Identification of Known Mutation in LDL Receptor Gene Underlying Severe FH Phenotype in Thai Patient: A Case Report

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ABSTRACT

Objective: Familial hypercholesterolemia (FH) is associated with atherosclerosis coronary artery disease (CAD). The aim of this study is to identify a mutation in the LDL receptor gene that underlined the FH phenotype in a female patient and her family.
Methods: The LDL receptor gene was screened by Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP), direct DNA sequencing and was subsequently confirmed by PCR-RFLP.
Results: The screening of the entire LDL receptor gene revealed a 5' donor splice site mutation of the first base of intron 3, i.e., 313+1G→T mutation in one allele. This mutation was previously reported in a Danish patient with severe hypercholesterolemia.
Conclusion: This case report illustrates the use of DNA diagnosis of a female heterozygous FH case and her family members, which is more accurate than clinical diagnosis especially when clinical phenotype is variable or when the individual who is at high risk is still a normolipidemic at his/ her young age. DNA diagnosis is now used as a tool to find or diagnose FH. Accurate and/or early diagnosis is important for prevention and treatment of FH patients in order to avoid the development of CAD in these patients.
Keywords: Familial hypercholesterolemia; FH; LDL receptor; Mutation

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Familial hypercholesterolemia (FH) is a relatively common lipoprotein disorder with autosomal dominant mode of transmission. FH is clinically characterized by high level of blood LDL-cholesterol, xanthoma, premature coronary artery disease (CAD) and family history of one or more of these features.[1] The genetic disease is caused by a mutation in the LDL receptor gene which encodes a cell surface glycoprotein, i.e., LDL receptor. The function of the LDL receptor is to uptake LDL from blood circulation in order to supply and simultaneously maintain cellular cholesterol. Thus, the function of the LDL receptor is to maintain normal level of blood LDL-cholesterol. The LDL receptor gene is located on chromosome 19 which is consisting of 18 exons and 17 introns.[2,3] Mutations in the LDL receptor gene causing FH are heterogeneous at the DNA level and more than 900 FH-related mutations in the LDL receptor are characterized worldwide.[4] We here report a DNA analysis to identify an FH-causing mutation in the LDL receptor gene in a female patient and members of her family with the clinical features of familial hypercholesterolemia.

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CASE REPORT

A 39-year-old female patient was detected as having hypercholesterolemia since 30 years of age. Her mother and all of her sisters had relatively severe hypercholesterolemia and some with xanthelasma, arcus corneae, and xanthoma over her elbows, hands, knees, Achilles tendon and feet. One of her sisters died suddenly from coronary event at the age of 24. Another sister of hers had surgery bypassed operation when she was 42 years old. One of her uncles had both severe hypercholesterolemia and coronary artery disease which were simultaneously detected at the age of 55 years old when he first encountered myocardial infarction and had to undergo a bypass operation. Her mother, two of her sisters and the index case, herself, were all asymptomatic of CAD and this may be attributed by the fact that all of these patients were under medications since the initial detection of the hypercholesterolemic condition.

To identify the mutation underlying the hypercholesterolemic condition in the index patient, all exons and promoter region of the LDL receptor gene was primarily amplified by Polymerase Chain Reaction (PCR) using the oligonucleotide primers previously designed by Leitersdorf et al.[5] For exon 3, the amplification condition was 95°C 1

TABLE 1. Clinical features of the index case and her family members with the 313+1G→T mutation at the time before any lipid-lowering medication (concentrations of all lipid were in mg/dl)

X : xanthomas, XL : xanthelasma, AC : arcus corneae; CAD : coronary artery disease

Subject	Age	Sex	TC	LDL-C	HDL-C	TG	X	XL	AC	CAD	Remarks
Index case	39	F	449	373.3	44.1	158	+	+	+	-	Under medication
Mother	69	F	400	364	60	120.9	+	+	+	-	Under medication
Uncle	68	M	386	299	39	236	+	+	+	+	CABG at 59 yr
Sister	45	F	453	361	53	69	+	+	+	+	CABG at 42 yr
Sister	43	F	301	229	57	75	+	+	+	-	Under medication

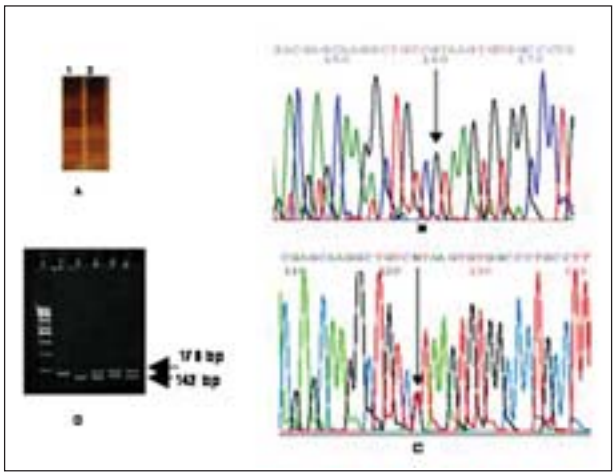

Fig 1. Mutation 313+1G→T. (A) PCR-SSCP analysis of exon 3 and breaking sequence, mobility shift in exon 3 in the index patient (lane 2) as compared with control (lane 1); (B) Partial DNA sequence from wild-type (upper panel); (C) Partial DNA sequence from the index case (lower panel); The heterozygous transversion G to T at the first base of intron 3 was indicated with N in the mutant DNA sequence and the position of the changed base was marked with arrow in both wild-type and mutant DNA sequences. (D) PCR-RFLP analysis for confirmation of the DNA sequence analysis. Lane 1: 100-bp DNA ladder marker, lane 2 : undigested PCR product of exon 3, lane 3 : RsaI-digested PCR product of wild-type DNA, lanes 4, 5, and 6 : RsaI-digested PCR products from the index case and two of her family members with 313+1G→T mutation (i.e., her mother and uncle). In this analysis, the PCR fragment was 178 bp. The wildtype DNA fragment contained a RsaI site and the RsaI digestion yielded two fragments of 142 and 36 bp (in this figure, only the 142 bp fragment was apparent). In the RFLP analysis, the normal control subject revealed one band of 142 bp since both alleles were able to be digested with RsaI enzyme whereas the index patient and her family members revealed both 178 and 142 bp fragments confirming that one allele of these patients possessed the heterozygous G to T mutation at the first base of intron 3.

min, 58°C 1 min, and 72°C 1 min for 36 cycles in a DNA thermal cycler (Gene Amp PCR system 2400, Perkin-Elmer, USA). The denaturation and extension were extended for 5 min for the first and final cycle, respectively. The PCR products were subsequently analyzed by single strand conformation polymorphism (SSCP) technique, previously described by Orita et al.[6] The SSCP gels were detected for mobility shift patterns by silver staining method.[7] Fragments with mobility shift compared with the wild-type were subjected to automated DNA sequencing in an ABI DNA sequencer (Model 377) with a dye terminator kit (Big Dye™ Terminator Cycle Sequencing V2.0). The DNA sequence was confirmed by Polymerase Chain Reaction-Restriction Fragment Length Polymor-phism (PCR-RFLP) method by virtue of the presence of RsaI restriction site in the wild-type allele but not in the mutant allele.

The DNA analysis using the PCR-SSCP, DNA sequencing and PCR-RFLP techniques described above identified a known splice site mutation in intron 3, namely, 313+1G→T in one allele of the LDL receptor gene in the index patient. The index patient was thus a heterozygous FH. This mutation was not observed in all normolipidemic subjects (n =100). All patients and normal control subjects signed their informed consent form and this study had been reviewed and approved by the ethics committees of Mahidol University and the Faculty of Medicine Siriraj Hospital. Clinical features of this FH case and members of her family with the 313+1G→T mutation are shown in Table 1. The results of DNA analysis are shown in Fig 1.

DISCUSSION

By PCR-SSCP and DNA sequencing, a mutation 313+1G→T was identified in a female heterozygous FH patient. The mutation was confirmed by PCR-RFLP and this method was then used to identify 313+1G→T mutation in the family members of the index case. The family analysis revealed that this mutation co-segregated with FH phenotype in the family of the index case. The mutation was also screened in normal control subjects (n = 100) using PCR-RFLP method to exclude the possibility of the presence of this mutation in normal subjects. The screening indicated that this mutation was absent in normal subjects. The mutation had been reported in one Danish FH patient.[8] The pathogenic effect of this mutation was expected to cause a direct splicing of exon 2 to exon 4 resulting in a deletion of 41 amino acids in the ligand binding domain of the mutant LDL receptor protein.[8]

Although the concept of the control of cholesterol levels in clinical practice has now been well established, the diagnosis of FH for appropriate treatment in cases and for prevention of CAD in high-risk individuals is not yet accurate. The report presented here was a rare case which manifested all features of FH that can be used for clinical diagnosis. However, most FH cases seem not to have any xanthoma and hypercholesterolemia may be expressed at older age. In these FH cases, accurate diagnosis may be missed until coronary event occurs without a chance of prevention. It is now accepted that unequivocal diagnosis of FH can be made by DNA-based tests.[9] Such DNA tests can be used for early diagnosis especially in those individuals who are at high risk of developing CAD. Early diagnosis is important since early treatment with statins and/or appropriate dietary control can substantially reduce cardiovascular risks.[10-14] This case report exemplified an exploitation of DNA analysis for diagnosis of FH case as well as to identify carriers among family members.

CONCLUSION

The molecular genetics underlying FH in Thai patients is largely unknown at present. In this study, molecular analysis of the LDL receptor gene revealed a known mutation, 313+1G→T that underlies severe FH phenotype in a patient and some members of her family. DNA diagnosis is more accurate than clinical diagnosis especially when clinical phenotype is variable or when an individual at high risk is still normolipidemic at his/her young age. DNA diagnosis is now used as a tool to find or diagnose FH. Accurate and/or early diagnosis is important for prevention and treatment of FH in order to avoid the development of CAD.

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บทคัดย่อ

การพบการกลายพันธุ์ในยีน LDL receptor ที่ทำให้เกิดโรคพันธุกรรม Familial Hypercholesterolemia ในคนไทย

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วัตถุประสงค์: โรคพันธุกรรม Familial Hypercholesterolemia (FH) เป็นปัจจัยเสี่ยงที่สำคัญของโรคหลอดเลือดหัวใจอุดตัน การกลายพันธุ์ในยีน LDL receptor เป็นต้นเหตุที่ก่อให้เกิดโรคพันธุกรรม FH จุดประสงค์ของการศึกษานี้คือการค้นหาการกลายพันธุ์ในยีน LDL receptor ในผู้ป่วยคนไทยและครอบครัวที่มีภาวะโคเลสเตอรอลสูงและมีลักษณะของโรคพันธุกรรม FH เด่นชัด

วิธีการ: การค้นหาการกลายพันธุ์ในยีน LDL receptor ใช้เทคนิค Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP), Direct DNA sequencing และ Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

ผลการศึกษา: การค้นหาการกลายพันธุ์ ตลอดทั้งยีน LDL receptor ได้พบการกลายพันธุ์ในเบสแรกของ intron 3, คือ การกลายพันธุ์ 313+1G>T การกลายพันธุ์ตำแหน่งนี้ได้มีรายงานแล้วว่าทำให้เกิดโรคพันธุกรรม FH ในผู้ป่วยชาวเดนนิช 1 ราย

สรุป: การศึกษานี้ได้แสดงให้เห็นว่าการตรวจวินิจฉัยผู้ป่วย FH อาจใช้เทคนิคทางด้าน DNA ซึ่งขณะนี้ได้มีหลักฐานเป็นที่ยอมรับและให้ความถูกต้องแม่นยำกว่าการวินิจฉัยทางคลินิคเพียงอย่างเดียว โดยเฉพาะในกรณีที่ผู้ป่วยมีประวัติหรืออาการทางคลินิคไม่ชัดเจน เช่นในผู้ป่วยที่อายุยังน้อยและยังมีระดับโคเลสเตอรอลไม่สูงเด่นชัด การตรวจวินิจฉัยที่ถูกต้องและ/หรือการตรวจวินิจฉัยแต่เนิ่นๆจะเป็นประโยชน์ต่อการป้องกันและการรักษาภาวะโคเลสเตอรอลสูงในผู้ป่วย FH และจะช่วยให้สามารถชะลอการเกิดโรคหลอดเลือดหัวใจอุดตันในผู้ป่วยเหล่านี้ได้