE 3. Mutations in the human HNF4α protein. The amino acid sequence of the human HNF4α2 protein is given (Drewes *et al*. 1996) and the zinc finger domain (orange) as well as the 12 helices (green boxes) and the β-sheets (blue boxes) of the potential ligand binding domain are indicated. The position of the helixes and β-sheets deduced from a comparison with the known structure of the progesterone receptor are as reported by Bogan *et al*. (2000). In helix H12 the highly conserved motif essential for the activation function AF-2 is boxed in (Dell & Hadzopoulou-Cladaras 1999, Hadzopoulou-Cladaras *et al*. 1997, Iyemere *et al*. 1998). Below the human sequence, amino acids are listed that are different in the corresponding protein in the rat (Sladek *et al*. 1990), mouse (Hata *et al*. 1995), chipmunk (Kojima *et al*. 2000) and *Xenopus* HNF4α (Holewa *et al*. 1996) proteins. Above the human amino acid sequence, the polymorphic sites (green letters) as well as the mutated sites (boxed in) are given. Nonsense mutations and frameshift mutations leading to truncated proteins are marked in red boxes. A question mark indicates that the corresponding mutation may not be responsible for MODY but rather may represent some association with diabetes II or a rare polymorphism. The mutation D69A (encircled) was found in the human HepG2 cell line (Lausen *et al*. 2000) and an association to diabetes is unknown. The mutations and in some cases their functional properties have been described in the following papers: D69A (Lausen *et al*. 2000), F75fsdelT (Moller *et al*. 1999), K99delAA (Lehto *et al*. 1999 *a,b*), G115S (Malecki *et al*. 1999), R127W (Furuta *et al*. 1997, Navas *et al*. 1999, Bulman *et al*. 2000, Lausen *et al*. 2000, Yang *et al*. 2000), T/I130 (Yamagata *et al*. 1996, Moller *et al*. 1997, Malecki *et al*. 1999, Rissanen *et al*. 2000), R154X (Lindner *et al*. 1997, 1999*b*, Laine *et al*. 2000, Lausen *et al*. 2000), V255M (Moller *et al*. 1997, Navas *et al*. 1999, Lausen *et al*. 2000), Q268X (Yamagata *et al*. 1996, Herman *et al*. 1997, Stoffel & Duncan 1997, Sladek *et al*. 1998, Ilag *et al*. 2000, Lausen *et al*. 2000, Shih *et al*. 2000), E276Q (Bulman *et al*. 1997, Navas *et al*. 1999, Suaud *et al*. 1999, Lausen *et al*. 2000), R324H (Price *et al*. 2000), V328–329ins (Lehto *et al*. 1999*b*), V393I (Hani *et al*. 1998), I454V (Malecki *et al*. 1999).

even claimed a complete loss of activity (Navas *et al*. 1999). Functional data for V255M (Lausen *et al*. 2000, Navas *et al*. 1999) and V393I showed only a small reduction in transactivation potential. Both these latter mutants of the HNF4α gene do not represent, by definition, MODY mutants as they were found in diabetes II (NIDDM) patients. They could represent rare polymorphisms or at most a susceptibility gene for diabetes, but the family members were not available for examination. None of the other mutations given in Fig. 3 have been analyzed for their functional properties.

Using strict criteria one would require full loss-of-function for a given mutant or at least two independent MODY families containing the same mutations to be confident that a change in the HNF4α sequence is relevant. On this basis, the two nonsense mutations and the two frameshift mutants most likely represent MODY1 mutations as well as the mutation R127W that has been found in two independent families (Furuta *et al*. 1997, Bulman *et al*. 2000). Taken together, it is clear that HNF4α represents the MODY1 gene, but for a given mutation in this gene the evidence may not be sufficient. This holds true also for the D69A mutation that was identified in the commonly used human hepatoma cell line HepG2 and alters the P-box of HNF4α, a hallmark of this gene family (Lausen *et al*. 2000). As inactivation of HNF4α is a frequent event in human renal carcinoma (Sel *et al*. 1996), one might speculate that it represents a tumor-associated mutation. However, a detailed analysis of renal carcinomas and hepatomas failed to detect any other mutation in the HNF4α gene associated with carcinogenesis (our unpublished data).

## POSSIBLE MECHANISMS IN THE DEVELOPMENT OF MODY

The multitude of mutations found in the HNF1α and HNF4α genes implies that the crucial character is a loss of gene function and thus haploinsufficiency may best explain the cause of MODY1 and MODY3. In the few cases where a dominant negative effect has been seen in transfection assays, one should remember that nonsense mediated decay (NMD) may affect the expression of the mutated allele. NMD is a well established process that degrades any RNA transcribed from a gene containing a nonsense mutation or a frameshift mutation that generates a stop codon 5′ to an exon–intron boundary (Hentze & Kulozik 1999). As all 5 nonsense and 20 frameshift mutations in the HNF1α gene as well as the two nonsense and two frameshift mutations in the HNF4α gene (Table 1) are of this type, one might expect that in all these cases the transcript of the mutated allele will be unstable and therefore any dominant negative effect can hardly be working, as the mutated factor will be present at too low a level. NMD has been well documented for other mutations in human diseases such as β-thalassemia (Culbertson 1999, Frischmeyer & Dietz 1999), but up to now no experimental data have been reported for the HNF1α and HNF4α genes. In this context, it would be important to compare the abundance of the transcript derived from the mutated allele to that expressed from the wild-type allele in β-cells of MODY patients, to see whether the mutated transcript is underrepresented, indicating NMD. However, such experiments cannot be done readily in human subjects and heterozygous mice cannot be used as a model since they do not develop MODY.

## POTENTIAL OTHER PROCESSES AFFECTED IN MODY1 AND MODY3 PATIENTS

It is quite astonishing that patients with a mutated HNF1α or HNF4α gene have no obvious clinically relevant defect in the other tissues expressing these transcription factors. This may indicate that the presence of one wild-type allele is sufficient for the normal function of these transcription factors in most cell types. However, a more careful analysis has shown that MODY1 patients with the mutated HNF4α gene also show impaired function of the pancreatic α-cells that have abnormal glucagon secretion (Herman *et al*. 1997) as well as of the PP-cells that are characterized by reduced pancreatic polypeptide secretion (Ilag *et al*. 2000). Furthermore, extrapancreatic abnormalities have been observed in the serum of MODY1 patients by lower concentrations of the apolipoproteins apoAII, apoCIII and possibly apoB (Lehto *et al*. 1999*a*, Shih *et al*. 2000). As these genes encoding apolipoproteins are target genes of HNF4α in the liver (Sladek & Seidel 2001), it seems likely that the low level of these apolipoproteins reflects insufficient HNF4α activity in hepatocytes. It has been postulated that the decreased expression of apolipoproteins could lead to an increased lipoprotein lipase activity and thus to the decreased plasma triglyceride concentration found in these MODY1 patients (Lehto *et al*. 1999*a*, Shih *et al*. 2000). As the level of these apolipoproteins was not reduced in early-onset diabetic patients with wild-type MODY1–5 genes (Shih *et al*. 2000), it seems plausible that HNF4α haploinsufficiency, and not a diabetes mediated event, is the primary cause for

defective lipoprotein metabolism in the liver of MODY1 patients. Taken together, there is good evidence that a heterozygous mutation of the HNF4α gene affects not only pancreatic cell function but also gene activities in hepatocytes.

In the case of the HNF1α gene only a few data are available. There is one report showing an impaired glucagon secretion in MODY3 patients indicative of an α-cell defect in the pancreas (Yoshiuchi *et al*. 1999). Most recently, it has been shown that MODY3 patients belonging to four families each affected by a different mutation in the HNF1α gene, are characterized by a reduced tubular reabsorption of glucose in the kidney (Pontoglio *et al*. 2001). This renal defect is clearly associated with MODY3, as it is not found in late onset type II diabetes patients. This renal defect in MODY3 patients corresponds to the renal glucose reabsorption defect found in HNF1α-deficient mice that is due to a reduced expression of the sodium dependent glucose cotransporter 2 (SGLT2). However, mice heterozygous for HNF1α deficiency do not show a reduced glucose reabsorption or reduced SGLT2 transcription. This corresponds to the lack of diabetes in heterozygous animals and further illustrates the limit of the mouse model for the human disease.

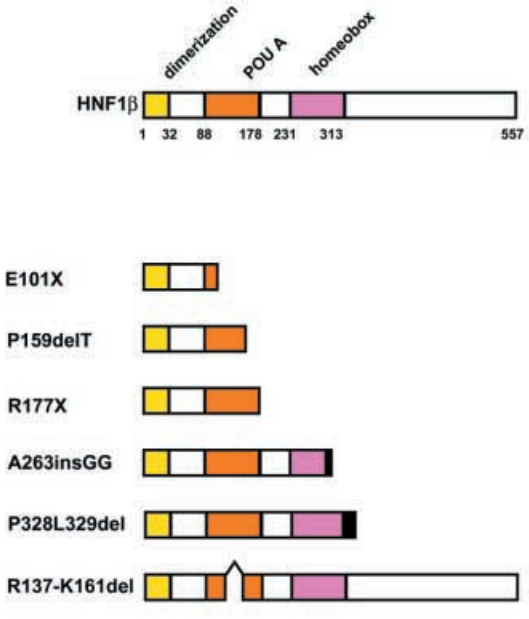
In contrast, MODY5 patients carrying a defective HNF1β gene have, in addition to the pancreatic dysfunction, severe renal and in some cases genital defects (see below).

## POTENTIAL EVENTS OCCURRING IN THE DEVELOPMENT OF MODY

Summarizing all the data available on mutated HNF1α, HNF1β and HNF4α genes in MODY patients it seems most likely that loss-of-function of one allele is the common defect leading to this disease. This would mean that haploinsufficiency is the most suitable explanation for this form of diabetes. An important open question is why people with MODY are born with apparently no metabolic defect and diabetes develops only later usually after 25 years of age. Considering the fact that all these transcription factors play an imminent role in development I propose that MODY does not result from a deregulation of a few specific genes but rather reflects some dysfunction of the corresponding transcription factors in maintaining the β-cell population of the pancreas. It is well established that β-cells are a cell population in the organism that is under a strict renewal process (Finegood *et al*. 1995). Assuming that MODY transcription factors play some role in this process, I speculate that the inactivation of the wild-type allele plays the key role in MODY. This would mean that a cell with an already mutated allele loses the function of the HNF transcription factor. Assuming that this event has a selective advantage for this cell, I would expect that the cell lacking functional HNF would, with time, overgrow the other cells. This leads to a growing percentage of cells in the β-cell population that lacks HNF function. These cells have an impaired function in insulin secretion as shown in the models for INS-1 cells overexpressing dominant negative mutants of the HNF transcription factors (Wang *et al*. 1998, 2000*a*,*b*) and thus a gradual appearance of defective β-cell function resulting in diabetes will occur. This scenario is quite speculative at the moment, but it predicts some specific properties of the HNF transcription factors and of the β-cell population in MODY patients that can be tested. First, HNF function in cells should influence not only the expression of specific genes as shown in many examples, but also cell multiplication either by affecting cell proliferation or apoptosis. Thus, we would expect that the loss of HNF function in a β-cell yields some growth advantage compared with cells expressing normal HNF levels. Secondly, my model predicts that β-cells of a MODY patient would contain a substantial number of cells without HNF function. This could be reflected, for instance, by loss of heterozygosity (LOH), a phenomenon most commonly observed in tumor cells, when in a heterozygous situation of a tumor suppressor gene the wild-type gene is lost (Fearon 1997). Alternatively, I would expect that in β-cells of MODY patients the activity of the wild-type HNF gene is inactivated at the transcriptional level. This could be measured by *in situ* hybridization of HNF expression in β-cells of MODY patients. Of course, such material is usually not available from human patients.

## HNF1β INVOLVEMENT IN INBORN KIDNEY DISEASE

As HNF1α and HNF1β are two closely related transcription factors, it is not surprising that mutations in HNF1β also lead to MODY and this diabetes is referred to as MODY5. This diabetes form is also characterized by reduced insulin secretion of the β-cells and thus demonstrates a β-cell dysfunction as the primary pathophysiological event (Bingham *et al*. 2000). However, the first patient identified with the mutant R177X seemed to have, in addition, a renal defect (Horikawa *et al*. 1997) and this has been a consistent feature in all six




| | clinical phenotype | | | molecular phenotype | | | |
|---|---|---|---|---|---|---|---|
| | | diabetes | renal defect | genital defect | dimerisation | DNA binding | transactivation | dominant negative |
| MODY | hypoplastic GCKD | − | nd | nd | nd | nd |
| type II | hypoplastic GCKD | − | nd | nd | nd | nd |
| MODY | nephropathy | − | + | − | − | −/+ |
| MODY | Cysts | − | + | − | − | + |
| MODY | Cysts, nephron agenesis | − | + | + | ++ | − |
| MODY | Oligomega-nephronia | + | nd | − | − | − |

FIGURE 4. Mutations in the HNF1β gene. The major clinical phenotypes of patients carrying mutated HNF1β alleles are summarized and compared with the functional analysis. Data taken for E101X and P159delT is from Bingham *et al*. (2001), for R177X from Horikawa *et al*. (1997), Iwasaki *et al*. (1998) and Tomura *et al*. (1999), for A263 insGG from Nishigori *et al*. (1998) and Tomura *et al*. (1999), for P328L329delCCTCT from Bingham *et al*. (2000) and Wild *et al*. (2000) and for R137-K161del from Lindner *et al*. (1999*a*). nd, not determined; +, present; ++, more than wild type; −/+, marginal; −, absent.

families with HNF1β mutations (Fig. 4). These renal defects are distinct from the diabetic nephropathy frequently occurring in MODY3 patients due to microvascular complications in the kidney that lead to progressive microalbuminuria, macroalbuminuria and renal failure strongly correlated to poor glycemic control (Isomaa *et al*. 1998). The nondiabetic renal disease identified in the MODY5 patients is most distinct between the six families but differs also to some extent between mutation carriers within a given family. Three separate histological types have been described (Fig. 4). The first is hypoplastic glomerulocystic kidney disease (GCKD) found in early onset diabetes patients carrying the E101X or P159delT mutations (Bingham *et al*. 2000). GCKD is defined by cortical glomerular cysts with dilatations of the Bowman spaces and primitive glomular tufts in at least 5% of the cysts. The hypoplastic subtype found in the HNF1β mutant carriers have typically small kidneys with abnormal calyces and papillae. The second histological type in MODY patients with the mutations A263insGG (Nishigori *et al*. 1998) or P328L329del (Bingham *et al*. 2000) is characterized by cystic dysplasia with the replacement of the renal parenchyma by cysts and cystic glomeruli and primitive tubules. The third form was described in the R137-K161del mutant carriers (Lindner *et al*. 1999*a*) as oligomeganephronia characterized by a reduced number of glomeruli and hypertropy of the remaining glomeruli and the proximal renal tubules. For the patients carrying the R177X mutation, no renal appearance or histology has been presented (Horikawa *et al*. 1997). Furthermore, a genital defect was seen in two of four R137-K161del female mutant carriers characterized by vaginal aplasia and rudimentary uterus (Lindner *et al*. 1999*a*). Surprisingly, all these mutants found in the HNF1β gene are nonsense or deletion mutants, a quite distinct situation compared with the HNF1α gene where some 70% of the mutants are missense mutants. As the two HNF1 genes are so similar, it is most likely that missense mutants occur in the HNF1β gene as well, but that they have no clinical phenotype. Consistent with this interpretation, MODY5 seems to be a less severe form of diabetes and thus may have been overlooked quite frequently. In fact, HNF1β mutants were more easily identified when patients with renal defects were analyzed (Bingham *et al*. 2000, 2001).

Four of the six HNF1β mutants have been characterized in their functional properties in *in vitro* and *in vivo* systems (Fig. 4). As predicted from the structure, only the P328L329del mutant

showed DNA binding as this mutant retains the two domains essential for DNA binding and it was thus also the only mutant having a transactivation potential in transfection experiments (Wild *et al*. 2000). Interestingly, this mutant was even more active at high expression vector input than the wild-type factor and thus might be considered as a gain-of-function mutation. All the other mutants have lost their transactivation potential and this most likely also holds true for the E101X and P159delT mutants that have not been tested but are also defective in the DNA binding domains. In all cases studied, the dimerization domain of the truncated proteins retained its ability to form heterodimers and in two cases an interference of the mutated factor with the wild-type factor activity has been reported that might be considered as a dominant negative effect. In summary, the data available suggest that the various mutants have quite distinct properties and it is tempting to speculate that these differences correlate with the distinct renal malformations. As patients with HNF1β mutations are born with renal defects and a defective kidney has been observed in a 17-week-old fetus (Bingham *et al*. 2000), it seems clear that HNF1β dysfunction interferes with kidney formation during embryogenesis. This assumption could be confirmed by introducing the HNF1β mutant P328L329fsdel into a *Xenopus* embryos (Wild *et al*. 2000). Overexpression of this mutated form of HNF1β induces severe defective development of the pronephros, the first type of kidney formed in vertebrates. The defect observed was either a complete lack of the pronephros or a substantial reduction of the tubular structure on the injected side of the larvae. A similar phenotype was observed by overexpressing wild-type HNF1β in *Xenopus* implying that the mutated form acts as a gain-of-function mutation. The effect was specific as overexpression of the related transcription factor HNF1α did not affect kidney organogenesis at all. As the introduction of the E137-K161 del mutant resulted in a much less severe phenotype, it will be important to analyze how the other HNF1β mutants affect pronephros formation thus allowing a comparison of the various mutants in a developmental system. This would allow one to deduce whether the mutants have distinct qualitative properties that are responsible for the various forms of kidney defects in humans. The *Xenopus* system also offers the opportunity to dissect the early molecular and developmental processes in kidney formation as not only are the basic structural events most similar in this lower vertebrate but also the same regulators play an essential role in this easy, amenable, developmental system (Brändli 1999, Carroll *et al*. 1999, Seufert *et al*. 1999).

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