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REVIEW

Regulation of Pancreatic β-cell Function by the HNF Transcription Network: Lessons from Maturity-Onset Diabetes of the Young (MODY)

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Introduction

TYPE 2 diabetes mellitus is a heterogenous group of disorders characterized by a high blood glucose level. Pancreatic β-cell dysfunction and insulin resistance of target tissues play a central role in the pathogenesis of diabetes. Although multiple genetic and environmental factors (e.g., physical inactivity and overnutrition) may contribute to the development of type 2 diabetes, about 2–5% of these patients suffer from a monogenic disease with autosomal dominant inheritance. This monogenic form of type 2 diabetes is called maturity-onset diabetes of the young (MODY). MODY is a clinically heterogenous disorder, which is characterized by autosomal dominance, an early age of onset, and a primary defect of pancreatic β-cell function. In 1996, we showed that heterozygous mutations in genes encoding hepatocyte nuclear factor (HNF)-1α and HNF-4α cause MODY3 and MODY1, respectively [1, 2]. HNF-1α and HNF-4α are functionally related transcription factors, and HNF-4α regulates the transcription of HNF-1α. Further studies of MODY patients have identified defects of three other genes encoding transcription factors: HNF-1β (MODY5) [3], insulin promoter factor-1 (IPF-1)/pancreatic duodenal homeobox 1 (PDX-1) (MODY4) [4], and neurogenic differentiation 1/β-cell E-box transactivation 2 (NeuroD1/BETA2) (MODY6) [5] (Table 1). All five transcription factors that have been shown to have a role in MODY are expressed in pancreatic β-cells and regulate the expression of insulin as well as other proteins involved in glucose metabolism and/or β-cell development. These findings suggest the importance of transcription factor for normal β-cell function and have led to the concept of "diabetes mellitus as a disorder of abnormal transcription factors". In addition, identification of mutations of the three HNF genes in diabetic patients with impaired insulin secretion has indicated the important role of HNF transcription factors in the normal regulation of insulin secretion. As shown by the term of "hepatocyte nuclear factor", the critical role of these factors in pancreatic β-cells had not been recognized before cloning of the MODY genes. Studies of HNF-related diabetes may give us some important clues towards better understanding of the mechanism of insulin secretion. In this review, I focus on the significance of HNFs with respect to glucose metabolism.

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HNF-1α diabetes

HNF-1α was initially identified as a transcription factor that was enriched in the liver, but its gene is also expressed in the pancreas, stomach, small intestine, and kidney. Both endocrine cells (glucagon-positive, insulin-positive, somatostatin-positive, and pancreatic polypeptide (PP) cells) and exocrine cells in the pancreas express HNF-1α from the developmental stage [6]. HNF-1α is composed of three functional domains: an amino-terminal dimerization domain, a DNA-binding domain with homeodomain-like and POU-like motifs, and a COOH-terminal transactivation domain. HNF-1α binds DNA as a homodimer or as a heterodimer with HNF-1β.

Mutations associated with diabetes have been found

Table 1. Comparison of the different types of MODY

	MODY1	MODY2	MODY3	MODY4	MODY5	MODY6
Gene	HNF-4α	glucokinase	HNF-1α	IPF-1	HNF-1β	NeuroD1
Chromosomal location	20q12-13.1	7p15-13	12q24	13q12.1	17q	2q32
Frequency	rare	relatively common (rare in Japan)	most common	rare	rare	rare
Severity of hyperglycemia	severe	mild	severe	mild~severe	mild~severe	mild~severe
Clinical features	diabetes with impaired insulin secretion	mild hyperglycemia with minor deterioration with age	diabetes with impaired insulin secretion	diabetes with impaired insulin secretion	diabetes and progressive renal dysfunction	diabetes with impaired insulin secretion

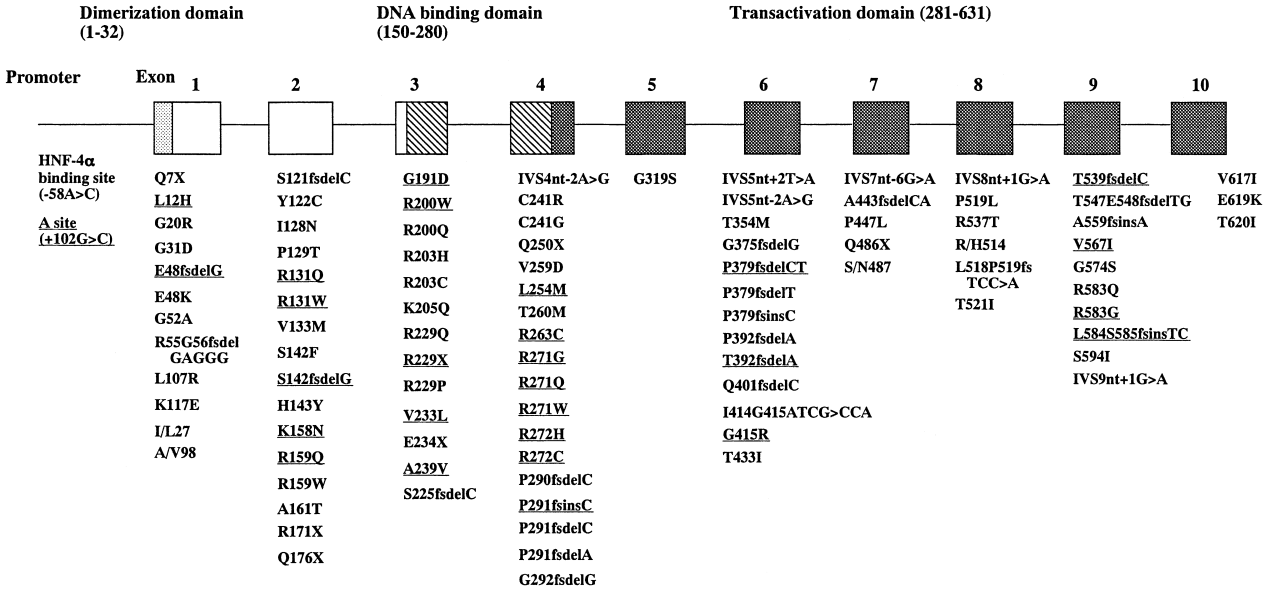

Fig. 1. Mutations and polymorphisms in the HNF-1α gene. Mutations found in Japanese subjects with diabetes are underlined.

in all regions of the HNF-1α gene, including the promoter. Mutation screening of the HNF-1α gene in different racial groups of MODY patients has shown that defects of this gene are a common cause of MODY in the UK (73%), France (25%), and Japan (10–15%) [7]. More than 90 distinct mutations of the HNF-1α gene have been reported so far, and the mutations and polymorphisms reported for this gene are shown in Fig. 1. The most common mutation of the HNF-1α gene is insertion of C in the poly C tract around codon 291 (P291fsinsC) and this mutation is considered to be due to slipped mispairing during DNA replication rather than a founder effect. This mutation is especially common in Caucasians. In Japanese, codons 271 and 272 may be vulnerable sites.

The chief clinical characteristic of HNF-1α diabetes is impaired insulin secretion by pancreatic β-cells [8, 9]. Mutation of the glucokinase gene also causes MODY associated with primary β-cell dysfunction [10]. In the case of glucokinase mutations, patients usually show mild hyperglycemia throughout life. In contrast, patients with HNF-1α diabetes show rapid deterioration of glycemia with age and most of them soon need treatment with oral hypoglycemic drugs or insulin [7]. In keeping with the rapid worsening of their glucose levels, subjects with HNF-1α mutations sometimes have severe diabetic microangiopathy. Insulin resistance is an important clinical feature of common type 2 diabetes. It has been reported, however, that insulin sensitivity is actually increased in

HNF-1α diabetes [7].

Mutations of the HNF-1α gene may cause diabetes through haploinsufficiency (simple loss of function) or by a dominant negative mechanism. We found that P291fsinsC-HNF-1α has a dominant negative effect, but most HNF-1α defects are due to simple loss-of-function mutations [11–13]. Since one allele of the HNF-1α gene is normal in these patients, the level of HNF-1α expression must play a critical role in determining β-cell function. We identified a mutation at the A site of the promoter region in a diabetic family and found that this mutation was associated with an increase of promoter activity. Thus, appropriate level of HNF-1α activity might be important for normal β-cell function.

Although mutations of the HNF-1α gene were originally identified in subjects with a clinical diagnosis of MODY, some mutations have been found in patients with a clinical diagnosis of type 1 diabetes, since the clinical features of severe HNF-1α diabetes are similar to those of type 1 diabetes [12, 14]. Interestingly, mutations of the HNF-1α gene have also been identified in subjects with typical type 2 diabetes. G319S is a missense mutation of the transactivation domain that reduces transactivation activity by nearly 50%. This mutation is found in about 40% of Canadian Oji-Cree Indians with type 2 diabetes. The mutation is strongly associated with type 2 diabetes in this population and has a very high specificity for predicting the onset of type 2 diabetes [15]. G191D, L254M, and R583Q mutations of the HNF-1α gene have also been identified in a small number of patients with late-onset type 2 diabetes [16–18]. Our *in vitro* studies showed that G191D is a weak loss-of-function mutation in HeLa cells, but has almost normal transactivation activity in β-cell-like MIN6 cells [13]. Mild HNF-1α mutations may contribute to the polygenic background and predispose some persons to type 2 diabetes. Mild mutations of the IPF-1/MODY4 gene also cause a predisposition to type 2 diabetes [19, 20].

Targets of HNF-1α in glucose metabolism

HNF-1α is critical for normal β-cell function, but its actual role in β-cells is not yet clear. Several targets of HNF-1α have been identified through various approaches. Glucose transporter type 2 (GLUT2) facilitates glucose transport in β-cells and L-type pyruvate kinase (PKL) is a rate-limiting enzyme of glycolysis that is expressed in β-cells. HNF-1α can activate both the GLUT2 gene and the PKL gene by binding to their promoter sequences [21, 22]. HNF-1α also transactivates the transcription of the aldolase B gene as an enhancer. The insulin gene may be an endogenous target of HNF-1α in rats [21]. However, the ability of HNF-1α to bind to the insulin gene in humans appears to be weaker [23] compared with its binding to the rat insulin gene. HNF-1α also regulates the expression of the mitochondrial 2-oxoglutarate dehydrogenase (OGDH) E1 subunit gene [24]. In addition to genes involved in glucose transport, glycolysis, and mitochondrial metabolism, HNF-1α can influence the expression of genes such as IGF-1, cyclin E, p27KIP1, and Bcl-xL, which regulate cell proliferation and apoptosis [25, 26]. A reduction of β-cell numbers and a decrease of the β-cell proliferation rate has been reported in HNF-1α knockout mice as well as in transgenic mice overexpressing dominant negative P291fsinsC-HNF-1α in pancreatic β-cells [22, 27]. It is well known that HNF-4α regulates the transcription of HNF-1α in hepatocytes, but HNF-1α conversely transactivates the expression of HNF-4α in the pancreas, indicating that HNF-4α is an important target of HNF-1α in β-cells [28]. The HNF-3γ and HNF-4γ genes are also targets of HNF-1α [28]. HNF-1α can bind to the regulatory element of the IPF-1/PDX-1 (MODY4) gene and activate its transcription *in vitro,* but little or no change of IPF-1/PDX-1 gene expression was found in the β-cells of HNF-1α-inactivated mice [6, 29]. In mice, HNF-1α expression is seen in most pancreatic epithelial cells on embryonic day 10.5 and such early expression suggests a role of HNF-1α in the development of the pancreas [6]. Indeed, the pancreatic islets of HNF-1α knockout mice and transgenic mice expressing dominant negative HNF-1α were found to be small and irregular in shape. HNF-1α appears to be involved in the organization of pancreatic islets by regulating the expression of E-cadherin, which is an adhesion molecule critical for cell-cell adhesion within the islets [22]. Thus, HNF-1α seems to have multiple roles in pancreatic β-cells.

HNF-4α diabetes

HNF-4α is a member of the steroid hormone re-

ceptor superfamily and is expressed in the liver, pancreatic islets, kidney, and small intestine. It binds to DNA as a homodimer and activates the transcription of various target genes. HNF-4α consists of several functional domains: an N-terminal transactivation domain (AF-1), a DNA-binding domain, and a functionally complex C-terminal region that forms a ligand-binding domain, a dimerization interface, and a transactivation domain (AF-2). Recent crystal structure studies have suggested that fatty acids are endogenous ligands for HNF-4α [30, 31].

HNF-4α diabetes is relatively uncommon, although 20 possible mutations have been identified to date (Fig. 2). In the case of HNF-1α, some mutations are dominant negative, but all of the HNF-4α mutations identified so far have been simple loss-of-function mutations. The HNF-4α gene has two distinct promoters (P1 and P2). Hepatocytes utilize the proximal P1 promoter, but the recently identified distal P2 promoter is specially utilized in pancreatic β-cells [29, 32]. Exon 1A is transcribed from the P1 promoter in the liver, but exon 1D is transcribed from the P2 promoter in pancreatic tissues. Using these two promoter systems, at least nine distinct isoforms are generated (HNF-4α1 to HNF-4α9). Pancreatic β-cells contain three isoforms, which are HNF-4α7 (corresponding to 4α1 in the liver), HNF-4α8 (corresponding to 4α2), and HNF-4α9 (corresponding to 4α3) [33]. The P2 promoter includes both an IPF-1-binding site and a HNF-1-binding site. Interestingly, mutations of these binding sites (HNF-1α; -181G to A, and IPF-1; -146T to C) in the P2 promoter cause MODY [32, 33]. In addition, hepatic expression of HNF-4α mRNA was normal in HNF-1α knockout mice, but its expression was abolished in the pancreas [28]. These results suggest the importance of the P2 promoter system for transcription of the HNF-4α gene in pancreatic β-cells. However, a genetic study has identified a mutation in the HNF-4α-binding site of the promoter region of HNF-1α in an Italian MODY family (-58A to C), indicating that HNF-4α-dependent transcription of HNF-1α is also required for normal pancreatic β-cell function [34]. Taken together, these findings indicate the existence of a positive feedback circuit involving HNF-1α and HNF-4α.

As suggested by the existence of cross-regulation between HNF-1α and HNF-4α, the clinical features of HNF-4α diabetes are similar to those of HNF-1α diabetes. Most patients show progressive deterioration of glycaemic control but some subjects remain well controlled on diet alone or oral hypoglycemic agents. In HNF-4α diabetic subjects, the onset of diabetes is also due to a primary defect of β-cell function. In addition, HNF-4α is known to play an important role in regulating the expression of various proteins involved in lipid metabolism. For example, the genes for ApoA-II, ApoA-IV, Apo-B, ApoC-II, and ApoC-III are all regulated by HNF-4α. It has been reported that the serum concentrations of triglycerides as well as apolipoproteins AII and CIII were reduced in some patients with HNF-4α diabetes [35].

T130I is a rare missense mutation that affects a conserved amino acid in a DNA-binding domain. This mutation can be found in the general population (0–5%), so it does not cause MODY alone. However, we recently found that this missense mutation is associated with late-onset type 2 diabetes mellitus in Japanese subjects [36]. Functional analysis suggested that it acts as a loss-of-function mutation in hepatocytes. Thus, the T130I mutation of HNF-4α may be a genetic risk factor for diabetes and may predispose to type 2 diabetes.

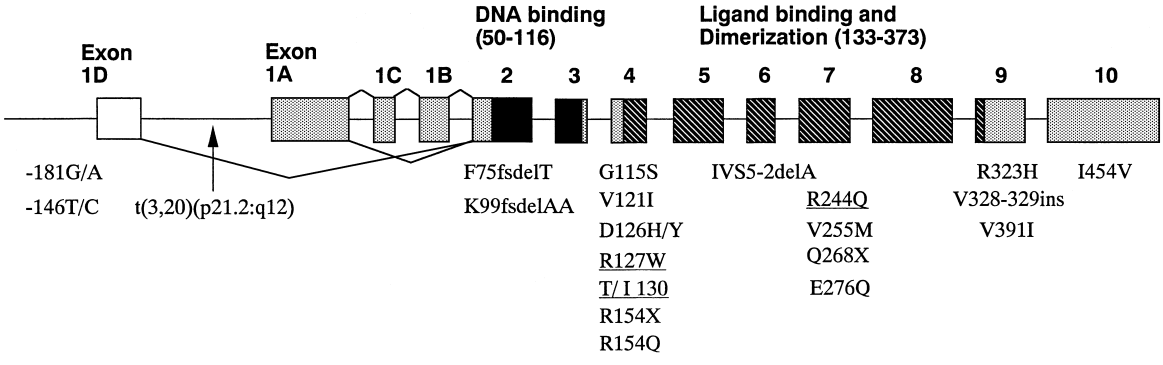

Fig. 2. Mutations and polymorphisms in the HNF-4α gene. Mutations found in Japanese subjects with diabetes are underlined.

Role of HNF-4α in pancreatic β-cells

The basic clinical characteristic of HNF-4α diabetes is impaired insulin secretion from pancreatic β-cells, but HNF-4α null mice suffer embryonic death that prevents further analysis of HNF-4α function in their β-cells [37]. Overexpression of a dominant negative HNF-4α mutant in INS-1 cells leads to the reduced expression of the GLUT2, PKL, aldolase B, and OGDH E1 genes, which are also targets of HNF-1α [38]. The similarity of the genes regulated by HNF-1α and HNF-4α might be due to the fact that HNF-4α is a downstream regulator of HNF-1α in pancreatic β-cells. Phosphoenol pyruvate carboxykinase (PEPCK), which is a key enzyme of gluconeogenesis, is an important target of HNF-4α in the liver [39]. It was reported that liver-specific HNF-4α knockout mice exhibit hepatomegaly along with reduced serum cholesterol and triglyceride levels and an increase of serum bile acids [40], but there have been no reports about pancreatic β-cell-specific HNF-4α knockout mice.

HNF-1β diabetes

HNF-1β is another homeodomain-containing transcription factor. HNF-1β functions as a homodimer or as a heterodimer with HNF-1α. There is a high level of HNF-1β expression in the kidneys, but it is also found in the pancreas, liver, stomach, and lung. HNF-1β regulates the expression of HNF-4α1 and HNF-1α in embryoid bodies [41], but the importance of such an action in pancreatic β-cells remains to be clarified. HNF-1β diabetes is also characterized by impaired insulin secretion. Interestingly, the clinical phenotype of HNF-1β diabetes is not confined to MODY, but also features progressive nondiabetic renal dysfunction [3, 42]. The renal phenotype varies between families, but kidney cysts are a common feature of the disease. Solitary functioning kidney, renal dysplasia, and glomerulocystic kidney disease have been reported in subjects with HNF-1β mutations. Female subjects with HNF-1β mutations occasionally have genital malformations. Fig. 3 shows a list of mutations/polymorphisms of the HNF-1β gene. N228K is a relatively common polymorphism found in Pima Indians and the A241T, G492S, and S465R mutations might be rare polymorphisms. We found the S36F mutation in a Japanese MODY family and functional analysis showed that it was a gain-of-function mutation [43]. It has been reported that overexpression of wild-type HNF-1β in Xenopus embryos leads to impaired development of the pronephros [44].

The HNF network in pancreatic β-cells

As described above, the transcriptional hierarchy of HNF in pancreatic β-cells differs from that in the liver. Fig. 4 shows a summary of the HNF network in pancreatic β-cells. Although HNF-1α expression is strictly restricted by HNF-4α in hepatocytes, HNF-1α mainly acts as an upstream regulator in pancreatic β-cells. CREB-binding protein (CBP) and thyroid hormone receptor interacting protein 3 (Trip3) interact with HNF-4α and augment its transactivation [45, 46], while small heterodimer partner (SHP) functions as a co-repressor of HNF-4α. SHP gene expression might, in turn, be regulated by HNF-4α [47]. SHP interacts with several nuclear receptors and generally represses their functions. Mutations of the SHP gene in humans are associated with mild obesity and insulin

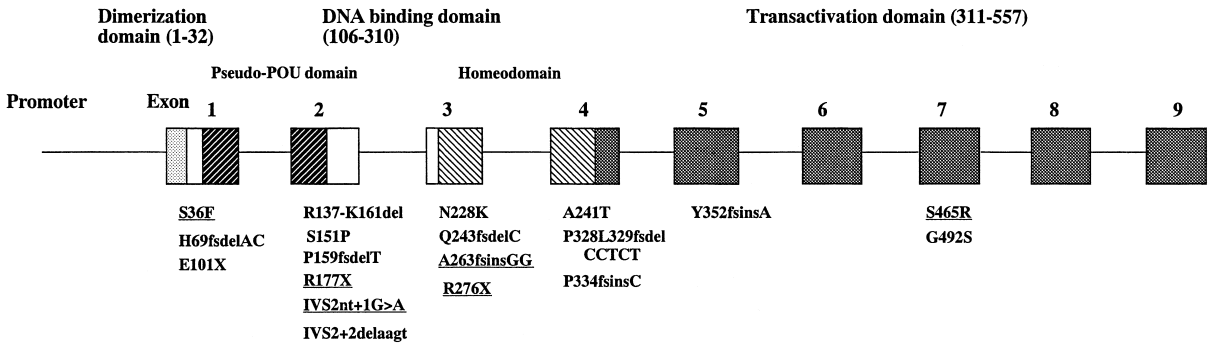

Fig. 3. Mutations and polymorphisms in the HNF-1β gene. Mutations found in Japanese subjects with diabetes are underlined.

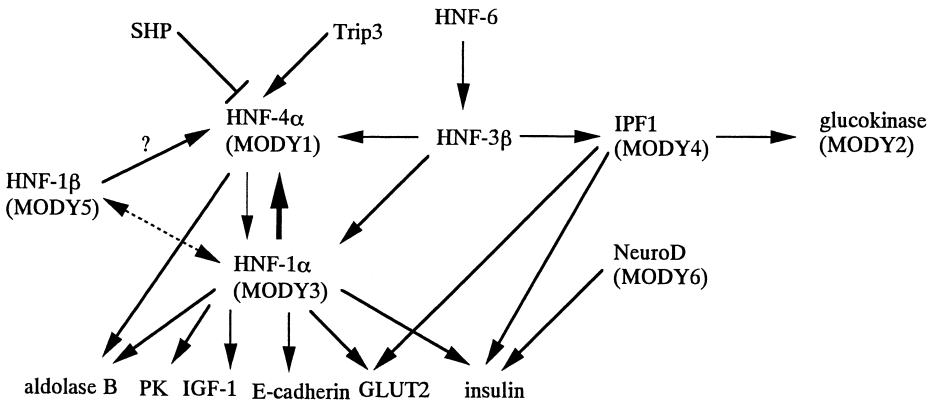

Fig. 4. HNF network in pancreatic β-cells. HNF-4α expression is mainly regulated by HNF-1α in pancreatic β-cells. HNF-1β functions as a homodimer or as a heterodimer with HNF-1α. SHP inhibits the function of HNF-4α. Trip3 augments the function of HNF-4α.

resistance [48]. We have shown that SHP is a co-activator of peroxisome proliferator-activated receptor γ (PPARγ) and that impaired activation of PPARγ due to defects of SHP might be involved in the insulin resistance observed in persons with SHP gene mutations [49]. HNF-3β is expressed in pancreatic islets, and has been suggested to act as the upstream transactivator of HNF-1α, HNF-4α, and IPF-1 in the transcriptional hierarchy. Although mutations of the HNF-3β gene are not a common cause of MODY, we have found a weak loss-of-function mutation (A86T) of this gene in association with late-onset type 2 diabetes [50]. Thus, weak mutations of the HNF-3β gene might predispose to type 2 diabetes. HNF-6 transactivates HNF-3β gene expression, but no mutations of the HNF-6 gene have yet been identified in subjects with MODY or type 2 diabetes [51, 52].

Future prospects

Identification of the HNF genes as diabetogenic genes has led to some degree of understanding as to the importance of these transcription factors in β-cell function. Recent studies have revealed part of the complex HNF transcription network, but we are still lacking information about the targets of HNFs. Better understanding of the whole network may lead to new therapeutic approaches and agents for MODY. Such knowledge may also help to develop treatment for impaired insulin secretion in the common polygenic form of type 2 diabetes and may even lead to the creation of artificial β-cells from non-β-cells such as ES cells.

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