Regulation of human insulin, IGF-I, and multidrug resistance protein 2 promoter activity by hepatocyte nuclear factor (HNF)-1β and HNF-1α and the abnormality of HNF-1β mutants

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Abstract

Mutations in hepatocyte nuclear factor-1β (*HNF-1β*) lead to type 5 maturity-onset diabetes of the young (MODY5). Moreover, mutations in the *HNF-1β* gene might cause multiorgan abnormalities including renal diseases, genital malformations, and abnormal liver function. The objective of this study was to investigate the molecular mechanism of diabetes mellitus, intrauterine growth retardation, and cholestasis observed in MODY5 patients. We analyzed the transactivity of wild-type and three mutant HNF-1β on native human insulin, IGF-I, and multidrug resistance protein 2 (MRP2) promoters in combination with HNF-1α, using a reporter-assay system in transiently transfected mammalian cells. In the human insulin gene promoter, we found that the cooperation of HNF-1α and HNF-1β is prominent. Absence of this cooperation was observed in all of the HNF-1β mutants. In the human IGF-I and MRP2 promoters, we found that the HNF-1β His153Asn (H153N) mutant had a mutant-specific repressive effect on both HNF-1α and wild-type HNF-1β transactivity. Absence of the cooperation of HNF-1β mutants with HNF-1α in the human insulin gene promoter might be one cause of defective insulin secretion. The H153N mutant-specific repression of HNF-1α and HNF-1β transactivity in human IGF-I and MRP2 promoters might explain the case-specific clinical features of growth retardation and cholestasis observed only in early infancy. We found differential property of HNF-1α/HNF-1β activity and the effect of HNF-1β mutants by the promoters. We consider that analyses of HNF-1β mutants on the intended human native promoters in combination with HNF-1α may be useful in investigating the molecular mechanisms of the various features in MODY5.

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Introduction

Maturity-onset diabetes of the young (MODY) is a form of diabetes characterized by the autosomal dominant mode of inheritance, early onset (usually diagnosed before the age of 25), and impaired glucose-stimulated insulin secretion (Fajans *et al.* 2001, Ryffel 2001). The type 5 form of MODY, MODY5, has been reported to be caused by heterozygous mutations in the homeodomain-containing transcription factor, hepatocyte nuclear factor-1β (*HNF-1β*) (Horikawa *et al.* 1997). Patients with *HNF-1β* mutation are most commonly characterized by renal diseases including renal cysts and dysplasia (Bingham & Hattersley 2004, Edghill *et al.* 2006). As more subjects have been reported, it has become apparent that mutations in the *HNF-1β* gene may cause a multisystem disorder. Additional features including genital malformations, abnormal liver function, hyperuricemia, and cholestasis differ in individuals (Bingham *et al.* 2002, 2003, Bingham & Hattersley 2004, Kitanaka *et al.* 2004).

HNF-1β is closely related to *HNF-1α*, a gene heterozygously mutated in MODY3 (Yamagata *et al.* 1996). HNF-1α and HNF-1β bind to the same consensus HNF-1 site and function as homodimers or heterodimers. Various target genes have been identified including insulin, albumin, glucose transporter-2, and α-fetoprotein for both HNF-1α and HNF-1β, and insulin growth factor-I (IGF-I), and L-type pyruvate kinase for HNF-1α. Transcriptional modification by the co-expression of HNF-1α and HNF-1β has been studied in some promoters and is known to differ with the promoter (Song *et al.* 1998, Boudreau *et al.* 2001, Kitanaka *et al.* 2004). Considered together with the changes in the ratio of HNF-1β expression to HNF-1α during development, it was suggested that HNF-1β has a regulatory role in HNF-1α and HNF-1β target gene regulation (Cereghini *et al.* 1992, Song *et al.* 1998, Boudreau *et al.* 2001).

The molecular mechanisms by which mutations in HNF-1β cause various clinical features have been only partly investigated. The transcriptional inhibition of genes including *Pkhd1* by HNF-1β mutants has recently been reported in mice, which might be related to the formation of renal cysts (Gresh *et al.* 2004, Hiesberger *et al.* 2004). The molecular mechanism of diabetes mellitus by HNF-1α mutations in MODY3 has been

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attributed to the decreased transcription of genes including insulin, glucose transporter 2, and L-type pyruvate kinase (Wang *et al.* 1998, 2000). However, although HNF-1β is known to be essential for normal glucose-stimulated insulin secretion from the analysis of β-cell-conditional *Hnf-1β* knockout mice (Wang *et al.* 2004) and MODY5 patients (Lindner *et al.* 1999, Bingham *et al.* 2000, Furuta *et al.* 2002, Bellanne-Chantelot *et al.* 2004, Kitanaka *et al.* 2004), the molecular mechanism of MODY5 diabetes has not been elucidated. Furthermore, although severe growth retardation was observed in liver- and bile duct-conditional *Hnf-1β* knockout mice (Coffinier *et al.* 2002), the molecular mechanism is unknown. Growth retardation was similarly observed in *Hnf-1α* knockout mice, most likely due to the loss of expression of IGF-I in the liver (Lee *et al.* 1998). Moreover, HNF-1β has been shown to play an important role in the development of the bile system from the analysis of liver- and bile duct-conditional *Hnf-1β* knockout mice (Coffinier *et al.* 2002). Cholestasis and conjugated hyperbilirubinemia have also been reported in MODY5 patients (Bellanne-Chantelot *et al.* 2004, Kitanaka *et al.* 2004). A single gene, organic anion transporting polypeptide 1 (*Oatp1*), was downregulated in *Hnf-1β* knockout mice (Coffinier *et al.* 2002). However, since OATP1 is a basolateral sinusoidal bilirubin transporter which mediates the hepatocellular uptake of unconjugated bilirubin, the molecular mechanism of conjugated hyperbilirubinemia could not be explained. Multidrug resistance protein 2 (MRP2), also known as the canalicular multispecific organic anion transporter, is indispensable for the excretion of conjugated bilirubin from hepatocytes to the bile duct. Mutations in the *MRP2* gene lead to Dubin–Johnson syndrome, a form of hereditary-conjugated hyperbilirubinemia.

We have previously reported a novel *HNF-1β* missense mutation, His153Asn (H153N), in a patient with MODY5 (Kitanaka *et al.* 2004). This patient had specific features of neonatal cholestasis and intrauterine growth retardation together with the common MODY5 features of diabetes mellitus and renal cysts. Interestingly, functional analyses revealed that HNF-1β H153N mutant had diminished transcriptional activity and a promoter-specific transcriptional repressive effect not only on wild-type HNF-1β but also on HNF-1α (Kitanaka *et al.* 2004). In this report, based on the presumption that the phenotype of patients with MODY5 might be related to the mutant HNF-1β property on each promoter, we analyzed the function of HNF-1β mutants on native human promoters in combination with HNF-1α to investigate the molecular mechanism of diabetes mellitus, intrauterine growth retardation, and cholestasis observed in our patient. We found that the cooperation of HNF-1α and HNF-1β is prominent in the regulation of human insulin gene transcription. Absence of this cooperation was observed in HNF-1β mutants, which might be one of the causes of defective insulin secretion. Moreover, we found that HNF-1β H153N mutant had a mutant-specific transcriptional repressive effect on both HNF-1α and wild-type HNF-1β transactivity in human IGF-I and in human MRP2 promoters.

Materials and Methods

Construction of plasmids

The constructs of wild-type (WT) HNF-1β expression plasmid pCMV-1βWT and HNF-1α expression plasmid pCMV-1α in pCMV6b were described previously (Tomura *et al.* 1999). His153Asn (H153N), Glu101Ter (E101X), and Arg177Ter (R177X) mutations in pCMV-1β were introduced with a Quick Change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). Construction of the luciferase reporter plasmid with the human insulin gene promoter region (−362 to +27 relative to the cap site) and human MRP2 gene promoter region (−179 to +248 relative to the major transcription initiation site) was described previously (Okita *et al.* 1999, Tanaka *et al.* 1999). Human IGF-I reporter plasmid was constructed by subcloning a fragment (−488 to +54 relative to the major transcription site) amplified by PCR using sense primer 5′-GGG*GCTAGC*TGTCTTCCAATCTACTTTAC-3′ and antisense primer 5′-GGG*AAGCTT*TATTCCATTGCG-CAGGC-3′ (restriction sites are in italics) into NheI and HindIII site of pGL3-basic (Promega). The constructs were checked by sequencing.

Cell transfection and luciferase assay

HeLa cells and HepG2 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS. Cells cultured in six-well plates were transfected with 100 ng of the reporter gene, the indicated amounts of each HNF-1 expression plasmid, and 0·5 ng pRL-CMV (Promega) using LipofectAMINE PLUS (Invitrogen Corp.) according to the manufacturer's protocol in a culture medium of 200 μl. The total amount of the plasmids was adjusted to 250 ng by empty pCMV vector. After 24 h, transcriptional activity was assayed using a Dual-Luciferase Reporter Assay System (Promega). The relative luciferase activity (Firefly/Renilla) was measured by five independent experiments and is presented as the mean ± S.E.M. The statistical significance of differences between the means was evaluated by one-way ANOVA followed by *post hoc* Bonferroni/Dunn's test (Fig. 1A) or Dunnett's test (others). A P value of $<0{\cdot}05$ was considered significant.

Results

Regulation of human insulin promoter activity by HNF-1β and HNF-1α

Human insulin promoter has an HNF-1 site and has been reported to be activated by HNF-1α or HNF-1β (Okita *et al.* 1999); however, transcriptional activity by the co-expression of HNF-1α and HNF-1β is unknown. We first analyzed the regulation of human insulin promoter activity by the co-expression of HNF-1α and HNF-1β. Transfection of half

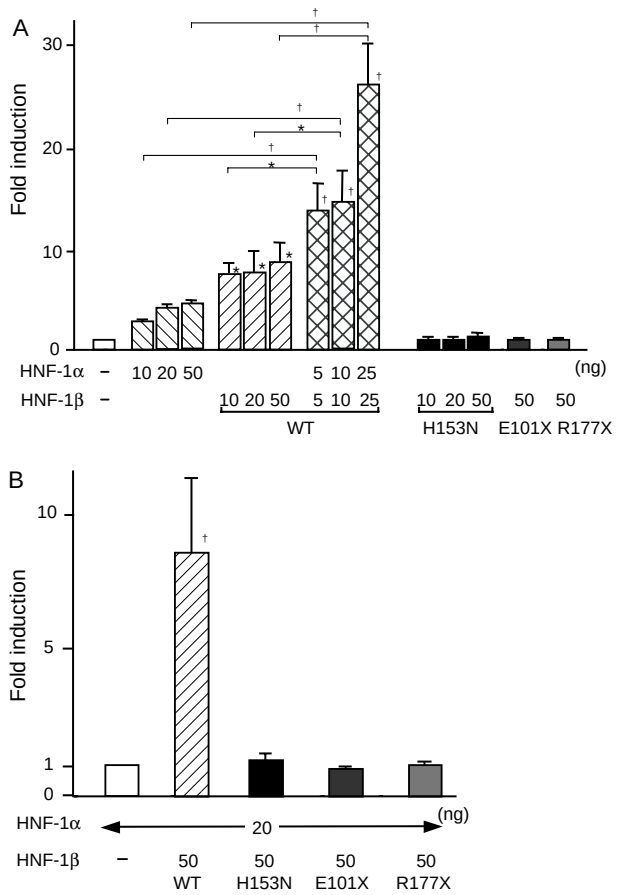


Figure 1 Regulation of human insulin promoter activity by HNF-1α and HNF-1β and the activity of HNF-1β mutants. (A) The indicated amounts of pCMV-1α, pCMV-1βWT, pCMV-1β mutants (H153N, E101X, R177X) were transfected with 100 ng human insulin reporter plasmid into HeLa cells. Fold induction refers to the activity without any HNF-1α or HNF-1β. (B) The indicated amounts of pCMV-1βWT or pCMV-1β mutants (H153N, E101X, R177X) were co-transfected with 20 ng of pCMV-1α, 100 ng human insulin reporter plasmid into HeLa cells. Fold induction refers to the activity with 20 ng pCMV-1α. The relative luciferase activity (Firefly/Renilla) was measured by five independent experiments. The mean ± S.E.M. is shown. $^{*}P<0{\cdot}05$, $^{\dagger}P<0{\cdot}01$ compared with no HNF-1α/HNF-1β or as indicated (A), or compared with no HNF-1β (B).

amounts of HNF-1α and HNF-1β showed higher transcriptional activity than individually (Fig. 1A). We next examined the activity of HNF-1β mutants on human insulin promoter. HNF-1β H153N mutant had no transcriptional activity on the insulin promoter (Fig. 1A). Another two mutants, E101X and R177X, reported to have diminished transcriptional activity on other promoters (Tomura *et al.* 1999, Bohn *et al.* 2003), also had no transcriptional activity.

We next examined the effect of HNF-1β mutants on HNF-1α transcriptional activity in the human insulin promoter. Wild-type HNF-1β showed an apparent cooperative effect on HNF-1α as already shown; however, no HNF-1β mutants showed any cooperative effect on HNF-1α (Fig. 1B). None of the mutants had a significant repressive effect either on HNF-1α or on wild-type HNF-1β (Fig. 1B and data not shown). All the results were similar in HepG2 cells (data not shown). Taken together, these results suggested that the cooperation of HNF-1α and HNF-1β is prominent in human insulin promoter, and all the HNF-1β mutants lack the cooperative effect.

Regulation of human IGF-I promoter activity by HNF-1β and HNF-1α

Our patient had intrauterine growth retardation (39 weeks, 2390 g) and catch-up growth was observed during infancy (Kitanaka *et al.* 2004). IGF-I is well known to be important in fetal growth (Nakae *et al.* 2001) and the human gene has been identified as one of the target genes of HNF-1α (Nolten *et al.* 1995). However, the regulation of human IGF-I by HNF-1β and by the co-expression of HNF-1α and HNF-1β are unknown. We first examined the transcriptional activity of HNF-1α and HNF-1β on human IGF-I promoter. Transfection of wild-type HNF-1β showed dose-dependent transcriptional activity (Fig. 2A). The co-expression of HNF-1α and HNF-1β showed intermediate activity between them, suggesting that they do not have a cooperating effect on the human IGF-I promoter. HNF-1β mutants H153N, E101X, and R177X had no or negligible transcriptional activity (Fig. 2A).

We next examined the effect of wild-type and mutant HNF-1β on HNF-1α transcriptional activity in the human IGF-I promoter. Wild-type HNF-1β had slight but not significant additional transcriptional activity (Fig. 2B); however, HNF-1β H153N mutant showed significant repressive effect on HNF-1α transcriptional activity. Other mutants showed no repressive effect, suggesting that this repression was specific to the H153N mutant.

Since HNF-1β mutation in MODY5 is heterozygous, we next examined the dominant-negative effect of HNF-1β mutants on wild-type HNF-1β transactivity. Transfection of equal amounts of the HNF-1β H153N mutant showed a significant repressive effect on wild-type HNF-1β (Fig. 2C); however, other mutants had no repressive effect. All of the results were similar in HepG2 cells (data not shown). Taken together, these results indicated that the HNF-1β H153N mutant has a mutant-specific repressive effect both on HNF-1α and wild-type HNF-1β in the human IGF-I promoter.

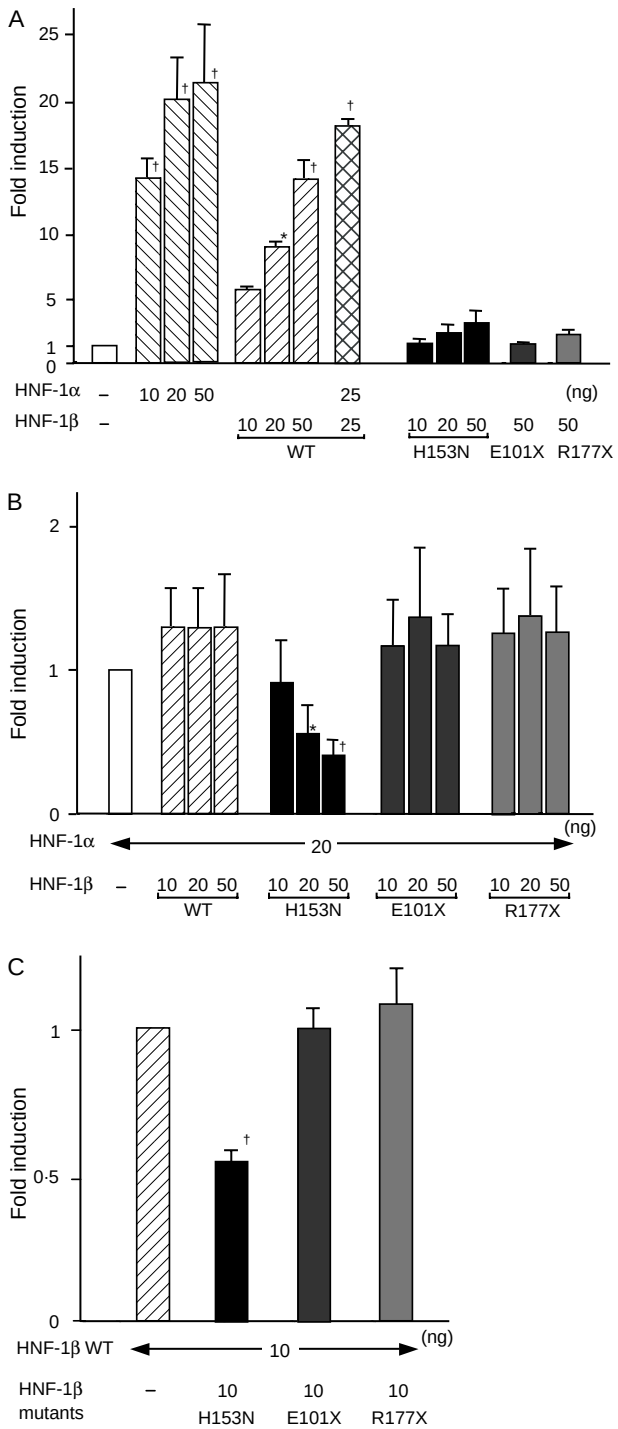
Regulation of human MRP2 promoter activity by HNF-1β and HNF-1α

Our patient had neonatal hyperbilirubinemia and improved spontaneously by 9 months of age (Kitanaka *et al.* 2004). The hyperbilirubinemia and histological findings were similar to the liver- and bile duct-conditional *Hnf-1β* knockout mice (Coffinier *et al.* 2002). The conjugated hyperbilirubinemia in the knockout mice and in our patient suggested that the abnormality lay in the process after the conjugation of

bilirubin in the hepatocytes. Human MRP2 promoter has an HNF-1 site near one of the transcription initiation sites (Tanaka *et al.* 1999); however, the regulation of MRP2 promoter by HNF-1α and HNF-1β are unknown. Thus, we first analyzed HNF-1α and HNF-1β transactivity on the human MRP2 promoter. HNF-1α had high transcriptional activity and wild-type HNF-1β also had dose-dependent transcriptional activity (Fig. 3A). This result indicated that the human *MRP2* gene is regulated mainly by HNF-1α and also by HNF-1β. None of the HNF-1β mutants had transcriptional activity (Fig. 3A).

We next examined the effect of wild-type and mutant HNF-1β on HNF-1α transcriptional activity in the human MRP2 promoter. Wild-type HNF-1β had a slightly repressive effect on HNF-1α transcriptional activity (Fig. 3B). Among the mutants, HNF-1β H153N showed more repressive effect than wild-type HNF-1β; this repression was specific to the H153N mutant.

We next examined the dominant-negative effect of HNF-1β mutants on wild-type HNF-1β transactivity. Transfection of equal amounts of HNF-1β H153N mutant showed an apparent repressive effect on wild-type HNF-1β (Fig. 3C); however, other mutants had no repressive effect. All the results were similar in HepG2 cells (data not shown). Taken together, these results indicated that the HNF-1β H153N mutant has a mutant-specific repressive effect on both HNF-1α and wild-type HNF-1β in the human MRP2 promoter.



Discussion

In this report, we found that not only HNF-1α and HNF-1β independent activity, but also the activity by their co-expression differ among the analyzed promoters (i.e. insulin, JGF-1 and MRP2). The most differential property was observed in the co-expression activity; the results of adding HNF-1β to HNF-1α ranged from prominent cooperation in human insulin promoter to repression in human MRP2 promoter. The repression of HNF-1α activity by HNF-1β has been reported in other promoters (Song *et al.* 1998, Boudreau *et al.* 2001); however, high cooperation observed in human insulin promoter has not been reported in any other promoters and may be specific to the human insulin promoter. Our findings assist the concept that the relative abundance of HNF-1β to HNF-1α defines each promoter activity in a specific way.

Figure 2 Regulation of human IGF-I promoter activity by HNF-1α and HNF-1β, and the activity of HNF-1β mutants. (A) The indicated amounts of pCMV-1α, pCMV-1βWT, pCMV-1β mutants (H153N, E101X, R177X) were transfected with 100 ng human IGF-I reporter plasmid into HeLa cells. Fold induction refers to the activity without any HNF-1α or HNF-1β. (B) The indicated amounts of pCMV-1βWT or pCMV-1β mutants (H153N, E101X, R177X) were co-transfected with 20 ng pCMV-1α, 100 ng human IGF-I reporter plasmid into HeLa cells. Fold induction refers to the activity with 20 ng pCMV-1α. (C) The indicated amounts of pCMV-1β mutants (H153N, E101X, R177X) were co-transfected with 10 ng pCMV-1βWT, 100 ng human IGF-I reporter plasmid into HeLa cells. The ratio of mutant:wild-type was set to 1:1 reflecting the *in vivo* state. The relative luciferase activity (Firefly/Renilla) was measured by five independent experiments. The mean ± S.E.M. is shown. *$P<0{\cdot}05$, †$P<0{\cdot}01$ compared with no HNF-1α/HNF-1β (A), compared with no HNF-1β (B), or compared with no HNF-1β mutants (C).

We also found in this and in our previous report that the effect of HNF-1β mutants on wild-type HNF-1β and HNF-1α differs with the promoter (Kitanaka *et al.* 2004). We have reported that this difference may be caused by the difference in DNA binding affinity (Kitanaka *et al.* 2004). The dominant-negative effect of R177X mutant on wild-type HNF-1β has also been reported to differ with cells and promoters (Tomura *et al.* 1999, Barbacci *et al.* 2004, Gu *et al.* 2004). From these observations, we consider that analyses of the intended native promoters in combination with wild-type HNF-1β and HNF-1α are essential to investigate the function of HNF-1β mutants.

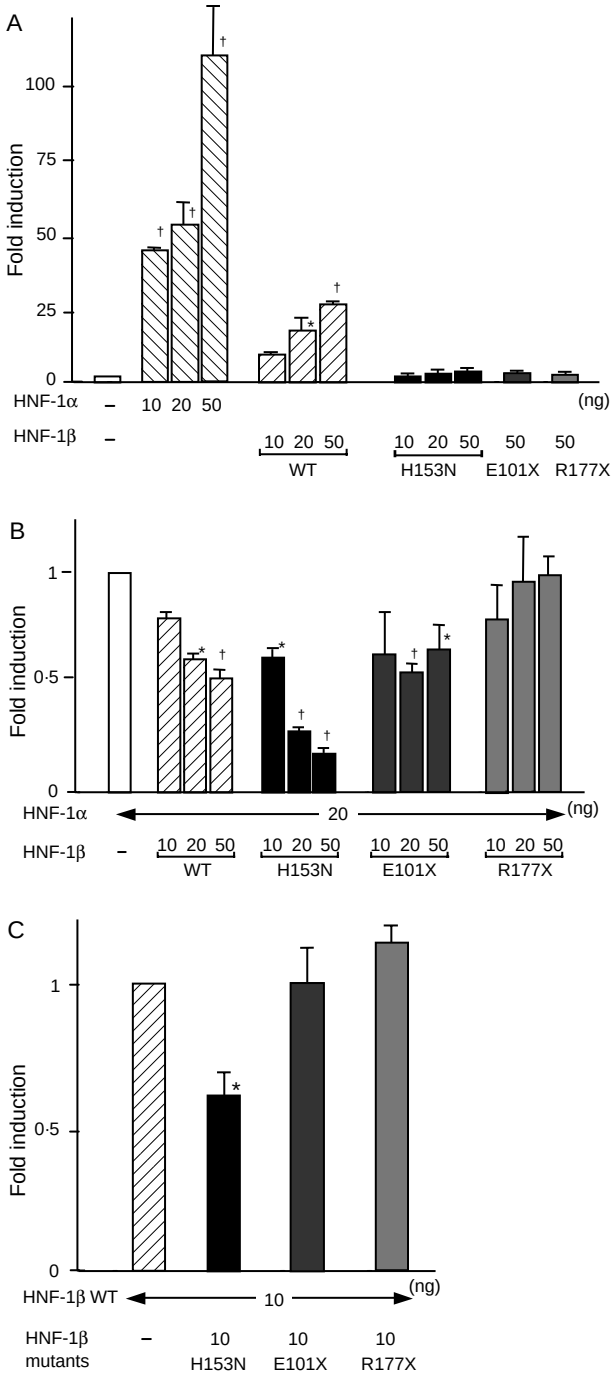


Figure 3 Regulation of human MRP2 promoter activity by HNF-1α and HNF-1β, and the activity of HNF-1β mutants. (A) The indicated amounts of pCMV-1α, pCMV-1βWT, pCMV-1β mutants (H153N, E101X, R177X) were transfected with 100 ng human MRP2 reporter plasmid into HeLa cells. Fold induction refers to the activity without any HNF-1α or HNF-1β. (B) The indicated amounts of pCMV-1βWT or pCMV-1β mutants (H153N, E101X, R177X) were co-transfected with 20 ng pCMV-1α, 100 ng human MRP2 reporter plasmid into HeLa cells. Fold induction refers to the activity with 20 ng pCMV-1α. (C) The indicated amounts of pCMV-1β mutants (H153N, E101X, R177X) were co-transfected with 10 ng pCMV-1βWT, 100 ng human MRP2 reporter plasmid into HeLa cells. The ratio of mutant:wild-type was set to 1:1 reflecting the *in vivo* state. The relative luciferase activity (Firefly/Renilla) was measured by five independent experiments. The mean ± S.E.M. is shown. *$P<0{\cdot}05$, †$P<0{\cdot}01$ compared with no HNF-1α/HNF-1β (A), compared with no HNF-1β (B), or compared with no HNF-1β mutants (C).

The mechanism of diabetes mellitus in MODY5 has not been fully elucidated. It is considered that the major cause of diabetes may be due to the abnormality in pancreas development from the findings of *Hnf-1β*-disrupted mice and of MODY5 patients (Bellanne-Chantelot *et al.* 2004, Haumaitre *et al.* 2005). However, decreased insulin response to glucose loading has been reported in several MODY5 patients and in β-cells-conditional *Hnf-1β* knockout mice, suggesting that HNF-1β has a function in insulin secretion (Lindner *et al.* 1999, Bingham *et al.* 2000, Furuta *et al.* 2002, Bellanne-Chantelot *et al.* 2004, Wang *et al.* 2004). In this study, we found that the co-expression of HNF-1α and HNF-1β had higher transcriptional activity than individual expression on human insulin promoter; mutant HNF-1β did not have such cooperation. Thus, we consider that cooperation of HNF-1α and HNF-1β is important and that abnormal HNF-1β may result in defective insulin gene transcription due to the lack of cooperation with HNF-1α. Although the expression of HNF-1β is less abundant than HNF-1α in the β-cells, small amounts of HNF-1β may have cooperative effect on HNF-1α from our preliminary results. However, further studies of the biological implications of our data are necessary considering the report of the differential expression of *Hnf-1α* and *Hnf-1β* genes in the β-cells of mice (Maestro *et al.* 2003).

IGF-I is essential in fetal growth and is produced mainly in the liver (Nakae *et al.* 2001). The human *IGF-I* gene is known as one of the target genes of HNF-1α (Nolten *et al.* 1995). Growth retardation was observed in *Hnf-1α* knockout mice, most likely due to the loss of IGF-I expression in the liver (Lee *et al.* 1998). Since the expression of HNF-1β precedes that of HNF-1α during development (Cereghini *et al.* 1992), we considered that HNF-1β abnormality might affect *IGF-I* gene transcription. We found that the human *IGF-I* gene is regulated by HNF-1β as well as by HNF-1α and that the HNF-1β H153N mutant had mutant-specific repression on

HNF-1α and wild-type HNF-1β activity. From these findings, we considered that the heterozygous HNF-1β H153N mutant repressed *IGF-I* gene transcription during the fetal period when HNF-1β is dominant and this repression was eliminated with the decreasing HNF-1β expression after birth. This expectation will explain the clinical findings that our patient had intrauterine growth retardation and that spontaneous catch-up growth was observed after birth. However, we do not know whether other MODY5 patients, especially those with E101X and R177X mutation, had intrauterine growth retardation.

Our patient had neonatal cholestasis of unknown etiology and recovered spontaneously in infancy. Another patient with MODY5 has also been reported to have cholestasis (Bellanne-Chantelot *et al.* 2004). It has been shown that HNF-1β is expressed in the biliary system from the fetal period to adulthood and that both HNF-1α and HNF-1β are important for normal bile production (Coffinier *et al.* 1999, 2002, Shih *et al.* 2001). The histological data of the liver in our patient were quite similar to liver- and bile duct-conditional *Hnf-1β* knockout mice, including cholestasis and a decreased number of intrahepatic bile ducts (Coffinier *et al.* 2002). HNF-1β might have a function in intrahepatic bile duct formation; however, the spontaneous improvement of cholestasis in our patient suggests that other factors are involved in the presentation of cholestasis.

In the liver- and bile duct-conditional *Hnf-1β* knockout mice, the expression of *Oatp1* was diminished among multiple bile-related genes (Coffinier *et al.* 2002); however, the clinical condition of high direct bilirubin could not be explained only by this abnormality. Therefore, we suspected that another gene involved in post-conjugation might be affected. We focused on MRP2 and found that the human MRP2 promoter is regulated by both HNF-1α and HNF-1β and that the HNF-1β H153N mutant had a mutant-specific repressive effect on both HNF-1α and HNF-1β. We consider from these results that MRP2 transcription may be repressed when the expression of HNF-1β is dominant, resulting in high direct bilirubin levels. Moreover, cholestasis may spontaneously improve by the decrement in HNF-1β expression. Alternatively, since cholestasis in *Hnf-1β* conditional knockout mice was observed in the homozygotes, the repressive effect only on HNF-1β may be the cause of cholestasis.

We found in this study that only the H153N missense mutant had a repressive effect and the other two mutants did not. All the mutants had an intact dimerization domain and defective DNA binding ability (Tomura *et al.* 1999, Bohn *et al.* 2003, Kitanaka *et al.* 2004). We have shown that the repression by H153N mutant may be caused by inhibiting the DNA binding of HNF-1α and HNF-1β in another promoter (Kitanaka *et al.* 2004). Thus, we consider that the repressive effect in this study is also caused by inhibiting the binding of HNF-1α and HNF-1β to the promoters, but left to be confirmed. Moreover, since the other two mutants are nonsense mutants, further analyses on other missense mutants are necessary to investigate whether this repressive effect is specific to this mutant.

Our study had several limitations. First, these analyses were reporter assays using a transfected reporter plasmid and not by chromosomal genes. There are many factors controlling the chromosomal structure in transcription, so we should be careful about the interpretation of the results on the model system. Secondly, we do not know how these genes are actually regulated by HNF-1α and HNF-1β *in vivo*. We do not know whether these gene expression was actually reduced in our patient and could not examine the cosegregation of the features and the mutation. Thus, it is speculated that the clinical features are caused by other known and unknown factors regulated or not by HNF-1β. Further, accumulation of patients and mutational analyses are necessary to uncover the mechanism of the clinical features.

In conclusion, we found that the cooperation of HNF-1α and HNF-1β is prominent in the regulation of human insulin gene transcription. Absence of this cooperation in HNF-1β mutants may be one cause of defective insulin secretion in MODY5 patients. Furthermore, we found that the HNF-1β H153N mutant had a mutant-specific transcriptional repressive effect on both HNF-1α and wild-type HNF-1β transactivity in human IGF-I and human MRP2 promoters. These observations might explain the variety of clinical features of MODY5. We consider that analysis of each human native promoter is essential to investigate the function of HNF-1β mutants.

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References

Barbacci E, Chalkiadaki A, Masdeu C, Haumaitre C, Lokmane L, Loirat C, Cloarec S, Talianidis I, Bellanne-Chantelot C & Cereghini S 2004 HNF1β/TCF2 mutations impair transactivation potential through altered co-regulator recruitment. *Human Molecular Genetics* **13** 3139–3149.

Bellanne-Chantelot C, Chauveau D, Gautier JF, Dubois-Laforgue D, Clauin S, Beaufils S, Wilhelm JM, Boitard C, Noel LH, Velho G *et al.* 2004 Clinical spectrum associated with hepatocyte nuclear factor-1β mutations. *Annals of Internal Medicine* **140** 510–517.

Bingham C & Hattersley AT 2004 Renal cysts and diabetes syndrome resulting from mutations in hepatocyte nuclear factor-1β. *Nephrology, Dialysis, Transplantation* **19** 2703–2708.

Bingham C, Ellard S, Allen LI, Bulman M, Shepherd M, Frayling T, Berry PJ, Clark PM, Linder T, Bell GI *et al.* 2000 Abnormal nephron development associated with a frameshift mutation in the transcription factor hepatocyte nuclear factor-1β. *Kidney International* **57** 898–907.

Bingham C, Ellard S, Cole TR, Jones KE, Allen LI, Goodship JA, Goodship TH, Bakalinova-Pugh D, Russell GI, Woolf A *et al.* 2002 Solitary functioning kidney and diverse genital tract malformations associated with hepatocyte nuclear factor-1β mutations. *Kidney International* **61** 1243–1251.

Bingham C, Ellard S, van't Hoff WG, Simmonds HA, Marinaki AM, Badman MK, Winocour PH, Stride A, Lockwood CR, Nicholls AJ *et al.* 2003 A typical familial juvenile hyperuricemic nephropathy associated with a hepatocyte nuclear factor-1β gene mutation. *Kidney International* **63** 1645–1651.

Bohn S, Thomas H, Turan G, Ellard S, Bingham C, Hattersley AT & Ryffel GU 2003 Distinct molecular and morphogenetic properties of mutations in the human HNF-1β gene that lead to defective kidney development. *Journal of the American Society of Nephrology* **14** 2033–2041.

Boudreau F, Zhu Y & Traber PG 2001 Sucrase-isomaltase gene transcription requires the hepatocyte nuclear factor-1 (HNF-1) regulatory element and is regulated by the ratio of HNF-1α to HNF-1β. *Journal of Biological Chemistry* **276** 32122–32128.

Cereghini S, Ott MO, Power S & Maury M 1992 Expression patterns of vHNF1 and HNF1 homeoproteins in early postimplantation embryos suggest distinct and sequential developmental roles. *Development* **116** 783–797.

Coffinier C, Barra J, Babinet C & Yaniv M 1999 Expression of the vHNF1/HNF1β homeoprotein gene during mouse organogenesis. *Mechanisms of Development* **89** 211–213.

Coffinier C, Gresh L, Fiette L, Tronche F, Schutz G, Babinet C, Pontoglio M, Yaniv M & Barra J 2002 Bile system morphogenesis defects and liver dysfunction upon targeted deletion of HNF1β. *Development* **129** 1829–1838.

Edghill EL, Bingham C, Ellard S & Hattersley AT 2006 Mutations in hepatocyte nuclear factor-1β and their related phenotypes. *Journal of Medical Genetics* **43** 84–90.

Fajans SS, Bell GI & Polonsky KS 2001 Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *New England Journal of Medicine* **345** 971–980.

Furuta H, Furuta M, Sanke T, Ekawa K, Hanabusa T, Nishi M, Sasaki H & Nanjo K 2002 Nonsense and missense mutations in the human hepatocyte nuclear factor-1β gene (TCF2) and their relation to type 2 diabetes in Japanese. *Journal of Clinical Endocrinology and Metabolism* **87** 3859–3863.

Gresh L, Fischer E, Reimann A, Tanguy M, Garbay S, Shao X, Hiesberger T, Fiette L, Igarashi P, Yaniv M *et al.* 2004 A transcriptional network in polycystic kidney disease. *EMBO Journal* **23** 1657–1668.

Gu N, Suzuki N, Takeda J, Adachi T, Tsujimoto G, Aoki N, Ishihara A, Tsuda K & Yasuda K 2004 Effect of mutations in HNF-1α and HNF-1β on the transcriptional regulation of human sucrase-isomaltase in Caco-2 cells. *Biochemical and Biophysical Research Communications* **325** 308–313.

Haumaitre C, Barbacci E, Jenny M, Ott MO, Gradwohl G & Cereghini S 2005 Lack of TCF2/vHNF1 in mice leads to pancreas agenesis. *PNAS* **102** 1490–1495.

Hiesberger T, Bai Y, Shao X, McNally BT, Sinclair AM, Tian X, Somlo S & Igarashi P 2004 Mutation of hepatocyte nuclear factor-1β inhibits *Pkhd1* gene expression and produces renal cysts in mice. *Journal of Clinical Investigation* **113** 814–825.

Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinokio Y, Cockburn BN, Lindner T, Yamagata K, Ogata M, Tomonaga O *et al.* 1997 Mutation in hepatocyte nuclear factor-1β gene (TCF2) associated with MODY. *Nature Genetics* **17** 384–385.

Kitanaka S, Miki Y, Hayashi Y & Igarashi T 2004 Promoter-specific repression of hepatocyte nuclear factor (HNF)-1β and HNF-1α transcriptional activity by an HNF-1β missense mutant associated with Type 5 maturity-onset diabetes of the young with hepatic and biliary manifestations. *Journal of Clinical Endocrinology and Metabolism* **89** 1369–1378.

Lee YH, Sauer B & Gonzalez FJ 1998 Laron dwarfism and non-insulin-dependent diabetes mellitus in the *Hnf-1α* knockout mouse. *Molecular and Cellular Biology* **18** 3059–3068.

Lindner TH, Njolstad PR, Horikawa Y, Bostad L, Bell GI & Sovik O 1999 A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1β. *Human Molecular Genetics* **8** 2001–2008.

Maestro MA, Boj SF, Luco RF, Pierreux CE, Cabedo J, Servitja JM, German MS, Rousseau GG, Lemaigre FP & Ferrer J 2003 Hnf6 and Tcf2 (MODY5) are linked in a gene network operating in a precursor cell domain of the embryonic pancreas. *Human Molecular Genetics* **12** 3307–3314.

Nakae J, Kido Y & Accili D 2001 Distinct and overlapping functions of insulin and IGF-I receptors. *Endocrine Reviews* **22** 818–835.

Nolten LA, Steenbergh PH & Sussenbach JS 1995 Hepatocyte nuclear factor 1α activates promoter 1 of the human insulin-like growth factor I gene via two distinct binding sites. *Molecular Endocrinology* **9** 1488–1499.

Okita K, Yang Q, Yamagata K, Hangenfeldt KA, Miyagawa J, Kajimoto Y, Nakajima H, Namba M, Wollheim CB, Hanafusa T *et al.* 1999 Human insulin gene is a target gene of hepatocyte nuclear factor-1α (HNF-1α) and HNF-1β. *Biochemical and Biophysical Research Communications* **263** 566–569.

Ryffel GU 2001 Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF)1 and HNF4 families: functional and pathological consequences. *Journal of Molecular Endocrinology* **27** 11–29.

Shih DQ, Bussen M, Sehayek E, Ananthanarayanan M, Shneider BL, Suchy FJ, Shefer S, Bollileni JS, Gonzalez FJ, Breslow JL *et al.* 2001 Hepatocyte nuclear factor-1α is an essential regulator of bile acid and plasma cholesterol metabolism. *Nature Genetics* **27** 375–382.

Song YH, Ray K, Liebhaber SA & Cooke NE 1998 Vitamin D-binding protein gene transcription is regulated by the relative abundance of hepatocyte nuclear factors 1α and 1β. *Journal of Biological Chemistry* **273** 28408–28418.

Tanaka T, Uchiumi T, Hinoshita E, Inokuchi A, Toh S, Wada M, Takano H, Kohno K & Kuwano M 1999 The human multidrug resistance protein 2 gene: functional characterization of the 5′-flanking region and expression in hepatic cells. *Hepatology* **30** 1507–1512.

Tomura H, Nishigori H, Sho K, Yamagata K, Inoue I & Takeda J 1999 Loss-of-function and dominant-negative mechanisms associated with hepatocyte nuclear factor-1β mutations in familial type 2 diabetes mellitus. *Journal of Biological Chemistry* **274** 12975–12978.

Wang H, Maechler P, Hagenfeldt KA & Wollheim CB 1998 Dominant-negative suppression of HNF-1α function results in defective insulin gene transcription and impaired metabolism-secretion coupling in a pancreatic β-cell line. *EMBO Journal* **17** 6701–6713.

Wang H, Antinozzi PA, Hagenfeldt KA, Maechler P & Wollheim CB 2000 Molecular targets of a human HNF1α mutation responsible for pancreatic β-cell dysfunction. *EMBO Journal* **19** 4257–4264.

Wang L, Coffinier C, Thomas MK, Gresh L, Eddu G, Manor T, Levitsky LL, Yaniv M & Rhoads DB 2004 Selective deletion of the Hnf1β (MODY5) gene in β-cells leads to altered gene expression and defective insulin release. *Endocrinology* **145** 3941–3949.

Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV *et al.* 1996 Mutations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young (MODY3). *Nature* **384** 455–458.

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