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Interaction between the LDL-receptor gene bearing a novel mutation and a variant in the apolipoprotein A-II promoter: molecular study in a 1135-member familial hypercholesterolemia kindred

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Abstract Lipid and lipoprotein concentrations in plasma generally reflect complex influences of multiple genetic loci. Even an autosomal dominant disorder, familial hypercholesterolemia (FH), is characterized by phenotypic heterogeneity, as low-density lipoprotein (LDL) levels vary widely within the same pedigree. Molecular screening for LDL receptor (*LDLR*) mutations among 75 patients with clinically apparent FH led to identification of a novel splice-site mutation (IVS14+1 G>A) shared by 14 patients. Genealogical research confirmed that all 14 carriers were part of the same 1135-member pedigree with a common ancestor. The mutation resulted in an abruptly truncated LDLR protein, reducing functional LDLR activity by half in heterozygous carriers of the mutant allele. Of the 208 members of the kindred who were screened for the presence of this *LDLR* mutation, we identified 94 carriers and 114 noncarriers. Nine principal apolipoprotein genes that might affect LDL cholesterol differentially according to LDL-receptor status were examined in this pedigree. Strikingly lower total cholesterol and LDL-cholesterol values were observed among the majority of the *LDLR* mutation carriers who were simultaneously homozygous for the −265C variant of *apoA-II* (total cholesterol: 324 ± 8 vs 244 ± 19 mg/dl, $P = 0.0015$; LDL-cholesterol: 237 ± 8 vs 155 ± 18 mg/dl, $P = 0.0008$). In vitro transfection assays showed that transcriptional activity of the *apoA-II* promoter was reduced by 30% in the −265C variant as compared with the −265T variant. We thus concluded that one variant of the *apoA-II* gene was associated with reduced plasma LDL cholesterol only in FH patients.

Key words Familial hypercholesterolemia · Apolipoprotein A-II · LDL receptor · Modifier gene · Gene interaction

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In memory of Professor Roger R. Williams, who died in an airplane accident on a MEDPED mission to the WHO, Geneva, Switzerland.

Introduction

Lipid and lipoprotein concentrations in plasma generally reflect the complex influences of multiple genetic loci (Zannis and Breslow 1985; Breslow 1987). Only a few examples of monogenic hyperlipidemia are known, familial hypercholesterolemia (FH) being the prototypic example (Goldstein et al. 1995). FH is a common autosomal dominant disorder, with a prevalence of 1/500 in the general population, and it is associated with high risk of cardiovascular disease (Goldstein et al. 1995). Usually, FH results from a mutation in the coding region of the low-density lipoprotein (LDL) receptor (*LDLR*) gene, with heterozygotes manifesting approximately twofold elevations in plasma LDL cholesterol. However, even FH is characterized by phenotypic heterogeneity, as LDL levels can vary widely within the same pedigree (Pullinger et al. 1992; Vega et al. 1991; Wu et al. 2000). FH patients may show increased plasma levels of triglyceride-rich lipoproteins in a variable spectrum that depends, in part, on the *apoE* genotype (Emi et al. 1991; Hopkins et al. 1991; Carmena et al. 2000). Potential influences of additional genetic factors on the variability and severity of FH, such as modifier genes interacting with the *LDLR* mutation, have been assumed but rarely demonstrated (Emi et al. 1991; Hopkins et al. 1991).

Through a combined genealogical and molecular approach, we ascertained, to our knowledge, the largest known genealogically connected FH kindred (K653), with over 60 000 estimated members descended from a com-

mon ancestor in the 18th century. This pedigree provided a unique opportunity to examine effects on plasma lipoprotein levels of genetic variations among genes involved in lipoprotein metabolism, including the LDL receptor and nine apolipoprotein loci.

Subjects and methods

Patients and family members

Probands of 75 hypercholesterolemic families with clinically apparent FH were identified by the Cardiovascular Genetics Research Clinic at the University of Utah, using criteria developed and utilized by the MEDPED program (Make Early Diagnosis, Prevent Early Death in MEDical PEDigrees) (Day and Hopkins 1998; Williams et al. 1993). MEDPED is a humanitarian organization whose goal is to identify and help persons with FH throughout the world. Of the 75 initial subjects, 14 were found to share a novel LDLR mutation. Many of the relatives of these 14 individuals had already been identified and diagnosed clinically through the efforts of the Utah MEDPED program. Indeed, branches of the pedigree were known to extend into ten western states. Guided by the results of a molecular LDLR-screening program and extensive genealogical research, we assembled an integrated pedigree, K653, which included all 14 identified carriers of the novel mutation and their screened relatives. Ultimately, genomic DNA was available from 208 members of this pedigree. Examinations included physicals, anthropometric measurements, medical history questionnaires for information on coronary heart disease and risk factors, and a blood draw. This study was approved by the Institutional Review Board of the University of Utah, and all participants provided informed consent.

Single-strand conformational polymorphism and reverse transcription-polymerase chain reaction

Isolation of genomic DNA and single-strand conformational polymorphism (SSCP) experiments were carried out as previously described (Nakazawa et al. 2001; Harada et al. 2001). Polymerase chain reaction (PCR) amplification of 20 ng of genomic DNA from each subject was carried out using primers designed to amplify each exon and the exon–intron boundaries of *LDLR*. (Nakazawa et al. 2001; Leitersdorf et al. 1990; Leren et al. 1993). The amplified products were analyzed by electrophoresis (Yoshida et al. 2001).

Leukocytes were isolated from 7.5 ml of peripheral blood from two K653 FH patients and three control subjects, using red blood cell lysis buffer (Gentra Systems, Minneapolis, MN, USA). RNA was isolated from these materials with TRIZOL Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RT-PCR experiments were performed as described previously (Mine et al. 2001) using gene-specific primers designed to amplify a fragment including exon 13 to exon 16 of the *LDLR* gene, as follows: forward primer 5′-TTTGGACAGATATCA TCAACGAAGC-3′ (exon 13), reverse primer 5′-AGTAGCGTGAGGGCTCTGTCC-3′ (exon 16).

Flow-cytometric functional analyses of the LDL receptor

Binding and uptake of fluorescently labeled LDL (DiI-LDL; Molecular Probes, Eugene, OR, USA) in primary cultured peripheral lymphocytes from two patients and three control subjects were analyzed by Fluorescence Activated Cell Sorter (FACS) flow cytometry (Hattori et al. 2002). Levels of LDL receptor protein on the surfaces of the lymphocytes were measured using a specific antibody and by flow cytometry, as described elsewhere (Hattori et al. 2002; Schmitz et al. 1993).

Genotyping of single-nucleotide polymorphisms on apolipoprotein genes

Nine major apolipoprotein genes were chosen as principal candidates for serving as "modifiers." For each gene, an appropriate single-nucleotide polymorphism (SNP) was selected from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/index.html) on the basis of its location, allele frequencies, and polymorphic characteristics (Table 1). SNP genotyping was performed by cycle resequencing of PCR products (Iwasaki et al. 2001).

Table 1. Associations between nine apolipoprotein genes and adjusted plasma lipid levels

Locus	/	SNP (NCBI dbSNP No.)	Allele frequencies	Adj TC	Adj LDL-c	Adj TG	Adj HDL-c
ApoA-I	/	−151C/T (rs5069)	0.72/0.28	NS	NS	NS	NS
ApoA-II	/	−265T/C (rs5082)	0.64/0.36	$P = 0.0015$	$P = 0.0008$	NS	NS
ApoA-IV	/	Q360H (rs5110)	0.78/0.22	NS	NS	NS	NS
ApoB-100	/	T98I (rs1367117)	0.77/0.23	NS	NS	NS	NS
ApoC-I	/	I16M (rs5112)	0.60/0.40	NS	NS	NS	NS
ApoC-II	/	K77Q (rs5126)	0.74/0.26	NS	NS	NS	NS
ApoC-III	/	−84C/T (rs5143)	0.66/0.34	NS	NS	NS	NS
ApoC-IV	/	L96R (rs5167)	0.65/0.35	NS	NS	NS	NS
ApoD	/	3′-UTR+70C>G (rs4651)	0.59/0.41	NS	NS	NS	NS

Differences between genotypic groups were assessed by Student's *t*-test and ANOVA with Sheffe's post hoc test
Adj, adjusted; TC, total cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; SNP, single-nueleotide polymorphism; NS, not significant

Plasmid construction

A 1006-bp fragment of the *apoA-II* promoter region that was sufficient to promote maximum transcriptional activity (Ribeiro et al. 1999) was PCR-amplified from peripheral leukocyte DNAs of individuals homozygous for either the −265T variant or the −265C variant. *Mlu*I recognition sites were introduced by oligonucleotide primers, in both 5′ and 3′ ends, and the promoter fragments were subcloned into the unique *Mlu*I site of the pGL2 basic vector (Promega, Madison, WI, USA). These two constructs were named pLUC−265T and pLUC−265C, respectively. The integrity and the sequence of each insert were verified by sequencing.

Luciferase assays

HepG2 cells were cultured according to regular protocols. One day prior to transfection, HepG2 cells were seeded in 6-well plates at 6.3×10^5 cells per well (7×10^4 cells/cm^2). Cells were transfected with 1.6 μg of plasmid DNA, using Polyfect reagent (Qiagen Valencia, CA, USA). Either one of the *apoA-II* promoter constructs, control pGL2-control, or pGL2-basic plasmids were used separately in each well. After 48 h of culture, cells were lysed with 100 μl of lysis buffer, and the total protein content was measured by Bradford's method for the supernatant. Luciferase activity was measured for the lysate containing equal amounts of total protein, using a LUMAT LB 9507 instrument (Berthold Technologies, Bad Wildbad, Germany). The promoter activity of each construct was evaluated by normalizing the luciferase activity in each experiment with that of cells transfected with empty pGL2-basic vector simultaneously cultured. Eight independent experiments were performed in duplicate, reproducibly. Results from two different preparations of plasmid DNA were identical.

Statistical analysis

Plasma lipoprotein levels were adjusted for sex and age of the subjects. Coefficients of skewness and kurtosis were calculated to test deviation from a normal distribution. Because the clinical and biochemical traits in each genotypic group were normally distributed, we applied an analysis of variance (ANOVA) with Scheffe's post hoc test and Student's *t*-test ($P < 0.05$). χ-squared tests were invoked to detect Hardy-Weinberg equilibrium. Data from assays of the *apoA-II* promoter were tested by Student's *t*-test, using the InStat 3 software package for Windows (GraphPad Software, San Diego, CA, USA).

Results

Extended FH kindred K653

The abridged pedigree shown in Fig. 1 indicates relationships among the examined pedigree members; the pedigree as a whole includes eight generations and 1135 individuals. We estimated the true size of the pedigree by calculating the average family size for each generation and adding the estimated number of descendants not displayed in the figure. To complete that effort, the roots of the pedigree were determined from genealogical records. Both members of the founding couple were born in the 1790s in the state of Vermont; they were married in 1813 and had 12 children, but the offspring of only four of those children (a total of 80 grandchildren) are included in Fig. 1.

Detection of an IVS14+1 G>A mutant of *LDLR*, causing aberrantly spliced transcripts, in 14 FH families

Of hypercholesterolemic probands from 75 Utah families with clinically diagnosed FH, 14 carried a G-to-A transition at the first nucleotide of the splicing-donor site of intron 14 (IVS14+1 G>A) of the *LDLR* gene. RT-PCR of the region from exon 13 to exon 16 in *LDLR* mRNA from these 14 patients revealed products that yielded an additional band of about 730 bp (Fig. 2A) as a result of insertion of a 214-bp intronic sequence between exons 14 and 15. This event involved activation of a cryptic splicing-donor site (GT) at IVS14+215,216 to the acceptor site of exon 15 (Fig. 2B), resulting in the reading-through of the intronic 214-bp sequence, creation of a premature stop codon at IVS14+99, and production of a truncated protein (724 amino acids) that lacked transmembrane and cytoplasmic domains. Assay of fluorescently labeled LDL and cell surface-bound LDL receptor in up-regulated peripheral lymphocytes revealed decreased LDL uptake (46%) and only 39% of the normal amount of cell-surface protein in heterozygous carriers of the novel mutation (Fig. 3). Most of these carriers presented high total cholesterol (316 ± 78 mg/dl) and LDL cholesterol levels (228 ± 75 mg/dl) but normal levels of high-density lipoprotein (HDL) cholesterol (41 ± 12 mg/dl) and triglyceride (185 ± 117 mg/dl). Corresponding data from noncarriers in K653 were normal (total cholesterol: 195 ± 43 mg/dl; LDL cholesterol: 117 ± 35 mg/dl; triglyceride: 150 ± 78 mg/dl; HDL cholesterol: 48 ± 13 mg/dl).

Mutational screening of 194 additional members of K653 identified a total of 94 heterozygous carriers of the same *LDLR* mutation, IVS14+1 G>A; the remaining 114 were verified to be noncarriers. Cosegregation and linkage of high plasma total cholesterol and LDL cholesterol levels with this *LDLR* mutation are indicated in the abridged pedigree chart (Fig. 1).

Intrafamilial association studies of potential modifier genes

We attempted an intrafamilial study to reveal potential allelic associations among various plasma lipoprotein variables, using amino acid-substituting or regulatory single-nucleotide polymorphisms (SNPs) present on nine principal apolipoprotein genes (Table 1). The determined genotypes of nine SNPs were analyzed logistically by testing

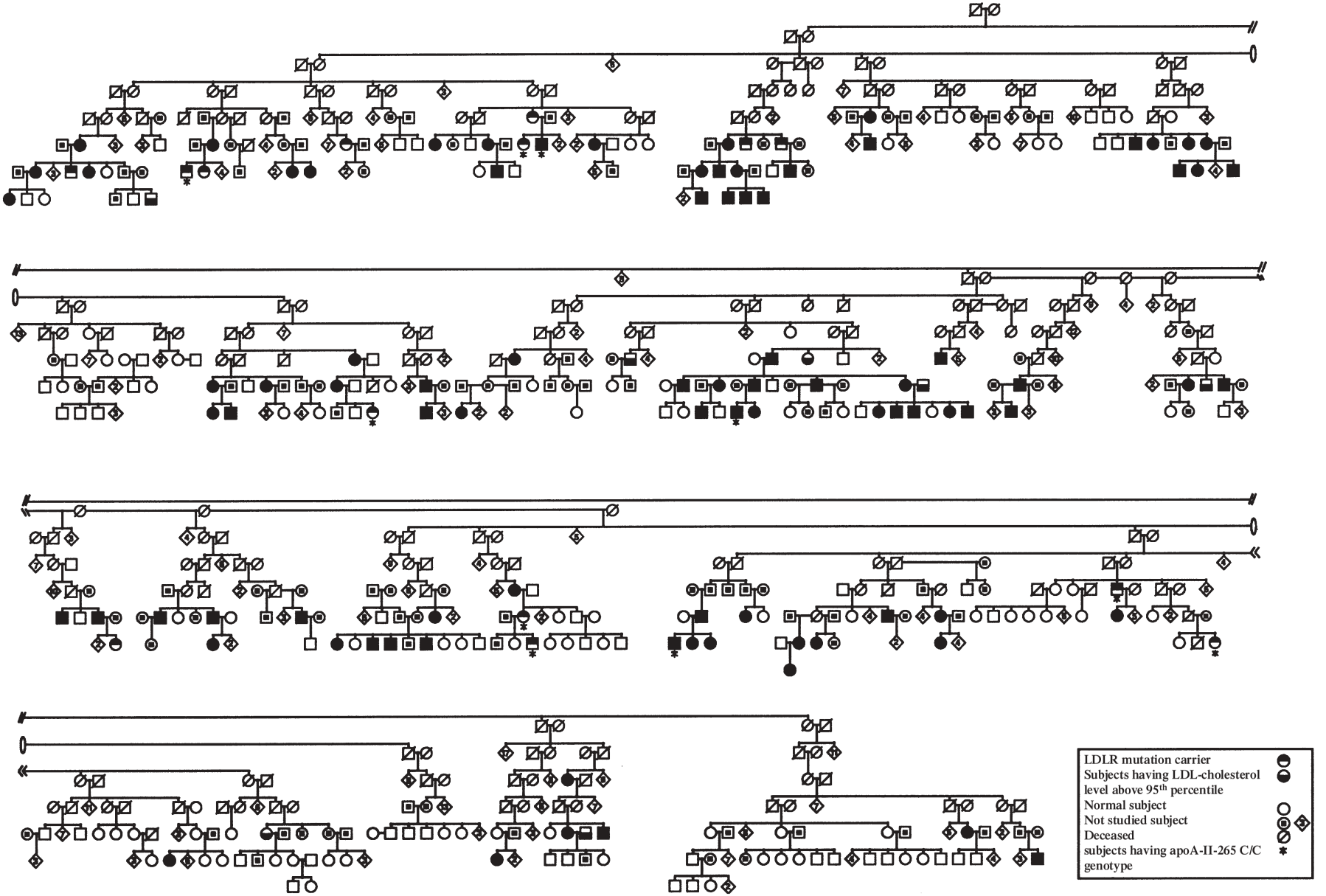

Fig. 1. Core of the 1133-member pedigree K653. *Filled symbols* (*circles* and *squares*) in the *upper half* indicate familial hypercholesterolemia (FH) patients diagnosed by phenotype, as defined by low-density lipoprotein (LDL)-cholesterol levels beyond the 95th percentile of the normal range. *Filled symbols* in the *lower half* indicate individuals found to carry a mutant allele of the LDL receptor (i.e., genotypically defined FH patients). *Asterisks* indicate ten *LDLR*-mutation carriers who were homozygous for the −265C variant of *apoA-II*

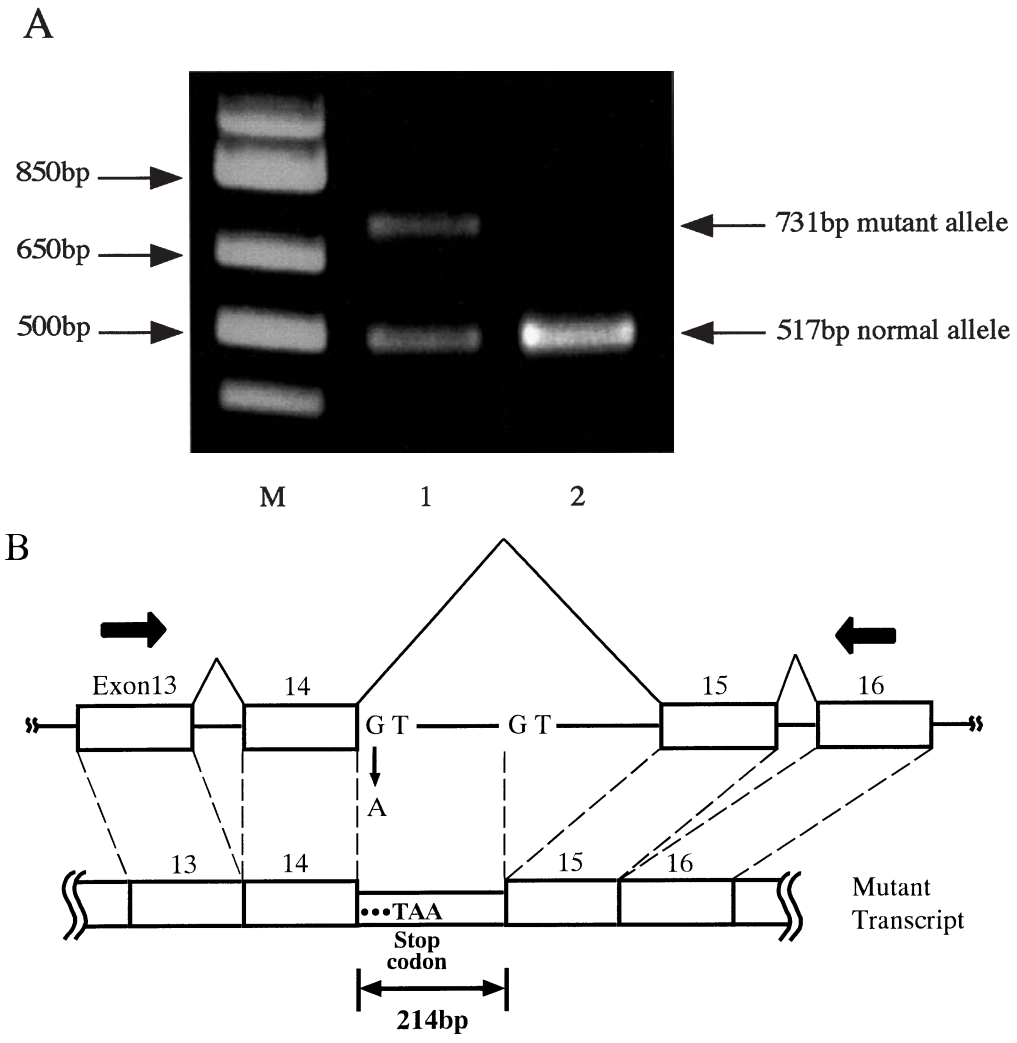

Fig. 2. **A** Reverse transcriptase-polymerase chain reaction (RT-PCR) products derived from exons 13 to 16 of the *LDLR* gene, represented as a single band (517 bp) for normal transcripts (*Lane 2*), and an additional, slower band (731 bp) for mutant transcripts (*Lane 1*). *M*, molecular size marker. **B** Schematic representation of the mutation, the abnormality in the transcript, and the structure of the mutant LDL receptor protein; the first nucleotide (G) in intron 14 is substituted by A (IVS14+1 G>A) in carriers of the mutant gene. *Arrows* indicate positions of the PCR primers. The abnormally spliced transcript and a *stop codon* (*TAA*) appearing at IVS+99 to +101nt are indicated below

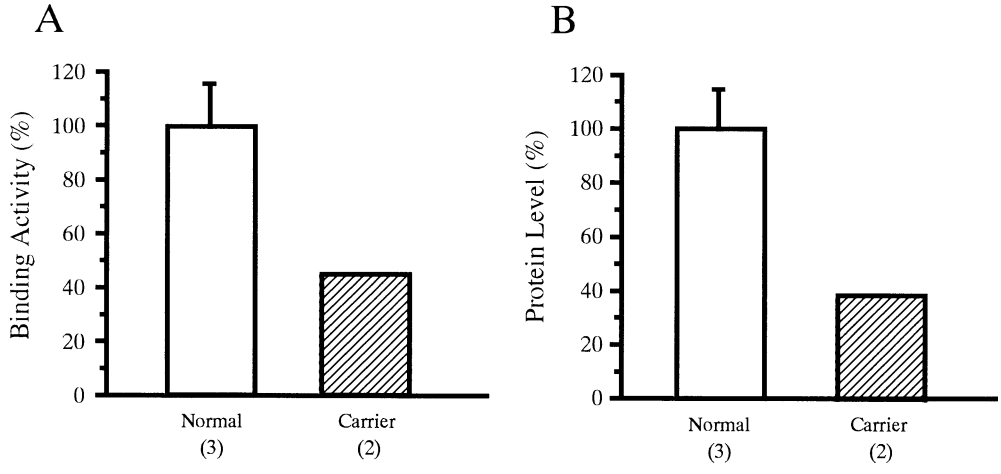

Fig. 3. LDL receptors expressed on peripheral lymphocytes from two carriers of the novel *LDLR* mutation, as analyzed by Fluorescence Activated Cell Sorter (FACS) flow cytometry. Relative binding activities (**A**) and relative protein levels (**B**) were calculated in percentiles for comparison with three normal subjects. Data were calculated by the following formula: Specific Fluorescence (%) = (Total Fluorescence 1 + Total Fluorescence 2) / 2 − Nonspecific Fluorescence

for correlation with plasma lipoprotein levels among the 94 carriers and 114 noncarriers of the *LDLR* mutation separately. Significant associations with total cholesterol and LDL cholesterol levels were identified for the T/C substitution at the −265 position in the regulatory region of *apoA-II* among the *LDLR*-mutation carriers. No association between remaining SNPs on the other eight candidate genes were observed with any lipoprotein variables, as shown in Table 1. When LDL cholesterol levels in the carriers were compared among the genotypic categories (C/C, C/T, T/T), the values were low among C/C homozygotes (mean ± SD = 155 ± 18 mg/dl), and high in T/T and T/C genotypic groups (242 ± 78 mg/dl) (Student's *t*-test; $P = 0.0008$) (Fig. 4A). Similarly, total cholesterol levels were low among

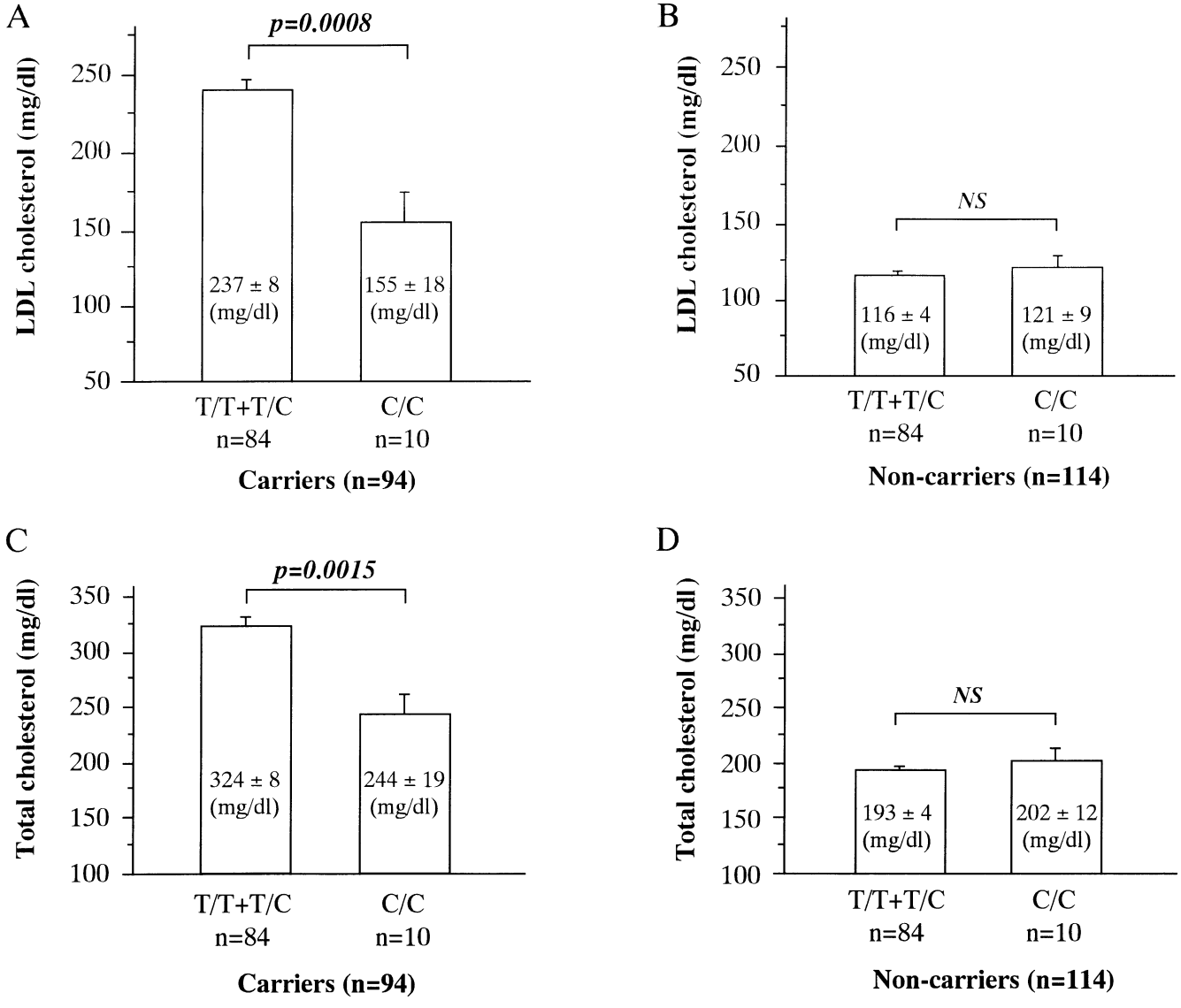

Fig. 4. Comparison of plasma cholesterol levels between *apoA-II* –265C/C carriers and noncarriers. Differences of LDL cholesterol (**A, B**) or total cholesterol (**C, D**) levels were analyzed by Student's *t*-test separately among *LDLR* mutation carriers (**A, C**) and among non-mutation carriers (**B, D**). *Bars* represent the mean ± standard error. *NS*, not significant

C/C homozygotes (244 ± 19 mg/dl), and high in T/T and T/C genotypic groups (324 ± 8 mg/dl) ($P = 0.0015$) (Fig. 4C). These data suggest a recessive effect of the *apoA-II* −265 C-allele. No such effect was observed among noncarriers of the *LDLR* mutation (Fig. 4B, 4D). Triglyceride and HDL cholesterol levels were not affected by the *apoA-II* SNP (Table 1). No significant difference in age, sex or body mass index (BMI) data was observed among three genotypically categorized subjects of the *LDLR* mutation carriers (age: 40 ± 18, 39 ± 20, 31 ± 18 years; female/male: 14/13, 33/24, 4/6; BMI: 28 ± 7, 23 ± 5, 32 ± 6 kg/m^2 for T/T, T/C, C/C subjects, respectively). Thus, the −265 variation in the *apoA-II* promoter strongly influenced LDL-related lipoprotein phenotypes among proven carriers of the novel *LDLR* mutation, but not among noncarriers, in an apparent gene–gene interaction.

Influence of the −265T/C variant on activity of the *apoA-II* promoter

To examine a functional difference of promoter activity related to the SNP (−265T/C), a luciferase assay using transiently transfected HepG2 cells was done. Two types of *apoA-II* promoter–luciferase constructs containing a 1006-bp 5′-flanking sequence (−745 to +261 from transcription start site) with the polymorphic nucleotide at the SNP site were used along with control pGL2-control plasmid. The luciferase activity of cells transfected with pLUC−265C was reproducibly lower than that of pLUC−265T-transfected cells in eight independent experiments. The statistical significance of the about 30% reduction was proved by analyzing mean values of normalized luciferase activities from the entire experiment (Student's *t*-test; $P = 0.02$) (Fig. 5).

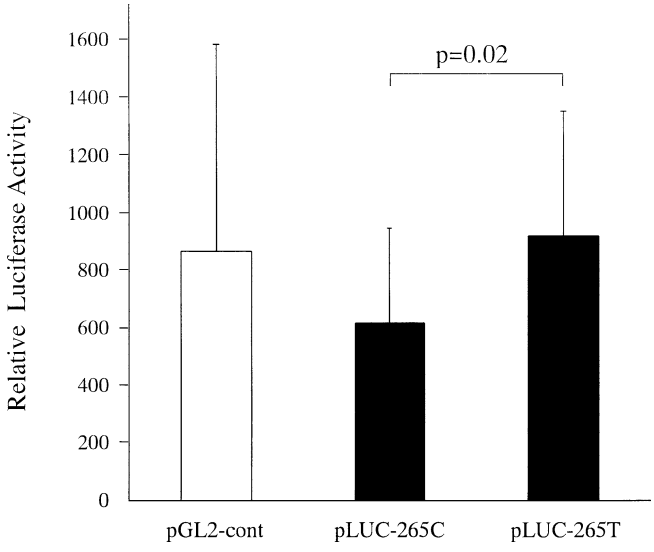

Fig. 5. Differential promoter activity of *apoA-II* promoter constructs, pLUC-265C, pLUC-265T, and pGL2-control plasmid. Luciferase activity of the each construct was normalized by total protein content and background luciferase activity of the pGL2-basic construct. *Bars* represent mean ± standard error (SE). *P* values were given by Student's *t*-test. pGL2-cont, pGL2-control; −265C, pLUC-265C; −265T, pLUC-265T

Discussion

We have reported here the finding of a novel splicing mutation in the *LDLR* gene, IVS14+1 G>A, accounting for dysfunction of the LDL receptor and elevated LDL cholesterol in a large FH kindred (K653). As far as we are aware, this is the largest FH pedigree known; it has been traced from the founding couple through the eighth generation. The size of this single pedigree provided a unique test population for examining gene–gene interactions involving the LDLR defect. We identified genetic interaction between an *apoA-II* variant (−265C); thus plasma levels of LDL cholesterol were nearly normal in FH patients who were homozygous for the *apoA-II* variant (C/C), and simultaneously heterozygous for the novel *LDLR* mutation. No decrease in LDL levels was seen in noncarriers of the *LDLR* mutation regardless of *apoA-II* status.

The present data suggest that the phenotypic effect of the *apoA-II* promotor variation manifests only under the stringent metabolic condition seen in FH patients who have a half-reduced ability to metabolize LDL cholesterol owing to heterozygous inactivation of the LDL receptor gene. We assume the threshold for manifestation of the *apoA-II* variant effect is the number of LDL receptors on the surface of body cells, so it will only become apparent in the special metabolic atomosphere where the metabolism rate of the LDL particle is half-reduced. A typical example of such a gene interaction where a gene variant effect only becomes apparent when accompanied by the defect of the other locus is found between defective LDL receptor and the *apoE2* allele. In normal individuals, apoE2 does not have the effect of elevating plasma cholesterol and triglyceride, rather the cholesterol level is slightly lowered among normal individuals. On the other hand, among FH patients carrying defective LDL receptor, the presence of the *apoE2* allele results in marked accumulation of very low-density lipoprotein (VLDL) remnant and elevated plasma cholesterol and tryglriceride levels, which is classified as type III hyperlipoproteinemia (Hopkins et al. 1991; Emi et al. 1991).

Other examples of gene–gene interactions in FH pedigrees have been hypothesized, but few have been demonstrated. We previously described four extended FH pedigrees in which type III hyperlipidemia occurred frequently among individuals who inherited both a defective *LDLR* allele and an apolipoprotein E2 allele. The *apoE2* allele had a striking effect on VLDL-remnant accumulation, expressed as the plasma VLDL cholesterol-to-triglyceride ratio, only among individuals who also carried defective LDL receptor alleles (Emi et al. 1991; Hopkins et al. 1991). More recently, we observed elevated LDL cholesterol segregating with a D92K mutation of the *LDLR* gene in another extended pedigree with multiple lipid phenotypes, but elevated plasma triglycerides did not cosegregate with the *LDLR* or *apoE* loci, suggesting that some other, unknown genetic factor influences triglyceride metabolism (Wu et al. 2000).

Multiple environmental and genetic factors influence phenotypic variation among FH patients. Lifestyle variations among patients in physical exercise, control of food-calorie intake, psychological awareness of the disease, and compliance, including medications, must have influenced lipoprotein variations among individuals belonging to a specific genotypic group. In addition, other unidentified genetic modifiers might have caused variability among the patients. One such instance is a striking LDL cholesterol-lowering effect of a defective *apoB* allele observed in LDL receptor carriers; we have previously described an *LDLR* mutation carrier in an FH pedigree who had normal cholesterol level with coinheritance of a truncated *apoB* (Pullinger et al. 1992). The other class of apolipoprotein, apoA-I and A-II are the major protein components of plasma HDL (Alaupovic 1998). Numerous studies have shown that high levels of apoA-I in plasma lessen the risk for chronic heart diseases. In contrast, functional roles of apoA-II are not defined yet, and only a few association studies have been reported (Duriez and Fruchart 1999; Sharrett et al. 2001). In the present study, the major modifying factor explaining the lowered cholesterol level among FH patients were obviously the *apoA-II* C allele, such that the majority of FH patients with lowered cholesterol levels were *apoA-II* C homozygotes. In addition to the *apoA-II* effect, individual variations among other environmental and genetic factors might have modified the cholesterol levels in the remaining FH patients with lowered cholesterol levels.

The genetic data presented in this report suggest that variation in the *apoA-II* gene or other genes adjacent to it may modify lipoprotein phenotype among carriers of the *LDLR* mutation. It is possible that the allelic genotype associated with the modified lipoprotein trait in our FH family may be in linkage disequilibrium with another unidentified variation in an adjacent gene. We have obtained informed consent and collected genomic DNA from 208

members who were classified molecularly as either affected by FH or unaffected by the presence or absence of a single *LDLR* mutation in a single large family. This strategy minimizes influence of variation in background of genetic and environmental factors, and makes possible an ideal genetic epidemiological study design to detect modifying factors by statistical analyses in a human population. The sample size in our study was relatively small for an association study. However, the above-mentioned advantages of using subjects from a single large pedigree with little genetic and environmental variation in their background might compensate for the sample size in terms of statistical power. With all these considerations, the finding of an LDL cholesterol-lowering effect among the *apoA-II* −265C homozygotes appears to be quite robust. However, a chance finding cannot, of course, be entirely ruled out, and further replication of this finding in other FH families when they become available for a DNA-based study in the future will be of great interest.

The functional importance of the *apoA-II* promoter variation was supported by an in vitro assay of promoter activity using transfected human cells. The *apoA-II* promoter is thought to be regulated by 14 elements (A to N elements) in a complex manner, but basically via two groups of those elements, distal enhancer (J to N; −911/−614) and proximal promoter (A and B; −65/−33) elements (Chambaz et al. 1991). The −265T/C variation occurs in element D, which has not received much research focus to date (Cardot et al. 1993, 1994). However, a recent independent study supports our finding concerning the effect of the −265T/C variation on the *apoA-II* promoter; that work revealed lower apoA-II, smaller waist circumference, and lower post-prandial lipidemia associated with the −265C allele (van't Hooft et al. 2001).

HDL function is known to depend on the content ratio of apoA-I/apoA-II, the major components of the particle. Decreased expression of *apoA-II* caused by the −265C allele is supposed to alter the HDL function that accelerates the reverse transport of cholesterol from peripheral tissues to the liver, resulting in lowered plasma LDL cholesterol levels. Presumably, the reverse transport system might be up-regulated only under the excess plasma cholesterol condition. In fact, a pedigree of familial apoA-II deficiency has been reported in which two members completely lack plasma apoA-II showed no apparent clinical symptoms, such as alterations in plasma lipoprotein levels and the manifestation of early coronary artery diseases (Deeb et al. 1990). ApoA-II deficiency might be compensated for by apoA-I in the normal state. Recent studies in transgenic mice further support the function of human apoA-II as required for increased VLDL secretion (Blanco-Vaca et al. 2001). A reduction in apoA-II synthesis may therefore be associated with reduced VLDL synthesis and lower LDL cholesterol in our K653 FH patients.

In conclusion, we have identified a functional SNP, −265T/C, in the promoter region of the *apoA-II* gene that appears to alter plasma LDL cholesterol levels in individuals carrying a newly described *LDLR* mutation. The gene interaction we observed might be specific to that particular mutation of *LDLR* in this single pedigree. Nevertheless, our study may lead to a better understanding of regulatory systems that control levels of plasma lipids, and to identification of new targets for clinical intervention in a variety of hypercholesterolemic syndromes.

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