hepatocyte nuclear factor-1α
hepatocyte nuclear factor - 1α

2000年 12月
没有自然阅读的文本。
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4. HNF-1α primer 487 · 13
5. HNF-1α primer 487 · 13

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hepatocyte nuclear factor-1α

non-insulin-dependent diabetes mellitus; MODY (maturity onset diabetes of young) 25%

MODY1 25%
MODY2 1-3%
MODY3 12%

hepatocyte nuclear factor-1α (HNF-1α) promoter

exon 1-10

polymerase chain reaction (PCR)
DNA sequencing

exon 1 17
CTC
CTG

12
5
silent mutation

exon 1 27
ATC

CTC

5
isoleucine
leucine

missense mutation

Exon 7 459

CTC

TTG
silent mutation
leucine
exon 7
487

leucine
asparagine
serine
mutated
exon 7

exon 1
promotor

exon 1
exon 7
silent mutation
missense mutation

polymorphism

HNF-1α

HNF-1α

MODY, Type 2 DM
hepatocyte nuclear factor-1α

I. 1

...
hepatocyte nuclear factor (HNF)-4α, MODY1, MODY2, MODY3, MODY4, and MODY5 can be involved in the pathogenesis of MODY. HNF-4α, HNF-1α, and glucokinase are key factors in the regulation of glucose metabolism. These factors play a role in the development of MODY. MODY2 accounts for 20% of MODY cases, MODY3 for 40%, MODY4 for 10%, and MODY5 for 10%. Insulin promoting factor-1 (IPF-1) is a key factor in the development of MODY. MODY1 and MODY5 can be involved in the pathogenesis of MODY. HNF-1β, MODY2, MODY3, and MODY5 are key factors in the regulation of glucose metabolism. These factors play a role in the development of MODY. MODY2 accounts for 25-50% of MODY cases, MODY3 for 15%, MODY4 for 25%, and MODY5 for 10%. Glucokinase is another key factor in the development of MODY. 1996 Yamagata et al. MODY3 and HNF-1α are key factors in the development of MODY.
### 1. MODY Comparison

<table>
<thead>
<tr>
<th></th>
<th>MODY1</th>
<th>MODY2</th>
<th>MODY3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal location</td>
<td>20q</td>
<td>7p</td>
<td>12q</td>
</tr>
<tr>
<td>Onset of hyperglycemia</td>
<td>Adolescence</td>
<td>Early childhood</td>
<td>Adolescence</td>
</tr>
<tr>
<td>Associating gene</td>
<td>HNF-4α</td>
<td>Glucokinase</td>
<td>HNF-1q</td>
</tr>
<tr>
<td>Severity of hyperglycemia</td>
<td>Progressive</td>
<td>Mild</td>
<td>Progressive</td>
</tr>
<tr>
<td>Microvascular complications</td>
<td>Frequent</td>
<td>Rare</td>
<td>Frequent</td>
</tr>
<tr>
<td>Pathophysiology</td>
<td>β cell dysfunction</td>
<td>β cell dysfunction</td>
<td>β cell dysfunction</td>
</tr>
<tr>
<td>Priming with hyperglycemia</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

HNF-4α: direct DNA sequencing

HNF-1α: single strand conformation polymorphism (SSCP)

HNF-1α: 40\% 30\% 40\% 40\% 30\% 40\% 40\% 30\% 40\% 30\%
II. Materials and Methods

1. General

2. Materials

2.1. Blood Collection: Blood samples were collected from healthy volunteers and patients with different conditions.

2.2. Genomic DNA Purification: The blood samples were centrifuged at 3,000 rpm for 15 minutes, and the plasma was removed. Then, the red blood cells (RBC) were lysed with a lysis buffer containing 1% polypropylene and 0.5 M EDTA. The lysis was followed by a wash step with RBC lysis buffer, followed by centrifugation at 3,000 rpm for 15 minutes. The supernatant was discarded, and the precipitate was resuspended in the lysis buffer. The DNA was then purified using the QIAamp Blood Kit (Qiagen Inc., Santa Clarita, Calif.).
3. oligonucleotide primer

HNF-1q promoter exon1 - 10 sequence specific oligonucleotide (Nature 384:455-458, 1996) PCR primer (2).

2. HNF-1q promoter primer

<table>
<thead>
<tr>
<th>exon</th>
<th>forward Primer</th>
<th>reverse Primer</th>
<th>product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>promoter</td>
<td>TCCCATCCAGGGCAGTTC</td>
<td>CCGGTCTGGGGTCAGTTT</td>
<td>385</td>
</tr>
<tr>
<td>exon1</td>
<td>GACGACGACTCTCGTGGG</td>
<td>GAAGGGGGGCTCGTTAGGAC</td>
<td>483</td>
</tr>
<tr>
<td>exon2</td>
<td>CATCCACAGTCCCCACCTTTCA</td>
<td>CTTCAGGGGGCTCCCTATTGAG</td>
<td>384</td>
</tr>
<tr>
<td>exon3</td>
<td>GACGACGACTCTCGTGGG</td>
<td>GAAGGGGGGCTCGTTAGGAC</td>
<td>483</td>
</tr>
<tr>
<td>exon4</td>
<td>CATCCACAGTCCCCACCTTTCA</td>
<td>CTTCAGGGGGCTCCCTATTGAG</td>
<td>384</td>
</tr>
<tr>
<td>exon5</td>
<td>GGCAAGGTCAGGGGATGGA</td>
<td>CAGCCCAGACCAAACCACAC</td>
<td>306</td>
</tr>
<tr>
<td>exon6</td>
<td>GCCGAGCACTCTCTCTCCAGCC</td>
<td>GTTCGGGGCCATTGGGCTACC</td>
<td>320</td>
</tr>
<tr>
<td>exon7</td>
<td>GAGCGATGCGCAGGGTGCTT</td>
<td>CCGCAATGCTGCCAGAAACC</td>
<td>345</td>
</tr>
<tr>
<td>exon8</td>
<td>TGCAATCACTCTCCAGGGTGCTT</td>
<td>CCGCAATGCTGCCAGAAACC</td>
<td>307</td>
</tr>
<tr>
<td>exon9</td>
<td>ACCAAACAGATAGTGCCC</td>
<td>AGTGACGGACAGCAACAGAA</td>
<td>332</td>
</tr>
<tr>
<td>exon10</td>
<td>GCTACCCCTACCACGGACAGGC</td>
<td>AAGCCCCAGACCTGAGCACA</td>
<td>247</td>
</tr>
</tbody>
</table>

4. (PCR) genomic DNA

Eppendorf tube genomic DNA(100ng), dNTP(dATP, dCTP, dGTP, dTTP), total volume 3 10x reaction

- 7 -
buffer [20 mM Tris-HCl (pH 8.0), 100 mM KCl, 20 mM Mg²⁺]. 1.5 μM forward primer, 1.5 μM reverse primer, 1 U Taq DNA polymerase (1 Unit), 14.5 μL distilled water (distilled water), 1 μL PCR cycle, 1 cycle. PCR cycle: 1 cycle of (denaturation) 95°C 5 min, (dwelling) 5 min, 38 cycles of 95°C 30 s, 62°C 30 s, 72°C 30 s (extension) 45°C 1 min, 1 cycle of 72°C 10 min (extension), 10 min of PCR.

5. PCR: Amplification of DNA

PCR: 1% Eppendorf tubes. Qiagen Quick Gel Extraction kit (Qiagen Co. Valencia, CA, USA) tubes to extract DNA.

6. Direct DNA sequencing

Gel extraction kit, DNA, 9 μL. Thermo Sequenase™ Cy5 Dye Terminator kit sequencing. ALFwin Sequence Analyser 2.00 (Amersham Pharmacia Biotech, Uppsala, Sweden) ABI Prism model 310 version 3.00 (Applied Biosystems Division, Perkin Elmer Corp., Foster city Calif.) to obtain sequences of DNA.
7. キュー
SAS システム (SAS system for Windows 6.12 TS level 0045）を使用した検定の結果は、Chi-square と Fischer の検定によって、P 値 0.05 の有意水準で有意差が認められた。
III. Table

1. Subject Information

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex (M/F)</th>
<th>Age of Onset (yr)</th>
<th>Mean Age (yr)</th>
<th>FBG (mg/dL)</th>
<th>HbA1c (%)</th>
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</thead>
<tbody>
<tr>
<td>12</td>
<td>4/8</td>
<td>29 ± 3</td>
<td>34.45 ± 18.13</td>
<td>193 ± 34</td>
<td>9.3 ± 3.4</td>
</tr>
<tr>
<td>10</td>
<td>8/2</td>
<td></td>
<td>29.42 ± 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FBG (fasting blood glucose): ≤ 115 mg/dL
HbA1c: ≤ 6%
2. Exon 1

<table>
<thead>
<tr>
<th></th>
<th>Position</th>
<th>Sequence</th>
<th>Mutation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>CTG</td>
<td>CTC</td>
<td>0.277</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>CTC</td>
<td>CTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>CTC</td>
<td>CTC</td>
<td>0.781</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>ATC</td>
<td>CTC</td>
<td>0.020</td>
</tr>
</tbody>
</table>

- Exon 7

<table>
<thead>
<tr>
<th></th>
<th>Position</th>
<th>Sequence</th>
<th>Mutation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>459</td>
<td>CTG</td>
<td>TTG</td>
<td>0.781</td>
</tr>
<tr>
<td></td>
<td>487</td>
<td>AAC</td>
<td>AGC</td>
<td>0.095</td>
</tr>
</tbody>
</table>

- Exon 1

HNF-1q exon 1

- Exon 7

Exon 7

- Exon 1

Exon 1
### Table 4.

<table>
<thead>
<tr>
<th>Exon</th>
<th>Codon</th>
<th>Type</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>Silent mutation</td>
<td>2/10</td>
<td>5/12</td>
<td>0.277</td>
</tr>
<tr>
<td>1</td>
<td>27</td>
<td>Missense mutation</td>
<td>0/10</td>
<td>5/12</td>
<td>0.020</td>
</tr>
<tr>
<td>7</td>
<td>459</td>
<td>Silent mutation</td>
<td>2/10</td>
<td>3/12</td>
<td>0.781</td>
</tr>
<tr>
<td>7</td>
<td>487</td>
<td>Missense mutation</td>
<td>5/10</td>
<td>2/12</td>
<td>0.095</td>
</tr>
</tbody>
</table>
1. Exon 1, codon 17: codon CTC → CTG, leucine → leucine, silent mutation.

2. Exon 1, codon 27: codon ATC → CTC, isoleucine → leucine, missense mutation.


IV. 

HNF-1α 1987 Courtois 1210 exon1 101 exon1 exon1 exon1 (transcription factor) A element

5. HNF-1α 1210 101 101 exon1 exon1 exon1 exon1 exon1 exon1 exon1 exon1 exon1 DNA-binding domain, -COOH terminal显露

HNF-1α 1210 101 101 101 NH terminal显露 101 exon1 encoding dimerization domain 234 exon1 exon1 exon1 exon1 exon1 exon1 DNA-binding domain, -COOH terminal显露 exon1 exon1 exon1 exon1 exon1 exon1 transcripional activating domain显露 (5) 2829 30 MODY显露. Alexandra显露 41210 exon1 exon1 exon1 exon1 exon1 exon1
HNF-1 homodimer (HNF-1α) is predicted to form 50% of the HNF-1 complex. HNF-1α heterodimer is predicted to form 50% of the HNF-1 complex. 

HNF-1α 

MODY

silent mutation

MODY

HNF-1 silent mutation

HNF-1 missense mutation

HNF-1 silent mutation

HNF-1 silent mutation

HNF-1 silent mutation
À¯ÀüÀÚÀûº´ÀÎÀ»¹àÈ÷´Âµ¥Áß¿äÇϸ®¶ó»ç·áµÇ°íÀÌ¿¡´ëÇÑ¿¬±¸°¡Áø
ÇàµÇ¸éÁ¶±â¹ß»ýÁ¦

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V. 

1. **HNF-1α** 17 codon CTC (leucine) → CTG (leucine), exon 1 etc. 40 codon CTG (leucine) → TTG (leucine), exon 7 etc.

2. Exon 1 17 codon CTC silent mutation 10 2, 20 12, 54 8, 100 12, 200 12, 400 12.

3. Exon 1 27 codon ATC missense mutation 10 2, 20 12, 54 8, 100 12, 200 12, 400 12.

4. Exon 7 459 codon CTG silent mutation 10 2, 20 12, 54 8, 100 12, 200 12, 400 12.

5. Exon 7 487 codon AAC missense mutation 10 2, 20 12, 54 8, 100 12, 200 12, 400 12.
HNF-1αがHNF-1βを抑制する関与が示唆されました。
8. Lindner T, Gagnoli C, Furuta H, Cockburn BN, Petzold C,


14. Beards F, Frayling T, Bulman M, Horikawa Y, Allen L, Appleton M et al. Mutations in hepatocyte nuclear factor 1β are not a
common cause of maturity-onset diabetes of the young in the U.K.


29. Mendel DB, Crabtree GR. HNF-1, a member of a novel class of


34. - 23 -
Abstract

Polymorphism of hepatocyte nuclear factor-1α gene in early-onset type 2 diabetes mellitus in Korea

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Brain Korea 21 Project for Medical Science
The Graduate School, Yonsei University

(Directed by Professor Young Soo Ahn)

Background: Non-insulin-dependent diabetes mellitus (NIDDM) affects 2-6% of the world population and is the major cause of morbidity and mortality of the old. Maturation-onset diabetes of the young (MODY) is a genetically heterogeneous subtype of NIDDM characterized by early onset, usually before 25 years of age, autosomal dominant inheritance and primary defect in insulin secretion. MODYs occur worldwide and would account for about 2 to 5% of all cases of type II diabetes. To the date five MODY genes have been identified, MODY 1 to MODY 5. There are many studies that HNF-1α mutation is the most common cause of MODY 3. This study was carried out to reveal whether HNF-1α gene is the common cause of early-onset type II diabetes and MODY in Korean population.

Method: Members of 12 pedigrees with MODY and early-onset of NIDDM were selected for the mutation detection. All of these
families have at least two members with NIDDM diagnosed before the age of 40 years, and diabetes was inherited as an autosomal dominant trait with at least two generations of diabetic subjects. Genomic DNAs were extracted from whole-blood samples. The 10 exons and promoter of \( \text{HNF-1} \) gene were amplified by polymerase chain reaction (PCR) using genomic DNA from each subject with sequence-specific primers (Nature 384: 455-458, 1996). The PCR products were electrophoresed on an 1% agarose gel and the DNA bands were excised and sequenced.

Result: In codon 17 of exon 1, 2 of 10 control subjects and 5 of 12 patients, have nucleotide replacement in which CTC nucleotide is replaced by CTG. But both CTC and CTG code same amino acid leucine, in other word, silent mutation \( (P=0.277) \). In codon 27 of exon 1, 5 patients have silent mutation in which codon ATC is replaced by CTC and amino acid change from isoleucine to leucine. There was no mutation in codon 27 of exon 1 in control group \( (P=0.020) \), but this is a known polymorphism site and is not a new site. In exon 7, codon 459 have silent mutation \( (\text{CTC} \rightarrow \text{TTG}) \) in 2 of control group and 3 of patient group \( (P=0.781) \). Both codon code leucine. Another missense mutation was observed in codon 487 of exon 7. Nucleotide AAC is replaced by AGC. AAC codes asparagine and AGC codes serine. This mutation was observed in 5 control subjects and 2 patients.

Conclusion: There was no significant or new mutation in \( \text{HNF-1} \) gene in this study. Therefore it could be concluded that \( \text{HNF-1} \) gene is not the major cause of early onset type 2 DM in Korea.
Keywords: hepatocyte nuclear factor-1α, HNF-1α, maturity onset diabetes mellitus, MODY, early onset diabetes mellitus, mutation