Biochemical and Biophysical Research Communications 383 (2009) 68–72

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Functional defect of truncated hepatocyte nuclear factor-1α (G554fsX556) associated with maturity-onset diabetes of the young

Suwattanee Kooptiwut [a,*], Jatuporn Sujjitjoon [b], Nattachet Plengvidhya [b,c], Watip Boonyasrisawat [b], Nalinee Chongjaroen [b], Prapapron Jungtrakoon [b], Namoiy Semprasert [a], Hiroto Furuta [d], Kishio Nanjo [d], Napatawn Banchuin [b], Pa-thai Yenchitsomanus [e,f]

[a] Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
[b] Department of Immunology and Immunology Graduate Program, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
[c] Division of Endocrinology and Metabolism, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
[d] The First Department, Wakayama Medical University, Japan
[e] Division of Medical Molecular Biology, Medicine Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
[f] Medical Biotechnology Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Bangkok, Thailand

ARTICLE INFO

Article history:
Received 5 March 2009
Available online 29 March 2009

Keywords:
Diabetes
Maturity-onset diabetes of the young
MODY
HNF-1α
Frameshift mutation
Dual-luciferase assay
Thai

ABSTRACT

A novel frameshift mutation attributable to 14-nucleotide insertion in *hepatocyte nuclear factor-1α* (*HNF-1α*) encoding a truncated HNF-1α (G554fsX556) with 76-amino acid deletion at its carboxyl terminus was identified in a Thai family with maturity-onset diabetes of the young (MODY). The wild-type and mutant HNF-1α proteins were expressed by *in vitro* transcription and translation (TNT) assay and by transfection in HeLa cells. The wild-type and mutant HNF-1α could similarly bind to human glucose-transporter 2 (*GLUT2*) promoter examined by electrophoretic mobility shift assay (EMSA). However, the transactivation activities of mutant HNF-1α on human *GLUT2* and rat *L-type pyruvate kinase* (*L-PK*) promoters in HeLa cells determined by luciferase reporter assay were reduced to approximately 55–60% of the wild-type protein. These results suggested that the functional defect of novel truncated HNF-1α (G554fsX556) on the transactivation of its target-gene promoters would account for the β-cell dysfunction associated with the pathogenesis of MODY.

Introduction

Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous monogenic form of diabetes characterized by an early onset (usually before 25 years), absence of ketosis, and an autosomal dominant pattern of inheritance [1–3]. Up to date, abnormalities of six known genes including *hepatocyte nuclear factor-4α* (*HNF-4α*), *glucokinase* (*GCK*), *HNF-1α*, *insulin promoter factor-1* (*IPF-1*), *HNF-1β*, and *neurogenic differentiation 1* (*NeuroD 1*), have been identified to be responsible for MODY subtypes 1–6, respectively [4]. The common subtype of MODY in Caucasian population is MODY3 which is caused by defects of *HNF-1α* [5] while that in French population is MODY2 which results from defects of *GCK* [6]. It has been observed that MODY with unknown genetic defects (MODY X) in Asian populations, accounting for 60–80% of MODY cases [7,8], is more prevalent than that in Caucasian populations. Our previous work indicated that genetic mutations of the six known MODY genes may not be a major cause of MODY and early-onset type 2 diabetes in Thais [9]. Nevertheless, mutations of *HNF-1α* were detectable with a low frequency in Thai population [9].

HNF-1α is a transcription factor that is expressed in liver, kidney, intestine, stomach, and pancreas [10–12]. HNF-1α protein is composed of three functional domains: an N-terminal dimerization domain (residues 1–32), a DNA-binding domain (residues 150–280) with POU-like and homeodomain motif, and a C-terminal transactivation domain (residues 281–631). It regulates expression of several proteins including amylin [13], insulin [14], GLUT2 [15] and L-type pyruvate kinase (L-PK), 3-hydroxy-3-methylglutaryl coenzyme A reductase, mitochondrial 2-oxoglutarate dehydrogenase (OGDH) E1 subunit [16]. It also plays an important role in glucose metabolism and insulin secretion [17,18].

Recently, our group identified a novel frameshift mutation with 14-nucleotide insertion at codon 554 in exon 9 of *HNF-1α* in a Thai family with MODY. This mutation resulted in a replacement of two amino acids (glycine and leucine by serine and glutamic acid) in the protein and an introduction of a stop codon (GTA) at the position 556 (G554fsX556). The encoded protein contained 555 amino acids with a truncation of C-terminus, compared to the normal

* Corresponding author. Fax: +66 2 4115009.
E-mail address: S_kooptiwut@hotmail.com (S. Kooptiwut).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.03.130

full-length HNF-1α protein. However, the functional effect of the truncated HNF-1α to the β-cell function and pathogenesis of MODY is unknown. We therefore, investigated into binding and transactivation activities of the mutant HNF-1α on two target-gene promoters in HeLa cells to understand its functional impact associated with the pathogenesis of MODY.

Materials and methods

Analysis of genes responsible for MODY. This project was approved by the Human Ethics Committee of the Faculty of Medicine Siriraj Hospital before its initiation. Fifty-one unrelated probands with MODY were recruited at the diabetic clinic, Siriraj Hospital, Thailand, according to the criteria as previously described [19].

Genomic DNAs were extracted from blood samples by a standard phenol/chloroform method. All exons, flanking introns, 5′ flanking and minimal promoter regions of six known genes, *HNF-4α*, *GCK*, *HNF-1α*, *IPF-1*, *HNF-1β* and *NeuroD1*, were analyzed by the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). The PCR product showing a mobility shift on the SSCP gel was subjected to direct sequencing by using ABI Prism BigDye™ (Applied Biosystems, CA, USA), as described in the manufacture's instructions.

Construction of a recombinant plasmid containing a novel frameshift mutation with 14-nucleotide insertion in HNF-1α cDNA. A full-length, human wild-type (WT) HNF-1α cDNA was cloned into pcDNA3.1 to create pcDNA3.1-HNF-1α-WT. The 14-nt insertion at codon 554 of HNF-1α (G554fsX556) was generated by using modified site-directed ligase-independent mutagenesis (SLIM) method described by Chiu et al. [20]. Briefly, two pairs of long-tailed (FL and RL) and short-tailed (Fs and Rs) primers were designed in order to insert 14 nucleotides at the codon 554 of the HNF-1α cDNA construct. The two long-tailed primers contained overhanging 14-nucleotides that would be inserted into the HNF-1α cDNA at their termini which were complementary to each other. The sequences of the two pairs of primers used for PCR were: FL 5′-AGTGAGTGAAGCCCGGGCTTCACACGCCGGCATCT-3′, Rs 5′-GG ACTCACTGGAAGCTTCAGTGTC-3′, and Fs 5′-GGGCTTCACACGCCGG CATCTCA G-3′, RL 5′-GGGCTTCACTCACTGGACTCACTGGAAGCTTCA GT-3′; the underlined regions were the complementary 14 nucleotides to be inserted.

The linear PCR products from amplifications of the whole plasmid containing the 14-nt insert were obtained from two separate reactions using two different pairs of primers as described. The PCR products from two reactions were pooled together, denatured and re-annealed to allow formation of double stranded DNA. The re-annealed PCR products were transformed into competent *Escherichia coli* cells. The *E. coli* colonies were screened for the presence of the recombinant plasmid containing 14-nt insert within *HNF-1α* cDNA, namely pcDNA3.1-*HNF-1α*-G554fsX556. The recombinant plasmid was purified and its nucleotide sequence was examined by sequencing.

Construction of pGL3-Basic luciferase reporter vector containing human GLUT2 promoter or rat L-PK promoter. Human *GLUT2* promoter region from nucleotides −1296 to +312 was isolated from human genomic DNA by PCR and cloned into pGL3-Basic luciferase reporter vector (Promega Corp., Madison, WI). Rat *L-type pyruvate kinase* (*L-PK*) gene was also regulated by HNF-1α [21,22]. Thus, the rat *L-PK* promoter, between nucleotides −189 and +37 was amplified by PCR and cloned into pGL3-Basic luciferase reporter vector. Sequences of these constructs were verified by automated DNA sequencing.

In vitro expression of wild-type and mutant HNF-1α. Wild-type HNF-1α and mutant HNF-1α (G554fsX556) proteins were synthesized by TNT assay (Promega) using pcDNA3.1-*HNF-1α*-WT and pcDNA3.1-*HNF-1α*-G554fsX556 constructs. The proteins were separated on 10% SDS–PAGE and transferred onto a nitrocellulose membrane by electroblotting. The membranes were incubated with anti-HNF-1α antibody (Santa Cruz Biotechnology), followed by horseradish peroxidase-conjugated anti-goat IgG (Santa Cruz Biotechnology), and the antibody binding was detected by using SuperSignal West Pico Chemiluminescence substrate (Pierce).

Expression of wild-type and mutant HNF-1α in HeLa cells. HeLa cells were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum and transfected with 2 μg each of pcDNA3.1, pcDNA3.1-*HNF-1α*-WT, or pcDNA3.1-*HNF-1α*-G554fsX556 construct using FUGENE® 6 transfection reagents (Roche Diagnostics). After 24 h, the transfected cells were lysed in M-PER reagent (Pierce) following the manufacturer's protocol. Then, 50 μg of protein were subjected to 10% SDS–PAGE and transferred by electroblotting onto nitrocellulose membrane. The expressed proteins were detected by Western blot method using anti-HNF-1α antibody as previously described.

Electrophoretic-mobility shift assay (EMSA). HeLa cells were transfected with either pcDNA3.1-*HNF-1α*-WT or pcDNA3.1-*HNF-1α*-G554fsX556 and cultured for 24 h. The nuclear proteins containing either wild-type or mutant HNF-1α were extracted by using NE-PER extraction reagents (Pierce) as described in the manufacturer's protocol. The nuclear proteins were incubated with double-stranded oligonucleotide containing HNF-1α-binding site from the human *GLUT2* promoter sequences [23]; one strand of the oligonucleotides was labeled at its 5′-end with biotin (biotin-5'-tcctccTGCAATGCATAACTAGGCCtaggc-3'). The oligonucleotide–protein binding reaction was performed following the manufacturer's protocol. The oligonucleotide–protein complexes were separated on 5% non-denaturing polyacrylamide gel and subjected to electrophoresis in 0.5× Tris–borate/EDTA. The complexes were transferred to the membrane, fixed, and detected by using LightShift Chemiluminescent EMSA kit (Pierce). To inhibit and confirm binding specificity, unlabeled double-stranded oligonucleotides was added into the binding reaction and then similarly processed. Electrophoretic mobility shift of the complexes, compared with the free biotinylated oligonucleotides that could be inhibit by unlabeled oligonucleotides, indicated the specific binding between the protein and biotinylated oligonucleotides.

Transactivation activities of wild-type and mutant HNF-1α determined by luciferase reporter assay. HeLa cells were transfected with 500 ng of either pcDNA3.1-*HNF-1α*-WT or pcDNA3.1-*HNF-1α*-G554fsX556 using FUGENE® 6 transfection reagent (Roche Diagnostics) along with 100 ng of either pGL3-human *GLUT2* or pGL3-rat *L-PK* promoter, and 10 ng of pRL-SV40 (to control transfection efficiency). After 24 h, the transactivation activities of the wild-type and mutant HNF-1α proteins were measured by means of the Dual-Luciferase Reporter Assay System (Promega). For each plasmid construct, the experiments were repeated three times.

Statistical analysis. The data were analyzed by using SPSS software (version 11.5; SPSS Inc.), expressed as mean ± SD, and tested for their difference by unpaired *t*-test. The p value < 0.05 was considered significant.

Results and discussion

A novel frameshift mutation attributable to an insertion of 14 nucleotides in exon 9 of *HNF-1α* introducing two amino-acid changes in the protein and creating an early stop codon at the position 556 (*HNF-1α*-G554fsX556) was identified in a Thai family with MODY (Fig. 1). Thus, the mutant HNF-1α protein was truncated by the deletion of 76 amino acids at its C-terminus. The mutation was observed in the male proband who had diabetes at the age of 12 years and also in his mother who had the disease

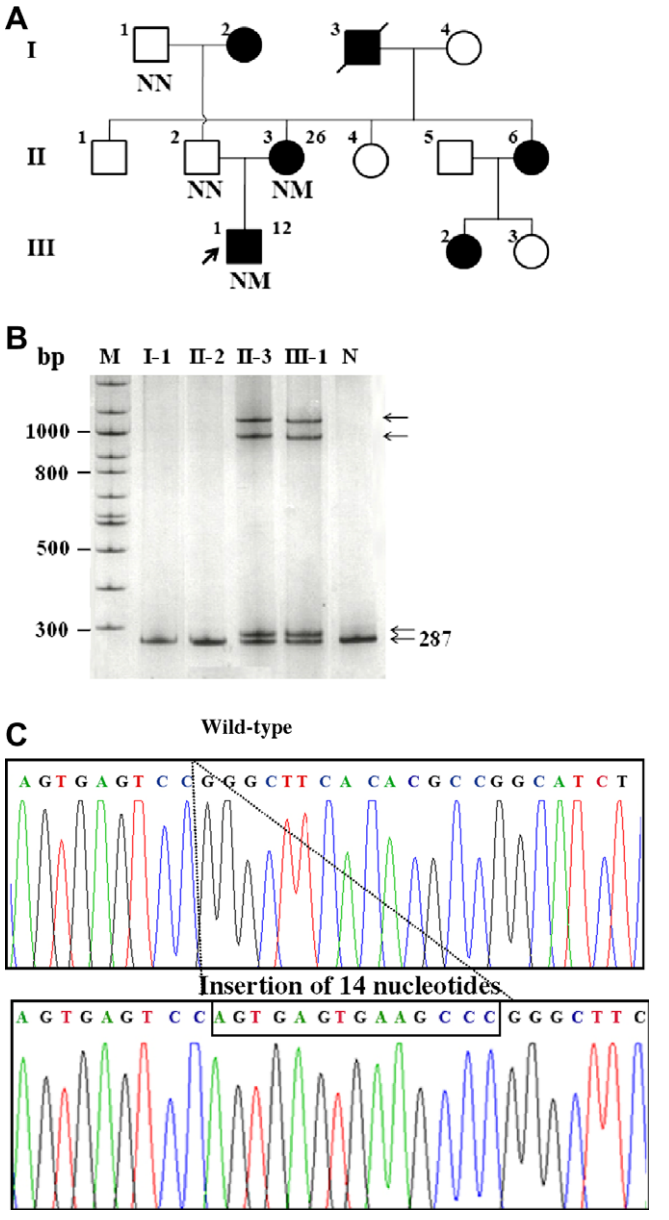

Fig. 1. (A) The pedigree of Thai family with MODY. *HNF-1α* G554fsX556 mutation was detected in the proband (III-1) and his affected mother (II-3) who had diabetes at the ages of 12 and 26 years, respectively. *Square* and *circular symbols* represent male and female members while *filled* and *open symbols* indicates diabetic and non-diabetic individuals, respectively. *Arrow* indicates the proband. *Roman numerals* on left site indicate the generation number and *Arabic numbers* on upper left of the symbols indicate individual numbers in the generation. Age at onset of diabetes is shown at the upper right of the symbol. The letters under the symbol indicate *HNF-1α* genotypes: *N*, normal; *M*, mutant. (B) The result of *HNF-1α* mutation screening (exon 9) by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). Positive screening results were found in the PCR products from the mother (II-3) and proband (III-1) showing the mobility shift and double bands (heteroduplexes) of DNA. Lanes are indicated above the gel: *M*, ladder of DNA markers; *I-1, II-2, II-3,* and *III-1* are the PCR products from the family members (the numbers are corresponding to those in the pedigree); *N*, normal. (C) Sequencing profiles of the exon 9 of *HNF-1α* in the mutation region: *top panel*, wild-type sequence; *lower panel*, mutant sequence with 14-nucleotide insertion indicated by box.

at the age of 26 years while his father who was healthy did not carry this mutation (Fig. 1). However, other members of the family were not available for the studies. This mutation was not observed in the screening of 214 non-diabetic subjects. Clinical findings of the proband, his mother and father were shown in Table 1.

Table 1
Clinical characteristics of the patient, affected mother, an healthy father.

	Proband	Mother	Father
Age (yrs)	18	50	52
Age at onset (yrs)	12	26	–
Duration (yrs)	6	24	–
BMI (kg/m^2)	20.41	22.51	18.34
Waist (cm)	79	81	77
Hip (cm)	95	93	89
Waist/Hip ratio	0.83	0.87	0.86
FPG (mmol/l)	13.20	11.71	6.33
Serum creatinine (μmol/L)	61.88	79.56	–
Total cholesterol (mmol/l)	3.55	4.82	–
Triglyceride (mmol/l)	0.43	0.723	–
LDL (mmol/l)	1.77	3.24	–
Treatment	OHA	OHA	–
Complications	–	Retinopathy mild neuropathy	–

Normal range of fasting plasma glucose < 5.6 mmol/l.
Normal range of serum creatinine: adult males, 70.72–123.76 mg/dl; adult females, 53.04–97.24 mg/dl.

To examine expression of the mutant HNF-1α (G554fsX556) protein and its functions in binding and transactivating target-gene promoters, we constructed the recombinant plasmid containing *HNF-1α* G554fsX556 cDNA for the studies. The protein expression was studied by TNT system and by HeLa cell transfection and detected by Western blot method. By both expression systems, the wild-type and mutant proteins were expressed to the predicted sizes of 68 and 59 kDa, respectively (Fig. 2A and B). In the transfected HeLa cells, we determined the expression of wild-type and mutant proteins in whole cell lysate in three independent experiments and found that the wild-type and mutant HNF-1α proteins had relatively similar quantities calculated as ratios with the densities of β-actin protein ($p = 0.219$) (Fig. 2B). When the cytoplasmic and nuclear compartments were separated, the wild-type and mutant proteins were found to be predominantly expressed in the nucleus (data not shown). This indicated that, similar to the wild-type HNF-1α, the mutant was stable and could migrate to localize in the nuclear compartment of the cells.

The binding abilities of the wild-type and mutant HNF-1α proteins expressed in HeLa cells to human *GLUT2* promoter containing HNF-1α binding site were determined by electrophoretic mobility shift assay (EMSA). The results showed that both wild-type and mutant proteins were able to bind and cause electrophoretic mobility shift of the biotinylated oligonucleotides (Fig. 2C), indicating that the binding ability of mutant protein to the HNF-1α binding site was still intact. In addition, their binding to the biotinylated oligonuleotides could completely be blocked by the addition of 200-fold excess of unlabeled oligonucleotide (Fig. 2C), confirming their binding specificity.

Since HNF-1α is a transcription factor that activates expression of *GLUT2* and *L-PK* genes [21,24], we examine whether the mutant HNF-1α protein, would exhibit any defect in activation of these two promoters. Human *GLUT2* or rat *L-PK* promoters cloned into pGL3-Basic luciferase reporter vector were co-transfected with either pcDNA3.1-*HNF-1α*-WT or pcDNA3.1-*HNF-1α*-G554fsX556 into HeLa cells, which has no endogenous HNF-1α [25]. The results showed that the wild-type HNF-1α could transactivate the human *GLUT2* and rat *L-PK* promoters, resulting in markedly increased luciferase activities. However, the mutant HNF-1α could not similarly transactivate these two promoters but only at the levels of 60% and 55% of that of the wild-type protein, respectively (Fig. 3A and B). The reduction of transactivation activity of the mutant protein is most likely due to the defect from the deletion of transactivation domain in its C-terminal region since similar amounts of wild-type and mutant proteins were expressed in the transfected HeLa cells as shown by the Western blot analysis

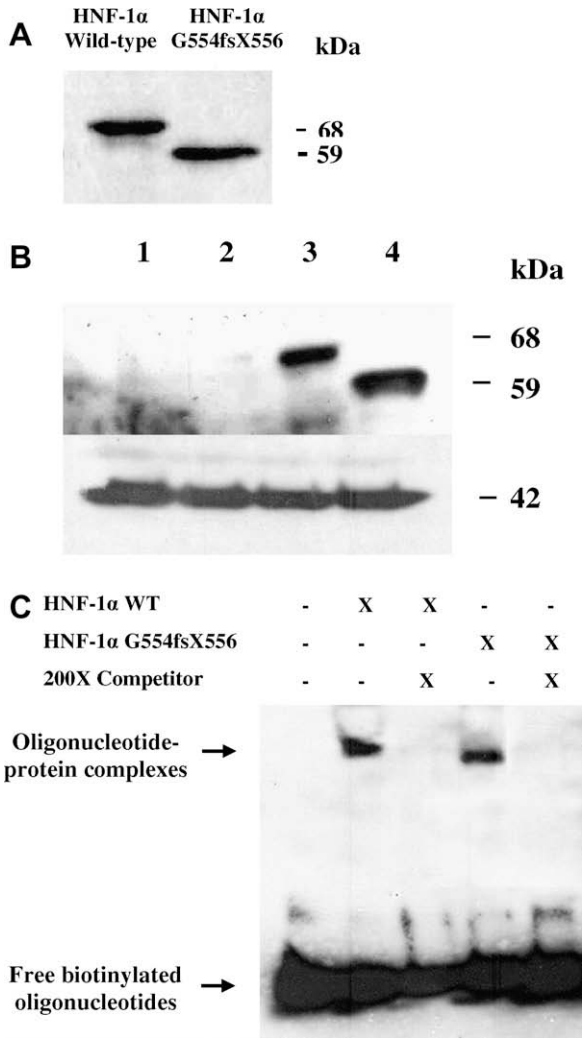

Fig. 2. (A) Western blot analysis of protein expression by TNT system. Equal amounts of proteins were loaded on SDS–PAGE, transferred onto nitrocellulose membrane, and detected by anti-HNF-1α antibody. The wild-type (HNF-1α WT) and mutant (HNF-1α G554fsX556) proteins were expressed at the molecular weights of 68 and 59 kDa, respectively. (B) Western blot analysis of protein expression in HeLa cells. Equal amounts of proteins were loaded on SDS–PAGE, transferred by onto nitrocellulose membrane, and detected by anti-HNF-1α antibody: *lanes 1* and *2*, empty vectors; *lane 3*, wild-type protein; *lane 4*, mutant protein. The wild-type and mutant proteins (in *lanes 3* and *4*) were expressed at the molecular weights of 68 and 59 kDa, respectively. *Lower panel* were β-actin protein with molecular weight of 42 kDa. (C) Electrophoretic mobility shift assay (EMSA) for analysis of the binding between wild-type or mutant HNF-1α protein to *GLUT2* promoter consisting of HNF-1α-binding sequence. The oligonucleotide–protein complexes were analyzed on 5% non-denaturing polyacrylamide gel. In the competitive assay, 200-fold excess of unlabeled double-stranded oligonucleotides (competitor) were added into the binding reaction. Both wild-type and mutant proteins (*lanes 2* and *4*) could bind to the biotinylated double-stranded oligonucleotides, which could be inhibited by the competitor (*lanes 3* and *5*).

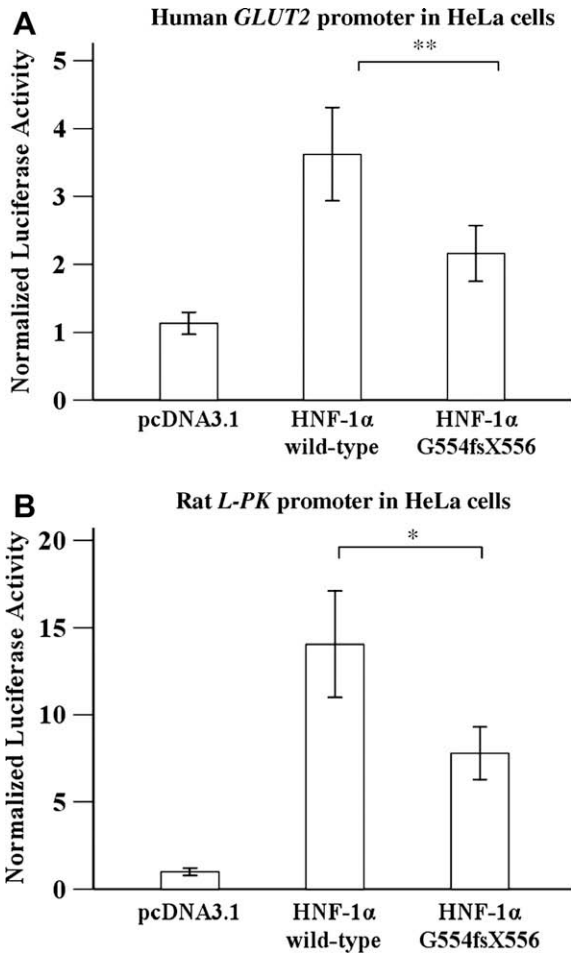

Fig. 3. (A) Transactivation activities of wild-type and mutant HNF-1α proteins on human *GLUT2* promoter in HeLa cells determined by dual-luciferase reporter assay. HeLa cells were transfected with pcDNA3.1-empty vector, pcDNA3.1-*HNF-1α* WT, or pcDNA3.1-*HNF-1α* G554fsX556 together with pGL3-human *GLUT2* vector and pRL-SV40. (B) Transactivation activities of wild-type and mutant HNF-1α proteins on rat *L-PK* promoter in HeLa cells determined by luciferase reporter assay. HeLa cells were transfected with pcDNA3.1-empty vector, pcDNA3.1-*HNF-1α* WT, or pcDNA3.1-*HNF-1α* G554fsX556 together with pGL3-rat *L-PK* vector and pRL-SV40.

(Fig. 2B) and both proteins could similarly attach to the *HNF-1α* binding site in the human *GLUT2* promoter (Fig. 2C).

The reduction in transcription activity of the mutant HNF-1α (G554fsX556) protein may down regulate the expression of its target genes such as *GLUT2* and *L-PK*. GLUT2 is a major glucose transporter in pancreatic β-cells and plays an important role in glucose-induced insulin secretion [26]. Therefore, decrease in *GLUT2* expression may lead to the impairment of insulin secretion [27–29]. On the other hand, L-PK is an important enzyme in the glycolytic pathway in liver, kidney, small intestine and pancreatic β-cells [30]. The defect in *L-PK* expression may lead to defect in glucose metabolism that involves in insulin secretion in pancreatic β-cells. Thus, the decrease in transactivation of the mutant HNF-1α (G554fsX556) protein on *GLUT2* and *L-PK* may result in the defect of insulin secretion and associated with MODY in the reported Thai family.

In conclusion, the *HNF-1α* G554fsX556 mutation identified in a Thai family with MODY should be pathogenic because although the mutant protein with truncation of the transactivation domain could be expressed and attach to the HNF-1α binding site, its transactivation activities on *GLUT2* and *L-PK* promoters was markedly decreased. This may explain the impairment of insulin secretion and insulin sensitivity leading to hyperglycemia in the patients with MODY caused by this pathogenic mutation.

Acknowledgments

This work was supported by Thailand Research fund (TRF), Siriraj Research Development Grant and Siriraj Chalearmprakiat Fund (to SK), Siriraj Graduate Scholarship (to JS), Research Career Development Grant of Thailand Research Fund (TRF) (to NP), and TRF Senior Research Scholar Grant (to PY). We thank to Nuanghathai Sawasdee, and Malika Churintaraphan for technical assistances.

References

[1] S. Fajans, G. Bell, D. Bowden, J. Halter, K. Polonsky, Maturity onset diabetes of the young (MODY), Diabet. Med. 13 (1996) S90–S95.

[2] G. Velho, P. Froguel, Maturity-onset diabetes of the young (MODY), MODY genes and non-insulin-dependent diabetes mellitus, Diabetes Metab. 23 (Suppl 2) (1997) 34–37.

[3] W.E. Winter, M. Nakamura, D.V. House, Monogenic diabetes mellitus in youth. The MODY syndromes, Endocrinol. Metab. Clin. North Am. 28 (1999) 765–785.

[4] S.S. Fajans, G.I. Bell, K.S. Polonsky, Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young, N. Engl. J. Med. 345 (2001) 971–980.

[5] F. Beards, T. Frayling, M. Bulman, Y. Horikawa, L. Allen, M. Appleton, G. Bell, S. Ellard, A. Hattersley, Mutations in hepatocyte nuclear factor 1beta are not a common cause of maturity-onset diabetes of the young in the UK, Diabetes 47 (1998) 1152–1154.

[6] J. Hager, H. Blanché, F. Sun, N.V. Vaxillaire, W. Poller, D. Cohen, P. Czernichow, G. Velho, J.J. Robert, N. Cohen, P. Froguel, Six mutations in the glucokinase gene identified in MODY by using a nonradioactive sensitive screening technique, Diabetes 43 (1994) 730–733.

[7] M.C. Ng, B.N. Cockburn, T.H. Lindner, V.T. Yeung, C.C. Chow, W.Y. So, J.K. Li, Y.M. Lo, Z.S. Lee, C.S. Cockram, J.A. Critchley, G.I. Bell, J.C. Chan, Molecular genetics of diabetes mellitus in Chinese subjects: identification of mutations in glucokinase and hepatocyte nuclear factor-1alpha genes in patients with early-onset type 2 diabetes mellitus/MODY, Diabet. Med. 16 (1999) 956–963.

[8] H. Nishigori, S. Yamada, T. Kohama, T. Utsugi, H. Shimizu, T. Takeuchi, J. Takeda, Mutations in the hepatocyte nuclear factor-1 alpha gene (MODY3) are not a major cause of early-onset non-insulin-dependent (type 2) diabetes mellitus in Japanese, J. Hum. Genet. 43 (1998) 107–110.

[9] N. Plengvidhya, W. Boonyasrisawat, N. Chongjaroen, P. Jungtrakoon, S. Sriussadaporn, S. Vannaseang, N. Banchuin, P. Yenchitsomanus, Mutations of maturity-onset diabetes of the young (MODY) genes in Thais with early-onset type 2 diabetes mellitus, Clin Endocrinol (Oxf). (2008) [Epub ahead of print].

[10] I. Bach, Z. Galcheva-Gargova, M.G. Mattei, D. Simon-Chazottes, J.L. Guenet, S. Cereghini, M. Yaniv, Cloning of human hepatic nuclear factor 1 (HNF1) and chromosomal localization of its gene in man and mouse, Genomics 8 (1990) 155–164.

[11] C.J. Kuo, P.B. Conley, C.L. Hsieh, U. Francke, G.R. Crabtree, Molecular cloning, functional expression, and chromosomal localization of mouse hepatocyte nuclear factor 1, Proc. Natl. Acad. Sci. USA 87 (1990) 9838–9842.

[12] J. Rey-Campos, T. Chouard, M. Yaniv, S. Cereghini, VHNF1 is a homeoprotein that activates transcription and forms heterodimers with HNF1, EMBO J. 10 (1991) 1445–1457.

[13] L.M. Shepherd, S.C. Campbell, W.M. Macfarlane, Transcriptional regulation of the IAPP gene in pancreatic beta-cells, Biochim. Biophys. Acta 1681 (2004) 28–37.

[14] K. Okita, Q. Yang, K. Yamagata, K.A. Hangenfeldt, J. Miyagawa, Y. Kajimoto, H. Nakajima, M. Namba, C.B. Wollheim, T. Hanafusa, Y. Matsuzawa, Human insulin gene is a target gene of hepatocyte nuclear factor-1alpha (HNF-1alpha) and HNF-1beta, Biochem. Biophys. Res. Commun. 263 (1999) 566–569.

[15] J.Y. Cha, H. Kim, K.S. Kim, M.W. Hur, Y. Ahn, Identification of transacting factors responsible for the tissue-specific expression of human glucose transporter type 2 isoform gene. Cooperative role of hepatocyte nuclear factors 1alpha and 3beta, J. Biol. Chem. 275 (2000) 18358–18365.

[16] H. Wang, P.A. Antinozzi, K.A. Hagenfeldt, P. Maechler, C.B. Wollheim, Molecular targets of a human HNF1 alpha mutation responsible for pancreatic beta-cell dysfunction, EMBO J. 19 (2000) 4257–4264.

[17] H. Wang, K. Hagenfeldt-Johansson, L.A. Otten, B.R. Gauthier, P.L. Herrera, C.B. Wollheim, Experimental models of transcription factor-associated maturity-onset diabetes of the young, Diabetes 51 (Suppl 3) (2002) S333–S342.

[18] K. Yamagata, T. Nammo, M. Moriwaki, A. Ihara, K. Iizuka, Q. Yang, T. Satoh, M. Li, R. Uenaka, K. Okita, H. Iwahashi, Q. Zhu, Y. Cao, A. Imagawa, Y. Tochino, T. Hanafusa, J. Miyagawa, Y. Matsuzawa, Overexpression of dominant-negative mutant hepatocyte nuclear fctor-1 alpha in pancreatic beta-cells causes abnormal islet architecture with decreased expression of E-cadherin, reduced beta-cell proliferation, and diabetes, Diabetes 51 (2002) 114–123.

[19] N. Plengvidhya, S. Kooptiwut, N. Songtawee, A. Doi, H. Furuta, M. Nishi, K. Nanjo, W. Tantibhedhyangkul, W. Boonyasrisawat, P.T. Yenchitsomanus, A. Doria, N. Banchuin, PAX4 mutations in Thais with maturity onset diabetes of the young, J. Clin. Endocrinol. Metab. 92 (2007) 2821–2826.

[20] J. Chiu, P.E. March, R. Lee, D. Tillett, Site-directed, Ligase-Independent Mutagenesis (SLIM): a single-tube methodology approaching 100% efficiency in 4 h, Nucleic Acids Res. 32 (2004) 174.

[21] M. Parrizas, M.A. Maestro, S.F. Boj, A. Paniagua, R. Casamitjana, R. Gomis, F. Rivera, J. Ferrer, Hepatic nuclear factor 1-alpha directs nucleosomal hyperacetylation to its tissue-specific transcriptional targets, Mol. Cell. Biol. 21 (2001) 3234–3243.

[22] K. Yamada, T. Noguchi, T. Matsuda, M. Takenaka, P. Monaci, A. Nicosia, T. Tanaka, Identification and characterization of hepatocyte-specific regulatory regions of the rat pyruvate kinase L gene. The synergistic effect of multiple elements, J. Biol. Chem. 265 (1990) 19885–19891.

[23] N. Ban, Y. Yamada, Y. Someya, K. Miyawaki, Y. Ihara, M. Hosokawa, S. Toyokuni, K. Tsuda, Y. Seino, Hepatocyte nuclear factor-1alpha recruits the transcriptional co-activator p300 on the GLUT2 gene promoter, Diabetes 51 (2002) 1409–1418.

[24] D.Q. Shih, S. Screenan, K.N. Munoz, L. Philipson, M. Pontoglio, M. Yaniv, K.S. Polonsky, M. Stoffel, Loss of HNF-1alpha function in mice leads to abnormal expression of genes involved in pancreatic islet development and metabolism, Diabetes 50 (2001) 2472–2480.

[25] A. Nicosia, R. Tafi, P. Monaci, Trans-dominant inhibition of transcription activator LFB1, Nucleic Acids Res. 20 (1992) 5321–5328.

[26] M.T. Guillam, P. Dupraz, B. Thorens, Glucose uptake, utilization, and signaling in GLUT2-null islets, Diabetes 49 (2000) 1485–1491.

[27] E. Chankiewitz, D. Peschke, L. Herberg, I. Bazwinsky, E. Mühlbauer, H. Brömme, E. Peschke, Did the gradual loss of GLUT2 cause a shift to diabetic disorders in the New Zealand obese mouse (NZO/Hl)? Exp. Clin. Endocrinol. Diabetes 114 (2006) 262–269.

[28] O. Laukkanen, J. Lindström, J. Eriksson, T. Valle, H. Hämäläinen, P. Ilanne-Parikka, S. Keinänen-Kiukaanniemi, J. Tuomilehto, M. Uusitupa, M. Laakso, F.D.P. Study, Polymorphisms in the SLC2A2 (GLUT2) gene are associated with the conversion from impaired glucose tolerance to type 2 diabetes: the Finnish Diabetes Prevention Study, Diabetes 54 (2005) 2256–2260.

[29] S. Reddy, M. Young, C. Poole, J. Ross, Loss of glucose transporter-2 precedes insulin loss in the nonobese diabetic and the low-dose streptozotocin mouse models: a comparative immunohistochemical study by light and confocal microscopy, Gen. Comp. Endocrinol. 111 (1998) 9–19.

[30] K. Yamada, T. Noguchi, Nutrient and hormonal regulation of pyruvate kinase gene expression, Biochem. J. 337 (1999) 1–11.