Familial Hypercholesterolemia Kindred in Utah with Novel C54S Mutations of the LDL Receptor Gene

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SUMMARY

In the course of investigations of coronary artery disease in Utah, we identified a family whose proband showed elevated plasma levels of LDL cholesterol. To determine the genetic etiology of the lipoprotein abnormalities, we screened DNA samples for mutations in all 18 exons and the exon-intron boundaries of the low-density lipoprotein (LDL) receptor gene. Novel point mutations were identified in the proband: a T-to-A transversion at nucleotide position 223, causing substitution of Ser for Cys at codon 54 in exon 3 of the receptor gene. This amino acid replacement would disrupt one of the disulfide bonds necessary for maintenance of the secondary structure of the repeat at the N-terminal of the receptor, prevent correct folding of the receptor, and result in defective intracellular transport of the receptor. (Jpn Heart J 1998; 39: 785–789)

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HETEROZYGOUS familial hypercholesterolemia (FH) results from inheritance of a single defective copy of a low-density lipoprotein receptor (LDLR) gene.1) The disease is recognized clinically by striking elevations of LDL cholesterol (about twice the normal range, or > 95th percentile) in children as well as adults. Affected families display bimodally distributed LDL cholesterol levels consistent with an autosomal dominant trait. In general, only LDL cholesterol levels are elevated in FH; elevated triglycerides or other lipid abnormalities may bring into question the diagnosis of FH and may suggest other inherited...
primary lipid disorders such as familial combined hyperlipidemia, or secondary lipid disorders.

The LDLR gene is large and structurally complex; it spans 45 kb of the human genome and consists of 18 exons and 17 introns.2) No mutational “hot-spot” has been identified; in fact, specific mutations rarely recur either among unrelated Caucasians or among families residing in mainland Japan.3,4) The LDLR mutations have been classified into the following five classes on the basis of biosynthetic and functional studies of fibroblasts derived from FH patients; null alleles (class 1 mutations), transport-defective alleles (class 2 mutations), binding-defective alleles (class 3 mutations), internalization-defective alleles (class 4 mutations), and recycling-deficient alleles (class 5 mutations).3-5) We describe here the identification of a novel mutation of the LDLR gene in a hypercholesterolemic kindred in Utah, that would result in defective intracellular transport of the LDL receptor.

**MATERIALS AND METHODS**

**Patients & lipoprotein measurement:** The proband is a patient who has been followed by the Cardiovascular Genetics Research Clinic at the University of Utah. Blood samples collected after 12–16 hours of fasting were prepared from each family member. Lipid and lipoprotein concentrations were measured by procedures described previously.6) In brief, plasma cholesterol and triglyceride concentrations were assayed enzymatically, and concentrations of HDL cholesterol were determined by the MgCl₂-dextran precipitation method.

**Single-strand conformational polymorphism (SSCP)** and sequencing analyses: Each PCR was carried out using 20 ng of genomic DNA extracted from lymphocytes, in a 10-microliter solution containing 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 μM of dNTP, 2 μCi of [alpha ³²P]-dCTP (3000 Ci/mmol, 10 mCi/ml), 2.5 pmol of primer, and 0.25 units of Taq polymerase. PCR primers for amplification of each exon of the LDLR gene and all exon-intron boundaries were described previously.8,9) Each of the 35 PCR cycles consisted of 30 sec at 94°C, 30 sec at 60°C, and 30 sec at 72°C. Reaction mixtures were diluted with 50 μl of 95% formamide dye and 20 mM EDTA, incubated at 85°C for 5 min, and applied (2 μl/lane) to 6% polyacrylamide gels containing 0.5X TBE (90 mM Tris-borate/2 mM EDTA) and 5% glycerol. Electrophoresis was performed both at room temperature and at 4°C. The gels were dried and autoradiographed with intensifying screens. When a variant band was revealed by SSCP analysis, the corresponding DNA fragment was sequenced directly on both strands to identify the nature of the mutation. The results were confirmed by two independent experiments.
**PCR-RFLP analysis:** PCR products were mixed with 10 microliters of a 2 × reaction mixture containing 5 units of the restriction enzyme *PvuII*, incubated at 37°C for one hour, electrophoresed on 3% agarose gel and the DNA visualized by ethidium bromide staining. The normal allele and the mutant alleles were distinguished by the different restriction digestion patterns on the gel after the *PvuII* cleavage.

**RESULTS**

The proband is a 27 year-old Caucasian female patient who is being fol-
followed by the Cardiovascular Genetics Research Clinic of the University of Utah. Pedigree analysis of the proband’s family showed a Mendelian mode of inheritance of the mutant allele. Plasma lipoprotein level of the proband at age 27 was total cholesterol 389 mg/dl, triglyceride 135 mg/dl, HDL cholesterol 58 mg/dl, and LDL cholesterol 298 mg/dl. Mutation screening of the LDL receptor gene in the proband’s genomic DNA identified an aberrant SSCP pattern in the PCR amplification product of exon 3 (Figure, A). Direct sequencing on both strands revealed heterozygosity for a T-to-A transversion at nucleotide position 223 of the LDL receptor cDNA (Yamamoto et al. 1984). This transversion would substitute serine for cysteine at codon 54 (C54S) (Figure, C).

The mutation abolishes a PvuII site, and therefore the absence of this site, diagnostic for the C54S mutation, can be assayed in a restriction fragment length polymorphism (RFLP) analysis. The normal allele was cleavable by PvuII and digestion produced 112 bp and 54 bp fragments. The C54S mutant allele was uncleavable by PvuII and digestion produced a 166 bp band. Figure, B shows that the proband was heterozygous for the mutant and normal alleles. C54S was the only sequence variation detected in the entire coding and splice-site consensus sequences of the LDL receptor gene of the proband. The proband’s mother and her maternal grandparents were negative for the C54S mutation. The proband’s father is deceased. The proband has no siblings or children. A group of 75 FH patients from Utah was screened for the presence of the C54S mutation. No additional index case was identified. This variant was not found in 96 control, normolipidemic individuals.

**DISCUSSION**

Environmental factors as well as genetic factors influence lipid levels in plasma. The nature of the LDL receptor mutation also contributes to clinical variability among FH patients: individuals with mutations that only partially impair receptor function tend to have lower plasma LDL cholesterol levels than do individuals whose genes produce totally defective receptors.1) In the present study, FH patients with the missense mutation C54S tended to show marked levels of total cholesterol and LDL cholesterol among same sex FH patients of a similar age.

Functionally, LDLR mutations were classified into five classes on the basis of studies that involved fibroblasts derived from FH patients.3-5) Class 2 alleles encode proteins that are blocked either completely or partially in transport between the endoplasmic reticulum and the Golgi apparatus. We observed a substitution of serine for cysteine at codon 54. Cys54 is the second critical cysteine of the second repeat of the ligand binding domain that consists of seven repeats of
40 amino acids; each repeat contains six cysteine residues that form three intra-repeat disulfide bonds. Thus, C54S mutation belongs to the transport-defective alleles (class 2 mutation), since disruption of one of the three disulfide bonds required for maintaining the secondary structure of the repeat would prevent correct folding of the receptor during synthesis and transport in the endoplasmic reticulum.

Previously, several other missense mutants occurring in the seven repeats of the ligand binding domain encoded by exon 2 through 6 have been functionally characterized. Since most of them encoded receptors that were blocked in transport between the endoplasmic reticulum and the Golgi apparatus (class 2; transport-defective alleles), we postulate that the C54S mutation reported here would cause the same class of deficiency on account of its location. Other possibilities cannot be ruled out, however, until proper functional tests are conducted for this particular mutation. Our study illustrates the feasibility of molecular genetic approaches in strengthening clinical diagnosis and invoking appropriate management of young members of families with FH, with a view toward early intervention in cases of predisposition to coronary heart disease.

REFERENCES