Mutation detection in hepatocyte nuclear factor (HNF)-1α gene in Korean early-onset type II diabetes mellitus

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Mutation detection in hepatocyte nuclear factor (HNF)-1α gene in Korean early-onset type II diabetes mellitus

Directed by Professor Hyun Chul Lee

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I would like to thank my mother and aunt for their supports in all aspects. I also thank my wife who always encouraged me and my son who always is my spiritual support and let me feel a responsibility which drives me to move forward without discontinuance.

By author

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Abstract

Mutation detection in hepatocyte nuclear factor (HNF)-1α gene in Korean early-onset type II diabetes mellitus

Mutations in the hepatocyte nuclear factor-1α (HNF-1α) gene have recently been shown to cause maturity-onset diabetes of the young (MODY) and early-onset type II diabetes mellitus. MODY is a genetically heterogenous subtype of type II diabetes mellitus characterized by early-onset, autosomal diminant inheritance and a primarily defect in insulin secretion. To examine the prevalence of the mutations in the HNF-1α gene in Korean subjects with MODY and early-onset type II diabetes, the whole regions of the genes encoding for HNF-1α, including 10 exons, flanking introns, and minimal promoter region, were screened in 59 subjects with early-onset type II diabetes, 10 sujects with MODY, 35 control subjects using polymerase chain reaction single strand conformation polymorphism (PCR-SSCP) analysis. The mutations were identified by directly sequencing the variant forms of samples detected in PCR-SSCP.

Among 59 subjects with early-onset type II diabetes and 10 subjects with MODY, only one silent mutation in exon 4(C900A) and four polymorphisms (Asn/Ser487, AAC→AGC; intron 2, nt –23; intron 7, nt +7; and intron 9, nt –24), which have been reported previously by others, were observed. However, there were no significant differences in frequencies of the four polymorphisms between the type II diabetes and control subjects. The mutation in exon 4 poly-C tract (codon 288), which had been noted to be a mutational “hotspot” in many previous studies of MODY3 and early-onset type II diabetes, was not found in these subjects, suggesting that poly-C tract following codon 288 may not be a mutational “hotspot” in Korean diabetic subjects as it is in other populations reported previously.

The main finding of the current study is that, in contrast to the previous reports, mutations of the HNF-1α gene are an uncommon cause of early-onset type II diabetes and MODY3 in Korean subjects.

Key Words: hepatocyte nuclear factor-1α (HNF-1α), maturity-onset diabetes of the young (MODY), early-onset type II diabetes mellitus, polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP), hotspot

Mutation detection in hepatocyte nuclear factor (HNF)-1α gene in Korean early-onset type II diabetes mellitus

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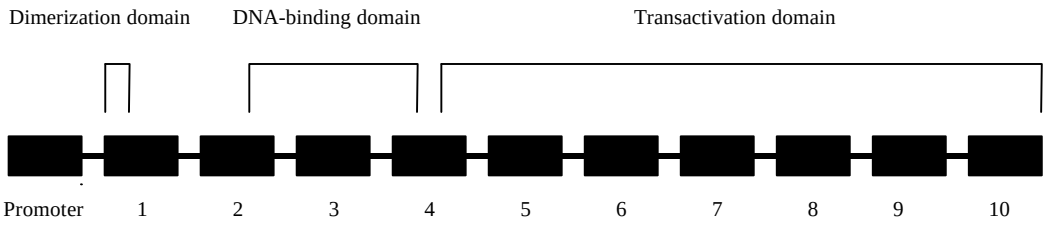
I. Introduction

Type II diabetes mellitus affects 2-6% of the world population and is a major cause of morbidity and mortality[1]. Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous subtype of type II diabetes characterized by early-onset, usually before 25 years of age, autosomal dominant inheritance[2] and a primary defect in insulin secretion[3-7]. MODY occurs world wide and could account for about 2 to 5% of all cases of type II diabetes[8]. To date five MODY genes have been identified: hepatocyte nuclear factor-4α (HNF-4α/MODY1) on chromosome 20q[9,10], glucokinase (GCK/MODY2) on chromosome 7p[11,12], hepatocyte nuclear factor-1α (HNF-1α/MODY3) on chromosome 12q[13,14] insulin promoter factor-1 (IPF1/MODY4) on chromosome 13q[15,16], and hepatocyte nuclear factor-1 β(HNF-1β/MODY5) on chromosome 17cen-q[17]. According to reports, at least one subtype of MODY still exists and remains to be identified.

HNF-1α is a transcription factor, which is required for the tissue-specific expression of a variety of genes in the liver, kidney, pancreas (including the islets of Langerhans), intestine, stomach, spleen, and thymus[18,19]. HNF-1α is composed of three functional domains[20]; an amino-terminal dimerization domain (amino acids 1-32), a DNA-binding domain with POU-like and homodomain-like motifs (amino acids 150-280) and a carboxy-terminal transactivation domain (amino acids 281-631) (**Figure 1**).

A.



B.

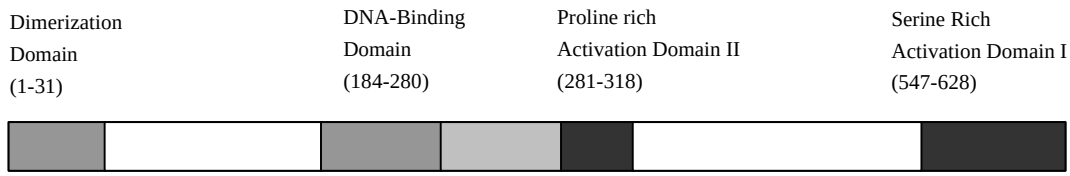


Figure 1. Figure A is the schematic structure of HNF-1α gene showing the Promoter and 10 exons. Figure B is the **s**chematic representation of the structure of HNF-1α protein showing the different functional domains depicted with hatched boxes. Amino acid residue numbers are indicated in parentheses

The functional form of HNF-1α is a dimer, and HNF-1α may form homodimers or heterodimers with the structurally related protein HNF-1β[21]. The mechanism by which mutations in one allele of HNF-1α gene impair pancreatic β cell function is unclear. It is possible that a partial deficiency of HNF-1α could lead to β cell dysfunction and diabetes. Alternatively, mutations in HNF-1α may cause diabetes by a dominant-negative mechanism[22] by interfering with the function of wild-type HNF-1α and other proteins that act together with HNF-1α to regulate transcription in the β cell and/or liver. All HNF-1α gene mutations so far identified would result in the synthesis of a mutant protein impaired in DNA binding or transactivation but not dimerization. These mutant proteins could form non-productive dimers with the product of normal HNF-1α allele or with other proteins such as HNF-1β and so impair the normal function of HNF-1α.

More recently, according to Okita K, *et. al*[23], both wild-type HNF-1α and HNF-1β bind to the oligonucleotide in the human insulin promoter and transactivate the insulin-luciferase reporter gene by 30- and 31-fold, respectively. These data suggest that the insulin gene is candidate target gene of HNF-1α/HNF-1β, and the impairment of insulin gene transcription by mutations in the HNF-1α gene might be involved in the

pathogenesis of MODY[23].

Mutation detection in UK[1], German[24], French[25], Danish[26], Italian[27], Finnish[28], North American[28], and Japanese[29] MODY pedigree confirmed that HNF-1α mutations are the most common cause of MODY. However, the prevalence of the mutations in the HNF-1α/MODY3 gene has not been surveyed in Korean population with MODY and early-onset type II diabetes. In addition, clinical studies have shown that the nonglucokinase forms of MODY are associated with the usual complications which occur in patients with diabetes of long duration[30,31]. Thus, the identification of patients with MODY or early-onset diabetes and the determination of the forms of MODY have implication for the treatment and prognosis of this disorder. Therefore, we examined the prevalence of the mutations in the HNF-1α/MODY3 gene in Korean subjects with early-onset type II diabetes.

II. Research design and methods

1. Subjects and blood sampling Sixty nine unrelated subjects diagnosed with early-onset type II diabetes (age of onset<35 yr) were recruited from among patients attending the out-patient clinic of the Diabetes Center, Yonsei University of Medical Center (**Table 1**).

Table 1. Clinical characteristics of MODY and Early-onset type II diabetic subjects

Subejcts (n)	69
Sex (M/F)	21/28
Age of Diabetes onset (yr)	29 ± 3
Duration (yr)	13 ± 2
Body Mass Index (kg/m^2)	22.5 ± 2.8
Fasting Blood Glucose (mg/dl)	193 ± 34
HbAIc (%)	9.3 ± 3.4
Fasting C-peptide (ng/ml)	1.43 ± 0.41
Postprandial 2hr C-peptide (ng/ml)	1.84 ± 0.34

Results are mean ± SD

Diagnosis criteria for MODY include 1) age of onset<25 years, 2) correction of fasting hyperglycemia without insulin for at least 2 years, 3) nonketonic disease, and 4) autosomal dominant mode of inheritance[32]. Sufficient family data were available to suggest a diagnosis of MODY for 10 of these subjects. There was not sufficient information on the remaining 59 subjects to allow a definitive diagnosis of MODY. The

average age at diagnosis was 29 ± 3 yr (range from 18 to 35 yr). Thirty five unrelated nondiabetic Korean subjects were tested for each mutation to determine whether the sequence change was private polymorphism or a disease-linked mutation[33]. After informed consent was obtained from all study participants according to the Helsinki Declaration, samples were obtained from the peripheral blood.

2. DNA isolation and PCR DNA was isolated from peripheral blood lymphocytes using the QIAGEN DNA extraction kit (Qiagen Inc., Valencia, CA, USA) according to the manufacture's instructions. The 10 exons, flanking introns, and the minimal promoter region of HNF-1α gene were amplified by polymerase chain reaction (PCR) using genomic DNA[24] from each subject selected and sequence-specific primers (Bioneer. Corp., Chungwon, Korea) (**table 2**).

Table 2. Sequences of primers used to amplify and directly sequence exons and flanking introns of the human HNF-1 alpha gene

Exon	Forward primer(5'-3')	Reverse primer(5'-3')	Product size(bp)
P	TCCCATCGCAGGCCATAGCTC	CCGTCTGCAGCTGGCTCAGTT	385
1	GGCAGGCAAACGCAACCCACG	GAAGGGGGGCTCGTTAGGAGC	483
2	CATGCACAGTCCCCACCCTCA	CTTCCAGCCCCCACCTATGAG	390
3	GGGCAAGGTCAGGGGAATGGA	CAGCCCAGACCAAACCAGCAC	304
4	CAGAACCCTCCCCTTCATGCC	GGTGACTGCTGTCAATGGGAC	397
5	GGCAGACAGGCAGATGGCCTA	GCCTCCCTAGGGACTGCTCCA	346
6	TGGAGCAGTCCCTAGGGAGGC	GTTGCCCCATGAGCCTCCCAC	322
7	GGTCTTGGGCAGGGGTGGGAT	CTGCAATGCCTGCCAGGCACC	347
8	GAGGCCTGGGACTAGGGCTGT	CCCCTGCATCCATTGACAGCC CTCTGTCACAGGCCGAGGGAG	229
9	CCTGTGACAGAGCCCCTCACC CAGAGCCCCTCACCCCCACAT	CGGACAGCAACAGAAGGGGTG	289
10	GTACCCCTAGGGACAGGCAGG	ACCCCCCAAGCAGGCAGTACA	248

p, minimal promoter required for tissue-specific expression.

PCR was performed in a 30-μl volume containing 100 ng genomic DNA, 10 mmol/l Tris-HCl, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 200 μmol/l dNTPs, 250 μmol/l each primer, 0.25 U Amplitaq Gold Taq polymerase (PerkinElmer, Inc., Boston, MA, USA), and 0.55 μCi [α-^{32}P] deoxycytidine triphosphate (NEN Life Science Products, Boston, MA, USA). The cycling conditions were 5 min at 95^{0}C followed by 38 cycles consisting of 30 s at 95^{0}C,

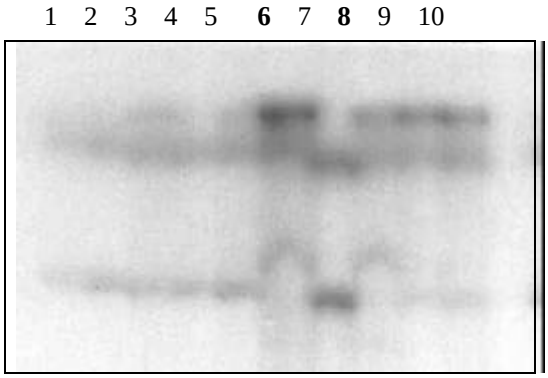
45 s at 62^0C, 45 s at 72^0C, and 10 min at 72^0C.

3. SSCP analysis and sequencing For the analysis of single stranded conformational polymorphisms (SSCP), denatured PCR products labeled with [α-^{32}P] deoxycytidine triphosphate were loaded onto a 5% polyacrylamide gel (MDE gel, FMC Bioproducts, Rockland, ME, USA) and subjected to electrophoresis at 5 W for from 6 through 12 hours. The gels were vacuum-dried, and autoradiographies were performed. Variants detected by analysis of SSCP were subjected to direct dideoxy sequence analysis with asymmetric PCR[34] and Sequenase Version 2.0 Kit (United States Biochemicals, Cleveland, OH, USA). Changes in bases were confirmed by the sequencing of the opposite strands and verified by restriction fragment-length polymorphism analysis on a 3% agarose gel (NuSieve GTG, FMC Bioproducts, Rockland, ME, USA).

III. Results

One mutation was found on screening the exon 4 in one of 69 Korean subjects with MODY or early-onset type II diabetes (**Figure 2, Table 3**).

A.



B.

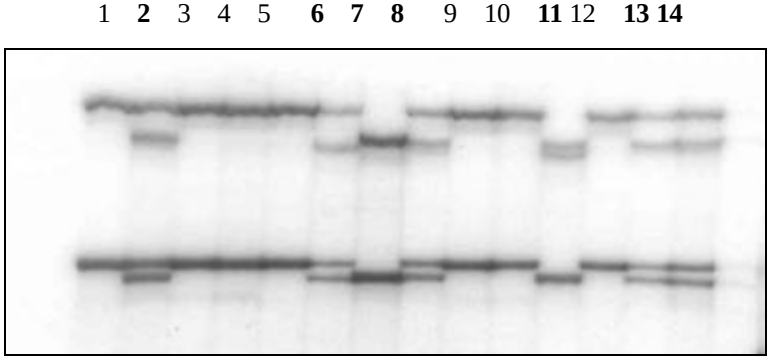


Figure 2. Figure A shows the mobility shifts of single stranded exon 4 fragment by PCR-SSCP analysis. Figure B shows the mobility shifts of single stranded exon 7 fragment by PCR-SSCP analysis. The lanes of the variants detected are showed with bold numbers.

Table 3. Nucleic acid variants from direct sequencing of early-onset subjects versus control subjects

Exon/Intron	Nucleic acid variant	Predicted amino acid variant	Early-onset type2 DM		Control	
			Variant allele frequencies			
Exon 4	C900A	None (Pro)	C=0.99,	A=0.01		
Exon 7	A1460G	Asn/Ser487	A=0.53,	G=0.47	A=0.59,	G=0.41
Intron 2	T-->C	nt -23	T=0.52,	C=0.48	T=0.58,	C=0.42
Intron 7	A-->G	nt +7	A=0.53,	G=0.47	A=0.59,	G=0.41
Intron 9	C-->T	nt -24	C=0.54,	T=0.46	C=0.62,	T=0.38

Results are mean ± SD

The mutation was located in the coding region of the gene and showed one nucleotide substitution (C900A). However, that was silent and did not change the amino acid (Pro300). No mutations were found in other exons and the minimal promoter of the HNF-1α gene, a region containing the binding sites for HNF-4α and HNF-3, both of which have been implicated in the regulation of HNF-1α expression[24]. No mutations were found in 35 control subjects.

Four polymorphisms were observed in our Korean subjects (Asn/Ser487, AAC→ AGC; intron 2, nt –23; intron 7, nt +7; and intron 9, nt –24) (**Table 3, Fgure 2**), which have been observed previously in other studies[24]. All the observed genotype frequencies were in Hardy-Weinberg equilibrium. The three substitutions found in introns and the one substitution in exon 7 have been described previously and were not associated with MODY[14]. There were no significant differences in frequencies of the four polymorphisms between the early-onset type II diabetic and control subjects. Among type II diabetic subjects, codon 487 variant showed no relationship to age at onset, BMI, FBG, HbAIc, basal C-peptide and 2hr C-peptide (data not shown).

IV. Discussion

Mutations in the HNF-1α gene have been observed to underlie the development of dominantly inherited MODY3 and early-onset type II diabetes in several populations worldwide. Whether the mutations in the HNF-1α are a common cause of MODY3 or early-onset type II diabetes in Korean population is unknown, we screened HNF-1α gene for variants in 69 Korean subjects with MODY and early-onset type II diabetes.

No specific mutations in HNF-1α gene for diabetic subjects were found, but one mutation (C900A) in exon 4 and four polymorphisms (Asn/Ser487, AAC→ AGC; intron 2, nt –23; intron 7, nt +7; and intron 9, nt –24) were observed. The mutation observed in exon 4 was found in only one among 69 diabetic subjects, and did not result in amino acid change (Pro300). The mutation in exon 4 poly-C tract (codon 288), which had been noted to be a "hotspot" because of recurrent insertions and deletions of a Cytosine following codon 288[1,24] in many previous studies of MODY and early-onset type II diabetes, was not found in these subjects. This finding suggest that poly-C tract following codon 288 may not be a mutational "hotspot" in Korean diabetic subjects as it is in other populations reported previously.

In this study we observed four polymorphisms (Asn/Ser487, AAC→ AGC; intron 2, nt –23; intron 7, nt +7; and intron 9, nt –24), which have been reported previously by other studies[24,29,36,37]. Some studies showed that some polymorphisms are associated with diabetes, e.g. the subjects carrying the codon 98 polymorphism in its heterozygous form significantly decreased serum C-peptide in the presence of normoglycemia at 30 min during the OGTT, compared with subjects homozygous for the common Ala allele[37]. However, the frequencies for the Asn/Ser487 polymorphism we observed in our study were not significantly different between 69 diabetic and 35 control subjects. Among 69 diabetic subjects, codon 487 variant showed no relationship to the age at onset of diabetes, BMI, FBG, HbAIc, basal C-peptide and 2hr C-peptide. These results were similar to those reported by others[37].

In this screening of 69 Korean early-onset type II diabetes, we identified one silent mutation and four polymorphisms in HNF-1α gene. However, there were no significant differences in frequencies of the four polymorphisms between type II diabetes and control subjects and the codon 487 variant showed no relationship to clinical characteristics in type II diabetic subjects. At present, a precise statement cannot be made on the frequency of these mutations and polymorphisms and their association with diabetes mellitus, but a lower frequency of mutation in HNF-1α in the Korean early-onset type II diabetes population was found than in other countries. The main finding of the current study is that, in contrast to the previous reports, mutations of the HNF-1α gene are an uncommon cause of early-onset diabetes or MODY in Korean subjects. These results may be due to both the ethnic differences between Korean subjects in which the mutation rate of HNF-1α gene is low and Caucasian subjects in which the mutation rate of HNF-1α gene is high and the method (SSCP) used in these studies is not sensitive enough to detect mutations which actually take place. However, this does not exclude a role for the HNF-1α gene in contributing to MODY3 susceptibility in

other populations. These studies therefore suggest that further study of HNF-1α gene mutations in other populations of MODY and early-onset diabetic patients should be assessed before broad conclusions are drawn about the contribution of this gene to diabetes and may give us more information about the pathogenesis of MODY3 and early-onset type II diabetes.

V. Conclusion

Mutation detection in 69 Korean subjects with MODY and/or early-onset type II diabetes showed that mutations of the HNF-1α gene are an uncommon cause of MODY and/or early-onset type II diabetes in Korean subjects.

References

1. Timothy MF, Michael PB, Sian E, Maffie A, Mark JD, Alasdair DR et al. Mutations in the hepatocyte nuclear factor-1 alpha gene are a nommon cause of maturity- onset diabetes of the young in the United Kingdom. Diabetes 1997; 46: 720-725.
2. Fajans SS, Bell GI, Bowden DW, Halter JB, and Polonsky KS. Maturity-onset diabetes of the young. Life Sci 1994; 55: 413-422.
3. Velho G, Froguel P, Clment K, Pueyo ME, Rakotoambinina B, Zouali H, et al. Primary pancreatic beta-cell secretory defect caused by mutations in the glucokinase in kindreds of maturity onset diabetes of the young. Lancet 1992; 340: 444-448.
4. Byme MM, Sturis J, Clement K, Vionnet N, Pueyo ME, Stoffel MM, et al. Insulin secretory abnormalities in subjects with hyperglycemia duo to glucokinase mutations. J. Clin. Invest.1994; 93: 1120-1130.
5. Herman WH, Fajans SS, Ortiz FJ, Smith MJ, Sturis J, Bell GI, et al. Abnormal insulin secretion, not insulin resistance, is the genetic or primary defect of MODY in the RW pedigree. Diabetes 1994;43: 40-46.
6. Byme MM, Sturis J, Fajans SS, Ortiz FJ, Stoltz A, Stoffel M, et al. Altered insulin secretory responses to glucose in subjects with a mutation in the MODY1 gene on chromosome 20. Diabetes 1995; 44: 699-704.
7. Byme MM, Sturis J, Menzel S, Yamagata K, Fajans SS, Dronsfield MJ, et al. Altered insulin secretory responses to glucose in diabetic and nondiabetic subjects with mutations in the diabeties mellitus susceptibility gene MODY on chromosome 12. Diabetes 1996; 45: 1503-1510.
8. Ledermann HM: Is maturity onset diabetes at young age (MODY) more common in Europe than

previously assummed? Lancet 1995; 345: 648(Letter).

9. Bell GI, Xiang KS, Newman MV, Wu SH, Wright LG, Fajans SS, et al. Gene for non-insulin – dependent diabetes mellitus (maturity onset diabetes of the young subtype) is linked to DNA polymorphism on chromosome 20q. Proc Natl Acad Sci USA 1991; 88: 1484-1488.
10. Yamagata K, Furuta H, Oda O. Mutations in the hepatocyte nuclear factor 4 alpha gene in maturity onset diabetes of the young (MODY1). Nature 1996; 384: 458-460.
11. Froguel P, Vaxillaire M, Sun F. Close linkage of glucokinase locus on chromosome 7p to early onset non-insulin-dependent diabetes mellitus. Nature 1992; 356: 162-164.
12. Frougel P, Zouali H, Vionnet N. Familial hyperglycaemea duo to mutations in glucokinase: definition of a subtype of diabetes mellitus. N Engl J Med 1993; 328: 697-702.
13. Vaxillaire M, Boccio V, Philippi A. A gene for maturity onset diabetes of the young (MODY) maps to chromosome 12q. Nat Genet 1995; 9: 418-423.
14. Yamagata K, Oda N, Kaisaki PJ. Mutations in the hepatocyte nuclear factor 1 alpha gene in maturity-onset diabetes of the young (MODY3). Nature 1996; 384: 455-458.
15. Stoffers DA, Ferrer J, Clarke WF, Habener JF: Early-onset Type-II Diabetes Mellitus (MODY4) linked to IPF-1. Nat Genet 1997; 17: 138-141.
16. Stoffers DA, Zinkin NT, Stanojevic V. Clarke WL, Habener JF: Pancreatic agenesis attribuable to a single nucleotide deletion in the human IPF-1 beta gene coding sequence. Nat Genet 1997; 15: 106-110.
17. Horikawa Y, Iwasaki N, Hara M. Mutation in the hepatocyte nuclear factor-1 beta gene (TCF2) associated with MODY. Nat Genet 1997; 17(4): 384-385.
18. Mendel DB, Crabtree GR: HNF-1 alpha, a member of a novel class of dimerizing homodomain proteins. J Biol Chem 1991; 266: 677-680.
19. Emens LA, Landers DW, Moss LG: Hepatocyte nuclear factor 1alpha is expressed in a hamster insulinoma line and transactivates the rat insulin gene. Proc Natl Acad Sci USA 1992; 89: 7300-7304.
20. Tronche F, Yaniv M. Bioessays 1989; 14: 579-587.
21. Mendel DB, Hansen LP, Graves, Conley PB, Crabtree GR. Genes Dev 1991; 5: 1042-1056.
22. Herskowitz I. Nature 1987;329: 219-222.
23. Okita K, Qin Y, Yamagata K, Kerstin A, Kajimoto Y, Claes B, et. al. Human insulin gene is a target gene of hepatocyte nuclear factor-1α and HNF-1β. Biochemical & biophysical research communications.1999; 263(2): 566-569.
24. Kaisaki PJ, Menzel S, Linder T. Mutations on the hepatocyte nuclear factor 1 alpha gene in MODY and early-onset NIDDM: evidence for a mutational hotspot in exon 4. Diabetes 1997; 45: 528-535.
25. Vaxillaire M, Rouard M, Yamagata K. Identification of nine novel mutations in the hepatocyte nuclear factor 1 alpha gene associated with maturity-onset diagetes of the young (MODY3).

Human Molecular Genetics 1997; 6: 583-586.

26. Hansen T, Eiberg H, Rouard M. Novel MODY3 mutations in the hepatocyte nuclear factor-1 alpha gene. Diabetes 1997; 46: 726-730.
27. Gragnoli C, Lindner T, Marozzi G, Andreani D. Distribution of the HNF-4 alpha promoter in an Italian family with MODY (Abstract). Diabetologia 1997; 40(suppl 1): A7.
28. Glucksmann MA, Lehto M, Tayber O. Novel mutatons and a mutational hotspot in the MODY gene. Diabetes 1997; 46: 1081-1086.
29. Iwasaki N, Oda N, Ogata M. Mutations in the hepatocyte muclear factor 1 alpha/MODY3 gene in Japanese subjects with early- and late-onset NIDDM. Diabetes 1997; 46: 1504-1508.
30. Fajans SS: Scope and heterogeneous nature of MODY. Diabetes Care 1990; 13: 49-64.
31. Velho G, vaxillaire M, Boccio V, Charpentier G, Froguel P: Diabetes complications in NIDDM kindreds linked to the MODY3 locus on chromsome 12q. Diabetes Care 1996; 19: 915-919.
32. Tattersall RB: Mild familial diabetes withy dominant inheritance. Q J Med Series 1974; XLIII: 339-357.
33. Bell GI, Horia S, Karam JH: A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. Diabetes 1984; 33: 176-183.
34. Shuldiner AR, Perfetti R. The polymerase chain reaction: applications to endocrine research. In: de Pablo F, Scanes CG, eds. Handbook of endocrine research techniques. San Diego, Calif: Academic Press 457-486, 1993
35. Miyako K, Hiroshi S, Kenshi H, Yasuo A, Yoshio Y, Masato K, et al. Detection of mutation in the human insulin gene by single strand conformation polymorphism. Journal of Clinical Endocrinology and metabolism. 1992; 74: 1027-1031.
36. Urhammer SA, Rasmussen SK, Kaisaki PJ, Oda N, Yamagata K, Moller AM, et al. Genetic variation in the hepatocyte nuclear factor-1α gene in Danish Caucasians with late-onset NIDDM. Diabetologia 1997; 40: 473-475.
37. Urhammer SA, Fridberg M, Hansen T, Rasmussen SK, Moller AM, Clausen JO, et al. A prevalent amino acid polymorphism at codon 98 in the hepatocyte nuclear factor-1α gene is associated with reduced serum C-peptide and insulin responses to an oral glucose challenge. Diabetes 1997; 46: 912-916.

국문요약

한국인 조기발병 제 2 형 당뇨병환자에서 HNF-1a 유전자 돌연변이에 대한 탐색

조 정 산
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최근 서구를 중심으로 hepatocyte nuclear factor-1α(HNF-1α)gene 의 돌연변이에 대한 많은 연구가 진행되어 왔는데 HNF-1α gene 의 돌연변이는 maturity-onset diabetes of the young (MODY) 또는 조기발병 제 2 형 당뇨병의 주요원인으로 알려졌다. MODY 는 제 2 형 당뇨병의 한 아형으로서, 조기(25 세 미만에) 발병, 상동체 우성유전, 인슐린 분비결핍 등을 특징으로 한다. 한국인 MODY 환자 또는 조기발병 제 2 형 당뇨병 환자에서 HNF-1α gene 의 돌연변이의 유병율을 를 알아보고자 본 연구를 시행하였다. 본 연구에서는 59 명 조기발병 제 2 형 당뇨병환자, 10 명 MODY 환자, 그리고 35 명 정상인을 대상으로 HNF-1α gene 을 구성하고있는 10 개 exon, flanking intron, minimal promoter 부위를 polymerase chain reaction (PCR)으로 증폭시킨 후 single-strand conformation polymorphism (SSCP) analysis 를 실행하여 돌연변이가 있는 sample 을 찾아낸 후 이 sample 을 다시 PCR 로 증폭하여 direct 한 sequencing 을 실행하여 돌연변이가 있는 여부를 확인하였다.

연구결과 exon 4 에서 한개의 silent mutation 이 발견되였고, 4 개의 유전자 다형성 (Asn/Ser487, AAC→AGC; intron 2, nt –23; intron 7, nt +7; intron 9, nt –24)이 발견되었다. 4 개 유전자 다형성은 많은 외국연구에서도 보고되었으나 MODY 나 또는 조기발병 제 2 형 당뇨병 발병에 관여하지는 않는 것으로 알려져있다. 서구에서 연구된 결과 exon 4 poly-C tract (codon 288) 부위는 돌연변이가 빈번하게 일어나, 이 부위를 돌연변이 "hotspot"로 지목하고있다. 그러나 한국인을 대상으로 한 본 연구에서는 exon 4 poly-C tract 부위에서 돌연변이를 발견하지 못하였으므로 이 부위에 돌연변이는 한국인 조기발병 제 2 형 당뇨병환자 또 MODY 환자의 주요원인으로 보기는 어렵다고 생각한다.

결론적으로 서구와 비교 할 때 HNF-1α gene 돌연변이는 한국인 MODY 또는 조기발병 제 2 형 당뇨병환자 발병에 주요원인으로 작용하지 않을 것으로 사료된다.

핵심되는 말: hepatocyte nuclear factor-1α(HNF-1α), maturity-onset diabetes of the young (MODY), 조기발병 제 2 형 당뇨병, polymerase chain reaction (PCR), single-strand conformation polymorphism (SSCP), hotspot