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Molecular genetics of monogenetic beta-cell diabetes

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ABSTRACT

Monogenic β-cell diabetes – a rare form of diabetes mellitus (DM) is caused by defects in a group of genes controlling pancreatic β-cell development and function. The diabetic symptoms are manifested within a short period after birth as neonatal diabetes mellitus (NDM), in childhood or early adulthood as maturity-onset diabetes of the young (MODY) and mitochondrial diabetes. Several etiologic genes for this form of DM have been identified in many patients. The common etiologic genes encode β-cell transcription factors and proteins involving in glucose-stimulated insulin secretion. Owning to their nature of genetic heterogeneity, monogenic β-cell diabetes presents the characteristics of variable age at onset, degree of severity, and occurrence of diabetic complications. The study of this form of diabetes has provided new knowledge and a better insight into the molecular mechanism controlling normal and pathological states of β-cells as reviewed in this article.

Keywords: Monogenic diabetes, β-cell dysfunction, neonatal diabetes mellitus, maturity-onset diabetes of the young, early-onset type 2 diabetes, mitochondrial diabetes, transcription factor, hepatocyte nuclear factor

INTRODUCTION

Diabetes mellitus (DM) is a group of common metabolic disorder that at present affects over 171 millions people worldwide. The disease is characterized by chronic hyperglycemia resulted from b-cell defects in insulin secretion, defects in insulin action, or combination of both. DM is generally recognized as a complex or multifactorial disease in which several genetic abnormalities together with environmental triggering are required for its development. However, 1%-5% of cases with DM are caused by single-gene (monogenic) defects, of which the genes controlling β-cell function is predominant. The monogenic β-cell diabetes is also found to be heterogeneous, comprising neonatal diabetes mellitus (NDM), maturity-onset diabetes of the young (MODY), and mitochondrial diabetes. Among these, MODY is the most intensively investigated. Currently, six different genes have been

identified to be responsible for MODY. While most of them encode transcription factors required for β-cell development and function, one encodes glucokinase – an enzyme in a rate-limiting step of glycolysis. Extensively heterogeneous clinical manifestrations of MODY are attributable to defects of distinct genes. While MODY is usually developed in childhood or young adult, the onset of NDM is at an early infancy. The most common causes of NDM are defects in genes encoding molecule involved in insulin secretion but a few cases are caused by mutations in the genes encoding transcription factors that are required for the β-cell development. Defects in some particular genes can cause either NDM or MODY. Mutation in mitochondrial DNA associated with DM is rare and can be differentiated from MODY by the presence of maternal transmission in conjunction with deafness. Due to their extensive heterogeneity in clinical presentations, it has recently been suggested that the terms of MODY and NDM are obsolete and new terminologies based on molecular genetic classification are proposed (Murphy *et al.*, 2008). The study of 'monogenic β-cell diabetes' has provided a better understanding in the etiology of β-cell dysfunction, which is also involved in other subtypes of DM. Knowledge of the molecular pathology of diabetes is required for appropriate treatment, prediction of disease progression, family-member screening and genetic counseling. Thus, the study into molecular genetics and pathophysiology of monogenic β-cell diabetes as well as other subtypes of DM will not only fulfill an image of complex biological networks maintaining glucose homeostasis but also lead to development of novel methods for therapeutic management.

Monogenic β-cell diabetes

Monogenic β-cell diabetes is caused by defects of single genes critically responsible for pancreatic β-cell development or function. The patients with monogenic β-cell diabetes may develop the disease since childhood, similar to type 1 diabetes (T1D), or they may develop it later in early adulthood. It can be differentiated from T1D by the absence of islet autoantibodies – a marker for autoimmunity. A markedly obesity, insulin resistance and acanthosis nigrican (a skin condition characterized by dark thickened velvety patches), generally presented in type 2 diabetes (T2D), are not observed in the patients with monogenic β-cell diabetes. NDM and MODY are two main forms of monogenic β-cell diabetes. NDM is a rare disease occurred and diagnosed within six months after birth. The clinical manifestations may be transiently observed (transient neonatal diabetes, TND) or permanently appear throughout the life (permanent neonatal diabetes, PND). MODY is usually developed in childhood or young adulthood and occurred from defects of different genes. Because of distinct genetic etiologies, monogenic β-cell diabetes presents with heterogeneous clinical manifestations. Four broad categories are proposed (Murphy *et al.*, 2008) including (*i*) neonatal diabetes with the disease diagnosed within the first 6 months of life, (*ii*) familial diabetes with mild fasting hyperglycemia, (*iii*) familial and young onset diabetes, and (*iv*) diabetes with extrapancreatic features. These categories provide more helpful guidance for clinical managements and new terminologies of different forms of monogenic β-cell diabetes also suggested.

Maturity-onset diabetes of the young

Definition and diagnostic criteria of MODY

Maturity-onset diabetes of the young (MODY) is originally described as an autosomal dominant inheritance of non-insulin dependent diabetes, now known as T2D, which is diagnosed before 25 years (Fajans *et al.*, 1960). However, according to a better understanding in its molecular etiology, MODY is now classified as a form of "other specific types of DM" which demonstrates monogenic defects in β-cell functions (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). The diagnostic criteria of MODY are as follows (Hattersley, 2003): *(i)* early onset of diabetes (usually less than 25 years), *(ii)* autosomal dominant

inheritance, *(iii)* rarely obese and non-ketotic diabetes, and *(iv)* diabetes results from β-cell dysfunction. The actual prevalence of MODY in general population is difficult to determine because its clinical phenotype is very heterogeneous and it is sometimes misdiagnosed as other types of DM. However, the studies in selected cohorts showed that it accounts for 1-5% of DM cases.

Genetic and clinical heterogeneity of MODY

Molecular genetics of MODY has been more progressively studied than other forms of DM. The autosomal dominant model of inheritance and the ability to collect large multi-generation pedigrees due to an early onset greatly advanced genetic study by linkage analysis. The studies by both candidate gene approach and positional cloning led to the identification of six different genes responsible for MODY (Table 1). These genes encode glucokinase enzyme (associated with MODY2) (Pearson *et al.*, 2001) and transcription factors expressed in pancreatic β-cells, including hepatocyte nuclear factor-4α (HNF-4α, MODY1) (Yamagata *et al.*, 1996a), hepatocyte nuclear factor-1α (HNF-1α, MODY3) (Yamagata *et al.*, 1996b), insulin promoter factor-1 (IPF-1, MODY4) (Stoffers *et al.*, 1997), hepatocyte nuclear factor-1β (HNF-1β, MODY5), and NeuroD1/BETA2 (MODY6) (Kristinsson *et al.*, 2001). The prevalence of each MODY subtype varies among ethnic groups. MODY2 are the most common cause of MODY in France, accounting for more than 60% of studied families, whereas its prevalence in United Kingdom and Germany were 11% and 8%, respectively. In general, MODY3 is most common in Caucasians but its prevalence varies from 21% to 64%. The other four types of MODY are rare. MODY with unknown genetic etiology (MODY-X) represents 16-45% of MODY cases in Caucasians and more than 90% of the cases in Asian populations.

Clinical phenotypes associated with defects of the six genes are distinct, requiring different treatments. Mild hyperglycemia in patients with *glucokinase* mutations (MODY2) is presented at birth and the patients are often asymptomatic at diagnosis. In addition, obesity, hypertension, dyslipidaemia and diabetic-associated complications

Table 1 Molecular genetics and clinical presentations of the different MODY subtypes (modified from Fajan *et al.*, 2000).

	MODY1	MODY2	MODY3	MODY4	MODY5	MODY6	MODYX
Gene	*HNF-4α*	*GCK*	*HNF-1α*	*IPF1*	*HNF-1β*	*NeuroD1/Beta2*	-
Gene locus	20q12-q13.1	7p15.3-p15.1	12q24.3	13q12.1	17cenq21.3	2q32	Heterogeneous?
Function	Orphan nuclear receptor	Glucose-phosphorylation enzyme	Homeodomain transcription factor	Homeodomain transcription factor	Homeodomain transcription factor	Homeodomain transcription factor	-
Distribution							
- Caucasians	2-4%	8-63%	21-64%	rare	0-1%	Rare	16-45%
- Asians	2%	2.5%	8%	0%	0.8-2%	0%	>90%
Diagnosis age	Post-pubertal	Childhood	Post-pubertal	Post-pubertal	Post-pubertal	Post-pubertal	Heterogeneous?
Primary defect	Pancreas/ liver	Pancreas/ liver	Pancreas/ Liver/kidney/	Pancreas/ others	Pancreas/liver/ Kidney/genitals	Pancreas/others	Pancreas/ Heterogeneous?
Associated phenotype	-	Low birth weight	Diminished renal glucose threshold	-	Renal morphologic abnormalities, renal insufficiency, pancreatic atrophy, genital abnormalities	-	-
Severity	Severe	Moderate	Severe	Moderate?	Severe	Severe?	Moderate/ Heterogenous?
Chronic complications	Frequent	Rare	Frequent	Rare	?	?	?

are uncommon. Patients carrying *HNF-4α* mutations (MODY1) exhibit a severe impairment in insulin secretion but may present mild diabetes. However, hyperglycemia tends to increase over time. Patients carrying *HNF-4α* mutations may exhibit the extra-pancreatic phenotypes; for instance, low serum levels of triglycerides, apoAII, apoCIII, and lipoprotein a (Shih *et al.*, 2000). Hyperglycemia in patients associated with HNF-1α (MODY3) is often symptomatic and progressive. Clinical features of MODY3 patients are highly variable from one family to another, or even within the same family. Both MODY1 and MODY3 patients are sensitive to hypoglycemic effect of sulfonylurea and insulin is required for treatment. Diabetic complications are frequently observed in MODY3 patients and some of them exhibited the extra-pancreatic phenotypes such as kidney dysfunction, renal tubulopathy, low serum concentration of apoM level, decreased renal reabsorption of glucose and glycosuria. Patients carried *HNF-1β* mutations (MODY5) is usually associated with disorder in other organs. These include renal dysfunction, pancreatic atrophy, abnormal liver function tests, familial glomerulocystic kidney disease, renal cysts, and genital malformations (Edghill *et al.*, 2006). The most common extra-pancreatic feature of *HNF-1β* mutations is renal cysts that, leads to a novel syndrome, namely "renal cysts and diabetes, RCAD". Clinical phenotypes of patients carrying *IPF1* (MODY4) and *NeuroD1* (MODY6) mutations are similar to other transcription factor-associated MODYs.

Pathophysiology of MODY

Five of six MODY subtypes are caused by mutations in the genes encoding transcription factors which are enriched in pancreatic β-cells (Fig. 1). The studies in knockout mice and humans indicated that these transcription factors coordinately play roles in embryonic development of pancreas and final differentiation to β-cells. In addition, they are involved in normal β-cell functions regulating gene expression in fully differentiated β-cells. *Insulin* and *glucose-transporter protein* (*GLUT2*) genes are important targets of their regulation. Insulin - a key hormone in maintaining glucose homeostasis is exclusively synthesized and secreted from pancreatic β-cells in response to glucose and other nutrient sensing. Once glucose is transported into the β-cell via a specific glucose-transporter protein (GLUT2) on β-cell cell membrane, it is catalyzed into glucose-6-phosphate by glucokinase, a rate-limiting enzyme in glycolysis pathway and associated with MODY2, before passing through the sequential steps of energy production. In turn, increasing of ATP and ADP ratio inhibits and closes the ATP-sensitive potassium channels, leading to depolarization of plasma membrane. As a result, membrane depolarization opens the voltage-dependent calcium channels. Increased intracellular calcium elicits movement of insulin-containing secretory vesicles to the plasma membrane and insulin is then secreted into the circulation (Fig. 1).

Hyperglycemia in MODY2 patients appears to result from a reduction in the activity of glucokinase which leads to decreased β-cell sensitivity to glucose. Since HNF-4α (MODY1) regulates genes involved in glucose transport and glycolysis (Stoffel *et al.*, 1997), the pathophysiology underlying MODY1 patients is described as an impairment of glucose-stimulated insulin secretion, similar to that of *glucokinase* mutations (MODY2). Because *HNF-1α* expression is regulated by HNF-4α, pathophysiology associated with *HNF-1α* mutations (MODY3) is occurred in the same manner. Not only in pancreas, but also in liver and kidney that HNFs play a role in tissue-specific gene expression. Therefore, mutations in *HNF-1α* (MODY3), *HNF-4α* (MODY1) and *HNF-1β* (MODY5) are associated with abnormalities in liver and kidney functions. The understanding of pathophysiology associated with *IPF-1* (MODY4) is based on the information from a single family whose the proband was an infant with neonatal diabetes and exocrine pancreatic insufficiency resulting from pancreatic agenesis (Wright *et al.*, 1993). Due to its seldom occurrence, the molecular pathogenesis

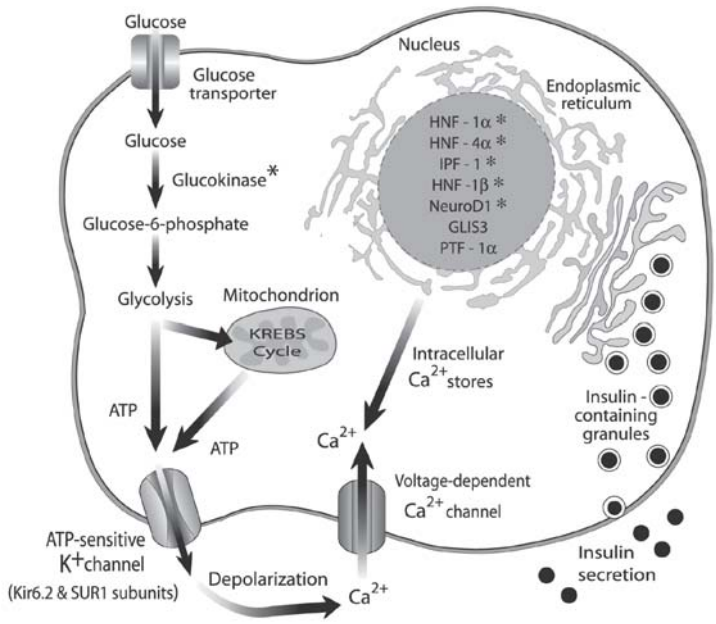


Figure 1 Schematic representation of the pancreatic β-cell and the proteins implicated in monogenic β-cell diabetes (modified from Fajan *et al.*, 2001). Proteins associated with MODY are marked with asterisk (*).

underlying mutation in *NeuroD1* (MODY6) is rarely examined.

Molecular pathology of MODY genes

MODY1 – *hepatocyte nuclear factor-4α* mutations

Hepatocyte nuclear factor-4α (*HNF-4A* or gene symbol *TCF14*) gene is located on chromosome 20q12-q13.1. This gene contains 13 exons with alternatively spliced exons 1A, 1B, 1C, and 1D to join with the sequence of exons 2-10 in RNA transcripts. Nine isoforms of HNF-4α are generated by two alternate P1 and P2 promoters, and by exonic splicing. The liver-specific P1 promoter drives the expression of transcripts HNF-4α1 to 6, while the pancreatic-specific P2 promoter regulates HNF-4α7 to 9. HNF-4α protein is composed of six domains, including A through F: A/B domain (amino acids 1-50), C domain (amino acids 50-116), D domain (amino acids 116-174), E domain (amino acids 174-370), and F domain (amino acids 370-465). HNF-4α is a liver-enriched transcription factor belongs to the nuclear receptor superfamily which normally expressed in liver, kidney, intestine, and pancreatic islets. It binds to DNA as a homodimer and activates the transcription of various target genes involved in embryogenesis, glucose and lipid metabolisms and glucose-stimulated insulin secretion (Lehto *et al.*, 1999). Its target genes include *insulin*, *GLUT2*, *aldolase B*, *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*), *pyruvate kinase* (*PK*), *fatty acid binding proteins* (*Fabp*), and *cellular retinol binding protein* (*CRBP*).

Heterozygous mutations in *HNF-4α* gene are responsible for MODY1 (Yamagata *et al.*, 1996a), a rare MODY subtype accounting for 2-5% of MODY cases. Up to now, more than 40 mutations have been reported (Fig. 2) and genetic variations near the P1 and P2 promoters may be susceptible to late-onset T2D. *HNF-4α* mutations including missense, nonsense, insertion/deletions are found throughout the gene and balanced translocation of *HNF-4α* gene is also

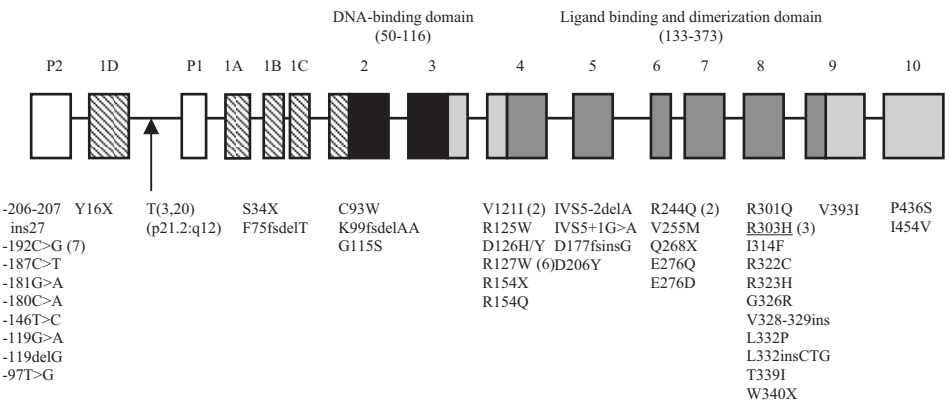


Figure 2 Mutations in *HNF-4α* gene. Location of *HNF-4α* mutations within the 10 exons, promoter, and splice sites of the gene. The functional domains of the HNF-4α protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets. Mutations with underlined were identified in Thai patients (modified from Yamagata *et al.*, 2003).

reported to cause MODY1. *HNF-4α* missense mutations are predominantly located in exon 8, encoding transactivation domain of the protein and affected the regions that are well conserved among species. Moreover, single nucleotide substitutions of P2, pancreatic β-cell promoter, were reported to be responsible for developing of late-onset T2D (Ek *et al.*, 2006).

MODY2 – *glucokinase* mutations

Glucokinase (*GCK*) gene is located on chromosome 7p15.3-p15.1, comprising 12 exons (spaning ~48,168 bp) and encoding glucokinase (or hexokinase IV) – a protein with 465 amino acids. GCK is expressed in pancreas, liver and brain. Three tissue-specific GCK isoforms are generated by using alternative promoters and transcription start sites. The isoforms in pancreatic β-cells and hepatocytes differ in their N-terminal sequences. GCK is a glycolytic enzyme that acts as a glucose sensor in pancreatic β-cells and plays important role in the regulation of insulin secretion. In turn, insulin can up-regulate the GCK expression in hepatocyte. Thus, β-cells can control glucose utilization in hepatocytes through the action of insulin that increases hepatic GCK concentrations.

Glucokinase was the first gene to be identified in MODY. MODY2 is the most common subtype in European Caucasians, particularly in French, Spanish, Italian, and is also common worldwide. In contrast, less than 5% of MODY2 were reported in Asian populations. Up to now, more than 210 different *GCK*-inactivating mutations causing MODY have been reported (Fig. 3). Missense, nonsense, frameshift, and splice site mutations have been identified and are distributed throughout the gene.

Homozygous *GCK* mutations result in a more severe phenotype, a complete deficiency of glucokinase, and are associated with permanent neonatal diabetes mellitus (PNDM). Moreover, heterozygous *GCK*-activating mutations cause familial hyperinsulinism and hypoglycemia (Glaser *et al.*, 1998).

MODY3 – *hepatocyte nuclear factor-1α* mutations

Hepatocyte nuclear factor-1α (*HNF-1α* or gene symbol *TCF1*) gene is located on chromosome 12q24.3, containing 10 exons, and encodes a protein with 631 amino acids. Using alternative splicing and polyadenylation sites, three isoforms (A, B and C) of HNF-1α protein are generated, differing in their

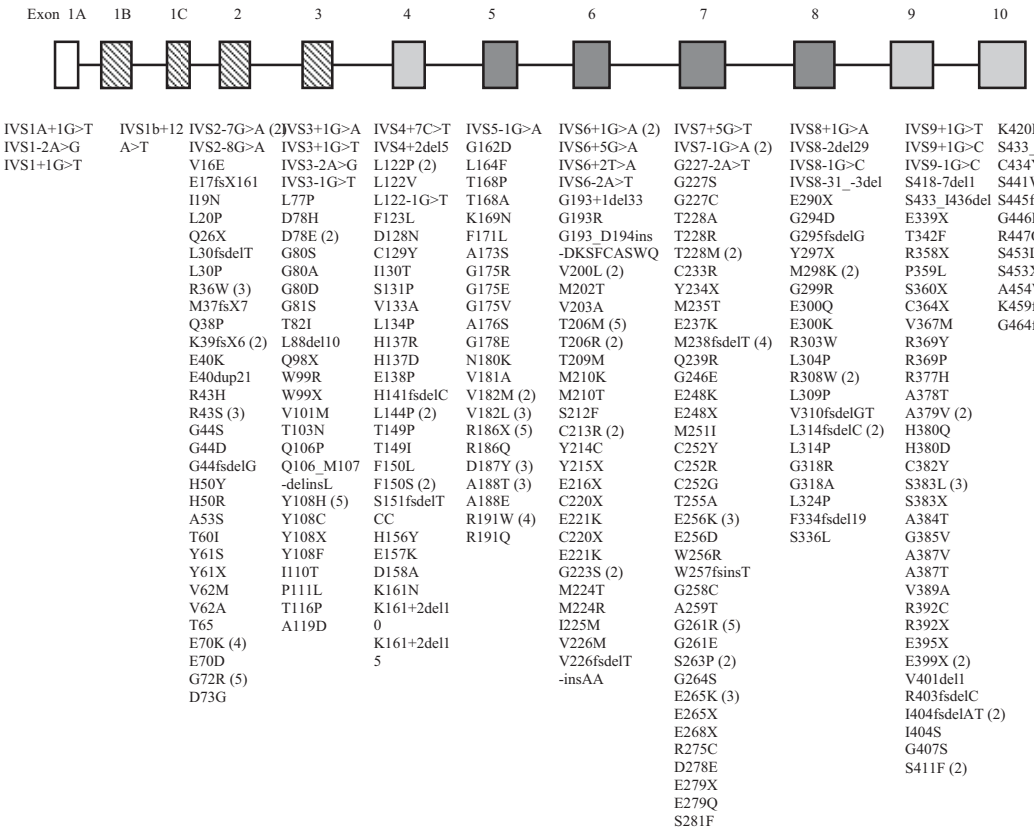
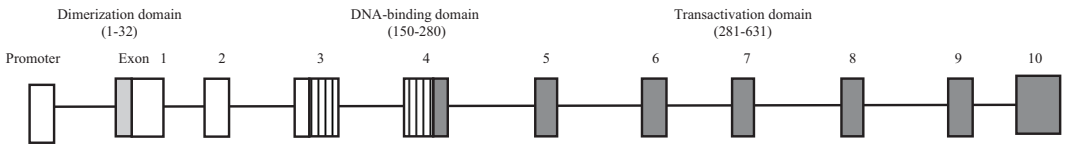


Figure 3 Mutations in *GCK* gene. Location of *GCK* mutations within the 10 exons, and functional domains of the GCK protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets (modified from Gloyn *et al.*, 2003).

tissue distribution patterns. HNF-1α protein is composed of three functional domains: N-terminal dimerization domain (amino acids 1-32), DNA-binding domain (amino acids 150-280) and C-terminal transactivation domain (amino acids 281-631). It is a liver-enriched transcription factor belongs to homeobox gene family and expressed in liver, kidney and pancreatic islets. It normally forms homodimers or heterodimer with HNF-1β and controls multiple genes implicating in pancreatic β-cell function, notably in metabolism-secretion coupling. Its target genes include *amylin*, *insulin*, *GLUT2* and *L-type pyruvate kinase* (*L-PK*), *HMG-CoA reductase*, *mitochondrial 2-oxoglutarate dehydrogenase* (*OGDH*) *E1*.

Over 300 different mutations in *HNF-1α* associated with MODY, T1D and T2D have been described so far. The prevalence of *HNF-1α* mutations (MODY3) is different among various ethnic groups. It is most common in Caucasian, but less frequent in Asian populations. The *HNF-1α* mutations including missense, nonsense, frameshift insertions/deletions, duplications, promoter region mutations, and splice site mutations are located throughout the gene (Fig. 4). Among these, missense mutations are most common, spreading throughout the entire gene, and are concentrated in the dimerization and DNA-binding domains (Bellanne-Chantelot *et al.*, 2007). The truncated HNF-1α proteins are generated by nonsense mutation, or more

frequently a nucleotide deletion/insertion resulting in a frameshift encoding an altered amino acid sequence downstream of the mutational event and an introduction of a new stop codon. About 62% of truncating mutations are found in the C-terminal transactivation domain (Bellanne-Chantelot *et al.*, 2007). There is a *HNF-1α* mutational hotspot at codon 291 (P291fsinsC) resulted from insertion of C nucleotide in the poly-C tract of exon 4 which has been reported in more than 70 MODY families worldwide. A few cases of MODY3 patients have mutations outside the protein coding region, such as in promoter region and



+102G>C
-8G>A
-62C>G
-97T>G
-119G>A
-119delG
-128T>G
-187C>T
-207, -206 fsin27
-218T>C
-233-234 delAT
-283A>G
-462G>A
-527C>T

IVS1-1G>A
M1L
Q7X
L8M (2)
Q9P
T10M
L12F
L12H
A14V (2)
L17V
L17Q
G20R (2)
G20A
E24fsdelGA
A25P
L26P (2)
I27T
I27S
Q28X
G31D (6)
P33L
L44fsdelCCinsA
L44fsdelC (2)
G47E
E48K (2)
E48fsdelG
R54X
R55G56fsdel
-GAGGG
N62S
E66fsinsG
R68W (2)
G69fsdelG
E73X
D75E
T82M
K87X
E95X
L107I (2)
L107V
L107R (2)
Q109fsdelA (2)

IVS2+1G>A
IVS2+1G>C (4)
IVS2+5G>A
IVS2+21G>A (2)
P112L (6)
W113X
V115L
A116T
A116V (2)
K117E (2)
S121fsdelC (3)
Y122C (2)
L123R
Q124X
N127delAAC
I128N
P129T
Q130H
R131W (11)
R131Q (9)
A132K
E132D
V133M (5)
V133L
V133fsdel
-GGTCGAT
D135N
D135fsdelA
T137S
G138S
S142F
H143Y (3)
H143fsdelC
S145F
Q146K;H147R
Q146X
L148I
N149K
K150N
M154V
M154T
M154R
K158N
K158X
R159W (4)
R159Q (8)
A161T
R168P
R171X (8)
V173M
A174V
Q175X
Q176X

IVS3+1G>A
IVS3-1G>A
G191D
T196A
T196fsdelCCAA
R200W (4)
R200G
R200Q (4)
R203C (2)
R203H (8)
K205Q
K205fsdelA (2)
G207D (2)
G207fsinsG
A209fsins10
S210fsinsGGG
-GCCAGC
A217G
Y218C (3)
P224S
S225fsdelC
K226E
E228K
E228fsinsG
R229Q (7)
R229X (6)
R229P (2)
E230fsdelGA
V233L
V233fsinsA
E234X
C236X
C236fsinsA
R228fs

IVS4+2T>C
IVS4-2A>G
A239T
A239V
E240Q
C241R (3)
C241G (5)
C241Y
Q243E
R244S
V246L
V246G
S246fsdelGT
S247fsdelAG (2)
Q250X
Q250fsinsC
G255S
S256T
V259F
V259D (2)
T260M
E261K
R263C (2)
R263fsinsT
R263L
R263H
V264F
N266K
W267R
W267X
F268S
R271W (7)
R271G (3)
R271Q
R271fsdelC
R272fsdelGC
R272S (2)
R272C (3)
R272H (11)
K273N
E275fsdelGAAG
A276G
A276D
P289fsinsG
L281P (2)
G288C
P289S
P291T
P291fsinsC (76)
P291fsdelC (5)
P291fsdelA
G292fsdelG
A301T
G307fsdelC
P308L
A311D
S315fsinsA

IVS5+2T>A (2)
IVS5+4.5insA
IVS5-2A>G
IVS5-1delTAG
R321H
Y322C (2)
Y322fs
Y322X
Q324X
S328R
L341X
T343fsdelCA
V351fsdelG
T354M
T354fsinsC
P353delACGG
-GCCTGGAGC
S361fs

A373G (2)
G375fsdelG
P379T (5)
P379A (8)
P379S (2)
P379R
P379H
P379fsdelCC
P379fsdelCT (12)
P379fsdelC (2)
P379fsdelT (4)
P379fsinsT
P379fsinsC
L383fsinsA
L389V (5)
T392fsdelA
P393fsdelC
Q399X
Q401fsdelC
P409H
M412T
I414G415;
ATCG>CCA (2)
G415R
P424L
S432C
T433I

IVS7-6G>A
IVS7-2A>G
IVS7-2A>T
Q442X
R442fsX456
A443fsdelCA (3)
S445fsdelAG
P447L (5)
T457I
V462fs
Q463X
S465F
Q466X
P467L (2)
Q474X
P475L
P475fsomsA
S482fsdelAG (2)
Q486X (2)
P488fsdelC
F489V
T492I
S498R
P499S
H500N
A501T

IVS8+1G>A
IVS8+2T>C
Y503X (3)
H505N (3)
E508K
T513S
R514H (2)
T515K
L518fsdel
-TCCinsA (3)
P519L
T521I
M522V
T525S
T525I
D526N
D526Y
T528I
D529fsinsA
S531T
T537R
P538fs
T539fsdelC

IVS9-1G>A
IVS9+1G>A
D546G
D546fsdel
-GACA
E548fsdelTG
G554fsX556
L555F
A559fsinsC
A559fsinsA
V567I
R583G (2)
R583Q (3)
S585fsinsTC
P588S
V590fsinsA

S608fsdelAG
D614fsdelAA
V617I
I618M
I618fsdelTC
E619K
T620I (2)

Figure 4 Mutations in *HNF-1α* gene. Location of *HNF-1α* mutations within the 10 exons, promoter, and splice sites of the gene. The functional domains of the HNF-1α protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets. Mutations with underlined were identified in Thai patients (modified from Yamagata *et al.*, 2003).

5′-UTR. The mutations in promoter region may affect binding sites of other transcription factors including HNF-4α (Gragnoli *et al.*, 1997), NF-Y, C/EBP, HNF-3, and AP-1. In addition, deletions of partial and whole *HNF-1α* gene have also been identified in some MODY cases (Pearson *et al.*, 2001).

MODY4 – *insulin promoter factor-1* mutations

Insulin promoter factor-1 (*IPF-1*, also known as *PDX-1*, *IDX-1* and *STF-1*) gene is located on chromosome 13q12.1. It contains 2 exons, spaning about 6 kb, and encodes a protein with 283 amino acids. IPF-1 protein has two functional domains: transactivation domain (1-38 amino acids) and DNA-binding domain (146-206 amino acids). It is expressed in pancreas, duodenum, and pylorus and is a homeodomain-containing transcription factor that plays crucial roles in pancreatic development and in regulation of various target genes including *GLUT2*, *GCK*, *insulin*, *somatostatin* and *islet amyloid polypeptide* (*IAPP*).

Mutations of IPF-1 cause MODY4 (Fig. 5) which is a rare form of MODY in various ethnic groups. Homozygous *IPF-1* mutation results in pancreatic agenesis while its heterozygous mutations are responsible for MODY4 phenotype (Stoffers *et al.*, 1997) and may contribute to susceptibility in late-onset T2D. A previous report has described a family which a proband carried homozygous mutation of *IPF-1* (P63fsdelC) who had pancreatic agenesis. Both parents who had diabetes were heterozygous for the same mutation. Moreover, *IPF-1* mutations have also been reported in gestational diabetes and predisposition to T2D (Gragnoli *et al.*, 2005).

MODY5 – *hepatocyte nuclear factor-1β* mutations

Hepatocyte nuclear factor-1β (*HNF-1β* or gene symbol *TCF2*) gene is located on chromosome 17q12-q21. It contains 9 exons and encodes a protein with 557 amino acids. HNF-1β protein comprises three functional domains: dimerization domain (1–32 amino acids), DNA binding domain (90–311 amino acids), and transactivation domain (312–557 amino acids). It is a homeodomain transcription factor that shares structural homology with HNF-1β in their dimerization and DNA-binding domains. It is expressed in liver, kidney, stomach, uterus and pancreas and plays crucial roles in the embryonic development of these organs. It can form homodimer or a heterodimer with HNF-1β (Rey-Campos *et al.*, 1991) which recognizes the same binding site on target promoters. HNF-1β acts as transcriptional activator of the target genes including: *insulin*, *albumin*, *glucose transporter-2*, *L-type pyruvate kinase* and *α-fetoprotein*.

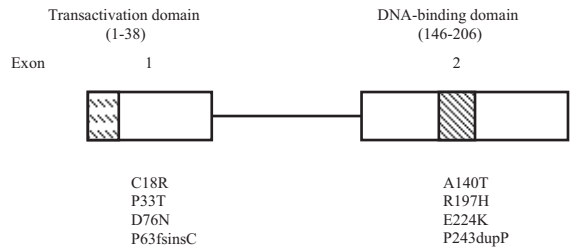


Figure 5 Mutations in *IPF-1* gene. Location of *IPF-1* mutations within the 2 exons, and functional domains of the IPF-1 protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets (modified from Winter *et al.*, 2000).

Mutations in *HNF-1β* are associated with MODY5 (Horikawa *et al.*, 1997), an uncommon subtype of MODY accounting for less than 1% of MODY cases. More than 40 different *HNF-1β* mutations have been reported in 46 families (Edghill *et al.*, 2006). These mutations, including missense, nonsense, frameshift insertion/deletions, and splice site mutations, were identified throughout the gene (Fig. 6). The majority of these mutations are private but there is a hotspot of mutation at the intron 2 splice donor site. The mutations are predominantly clustered in the first four exons, encoding for the dimerization and DNA-binding domain (Edghill *et al.*, 2006). The high proportion of whole gene deletions (one third of adult MODY5 patients), single exonic deletions (Bellanne-Chantelot *et al.*, 2005), and duplication of *HNF-1β* were also reported (Carette *et al.*, 2007). Several molecular pathologies of *HNF-1β* causing MODY5 were identified but the explanation for their different mechanisms was unclear. It was proposed, however, that the duplication might lead to genomic instability due to an unusual genomic architecture.

MODY6 – *NeuroD1* mutations

NeuroD1 (also known as *BETA2*) gene is located on chromosome 2q32 (Tamimi *et al.*, 1996). It contains two exons; exon 1 encodes part of the 5′-UTR of mRNA and exon 2 encodes for 11 nucleotides of the 5′-UTR and the protein with 356 amino acids. NeuroD1 protein is expressed in pancreatic islets, intestine, and brain. It belongs to the basic helix-loop-helix (bHLH) family of transcription factor and functions as transcriptional activators by forming heterodimer with the ubiquitous HLH protein E47. NeuroD1 regulates *insulin* gene transcription by binding to an E-box motif in the *insulin* promoter (Naya *et al.*, 1995; Sharma *et al.*, 1999).

Mutations in *NeuroD1* cause MODY6 – a rare MODY subtype. Only 4 mutations in *NeuroD1* were described in 4 MODY families (Fig. 7). The R111L and P260fsinsC mutations were firstly identified as *NeuroD1* mutations-associated with MODY6. The E110K and S159P mutation were identified in an Iceland MODY family (Kristinsson *et al.*, 2001) and a Chinese proband with early-onset type 2 diabetes, respectively (Liu *et al.*, 2007).

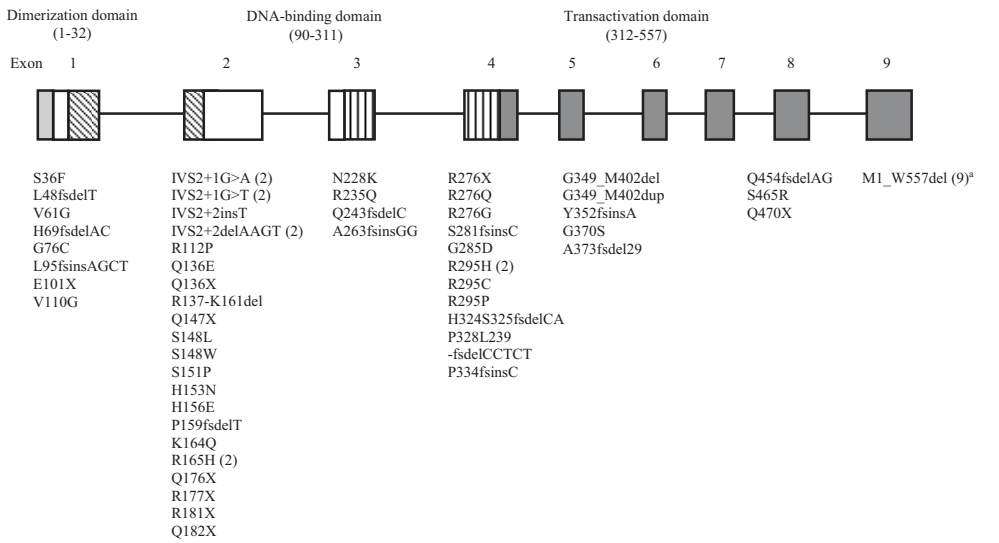


Figure 6 Mutations in *HNF-1β* gene. Location of *HNF-1β* mutations within the 9 exons, and functional domains of the HNF-1β protein are shown; the numbers in brackets refer to codons. Mutations that have been reported in more than one family are indicated by a number in brackets. [a] indicated whole gene deletions of *HNF-1β* (modified from Yamagata *et al.*, 2003).

MODY-X

MODY-X is denominated for MODY with unknown genetic etiology. It accounts for 20-25% of MODY cases in Caucasians and as many as 60-80% in Chinese, Japanese and Korean families. In Thai ethnic origin, Siriraj Diabetes Research Group (SiDRG) investigated genetic variations in the six known MODY genes in patients with MODY and early-onset T2D and found that the six known MODY genes account for a small proportion of both classic MODY (19%) and early-onset T2D patients (10%), suggesting that the majority of cases are MODY-X (Plengvidhya *et al.,* 2008), which is similar to the reports of other Asian ethnic groups.

Attempts have been made to identify unknown MODY genes. The results of genome-wide scan in European (Pearson *et al*., 2001) and American. (Kim *et al*., 2004) families with MODY-X suggested the existence of MODY-X loci on several chromosomes. A number of candidate genes involved in pancreatic β-cell transcription network as well as insulin secretion process have been examined in MODY-X families but none has been conclusively shown to cause MODY in the studied families. Recently, SiDRG investigated the role of *PAX4,* encoding transcription factor that plays a crucial role for β-cell development, in Thai patients with MODY-X (Plengvidhya *et al.*, 2007). A novel missense mutation, R164W, has been identified and found to be segregated with diabetes in the affected family. The mutant Pax 4 protein showed reduced repressor activities on insulin and glucagon promoters as compared to the wild-type protein. Therefore, mutation in *Pax4* could be a cause of MODY in the patients studied.

Functional studies of mutant genes responsible for MODY

***Glucokinase* mutations**

A majority of *glucokinase* mutations results in alteration of enzyme kinetics. The overall effect of inactivating mutations is the reduction of phosphorylation potential of the enzyme, which may lead to reduce glucose consumption in the β–cells and reduce insulin secretion, finally resulting in hyperglycemia. However, in more details, different *glucokinase* mutations impair enzymatic function through different mechanisms such as enzymatic activity, protein stability, and increased interaction with glucokinase regulator (GCKR). These data promote the understanding of relationship between glucokinase structure and function (Garcia-Herrero *et al*., 2007). For examples, an insertion of asparagine residue N161 fully inactivates glucokinase whereas M235V and R308W mutations only partially impair enzymatic activity. However, glucokinase kinetics was almost unaffected by R397L mutation (Garcia-Herrero *et al*., 2007).

Mutations of transcription factor-encoding genes

The transcription factors play roles in the regulation of β-cell function, insulin production, and glucose-stimulated insulin secretion. The functions of the wild-type and mutant transcription factors are

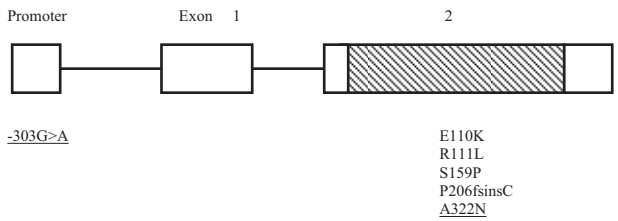


Figure 7 Mutations in *NeuroD1/BETA2* gene. Location of *NeuroD1* mutations within the 2 exons, and the coding region of NeuroD1 protein is entirely localized to exon 2 as shown in dotted area. Mutations found in Thais are shown with underlined and the numbers in brackets refer to codons.

extensively investigated, particularly in regulation of β-cell function using *in vivo* and *in vitro* models. The most simple and popular technique for studying mutant transcription factor proteins is *in vitro* promoter assay. In general, the promoter assay requires creations of three different constructs in plasmid vectors including protein expression construct, reporter construct, and internal control construct. The gene encoding transcription factor is cloned into the protein expression construct and the promoter region of a target gene of interest is combined with a reporter gene in the reporter construct. These two recombinant plasmid constructs are introduced with the internal control construct into an appropriate cell line. If the reporter system is well chosen, then the level of reporter gene expression will correlate with the transcriptional activity of the introduced transcription factor.

The transcriptional activity on target promoters may be used as a criteria for classification of the mutant proteins into several groups, for examples, reduced transcriptional activity, completed loss-of-function with or without dominant-negative effects, and gain-of-function. *In vitro* functional studies of *HNF-4α*, *HNF-1α*, *IPF-1*, *HNF-1β*, and *NeuroD1* mutations revealed that most of mutations may cause the defects through the mechanism of haploinsufficiency associated with loss-of-function and/or gene dosage effect. Loss-of-function mutations may occur in the regions encoding dimerization domain, DNA-binding domain, transactivation domain of the protein, and also occur in the promoter region of the gene. The effects of loss-of-function mutations were variable, ranging from absolute aberration of transactivation potential caused by nonsense mutations to partial loss of transactivation potential caused by missense mutations. Mutations in the promoter region may result in reduced protein expression levels, in turn, reduction of genes relevant to insulin secretory pathway. The reduction in transactivation activity of mutant proteins may be due to several reasons, for instances, reduction in protein stability and/or DNA-binding ability, impairment of nuclear import and defect in cooperative transactivation with its heterodimeric partner HNF-1β or coactivator p300 (Yang *et al.*, 1999; Kim *et al.*, 2003).

Dominant-negative effect was reported in some of the HNF-1α, HNF-1β, and also IPF-1 mutant proteins. Since HNF-1α can form a dimer, it is not surprising that mutant proteins may possess dominant-negative effect but their significance is not yet clear. These mutant proteins with intact dimerization domains but which are unable to bind DNA exhibited a much more drastic effect; the mutation not only abolished transactivation but also may form nonproductive dimers with wild-type protein thereby inhibiting the wild-type activity.

Gain-of-function mutants are defined as protein with enhanced activity to transactivate expression of target genes. It is a possible mechanism to cause some cases of MODY3 and MODY5. The HNF-1α gain-of-function mutants showed the differential effects in enhancing the wild-type activity. The downstream molecular mechanism of HNF regulatory network is important in determining pancreatic β-cell function. Thus, mutations in any MODY genes resulting in breakdown and/or disruption of this regulatory network may lead to impaired insulin secretion and hyperglycemia. Studying of functional properties of mutant proteins may provide a better understanding in pathogenesis of MODY and other types of DM.

Neonatal diabetes mellitus

Neonatal diabetes mellitus (NDM) is a rare form of DM occurred at an early infancy. The disease can be transiently developed (transient neonatal diabetes, TND) or permanent throughout the life (permanent neonatal diabetes, PND). NDM is caused by mutations of the genes involving in β-cell development and function. The most common causes of NDM are mutations of *ATP binding cassette, subfamily C, member 8* (*ABCC8*) and *potassium inwardly rectifying channel, subfamily J, member 11* (*KCNJ11*) genes, which encode sulfonylurea receptor

(SUR1) and $K_{ir}6.2$, respectively. Both SUR1 and $K_{ir}6.2$ are essential subunits of pancreatic ATP-dependent potassium channel (Fig. 1). Mutations in these two genes lead to the abnormalities of SUR1 and $K_{ir}6.2$ and reduction of response of potassium channel to ATP. Patients carried mutations in *ABCC8* and *KCNJ11* showed similar clinical features, in which marked hyperglycemia and ketoacidosis are presented. However, mutations in *KCNJ11* are more frequently found in PND and 20% of PND express neurological features, while mutations in *ABCC8* are common among TND. Mutations of *glucokinase* gene are involved in β-cell dysfunction. Homozygous *glucokinase* mutations completely abolish the enzyme activity. Therefore, the patients with neonatal diabetes due to homozygous *glucokinase* mutation require insulin treatment while the patients with MODY2 resulted from heterozygous *glucokinase* mutations do not. Mutations in *IPF1* (MODY4) and *HNF-1β* (MODY5) can also cause NDM. However, mutations in other genes encoding transcription factors, including *pancreas specific transcription factor-1α* (*PTF-1α*) and *GLIS family zinc finger 3* (*GLIS3*) (Fig. 1) are more frequently found to result in NDM (Hattersley *et al.*, 2006).

Mitochondrial diabetes mellitus

Mitochondrial diabetes or maternally inherited-diabetes with deafness (MIDD) is a specific maternally inherited form of DM, accounting for approximately 1% of DM cases (Kobayashi *et al.*, 1997). The presences of maternal transmission with bilateral hearing impairment allow discrimination of MIDD from other monogenic β-cell diabetes. The most common cause of MIDD occurred from A3243G mutation in the mitochondrial gene encoding $tRNA^{Leu(UUR)}$ (Kadowaki *et al.*, 1994). Pathophysiology underlying the disease is probably due to a depletion of ATP level in β-cell cytoplasm supporting a crucial role of β-cell respiratory-chain in maintenance of glucose homeostasis.

CONCLUSIONS

Molecular genetic studies of monogenic β-cell diabetes have provided an invaluable insight into molecular pathogenesis and mechanisms for this and other types of DM. The knowledge of molecular defects in monogenic β-cell diabetes is useful not only for selection of effective treatment, but also for further development of new drugs. The successful treatment of MODY3 and PND patients with sulfonylurea is a well-illustrated example. The evidence that defects in only one point of a particular gene is sufficient to produce clinical phenotypes indicate a central role of this particular gene in pancreatic β-cell development and function. Defects in several genes that have these roles have been identified as a cause of MODY, NDM, and MIDD. However, there are a number of patients with monogenic β-cell diabetes, especially in the Asian ethnic origins, that the causative genes are still unknown. Further study to identify these unknown causative genes and other genes that play interactive roles will provide a better understanding of a complex biological network underlying glucose homeostasis and pathogenesis of DM.

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