The Mutation Underlying Familial Hypercholesterolemia in a Taiwanese Family

Wen-Lang Lin[1], Der-Yan Tai[2], Eric Lo[1], Guey-Jen Lee-Chen[1]*

[1] Department of Biology, National Taiwan Normal University
Taipei, Taiwan

[2] Department of Internal Medicine, Wei Gong Memorial Hospital, Tou Fen
Miaoli, Taiwan

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ABSTRACT

DNA screening for low-density lipoprotein (LDL) receptor mutations was performed in a clinically diagnosed six-year-old familial hypercholesterolemia (FH) patient. The promoter region and the coding sequence of the LDL receptor gene were amplified by means of polymerase chain reaction and subjected to DNA sequencing to study the molecular lesion. A G→A transition in codon 207, changing Glu_{207} to Lys (E207K) in the ligand-binding domain of the LDL receptor, was identified. Analysis with *Mnl*I endonuclease digestion showed this mutation to be inherited from paternal grandmother. The proband's two siblings also carried the mutation. Identification of the disease-causing mutation can clearly define the cause of patient's hyperlipidemia and allow appropriate early treatment and antenatal and family studies.

Key words: familial hypercholesterolemia, LDL receptor mutation, DNA diagnosis

Introduction

The low-density lipoprotein (LDL) receptor is a 160 kDa transmembrane glycoprotein ubiquitously distributed, playing a major role in cholesterol homeostasis (Brown and Goldstein, 1986). Impairment of LDL receptor activity results in the accumulation of LDL cholesterol in the circulatory system leading to familial hypercholesterolemia (FH). In Western societies, individuals heterozygous for the disorder frequently display tendon xanthomata and premature symptomatic coronary heart disease (CHD) in addition to high concentration of plasma LDL (Goldstein *et al.*, 1995). FH is a common autosomal dominant disease that affects about 1 in 500 in most populations. The rare FH homozygotes occur about 1 in 1 million and have more severe symptoms than heterozygotes (Goldstein *et al.*, 1995).

The LDL receptor gene lies on the short arm of chromosome 19 (19p13.1-13.3) (Lindgren *et al.*, 1985). It contains 18 exons encoding the six functional domains of the mature protein: signal peptide, ligand-binding, epidermal growth factor (EGF) precursor like, O-linked sugar, transmembrane and cytoplasmic domains (Yamamoto *et al.*, 1984; Südhof *et al.*, 1985). To date, more than 800 mutations including deletions, insertions, point mutations, and splice-site mutations scattered over the LDL receptor gene have been identified (Villeger *et al.*, 2002). Mutations in the LDL receptor gene are known in Chinese FH patients in China, Hong Kong, Canada, and Southeast Asia (Sun *et al.*, 1994; Mak *et al.*, 1998; Pimstone *et al.*, 1998; Khoo *et al.*, 2000). No definite common mutation in Chinese populations due to a founder effect was reported.

In this study, a clinically diagnosed FH patient was screened by polymerase chain reaction (PCR) and DNA sequencing to detect mutation in the LDL receptor gene. One substitution mutation in the ligand-binding domain was found allowing for

*Corresponding author: Guey-Jen Lee-Chen; FAX: 886-2-29312904; E-mail: t43019@cc.ntnu.edu.tw

The first two authors contributed equally to this work.

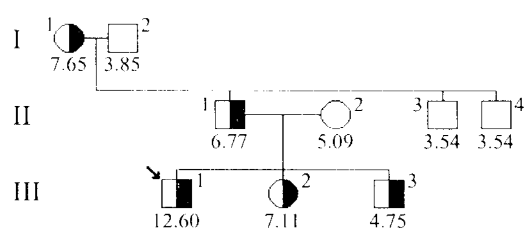

Figure 1. Family pedigree of Taiwanese FH patient. Unaffected individuals indicated with open symbols; those affected (E207K heterozygotes) shown with half-filled symbols. Arrow indicates the proband. The total plasma cholesterol concentration (mmol/L) for each individual shown below symbol.

accurate carrier detection in other members of this family.

Materials and Methods

Subjects and lipids

A clinically diagnosed six-year-old FH patient and family members were included in this study (Figure 1). The patient had cholesterol concentration 12.60 mmol/L and displayed tendon xanthomas. Family members of the patient were recruited for study with their consent. Blood samples from fasting subjects were drawn for DNA extraction and lipid measurements as described in Tai *et al.* (1998).

PCR amplification

Genomic DNA samples were amplified by PCR using primers (Leitersdorf *et al.*, 1990; Jensen *et al.*, 1996) specific for the promoter region and 18 exons of the LDL receptor gene (Table 1). A 25 μL reaction contained 100 ng DNA, 0.4 μmol/L of each primer, 0.2 mmol/L deoxynucleotide triphosphates, 1.5 mmol/L $MgCl_2$, and 0.5 U *Taq* DNA polymerase (Promega). Amplification for exon 4 was set as denaturation at 94℃ for 1 min and primer annealing and extension at 68℃ for 5 min. Amplification for the promoter region and other exons was set as denaturation at 94℃ for 1 min, primer annealing at 55℃ for 1 min and primer extension at 72℃ for 1 min.

Table 1. Primers for PCR amplification of LDL receptor exons.

Amplified region	Forward primer	Reverse primer	Frag. size (bp)
Promoter	CAGCTCTTCACCGGAGACC	ACCTGCTGTGTCCTAGCTGG	287
Exon 1	CACATTGAAATGCTGTAATGACG	CTATTCTGGCGCCTGGAGCAAGCC	215
Exon 2	TTGAGAGACCCTTTCTCCTTTTCC	GCATAATCATGCCCAAAGGGG	183
Exon 3	TTCCTTTGAGTGACAGTTCAATCC	GATAGGCTCAATAGCAAAGGCAGG	196
Exon 4	TGGTCTCGGCCATCCATCCCTGCAG	ACGCCCCGCCCCCACCCTGCCCCGC	437
Exon 5	AGAAAATCAACACACTCTGTCCTG	GGAAAACCAGATGGCCAGCG	180
Exon 6	TCCTCCTTCCTCTCTCTGGC	TCTGCAAGCCGCCTGCACCG	179
Exon 7	GGCGAAGGGATGGGTAGGGG	GTTGCCATGTCAGGAAGCGC	234
Exon 8	CCAAGCCTCTTTCTCTCTCTTCCAG	CCACCCGCCGCCTTCCCGTGCTCAC	176
Exon 9	CCTGACCTCGCTCCCCGGACCCCCA	GGCTGCAGGCAGGGGCGACGCTCAC	223
Exon 10	ATGCCCTTCTCTCCTCCTGCCTCAG	AGCCCTCAGCGTCGTGGATACGCAC	278
Exon 11	CAGCTATTCTCTGTCCTCCCACCAG	TGGCTGGGACGGCTGTCCTGCGAAC	173
Exon 12	TCTCCTTATCCACTTGTGTGTCTAG	CTTCGATCTCGTACGTAAGCCACAC	190
Exon 13	GTCATCTTCCTTGCTGCCTGTTTAG	GTTTCCACAAGGAGGTTTCAAGGTT	219
Exon 14	CCTGACTCCGCTTCTTCTGCCCCAG	ACGCAGAAACAAGGCGTGTGCCACA	204
Exon 15	AGAAGACGTTTATTTATTCTTTC	GTGTGGTGGCGGGCCCAGTCTTT	221
Exon 16	CCTTCCTTTAGACCTGGGCC	CATAGCGGGAGGCTGTGACC	173
Exon 17	TGACAGAGCGTGCCTCTCCCTACAG	TGGCTTTCTAGAGAGGGTCACACTC	210
Exon 18	GCCTGTTTCCTGAGTGCTGG	TCTCAGGAAGGGTTCTGGGC	135

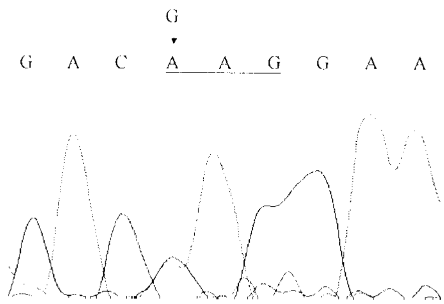

Figure 2. DNA sequence analysis of the E207K mutation. A G→A transition resulting in a glutamate (GAG) to lysine (AAG) substitution at codon 207 (E207K) is noted.

DNA sequencing

After gel purification, PCR products were sequenced directly using the DYEnamic ET Dye Terminator Kit and MegaBACE Analyzer (Molecular Dynamics, Division of Amersham Pharmacia Biotech). The identified E207K mutation was verified by *Mnl*I restriction and 2% agarose gel electrophoresis of PCR amplified product.

Haplotype analysis

Five markers were analyzed to establish haplotypes at the LDL receptor locus. These markers were as follows: polymorphic *Sma*I site in intron 7, *Bst*UI site in exon 11, *Hin*cII site in exon 12, *Ava*II site in exon 13, and *Msp*I site in exon 15 of the LDL receptor gene (Villeger *et al.*, 2002). The *Bst*UI site was generated by amplification created restriction site PCR using forward exon 11 primer and a mismatch reverse primer 5'-TCAGGCCCCCTTTCTTGATCTTCG-C. PCR-amplified products were digested with *Sma*I (for intron 7 polymorphism), *Bst*UI (for exon 11 polymorphism), *Hin*cII (for exon 12 polymorphism), *Ava*II (for exon 13 polymorphism), and *Msp*I (for exon 15 polymorphism) restriction enzymes and separated on 2.0% agarose gel.

Results

Identification of mutation

The promoter region and 18 exons of the LDL receptor gene of the patient were amplified and sequenced. We found a G→A substitution at nucleotide 682, resulting in the substitution of a glutamate for a lysine residue (E207K) in the ligand-binding domain of LDL receptor (Figure 2). This change abolishes a recognition site for the restriction enzyme *Mnl*I. Therefore, on *Mnl*I digestion, the mutant DNA retained its original size of 294 bp, whereas the wild type DNA was cut into fragments of 242 and 52 bp. As shown in Figure 3, the proband (III-1) inherited the mutation from his paternal grandmother (I-1); his two siblings (III-2 and III-3) also carry the mutation.

Haplotype analysis on the mutant allele

The genotypes at five polymorphic loci were examined in the proband and recruited family members. As shown in Table 2, a total of 18 chromosomes yielded four independent haplotypes. The E207K allele was associated with haplotype B: *Sma*I- / *Bst*UI- / *Hin*cII- / *Ava*II+ / *Msp*I+.

Lipoprotein concentrations

The cholesterol concentrations of the proband and recruited family members are shown in Figure 1. The plasma total cholesterol levels of E207K heterozygotes were 7.65 (I-1, 60-year old grandmother), 6.77 (II-1, 34-year old father), 7.11 (III-2, 4-year old sister) and 4.75 mmol/L (III-3, 3-year old brother). None of them showed evidence of tendon xanthomas or atherosclerotic disease. The 6-year old proband (III-1), however,

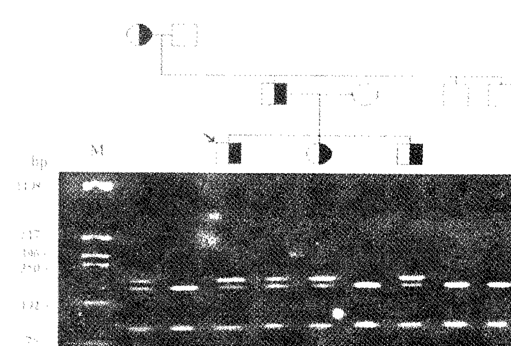

Figure 3. Restriction enzyme analysis of the E207K mutation. Upon *Mnl*I digestion, the wild type DNA was cut into fragments of 242, 145 and 52 bp. The E207K mutation converted the 242 and 52-bp fragments into 294-bp fragment. The marker (M) used was *Hin*fI digest of pGEM4 DNA.

Table 2. Haplotype of the proband (III-1) and recruited family

Subject	Haplotype	Haplotype marker[a]				
		*Sma*I	*Bst*UI	*Hinc*II	*Ava*II	*Msp*I
I-1[b]	A/B	+/-	+/-	+/-	-/+	+/+
I-2	C/C	-/-	+/+	-/-	+/+	+/+
II-1[b]	C/B	-/-	+/-	-/-	+/+	+/+
II-2	D/D	+/+	+/+	+/+	-/-	+/+
II-3	A/C	+/-	+/+	+/-	-/+	+/+
II-4	A/C	+/-	+/+	+/-	-/+	+/+
III-1[b]	D/B	+/-	+/-	+/-	-/+	+/+
III-2[b]	D/B	+/-	+/-	+/-	-/+	+/+
III-3[b]	D/B	+/-	+/-	+/-	-/+	+/+
	A	+	+	+	-	+
	B[c]	-	-	-	+	+
	C	-	+	-	+	+
	D	+	+	+	-	+

[a]+ or -, presence or absence of restricted site.
[b]E207K heterozygotes.
[c]Haplotype associated with the E207K mutation.

had tendon xanthomas, in addition to elevated level of cholesterol (12.60 mmol/L).

Discussion

In this study, a single amino acid substitution E207K in the LDL receptor gene was found in a clinically diagnosed FH patient. Although the patient was only six years old, he was severely affected, displayed tendon xanthomas and high levels of cholesterol. On the contrary, other FH heterozygotes in this family are not so severely affected. Both genetic and environmental factors may contribute to this. Lack of clinical presence was usual in FH heterozygotes in China (Cai *et al.*, 1985; Sun *et al.*, 1994). The clinical severity of FH heterozygote varies (Goldstein *et al.*, 1995). Therefore, conventional methods for diagnosis of FH, based on lipid determinations and a family history of CHD, may not always allow an accurate diagnosis of the disease.

The G→A substitution at nucleotide 682 causes the mutation E207K. This mutation occurs at a CpG hot spot in which G is mutable to A, C, or T (Cooper and Youssoufian, 1988). This mutation has been detected in different ethnic groups, including Canadian, Mexican, Italian, French, and Chinese (Leitersdorf *et al.*, 1990; Hobbs *et al.*, 1992; Mak *et al.*, 1998; Pimstone *et al.*, 1998). Due to the geographical distance and historic ethnic differences, this mutation may have evolved independently in different ethnic groups. The E207K allele in our patient was associated with haplotype *Sma*I- / *Bst*UI- / *Hinc*II- / *Ava*II+ / *Msp*I+. The lack of haplotype reported with the E207K alleles in two other Chinese patients (Mak *et al.*, 1998; Pimstone *et al.*, 1998) precludes the prediction of mutation origin in the Chinese.

The E207K mutation is located in the fifth cysteine-rich repeats in the binding domain. The missense mutation replaces one of the amino acids in the negatively charged Ser-Asp-Glu triplet, which is conserved at the COOH-terminus of all ligand-binding repeats (Südhof *et al.*, 1985). The substitution results in defective transport, similar to other missense mutations that affect the Ser-Asp-Glu triplet (Villeger *et al.*, 2002).

In summary, among the more than 30 mutations noted in the Chinese, E207K is one of eleven recurrent point mutations identified in unrelated patients. The rapid restriction enzyme detection method for most recurrent point mutations may provide a practical approach for further studies of hyperlipidemic Chinese patients. To clearly define gene defects in affected families can optimize treatment and prevention of CHD.

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臺灣一家族性高膽固醇血症患者低密度脂蛋白受體基因突變分析

林文郎[1] **戴德炎**[2] **羅偉軒**[1] **李桂楨**[1]*
[1]國立臺灣師範大學生物學系
[2]苗栗爲恭紀念醫院內科部

摘　要

本研究係藉聚合酶連鎖反應及 DNA 定序，來檢視一位六歲的家族性高膽固醇血症(FH)患者，其低密度脂蛋白(LDL)受體基因的起動子及表現子序列，以了解其分子致因。結果發現患者的 LDL 受體的配體結合部位上發生 E207K 突變，即第 207 個胺基酸由麩胺酸(Glu)轉變爲離胺酸(Lys)。進一步的 *Mnl*I 限制酵素切割檢測顯示，患者的 E207K 突變係遺傳至祖母，其妹妹弟弟亦帶有此突變。LDL 受體基因突變的確認可明確的定義患者的高血脂原因，以利早期安排適當的治療、進行出生前診斷、家族分析等。

關鍵詞：家族性高膽固醇血症，LDL 受體突變，DNA 分析

*通信作者: 李桂楨(Guey-Jen Lee-Chen); FAX: 886-2-29312904; E-mail: t43019@cc.ntnu.edu.tw