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A novel complex mutation in the LDL receptor gene probably caused by the simultaneous occurrence of deletion and insertion in the same region

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Abstract. A novel complex mutation with the presence of both deletion and insertion in very close proximity in the same region was detected in exon 8 of the LDL receptor gene from two apparently unrelated Japanese families with familial hypercholesterolemia (FH). In this mutant LDL receptor gene, the nine bases from nucleotide (nt) 1115 to nt 1123 (AGGGTGGCT) were replaced by six different bases (CACTGA), and consequently the four amino acids from codon 351 to 354, Glu-Gly-Gly-Tyr, were replaced by three amino acids, Ala-Leu-Asn, in the conserved amino acid region of the growth factor repeat B of the LDL receptor. The nature of the amino acid substitution and data on the families suggest that this mutation is very likely to affect the LDL receptor function and cause FH. The generation of this complex mutation can be explained by the simultaneous occurrence of deletion and insertion through the formation of a hairpin-loop structure mediated by inverted repeat sequences. Thus this mutation supports the hypothesis that inverted repeat sequences influence the stability of a given gene and promote human gene mutations.

Introduction

Familial hypercholesterolemia (FH), a common disease that affects about 1 in 500 people in most populations, is an autosomal dominant disorder caused by mutations in the gene encoding the low-density lipoprotein (LDL) receptor (Goldstein and Brown 1989). Individuals with FH have an elevated LDL cholesterol level in the plasma and an increased risk of atherosclerosis. So far, many different LDL receptor mutations have been identified and characterized, demonstrating a high degree of allelic heterogeneity at this locus (Goldstein and Brown 1989; Hobbs et al. 1990, 1992).

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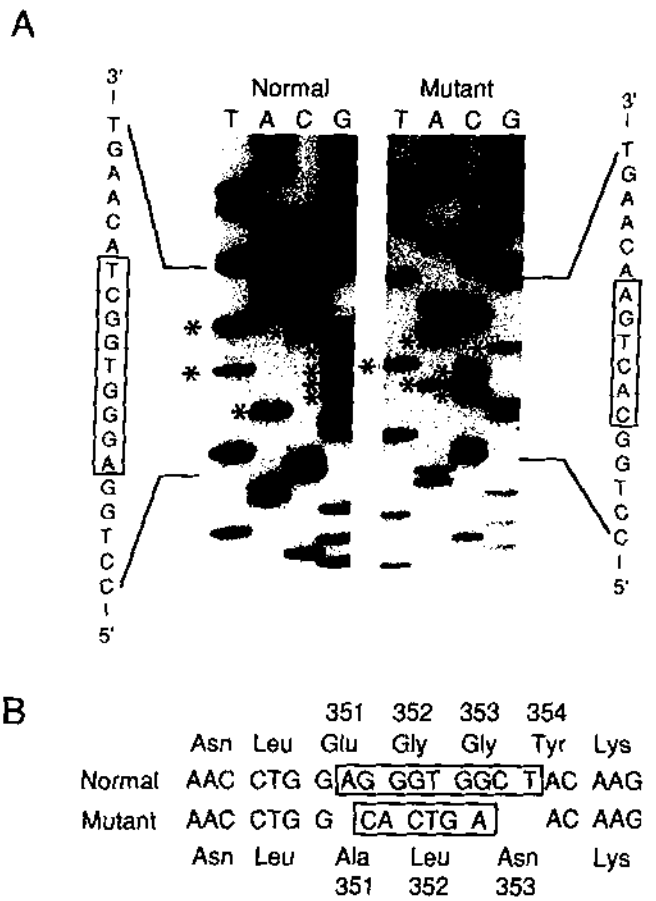


Fig. 1A, B. A complex mutation due to substitution of nine normal bases to six different bases in exon 8 of the LDL receptor gene. **A** Nucleotide sequence analysis of the region with the mutation from the clone containing the normal allele and the mutant allele, respectively. The nucleotide sequences differing between the normal allele and the mutant allele are indicated by *asterisks* and *boxes*. **B** The nucleotide and amino acid sequences of the normal and mutant allele in the region containing the nucleotide substitution. The changed nucleotides are *boxed*

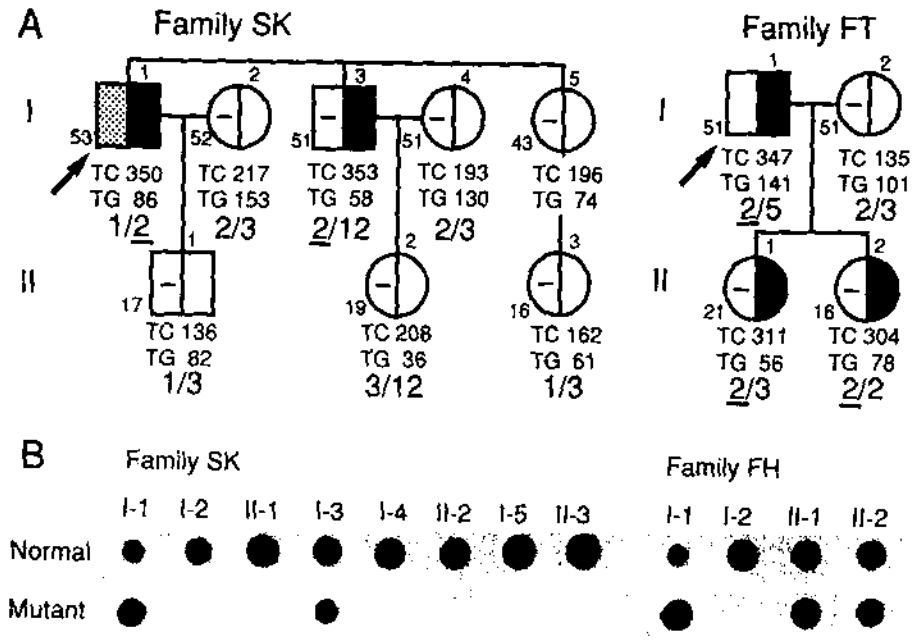


Fig. 2A, B. Pedigree analysis of the mutant LDL receptor gene. **A** The pedigrees of family SK and family FT. ◨◐, heterozygous familial hypercholesterolemia (FH); ◧◐, Achilles tendon xanthomas; ◫◑, no primary hypercholesterolemia (right) and no Achilles tendon xanthomas (left); ⊞⊕, not examined; *TC* total plasma cholesterol levels; *TG* total plasma triglyceride levels. The age is indicated to the left of each subject. Restriction fragment length polymorphism (RFLP) haplotypes are shown by *large Arabic numerals* below each subject. Haplotype 2 (*TaqI*+, *AvaII*–, *ApaLI5′*+, *PvuII*–, *NcoI*+, *PstI*–, *ApaLI3′*+) was associated with the mutant LDL receptor gene in both families. **B** Detection of the mutant allele in the members of families SK and FT by dot blot hybridization of the amplified DNA fragments of exon 8 using two allele specific oligonucleotide (ASO) probes, specific for the normal sequence and the mutant sequence, respectively. In family SK, the proband and his hypercholesterolemic brother (I-1, I-3) are heterozygous for this mutant allele and the normal allele. The normocholesterolemic son, sister, and two nieces of the proband (II-1, I-5, II-2, II-3) have only the normal allele. In family FT, the proband (I-1) and his two hypercholesterolemic daughters (II-1, II-2) are heterozygous for this mutant allele

It has been reported that the human gene mutations causing genetic disease occur non-randomly and that the location and nature of the mutational event are largely dependent upon the surrounding DNA sequences (Cooper and Krawczak 1990, 1991; Krawczak and Cooper 1991). Characterization of the mutations in the LDL receptor gene is advantageous for the study of the generative mechanisms that underlie naturally occurring deleterious gene mutations, because mutant LDL receptor genes are retained in the general population with a high frequency. Indeed, many large size deletions and insertions that involve Alu sequences at the junctions of rearrangements have been found in the LDL receptor gene (Hobbs et al. 1990, 1992). In addition, we have detected two frameshift mutations associated with the presence of the direct repeats of the LDL receptor gene (Yamakawa-Kobayashi et al. 1993). We describe here a novel complex mutation in exon 8 of the LDL receptor gene detected in two apparently unrelated Japanese FH families, in which both deletional and insertional mutations have occurred in exactly the same region. Although many deletional and insertional mutations causing human genetic disease have been reported, it is very rare that both deletional and insertional events have occurred in the same region in one gene (Cooper and Krawczak 1991; Krawczak and Cooper 1991). We discuss a possible mechanism that generated this mutant LDL receptor gene.

Subjects and methods

In family SK and family FT in which a mutant LDL receptor gene was analyzed, primary hypercholesterolemia (plasma cholesterol levels above 300 mg/dl) cosegregated with a restriction fragment length polymorphism (RFLP) haplotype of the LDL receptor gene. Achilles tendon xanthoma (11-mm thickness in radiography) was observed in the proband of family SK. These two families are apparently unrelated but live within the same prefecture near Tokyo.

Each exon of the LDL receptor gene was amplified from the genomic DNA using the polymerase chain reaction (PCR) technique (Saiki et al. 1988) with the oligonucleotide primers proposed by Leitersdorf et al. (1990). Single strand conformation polymorphism (SSCP) analysis was carried out essentially according to the method described by Orita et al. (1989). PCR products containing exon 8 of the proband of family SK and a normal control subject were subcloned into the pCR vector using a TA Cloning Kit (Invitrogen), and both strands from several independent clones were sequenced by the dideoxy chain-termination method (Sanger et al. 1977). Allele specific oligonucleotide (ASO) hybridization was performed using two 22-mer probes, one specific for the normal sequence (5′-AACCTGGAGGGTGGCTACAAGT-3′), the other specific for the mutant sequence(5′-GAACCTGGCACTGAA-CAAGTGC-3′). Membranes with dot-blotted amplified DNA fragments containing exon 8 were hybridized to (^{32}P)-end-labeled ASO probes in 6× SSC, 5× Denhardt's solution, 0.5% SDS, and 200 μg/ml salmon sperm DNA at 55°C for 4 h, and then washed in 6 × SSC, 0.1% SDS at 65°C for 15 min. The RFLP haplotypes at

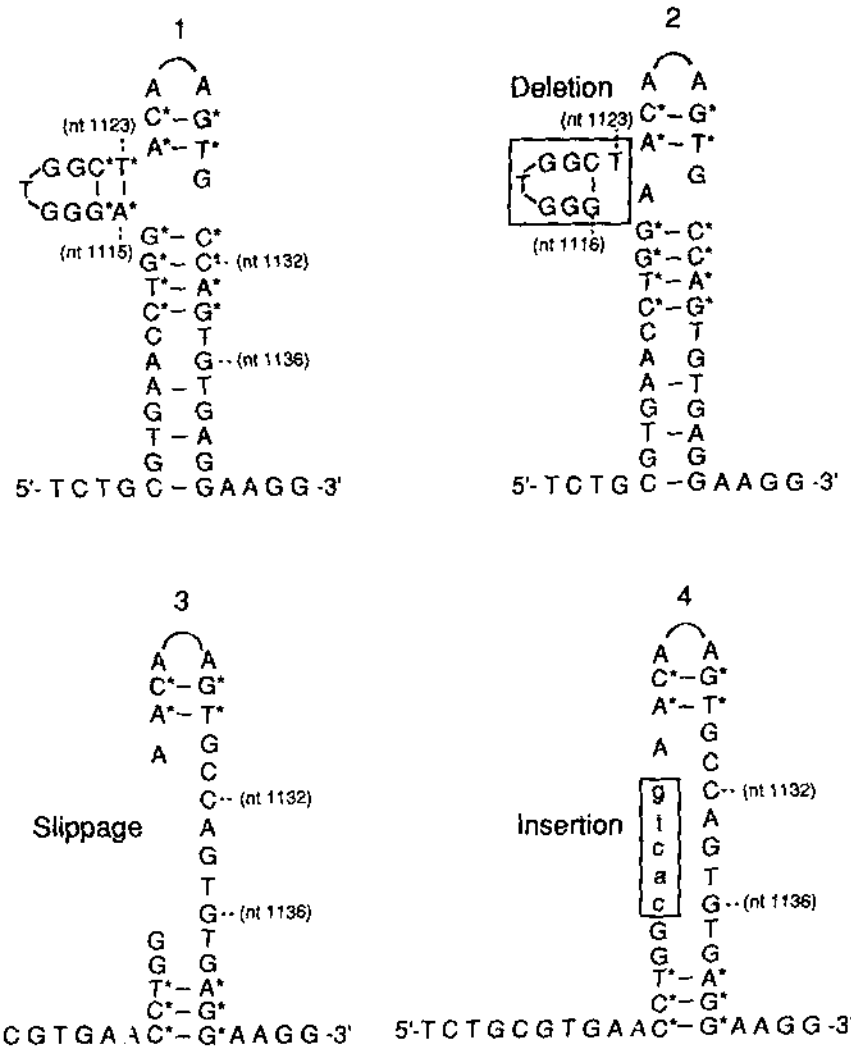


Fig. 3. Proposed mechanism for the generation of the mutant LDL receptor gene. *1* Hairpin-loop structure formation mediated by some inverted repeat sequences. *2* Deletion of eight bases within the hairpin-loop structure. *3* Occurrence of a five base slippage within the hairpin-loop structure. *4* Insertion of five bases in the hairpin-loop structure. The deleted bases and the inserted bases are surrounded by a *box*. The inverted repeat sequences are indicated by *asterisks*. The numbering of the nucleotides is according to Yamamoto et al. (1984)

the LDL receptor locus were determined according to the method of Yamakawa et al. (1991). Concentrations of fasting plasma cholesterol, triglyceride and HDL cholesterol were measured enzymatically. Achilles tendon thickness was measured radiographically by the method of Blankenhorn and Meyers (1969).

Results and discussion

When the LDL receptor genes from the two probands of family SK and family FT were analyzed by SSCP analysis, the same electrophoretic mobility shift was detected in the DNA fragments containing exon 8, but this could not observed in 23 other probands of heterozygous FH families (data not shown). To identify the nucleotide change responsible for the electrophoretic mobility shift, DNA fragments containing exon 8 of the proband from family SK and a normal control subject were amplified by PCR, cloned in the pCR vector, and sequenced. In two of five independent clones from the proband of family SK, a complicated nucleotide substitution was detected. Figure 1 shows a part of the results of the nucleotide sequence analysis in exon 8 from clones containing the mutant allele and the normal allele, respectively. In the mutant LDL receptor gene, the nine normal bases from nucleotide (nt) 1115 to nt 1123 (AGGGTGGCT) are replaced by six different bases (CACTGA), and consequently the four amino acids from codons 351 to 354, Glu-Gly-Gly-Tyr, are replaced by three amino acids, Ala-Leu-Asn.

Exon 8 encodes the second cysteine-rich growth factor repeat (repeat B) in the EGF precursor homology domain of the LDL receptor gene, and the amino acid substitutions of this mutant LDL receptor occur within a conserved 12 amino acid region in repeat B, which is proposed to contain a potential aspartate and/or aspargine hydroxylation site (Esser et al. 1988). In addition, previous studies on the mutant LDL receptor have suggested that the glycines in the EGF precursor homology domain are important for the folding of the LDL receptor (Esser and Russell 1988; Hobbs et al. 1990). The mutation in the LDL receptor gene detected in the proband of family SK causes the exchange of two glycine residues to other amino acids in addition to one amino acid deletion. This mutation was also detected in the proband of family FT.

The ASO hybridization analysis revealed that only hypercholesterolemic members were heterozygous for the mutant allele and vice versa in families SK and FT (Fig.

2). Furthermore, this mutation was not detected in 86 unrelated Japanese normocholesterolemic subjects. These findings suggest that this complex mutation in the LDL receptor gene causes FH in families SK and FT. Analysis of seven RFLPs at the LDL receptor gene locus revealed that haplotype 2 designated by us (Yamakawa et al. 1991) was associated with the mutant LDL receptor gene in both families (Fig. 2A), suggesting that the mutant LDL receptor genes of these two families have a common origin.

There are several inverted repeat sequences around the region of this complex nucleotide substitution of the mutant LDL receptor gene (Fig. 3). As a generative mechanism for this complex mutation, we propose that these inverted repeat sequences formed a hairpin-loop structure during replication and that both the deletional and the insertional events happened within this hairpin-loop structure (Fig. 3) In the proposed mechanism, the nucleotide exchange of the nine normal bases to the six different bases occurred as the combination of two events, an 8-bp deletion and a 5-bp insertion. In our model the deletion of the eight bases from nt 1116 to nt 1123 (GGGTGGCT) occurred by the formation of a small side loop structure, although the A residue at nt 1115 has not been deleted. Since the inserted sequence (CACTG) corresponds to the inverted sequence of that from nt 1132 to nt 1136 (CAGTG), these sequences from nt 1132 to nt 1136 could template the misincorporation of the novel five bases (CACTG) when the slippage mediated by the other inverted sequences occurred within this hairpin-loop structure (Fig. 3).

The data described in the present study seem to support the hypothesis that the hairpin-loop structure formation mediated by inverted repeat sequences within the same DNA strand influences the stability of a given gene and promotes the generation of human gene mutations (Cooper and Krawczak 1991; Krawczak and Cooper 1991). The molecular characterization of the many mutant LDL receptor genes will give us information regarding the generative mechanism that underlies naturally occurring human gene mutation.

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References

Blankenhorn DH, Meyers HI (1969) Radiographic determination of Achilles tendon xanthoma size. Metabolism 18:882–886

Cooper DN, Krawczak M (1990) The mutational spectrum of single base-pair substitutions causing human genetic disease: patterns and predictions. Hum Genet 85:55–74

Cooper DN, Krawczak M (1991) Mechanisms of insertional mutagenesis in human genes causing genetic disease. Hum Genet 87:409–415

Esser V, Russell DW (1988) Transport-deficient mutations in the low density lipoprotein receptor. J Biol Chem 263:13276–13281

Esser V, Limbird LE, Brown MS, Goldstein JL, Russell DW (1988) Mutational analysis of the ligand binding domain of the low density lipoprotein receptor. J Biol Chem 263:13282–13290

Goldstein JL, Brown MS (1989) Familial hypercholesterolemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th edn. McGraw-Hill, New York, pp 1215–1250

Hobbs HH, Russell DW, Brown MS, Goldstein JL (1990) The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. Annu Rev Genet 24:133–170

Hobbs HH, Brown MS, Goldstein JL (1992) Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat 1:445–466

Krawczak M, Cooper DN (1991) Gene deletions causing human genetic disease: mechanisms of mutagenesis and the role of the local DNA sequence environment. Hum Genet 86:425–441

Leitersdorf E, Tobin EJ, Davignon J, Hobbs HH (1990) Common low-density lipoprotein receptor mutations in the French Canadian population. J Clin Invest 85:1014–1023

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467

Yamakawa K, Yanagi H, Saku K, Sasaki J, Okafuji T, Shimakura Y, Kawai K, Tsuchiya S, Takada K, Naito S, Arakawa K, Hamaguchi H (1991) Family studies of the LDL receptor gene of relatively severe hereditary hypercholesterolemia associate with Achilles tendon xanthomas. Hum Genet 86:445–449

Yamakawa-Kobayashi K, Kobayashi T, Saku K, Arakawa K, Hamaguchi H (1993) Two novel frameshift mutations associated with the presence of direct repeats of the LDL receptor gene in familial hypercholesterolemia. Hum Genet 92:331–335

Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russell DW (1984) The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA. Cell 39:27–38