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Novel and Recurrent Mutations of the LDL Receptor Gene in Korean Patients with Familial Hypercholesterolemia

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We have identified 16 different mutations of the low-density lipoprotein receptor (*LDLR*) gene in 25 unrelated Korean patients with heterozygous familial hypercholesterolemia (FH), including five novel mutations, C83Y, 661del17, 1705insCTAG, C675X, and 941-1G>A. The 1705insCTAG mutation in which the four 3′-terminal nucleotides of exon 11 are duplicated was found to prevent splicing of exon 11 and would therefore generate a truncated polypeptide. The in-frame 36-bp deletion (1591del36) in exon 11, which had been reported only in one Korean FH patient, was also found. We showed that this change affects transport of the LDL receptor from the endoplasmic reticulum to the cell surface. In addition, we found 8 mutations (-136C>T, E119K, E207K, E207X, F382L, R574Q, 1846-1G>A, and P664L) that had been described in other ethnic groups but not in Koreans, and 2 mutations (R94H and D200N) that had been described in Koreans as well as other ethnic groups. 5 mutations (1591del36, E119K, E207X, E207K, and P664L) were found more than once in the Korean FH samples. Identification of the novel and recurring *LDLR* mutations in Korean FH patients should facilitate prenatal and early diagnosis in families at high risk of FH.

Keywords: Familial Cholesterolemia; Korean Patients; LDL Receptor; Novel Mutations; Recurrent Mutations.

Introduction

Familial hypercholesterolemia (FH, MIM# 143890) is one of the most common single gene disorders, occurring at a frequency of about 1 in 500 in most populations (Brown and Goldstein, 1986). It is caused by defects in the low-density lipoprotein (LDL) receptor (Goldstein and Brown, 1973) that regulates plasma cholesterol by binding to LDL and inducing its endocytosis, mainly in liver. FH heterozygotes develop tendinous xanthomas, corneal arcus, and coronary artery disease. The coronary artery disease usually becomes evident in the fourth or fifth decade. FH homozygotes, which occur at a frequency of about 1 in a million, develop these features at an accelerated rate in addition to planar xanthomas, and often die of heart attacks in childhood (Hobbs *et al*., 1990). More than 900 different mutations of the LDL receptor (*LDLR*) gene have been described worldwide (Villeger *et al*., 2002). Databases for the mutations are accessible at http://www.ucl.ac.uk/fh (UK database) and http://www.umd.necker.fr (French database). In addition, familial defective apolipoprotein B100 (ApoB) (MIM# 144010) that is caused by defects in the ligand (ApoB) of the LDL receptor, is identical to FH phenotypically. Only a few mutations of the *ApoB* gene are known, including R3500Q (Soria *et al*., 1989), R3500W (Gaffney *et al*., 1995), R3531C (Pullinger *et al*., 1995), R3480P (Nissen *et al*., 1995), R3480W (Boren *et al*., 2001), and

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Abbreviations: ApoB, apolipoprotein B100; DHPLC, denaturating high performance liquid chromatography; FH, familial hypercholesterolemia; LDL, low-density lipoprotein; PAGE, polyacrylamide gel electrophoresis; SSCP, single-strand conformation polymorphism.

T3492I (Bednarska-Makaruk *et al*., 2001).

The mutations underlying FH in Koreans are largely unknown because there have been only a few studies (Chae *et al*., 1997; 1999; Kim *et al*., 1999; Shin *et al*., 2000). Here we describe 16 different mutations of the *LDLR* gene, including 5 novel mutations, identified in 25 unrelated Korean FH heterozygotes.

Materials and Methods

Subjects Heterozygous FH was diagnosed when a subject over 40 had total cholesterol above 290 mg/dl or when a subject below 20 had total cholesterol above 220 mg/dl, with tendon xanthoma and/or coronary heart disease, or with first-degree relatives who had hypercholesterolemia. Coronary heart disease was diagnosed when the subject had significant stenosis (> 50%) of at least one vessel on coronary angiography, or documented myocardial infarction. All patients and their relatives gave informed consent prior to this study.

DNA analysis Genomic DNA was extracted from whole blood (Sambrook *et al*., 1989). An optimized set of primers and PCR conditions was used to amplify the promoter region from base –244 relative to the translation start codon of the *LDLR* gene, as well as the 18 exons with their flanking intron sequences. PCR products were screened for the presence of mutations by non-isotopic simultaneous detection of single-strand conformation polymorphism (SSCP) and by heteroduplex formation (Lee *et al*., 1995). PCR products that gave aberrant electrophoresis patterns were analyzed by fluorescence cycle sequencing followed by sequencing on a Perkin Elmer ABI PRISM 310 sequencer (Applied Biosystems, USA). When a specific mutation changed the site of a restriction enzyme, it was confirmed by restriction enzyme analysis of an independent PCR product. If a specific mutation did not change a restriction enzyme site, the mutation was confirmed by SSCP/heteroduplex formation or denaturating high performance liquid chromatography (DHPLC) (Bodamer *et al*., 2002) of the PCR product.

Analysis of splicing of *LDLR* intron 11 in skin fibroblasts Skin fibroblasts were cultured from skin biopsy samples of patient P10 and a normal control (Delanian *et al*., 1998). cDNA was made by reverse transcription of polyadenylated RNA from the fibroblasts (Kim *et al*., 2001). A cDNA fragment neighboring intron 11 was amplified by PCR using the primer pair 5′-AAACATTCAGTGGCCCAATG-3′ (nt 1674-1693; the first nucleotide of the translation start codon being designated +1 in human *LDLR* cDNA) in exon 11 and 5′-GGAGTCAACCCAGTAGAGGC-3′ (nt 1740-1721) in exon 12, and the P10 fibroblast cDNA mixture as template. A fragment of the splicing junction of exon 11 and intron 11 was PCR-amplified using primer pair, 5′-AAACATTCAGTGGCCCAATG-3′ (nt 1674-1693) in exon 11 and 5′-TCTGTCCTCCAGCCTGTGCC-3′ (nt IVS11+55 to IVS11+36) in intron 11, and P10 genomic DNA as template. The PCR products were analyzed by 8% polyacrylamide gel electrophoresis (PAGE). An aberrant band formed in the PCR reaction with cDNA of patient P10 was cloned into pGEM-T vector (Promega, USA). Two independent clones were sequenced in both directions.

Site-directed mutagenesis and transient expression of LDL receptors in CHO-ldlA7 cells The 1591del36 mutation was introduced into pLDLR-4, an expression construct for the wild type LDL receptor (Yamamoto *et al*., 1984). The 5′-cDNA fragment (nt 673-1645), which contains a unique *Eco*RI site (nt 718) and the 36-nt deletion, was amplified by PCR using pLDLR-4 as template, *Pfu* DNA polymerase, and the primer pair, 5′-AAATCTGACGAGGAAAACT-3′ (nt 673-691) and 5′-CATTCAGGCCCCCTTTCTTGAAGCCATGAACA-3′ (nt 1645-1627 and 1590-1578). The 3′-cDNA fragment (nt 1572-1740) that contains the 36-nt deletion and a unique *Bgl*II site (nt 1704) was PCR-amplified using primer pair, 5′-GGATCCTGTTCATGGCTTCAAGAAAGGGGGCCTGAATG-3′ (nt 1572-1590 and 1627-1645 with the 36-nt deletion) and 5′-GGAGTCAACCCAGTAGAGGC-3′ (nt 1740-1721). The two PCR products have a 38 nucleotide overlap; they were mixed and PCR-amplified using the primer pair at their extremities. The PCR product (nt 673-1740) containing the 1591del36 mutation was digested with *Eco*RI and *Bgl*II and ligated into pLDLR-4 digested with the same restriction enzymes. The fragment inserted into the resulting plasmid, pLDLR-4-1591del36, was sequenced to avoid possible PCR errors.

LDL receptor-deficient CHO-ldlA7 cells (Krieger, 1983) were transiently transfected with pLDLR-4 and pLDLR-4-1591del36 using FuGene 6 (Roche Diagnostics, Switzerland). Two days after transfection the cells were fixed with a 1:1 mixture of acetone and methanol, and stained with a 1:50 dilution of a mouse monoclonal antibody against human LDL receptor (Cat. number P02, Oncogene, USA) and a 1:100 dilution of FITC-conjugated secondary antibody. LDL receptor proteins were examined by fluorescence microscopy (Model Axioskop; Zeiss, Germany) (Kim *et al*., 2004).

Statistical analysis We used the group t-test for comparing age, triglyceride, and total cholesterol, and the chi square test for sex ratio (SAS version 8.01; SAS Institute, USA), and accepted significance at an α level of 0.05.

Results

Identification of *LDLR* mutations in Korean FH patients Thirty-one unrelated Korean individuals diagnosed with FH were analyzed for defects of the *LDLR* gene by SSCP/heteroduplex analysis and DNA sequencing. Clinical and biochemical features of 25 patients, and their family members, in whom the molecular defects were characterized are shown in Table 1. The sixteen different mutations of the *LDLR* gene identified are shown in Table 2.

Table 1. Clinical and biochemical features of FH patients and their family members.

FH No.	Sex	Age	Position	T-Chol	TG	TX	CHD	Clinical Dx
P10	M	38	Proband	340	73	-	+	Affected
P11	F	37	Proband	378	117	+	-	Affected
P12	M	44	Proband	319	82	-	-	Affected
P12-1	M	12	Son	327	ND	-	-	Affected
P12-2	F	10	Daughter	217	ND	-	-	Affected
P12-3	M	20	Nephew	370	222	-	-	Affected
P13	M	38	Proband	348	236	-	-	Affected
P14	F	71	Proband	305	237	+	-	Affected
P15	M	76	Proband	350	69	+	+	Affected
P15-1	F	73	Wife	ND	ND	-	-	Unaffected
P15-2	F	55	Daughter	200	48	-	-	Unaffected
P15-3	M	35	Son	164	60	-	-	Unaffected
P15-4	F	39	Daughter	333	90	+	-	Affected
P15-5	F	40	Daughter	434	115	+	-	Affected
P15-6	M	20	Grandson	162	54	-	-	Unaffected
P15-7	F	22	Granddaughter	ND	ND	-	-	Unaffected
P15-8	F	24	Granddaughter	352	65	+	-	Affected
P16	F	21	Proband	262	95	+	-	Affected
P16-1	F	19	Sister	363	73	-	-	Affected
P16-2	F	48	Mother	235	49	-	-	Unaffected
P17	F	26	Proband	312	55	-	+	Affected
P17-1	F	55	Mother	227	180	-	-	Unaffected
P17-2	F	21	Sister	316	92	-	-	Suspected
P17-3	M	19	Brother	284	123	-	-	Suspected
P21	M	58	Proband	319	136	+	+	Affected
P21-1	F	54	Wife	202	164	-	-	Unaffected
P25	M	62	Proband	300	ND	+	+	Affected
P25-1	M	57	Brother	337	274	+	+	Affected
P25-2	F	32	Niece	407	102	+	-	Affected
P26	F	48	Proband	341	63	+	+	Affected
P26-1	F	24	Daughter	185	33	-	-	Unaffected
P28	M	47	Proband	352	197	+	+	Affected
P29	M	44	Proband	301	240	+	+	Affected
P31	M	52	Proband	424	115	+	-	Affected
P34	M	64	Proband	309	274	+	+	Affected
P35	F	59	Proband	316	ND	+	-	Affected
P35-1	M	24	Son	137	80	-	-	Unaffected
P40	F	53	Proband	306	124	+	-	Affected
P40-1	M	55	Husband	174	170	-	-	Unaffected
P40-2	M	30	Son	246	125	-	-	Affected
P40-3	M	29	Son	214	213	-	-	Suspected
P40-4	F	58	Sister	191	157	-	-	Unaffected
P41	F	54	Proband	307	45	-	-	Affected
P42	F	47	Proband	320	116	+	-	Affected
P45	F	54	Proband	346	69	-	-	Affected
P47	M	33	Proband	322	198	+	+	Affected
P48	M	43	Proband	295	114	-	+	Affected
P48-1	F	47	Sister	258	85	-	-	Affected
P48-2	F	19	Daughter	173	170	-	-	Unaffected
P48-3	M	16	Son	210	90	-	-	Affected
P48-4	M	23	Nephew	150	72	-	-	Unaffected
P50	M	35	Proband	343	68	+	-	Affected
P51	F	60	Proband	360	260	+	+	Affected
P51-1	M	35	Son	356	85	-	-	Affected
P51-2	M	37	Son	206	74	-	-	Unaffected
P52	F	33	Proband	249	107	+	-	Affected

T-Chol, total cholesterol (mg/dl); TG, triglyceride (mg/dl); Clinical Dx, clinical diagnosis; TX, tendon xanthoma; CHD, coronary heart disease. ND, not determined.

Table 2. Summary of the *LDLR* mutations identified in this study.

Group[1]	Designation[2]	Nucleotide change[3]	Probands and family members	Second detection[4]	Reference
I	C83Y	311G>A	P29	Other	-
	661del17	661.677del	P41	Size	-
	941-1G>A	IVS6-1G>A	P15, P15-4, P15-5, P15-8	*Nla*IV (-)	-
	1705insCTAG	1702.1705dupCTAG	P10	Size	-
	C675X	2088C>A	P50	*Dde*I (+)	-
II	1591del36	1591.1626del	P12, P12-1, P12-2, P12-3, P17, P17-2, P17-3, P28, P35	Size	Chae *et al*. (1999)
III	–136C>T	-43C>T	P52	Other	Koivisto *et al*. (1994)
	E119K	418G>A	P16, P16-1, P40, P40-2, P51, P51-1	*Mnl*I (-)	Jensen *et al*. (1994)
	E207K	682G>A	P11, P14	*Mnl*I (-)	Leitersdorf *et al*. (1990)
	E207X	682G>T	P25, P25-1, P25-2, P31, P48, P48-1, P48-3	*Mnl*I (-)	Hobbs *et al*. (1992)
	F382L	1207T>C	P42	Other	Hattori *et al*. (1999)
	R574Q	1784G>A	P47	*Hpa*II (-)	French db
	1846-1G>A	IVS12-1G>A	P26	*Mse*I (+)	Jensen *et al*. (1996)
	P664L	2054C>T	P13, P21	*Pst*I (+)	King-Underwood *et al*. (1991)
IV	R94H	344G>A	P45	Other	French db; UK db
	D200N	661G>A	P34	Other	Ebhardt *et al*. (1999); UK db

[1] I, Novel; II, Described in Korean but not in other ethnic groups; III, Described in other groups but first in Korean; IV, Described in Korean and other groups.

[2] Named according to the amino acid numbering of Yamamoto *et al*. (1984).

[3] Nomenclature at the DNA level, according to international nomenclature and the Nomenclature Working group (Villeger *et al*., 2002).

[4] Mutations were confirmed by restriction enzyme digestion (+ or – indicating presence or absence of the restriction site), sizing (Size) in a gel electrophoresis, and other methods (Other) including SSCP, DHPLC, and sequencing of a second PCR product.

Novel mutations of the *LDLR* gene Among the *LDLR* mutations, 5 (C83Y, 661del17, 941-1G>A, 1705insCTAG, C675X) were novel, having not been described in other populations (Group I of Table 2). The C83Y missense mutation found in exon 3 of P29 was confirmed by an abnormal pattern in the DHPLC analysis of an independent exon 3 PCR product (Fig. 1A). The 661del17 mutation found in exon 4 of P41 was confirmed by the size of the PCR product corresponding to the 3′ half of exon 4 (Fig. 1B). The 941-1G>A mutation was located at the 3′-splice junction of the intron 6-exon 7 boundary in P15. Since it abolishes an *Nla*IV restriction enzyme site, *Nla*IV RFLP analysis was used to confirm the presence of the mutation, and the affected family members were successfully screened by this method (Fig. 1C). The 1705insCTAG mutation was found at the 3′-end of exon 11 in P10 and confirmed by the size of the exon 11 PCR product (Fig. 1D). The C675X nonsense mutation identified in exon 14 of P50 generates a *Dde*I restriction enzyme site, and was confirmed by *Dde*I RFLP analysis of the exon 14 PCR product of P50 (Fig. 1E).

The 1705insCTAG mutation in P10 has a 4-bp insertion at the 3′ end of exon 11. The abnormal allele of the patient was evident from the PCR products of the exon 11/intron 11 junction obtained with P10 genomic DNA as template, since there was a 91-bp band in addition to the 87-bp band from the normal allele (Fig. 2). We used RT-PCR to see whether the 4-bp insertion affected splicing of intron 11. As shown in Fig. 2A, amplification of the cDNA from P10 using exon 11 and exon 12 as primer pair produced an aberrant 0.7-kb band as well as the 67-bp band from the normal transcript. DNA sequencing showed that the aberrant band consisted of a 717-bp fragment containing the entire intron 11 (Fig. 2B). This result indicates that the 1705insCTAG mutation impairs the splicing of intron 11 in *LDLR* precursor RNA.

A recurrent *LDLR* mutation found only in Korean FH patients The 1591del36 mutation, which had been reported only in one Korean FH patient (Chae *et al.*, 1999), was detected in 4 patients (P12, P17, P28, and P35) in this study (Group II of Table 2). In an attempt to understand the molecular pathology of the mutation, LDL receptor-deficient CHO cells were transiently transfected with wild

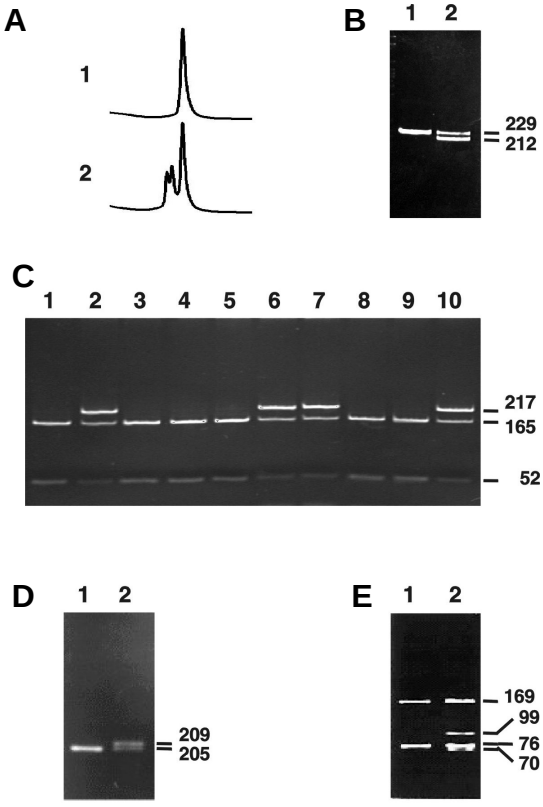

Fig. 1. Detection of five novel mutations in patients P29, P41, P15, P10, and P50. **A.** C83Y mutation. Exon 3 PCR products from a normal control (panel 1) and patient P29 (panel 2) were analyzed by DHPLC. **B.** 661del17 mutation. The 3′-half exon 4 PCR products from a normal control (lane 1) and patient P41 (lane 2) were analyzed by PAGE. The normal and mutant alleles appear as 229-bp and 212-bp fragments, respectively. **C.** 941-1G>A mutation. The 217-bp exon 7 PCR products from a normal control (lane 1) and patient P15 (lane 2) were cleaved with *Nla*IV and analyzed by PAGE. The normal allele forms 165-bp and 52-bp fragments while the mutant allele forms a 217-bp fragment. The *Nla*IV cleavage products of the exon 7 PCR products derived from P15 family members are presented (lanes 3–10; P15-1–8). **D.** 1705insCTAG mutation. The exon 11 PCR products from a normal control (lane 1) and patient P10 (lane 2) were analyzed by PAGE. The normal and mutant alleles generate 205-bp and 209-bp fragments, respectively. **E.** C675X mutation. The 245-bp exon 14 PCR products from a normal control (lane 1) and patient P50 (lane 2) were cleaved with *Dde*I and analyzed by PAGE. The normal allele forms 169-bp and 76-bp fragments, the mutant allele 99-bp, 76-bp, and 70-bp fragments.

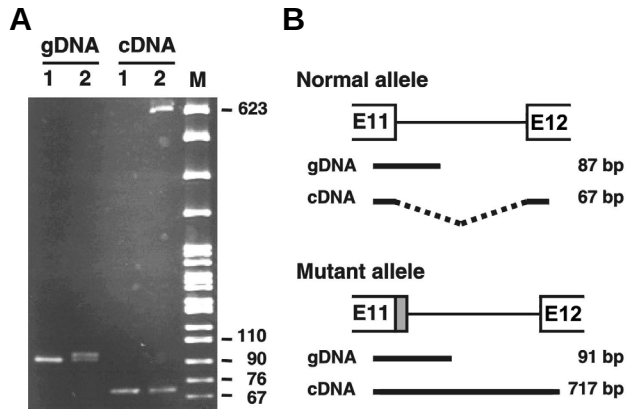

Fig. 2. Splicing of intron 11 in skin fibroblasts of patient P10. **A.** Analysis of the splicing of intron 11 by PCR. Genomic DNA (gDNA) from a normal control (lane 1) and patient P10 (lane 2) was amplified by PCR with a pair of primers located in exon 11 and intron 11. cDNAs from skin fibroblasts of a normal control (lane 1) and patient P10 (lane 2) were also amplified by PCR using primers in exons 11 and 12. The PCR products were analyzed by 8% PAGE. **B.** Schematic representation of the PCR products of the normal and mutant alleles of patient P10. Exons 11 and 12, the 4-bp insertion, and intron 11 are shown as open boxes, a shaded box, and a thin horizontal line, respectively. The thick horizontal lines represent the regions amplified by PCR. A dotted line indicates the spliced sequence.

type and mutant cDNAs and the intracellular location of the LDL receptor molecules was examined by immunofluorescence microscopy. The wild type LDL receptors were found primarily on the cell surface (Fig. 3A) whereas the mutant LDL receptor molecules formed a reticular pattern throughout the cytoplasm, with the highest intensity in the perinuclear region (Fig. 3B). This result suggests that the mutant LDL receptor molecules accumulate in the endoplasmic reticulum.

***LDLR* mutations reported in other populations** Eight mutations, -136C>T in the promoter, E119K, E207K, and E207X in exon 4, F382L in exon 9, R574Q in exon 12, 1846-1G>A at the junction of intron 12/exon 13, and P664L in exon 14, had been reported previously in other ethnic groups, but were found in Koreans for the first time in this study (Group III of Table 2). Two mutations, R94H and D200N in exon 4, which had been reported in Koreans as well as other ethnic groups, were detected once more in this study (Group IV of Table 2).

Statistical analysis of FH patients and their family members A total of 31 relatives of the 25 FH patients were available for genotyping. Of these, 15 harbored the same mutations as the probands of their families. Neither age nor male/female sex ratio differed significantly between patients and their affected relatives (n = 40), on the one hand, and the relatives (n = 16) in which the mutations were not present, on the other (average ± standard deviation, 40.5 ± 16.6 vs. 39.4 ± 17.3 years old, $p > 0.05$; range, 10–76 vs. 19–73 was significantly different between two groups (20:20 vs. 7:9, $p > 0.05$). Serum triglyceride levels were not significantly different (128.6 ± 68.6 vs. 108.9 ± 62.4 mg/dl, $p > 0.05$; range, 45–274 vs. 33–

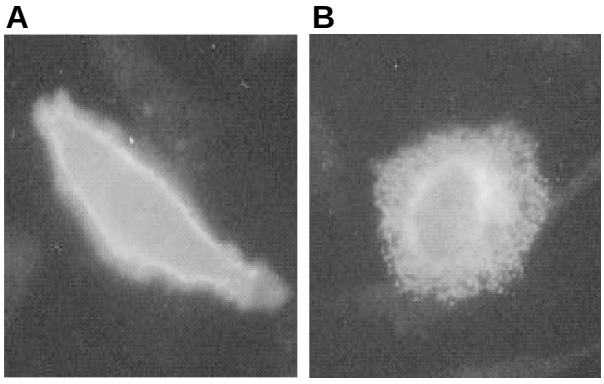

Fig. 3. Immunofluorescence staining of LDL receptor-deficient cells transfected with *LDLR* cDNA containing the 1591del36 mutation. Expression constructs for the wild type and 1591del36 LDL receptors were transiently transfected into LDL receptor-deficient CHO-ldlA7 cells, and the transfected cells fixed. The LDL receptor was stained with mouse anti-human LDL receptor monoclonal antibody (Cat. number LP02, Oncogene)/FITC-conjugated anti-mouse IgG antibody. Typical cells that express wild type LDL receptor (A) and the 1591del36 LDL receptor (B) are shown.

213 mg/dl), but the serum total cholesterol levels of the affected group were significantly higher than those of the unaffected group (323.4 ± 48.5 vs. 187.1 ± 28.8 mg/dl, $p < 0.0001$; range, 210–434 vs. 137–235 mg/dl) as expected.

Discussion

Five novel mutations were identified in our study of Korean patients with FH. The C83Y mutation, which was found in P29, was the only missense mutation among the novel mutations. The C83 residue is one of the 6 cysteines forming 3 intra-repeat disulfide bonds in repeat 2 of the ApoB-binding domain (Hobbs *et al.*, 1990), and the C83Y mutation substitutes the structurally important cysteine with a tyrosine residue. The C83F (311G>T) mutation that replaces the same cysteine residue with phenylalanine has been found as a pathological mutation in Britain, Northern Ireland, and Austria (UK and French databases). Presumably therefore the C83Y mutation is also damaging due to defective ApoB binding (Class 3 binding-defective mutation). The 661del17 mutation found in P41 deletes the 5 codons from D200 to K204 and two nucleotides of S205. Since the first codon generated by the frameshift is a TGA stop codon, the polypeptide would be truncated at P199. The 941-1G>A mutation in P15 occurs at the splice acceptor site at the intron 6/exon 7 boundary. Like other mutations at splice acceptor sites (Lombardi *et al.*, 1995; Nissen *et al.*, 1997), it should result in skipping exon 7. 1705insCTAG in P10 was found to have a defect in splicing of intron 11 (Fig. 2). Why does the 4-bp insertion at the 3′-end of exon 11 give rise to the splice mutation? We found that the insertion appeared to generate additional secondary structure in the pre-spliced *LDLR* RNA; ACCCTAG**CTAG**gt (uppercase and lowercase letters: 3′-end of exon 11 and 5′-end of intron 11, respectively; bold letters: the 4-bp inserted sequence, and underlines: nucleotides involved in the secondary structure). Thus, one possibility is that this secondary structure prevents base-pairing of the 5′-splice junction of intron 11 and U1 snRNA required for mRNA splicing (Mount *et al.*, 1983). The C675X mutation in P50 creates a premature stop codon at Cys675. Thus the polypeptides encoded by the 661del17, 941-1G>A, 1705insCTAG, and C675X alleles should each be truncated and non-functional.

The 1591del36 mutation previously reported only in a Korean patient (Chae *et al.*, 1999) was the most frequent mutation (16%) in this study. The polypeptide encoded by 1591del36 is expected to have an in-frame deletion of 12 amino acid residues (M510-I521) in the EGF spacer domain. As anticipated, it could be detected with anti-LDL receptor antibody. However, whereas the wild type protein was found primarily on the cell surface, the 1591del36 protein formed a pattern characteristic of ER localization (Fig. 3). Cells expressing the 1591del36 protein tended to be round, probably because of accumulation of 1591del36 in the ER. These results indicate that 1591del36 is a Class 2 transport-defective mutation.

In addition to 1591del36, mutations E119K (12%), E207X (12%), E207K (8%), and P664L (8%) that had not been reported in Koreans also occurred repeatedly, while R94H and D200N were identified only once in this study but had been previously reported in Korea (UK database). Therefore, these must be added to the mutations that recur in Koreans. Moreover, the F382L and R574Q mutations that were found in this study had been reported only in Japan (Hattori *et al.*, 1999; French database). E119K has been reported in Japanese as well as other ethnic groups (Maruyama *et al.*, 1995) and E207K in China as well as other countries (Mak *et al.*, 1998). It is thus presumed that these mutations not only recur in Koreans but also in other Oriental races.

In the Korean patients with FH in which *LDLR* mutations were not found, we screened for the presence of *ApoB* mutations. For this, a DNA fragment that encodes the S3455-K3541 region of ApoB-100 was amplified by PCR and examined by SSCP/heteroduplex analysis. However, we did not detect any mutations in the *ApoB* gene. The failure to detect mutations in these screened probands may be due to limitations of our screening methods, such as the occurrence of nucleotide changes outside the examined regions, or in other genes.

In this study we identified novel and recurring mutations of the *LDLR* gene in Korean patients with FH. Our results, together with genetic counseling, could lead to earlier diagnosis and better management of family mem-

bers at high risk of FH.

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