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Genetics

Familial hypercholesterolemia in a rhesus monkey pedigree: Molecular basis of low density lipoprotein receptor deficiency

(polymerase chain reaction/nonhuman primates/genetics)

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ABSTRACT **We have recently identified a family of rhesus monkeys with members exhibiting a spontaneous hypercholesterolemia associated with a low density lipoprotein receptor (LDLR) deficiency. By using the polymerase chain reaction, we now show that the affected monkeys are heterozygous for a nonsense mutation in exon 6 of the LDLR gene. This mutation changes the sequence of the codon for amino acid 284 (tryptophan) from TGG to TAG, thereby generating a nonsense codon potentially resulting in a truncated 283-amino acid protein, which needs documentation, however. This G → A mutation also creates a site for the restriction endonuclease *Spe* I. Using this site as a marker for this nonsense mutation, we have shown that the mutation is present in all of the affected members of the pedigree and absent in unaffected members and that the mutation segregates with the phenotype of spontaneous hypercholesterolemia through three generations. Quantitative analyses of RNA obtained from liver biopsies show that the abundance of the LDLR RNA is also reduced by about 50%. Thus, we have identified a primate model for human familial hypercholesterolemia which will be useful for studying the relationship between the LDLR and lipoprotein metabolism and for assessing the efficacy of diets and drugs in the treatment of human familial hypercholesterolemia.**

The low density lipoprotein receptor (LDLR) is a cell surface glycoprotein which binds to lipoprotein particles bearing apolipoproteins B100 or E and internalizes them by way of clathrin-coated pits. Mutations of the LDLR gene cause familial hypercholesterolemia (FH) (for review, see ref. 1). Because of the reduced numbers of functional receptors on the cell surface, FH heterozygotes and homozygotes have plasma low density lipoprotein (LDL) cholesterol levels approximately twice and six times the normal level, respectively. The human LDLR gene is comprised of 18 exons and spans a region of about 45 kilobases (kb). The mature mRNA is 5.3 kb long and encodes a protein of 860 amino acids. The mature receptor (without the signal peptide) is a 839-amino acid protein which can be divided into five functional domains: (*i*) a 292-amino acid ligand-binding region, (*ii*) a 400-amino acid region which is homologous to the precursor for epidermal growth factor and is required for dissociation of the receptor from the ligand in the lysosomes and recycling of the receptor to the plasma membrane, (*iii*) a 58-amino acid domain which is extensively glycosylated, (*iv*) the transmembrane region, and (*v*) the cytoplasmic domain which is required for targeting the protein to clathrin-coated pits for internalization (1).

A great deal of knowledge about LDLR function has been obtained by molecular analyses of mutations of the human LDLR gene (1). However, an animal model for studying lipoprotein metabolism in the presence and absence of functional LDLR genes would be of great value. At present, the only such model is the Watanabe rabbit (2). Although this model has proven to be very useful in the study of FH, the metabolism of plasma lipoproteins in the rabbit differs significantly from that of man, likely due to the phylogenetic distance between these two animal species. We have identified (3, 4) a family of rhesus monkeys in which some members present with a spontaneous elevation of total plasma and LDL cholesterol. This phenotype occurs in second and third generation animals and has an autosomal dominant mode of inheritance (3, 4). By ligand-blot and immunoblot analyses, as well as binding studies of ^{125}I-labeled LDL to skin fibroblast cell lines established from affected and unaffected monkeys, we also found that the abundance of LDLR protein was reduced in the affected animals (3). Moreover, the apparent molecular weight, rate of maturation, and the LDL binding affinity of the LDLRs from normal and affected animals were similar (3, 4), suggesting that if there is a mutation in the LDLR gene it is a class 1 mutation in which no receptor is synthesized.

To identify the putative mutation in the LDLR gene of these monkeys, we have used the polymerase chain reaction (PCR) (5, 6) to amplify several regions of the rhesus monkey LDLR gene. We show here that the hypercholesterolemic monkeys are heterozygous for a mutation which introduces a premature termination codon in exon 6 of the LDLR gene, potentially resulting in a truncated protein, yet to be documented. Quantitative analyses of RNA obtained from liver biopsies indicate that the abundance of LDLR RNA in cells from the affected animals is about 50% of that in unaffected animals.

METHODS AND MATERIALS

Isolation of Genomic DNA. Genomic DNA was isolated from 10 ml of whole blood by proteinase K digestion and phenol extraction as described by Bell *et al.* (7).

DNA Probes. The full-length human LDLR cDNA (pLDLR3) (8) was purchased from the American Type Culture Collection. For Southern blot analysis, we used the 2.8-kb *Hin*dIII–*Sma* I fragment, which contains all of the coding region, but lacks the 2.3-kb untranslated 3′ end as a probe. A 1.1-kb *Bam*HI fragment containing exons 1–7 was used as a probe for RNA analysis and for Southern blot detection of fragments containing the mutant *Spe* I site.

Analysis of LDLR RNA. Punch liver biopsies (≈150 mg of tissue) obtained at the Southwest Foundation for Biomedical Research were frozen immediately in liquid nitrogen, shipped to Chicago on dry ice, and stored at −80°C until use. The frozen tissue was homogenized in 2.8 ml of 4 M guanidinium isothiocyanate/25 mM sodium citrate/0.5% Sarkosyl/0.1 M 2-mercaptoethanol/0.1% Antifoam A, and total cell RNA

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Abbreviations: LDL, low density lipoprotein; LDLR, LDL receptor; FH, familial hypercholesterolemia; PCR, polymerase chain reaction.

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was isolated by centrifugation through a cushion of 5.7 M CsCl/25 mM sodium acetate, pH 5.0 at 25,000 rpm for 18 hr at 15°C in a SW 55 rotor (9). For slot blot analysis, samples were heated at 60°C for 15 min in 2.5 M formaldehyde/6× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0) and applied in triplicate to a ZetaProbe filter using a Minifold II apparatus (Scheicher & Schuell). After baking at 80°C for 2 hr, filters were prehybridized at 42°C in 50% (vol/vol) formamide/5× SSPE (1× SSPE = 0.18 M NaCl/20 mM NaH_2PO_4/1 mM EDTA)/5× Denhardt's solution (1× Denhardt's solution = 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin)/tRNA (100 μg/ml)/1% glycine/0.6% $NaDodSO_4$ and hybridized at 42°C for 18 hr with 5×10^6 cpm/ml in 50% formamide/5× SSPE/5× Denhardt's solution/tRNA (100 μg/ml)/0.6% $NaDodSO_4$/5% (wt/vol) dextran sulfate. The filters were washed twice at room temperature in 2× SSC/1× Denhardt's solution, washed twice for 30 min at 58°C in 0.1× SSC/0.1% $NaDodSO_4$, and exposed to film. Films were scanned with an LKB Ultrascan XL Densitometer. Filters were then stripped and rehybridized with a mouse β-actin cDNA probe (10).

Skin fibroblast cultures from affected and unaffected animals were grown as described (3).

Identification of the LDLR Mutation. The promoter region was amplified with the following primers: SP40, 5′-GGATCCCGACCTGCTGTGTCCTAGCTGGAAA-3′, which contains a *Bam*HI recognition site linked to positions −195 to −176 of the human LDLR promoter (numbered according to ref. 11), and SP54, 5′-GGATCCAATCAAGTCGCCTGCCCTGGCGACA-3′, which contains a *Bam*HI site linked to positions +35 to +54. Exons 3, 5, and 6 were amplified using the following primers: EX3A, 5′-TGACAGTTCAATCCTGTCTC-3′, and EX3B, 5′-GGCTCAATAGCAAAGGCAGG-3′; EX5A, 5′-AAAATCAACACACTCTGTCC-3′, and EX5B, 5′-GGGATGGAAAACCAGATGGC-3′; and EX6A, 5′-CCTTCCTCCTTCCTCTCTCT-3′ and EX6B, 5′-ACTCTGCAAGCCGCCTGCAC-3′, respectively.

One microgram of genomic DNA from monkey 7643, which is heterozygous for the mutation in the LDLR gene, was amplified using a GeneAmp DNA amplification kit and *Thermus aquaticus* DNA polymerase according to the directions of the manufacturer (Perkin–Elmer/Cetus). The DNA was denatured for 3 min at 94°C and amplified during 25 cycles of denaturation for 1 min at 94°C, annealing for 1.5 min at 55°C, and extension for 2 min at 70°C. The PCR products were then incubated at 70°C for 7 min to ensure complete extension of all the products. PCR products of the appropriate size were excised from 2% NuSieve GTG agarose (FMC BioProducts, Rockland, ME) gels, solubilized in 0.3 M NaCl/20 mM Tris, pH 7.4/10 mM EDTA/0.1% $NaDodSO_4$ at 60°C for 20 min, purified by phenol extraction and ethanol precipitation, and subjected to a second round of 15 cycles of PCR amplification. After gel purification, the PCR products were phosphorylated with T4 polynucleotide kinase, and the ends were made blunt with T4 DNA polymerase (12). After phenol extraction and ethanol precipitation, the PCR products were cloned into *Sma* I-digested M13mp10 DNA. Templates were purified and sequenced by the dideoxynucleotide method of Sanger *et al.* (13).

Screening of Other Members of the Pedigree for the Presence of the Mutation in Exon 6. Exon 6 was amplified by incubating DNA from each member of the pedigree with primers EX6A and EX6B and *Thermus aquaticus* DNA polymerase as described above. The reaction products were digested with *Spe* I according to the directions of the manufacturer (New England Biolabs) and electrophoretically separated on 3% NuSieve agarose gels.

Chemical Analyses. Plasma levels of total cholesterol, LDL cholesterol, and triglycerides were determined as described (3).

RESULTS

Table 1 summarizes the results of the analyses carried out on the three generation animals of the pedigree while on a cholesterol-free Purina Chow diet (3, 4). Four of the monkeys of the original family, the dam (766I), two male offspring (B1000 and 7643), and one female offspring (7587), had a significant elevation of their total plasma cholesterol and LDL cholesterol. An elevation of total plasma cholesterol and LDL cholesterol was also observed in monkeys 7099, 7139, and 7069 of the third generation obtained by mating 7643, one of the affected males, to six unrelated normocholesterolemic females. Another animal of the third generation, 7436, was mildly hypercholesterolemic. All of the hypercholesterolemic animals were found to be LDLR deficient by skin fibroblast studies (3, 4) except for 7587 and 7436 where the results were equivocal. All of the animals exhibited normal levels of plasma triglycerides.

The human LDLR gene is composed of 18 exons which span about 45 kb. Preliminary Southern blot analysis using a human LDLR cDNA probe containing the entire coding region (exons 1–17 and a small portion of exon 18) failed to identify restriction fragments unique to the affected animals, indicating that the mutant LDLR gene had not suffered a major deletion, insertion, or rearrangement. To identify a point mutation that might impair expression of the monkey LDLR gene, we used the PCR to amplify specific regions of the gene. We began with the promoter region and the ligand binding region, which is encoded by exons 2–6 in the human gene. We decided to amplify other exons, moving 5′ to 3′, as necessary. Given the close phylogenetic relationship between humans and monkeys, and the similarities in the sizes of their LDLR RNA and protein, we assumed that the organization and sequences of the human and monkey genes were very similar. We, therefore, used primers derived from

Table 1. Plasma lipids and LDL cholesterol of the rhesus monkeys in the pedigree

Monkey	Sex	Age, years	TC, mg/dl	LDL-C, mg/dl	TG, mg/dl	LDLR deficiency
			First generation			
431J (sire)*	M	19	146	67	40	−
766I (dam)	F	19	218	138	88	+
			Second generation			
B1000	M	6	272	185	38	+
7643	M	10	291	204	45	+
7587	F	2	215	137	52	Eq.
8204	M	13	171	106	36	−
8806	M	12	157	85	32	−
			Females bred to 7643			
6227	F	10	95	14	63	ND
6234	F	13	122	44	86	ND
6235	F	11	124	54	92	ND
6229	F	11	122	38	72	ND
6238	F	12	95	38	54	ND
6233*	F	11	176	55	105	ND
			Third generation			
7099	F	3	207	92	63	+
7139	M	3	227	132	76	+
7069	F	3	216	134	60	+
7436	F	2	182	96	82	Eq.
7700	M	2	134	70	58	−
7558	F	2	155	79	40	−
7489	M	2	141	62	62	−

TC, total cholesterol; LDL-C, LDL cholesterol; TG, triglycerides. The LDLR deficiency was assessed by the skin fibroblast studies. −, Absent; +, present; Eq., equivocal—animals where the LDLR deficiency could not be clearly established by binding studies (4); ND, not determined.
*Deceased.

the human LDLR gene for PCR amplification. DNA from monkey 7643 was used initially as a template. The amplified DNA was cloned into M13 and sequenced. At least eight clones were analyzed from each region of the monkey LDLR gene. The sequences of these clones were compared with each other and with the human gene, keeping in mind that possible differences might arise from (*i*) artifacts due to misincorporation by *Thermus aquaticus* DNA polymerase, which should occur at a very low frequency at any given position and should be observed no more than once in the clones we examined; (*ii*) species differences, which should be observed in 100% of the monkey clones; and (*iii*) mutations of the monkey gene, which should be observed in approximately 50% of the clones since the affected monkeys are expected to be heterozygous for the mutation in the LDLR gene.

Sequence of the Promoter Region of the Rhesus Monkey LDLR Gene. To identify mutations that might affect LDLR transcription, we amplified a 209-base-pair (bp) region from bp −175 to bp +34 relative to the major transcriptional initiation site (11). This region contains the three 16-bp repeats which have been shown to be important for basal level expression and sterol regulation of the human LDLR gene as well as the transcription initiation sites (11, 14, 15). Comparison of the human and rhesus sequences revealed differences in only 11 of the 209 bases. Two of the nucleotide changes observed were at the 3′ end of repeat 1: the rhesus monkey repeat 1 sequence was 5′-AAACTCCTCCTCTCA-3′ rather than 5′-AAACTCCTCCTCTTG-3′. Repeats 2 and 3 were 100% conserved. All of the sequence differences observed were present in 100% of the monkey clones analyzed, indicating that they were due to species differences rather than mutations of the LDLR gene.

Sequences of Exons 3, 5, and 6 of the Rhesus Monkey LDLR Gene. The 20-base oligonucleotide primers were used to amplify the specific exons of the rhesus monkey LDLR gene. We positioned these primers so that, in addition to the sequences of the exons, we would obtain at least 10 bases of the intron sequence flanking each exon, thus permitting detection of mutations at the splice junctions as well as the coding region. DNA from monkey 7643 was used as a template. Initially, we attempted to amplify exons 2 through 6, which in man encode the ligand-binding region (16). We amplified exons 3, 5, and 6 but had difficulty with exons 2 and 4, probably because of mismatch between the primers, which were based on the sequence of the human gene, and the rhesus gene. When we compared the sequences of exons 3, 5, and 6 of the monkey LDLR gene with those of the human gene (8), we found that these regions were highly conserved. We noted some differences between the sequences of the introns but the positions of the splice junctions were perfectly conserved. Of the 369 bp in the coding regions of exons 3, 5, and 6, we observed the following differences between the human and monkey genes: (*i*) eight silent changes resulting in the use of a different codon for the same amino acid; (*ii*) three conservative substitutions in the monkey sequence, Asp-75 → Glu, Val-250 → Ile, and Thr-270 → Ser; and (*iii*) three nonconservative substitutions in the monkey sequence, Gln-71 → Glu, Gly-82 → Asp, and Asn-226 → Thr.

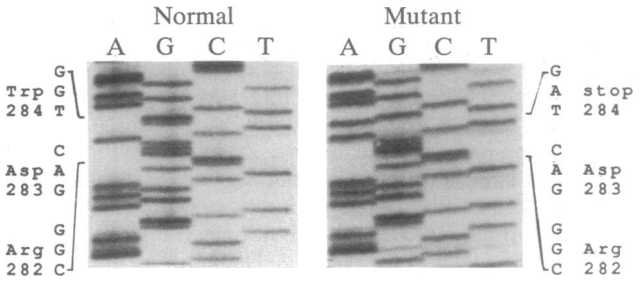

FIG. 1. Identification of the mutation in exon 6 of the rhesus monkey LDLR gene. Exon 6 was amplified from genomic DNA of monkey 7643. PCR products were cloned into M13 and sequenced. Five of 13 clones had the sequence TGG at a position corresponding to amino acid 284 of the human protein (*Left*). The remaining clones had a G → A transition which causes replacement of a tryptophan residue with a translation termination codon (*Right*).

Thus, in exons 3, 5, and 6, there is 95% identity in the amino acid residues of the human and monkey receptors. The rabbit LDLR is slightly more divergent: 83% of the amino acid residues in exons 3, 5, and 6 are identical to the human receptor. Like the monkey receptor, however, the rabbit LDLR has glutamic acid at residue 75, threonine at residue 226 and serine at residue 270 (17). All of the cysteine residues, which in the human LDLR are thought to be important in maintaining the tertiary structure of the ligand-binding region, are conserved, as are two potential sites for N-linked glycosylation (Asn-Xaa-Ser/Thr) at amino acids 76–78 and 251–253. The carboxyl terminus of each of the seven repeats in the ligand-binding region of the human LDLR contains a negatively charged sequence which matches the consensus sequence Asp-Cys-Xaa-Asp-Gly-Ser-Asp-Glu and is thought to be involved in binding to positively charged residues in apolipoproteins E and B100. This consensus sequence is retained in the rhesus monkey receptor as well as the rabbit LDLR.

Identification of a Mutation in the Monkey LDLR Gene. All of the sequence differences between the human and monkey genes described above were observed in 100% of the monkey clones. Thus we considered them to represent species differences rather than mutation of the LDLR gene. In contrast, 8 of the 13 clones of exon 6 had an adenosine residue at a position corresponding to nucleotide 914 of the human cDNA sequence (8). The remainder of the monkey clones had a guanosine at this position, as does the human LDLR gene. Thus, we concluded that the A → G transition would result in replacement of Trp-284 with a termination codon (Fig. 1) and that the protein product, if expressed, would be a 30-kDa peptide.

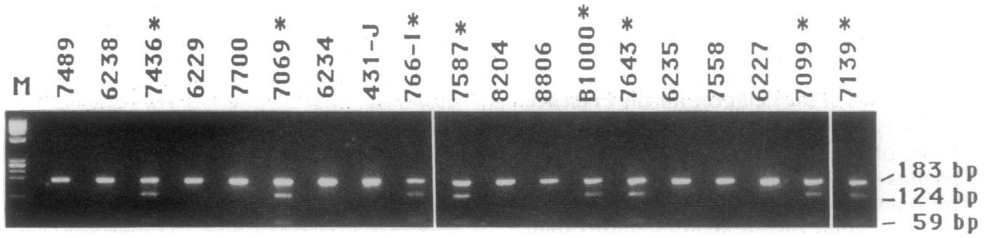

FIG. 2. Distribution of the mutation in exon 6 among members of the rhesus monkey pedigree. Exon 6 was amplified from genomic DNA of each member of the pedigree. The PCR products were size-fractionated, gel-purified, reamplified, and digested with *Spe* I. PCR products from the normal gene are not digested by *Spe* I. The mutation in exon 6 creates a new *Spe* I site. *Spe* I digestion of the PCR products from the mutant gene generates fragments of 124 and 59 bp. *, Indicates hypercholesterolemic monkeys. Cholesterol values are shown in Table 1. Numbers above the lanes identify the members of the pedigree shown in Table 1 and see Fig. 4.

Segregation of the Mutation in Exon 6. Analysis of the sequences of the normal and mutant genes revealed that the mutation in exon 6 introduced a new *Spe* I site. We took advantage of this observation to screen the rest of the pedigree for the mutation by the PCR reaction. The 183-bp PCR products were digested with *Spe* I and electrophoretically separated on Nu-Sieve agarose gels (Fig. 2). Only the 183-bp fragment was detected using DNAs from normocholesterolemic animals, indicating that these animals lacked the *Spe* I site and the mutation present in exon 6 of animals with hypercholesterolemia. We identified three fragments in the *Spe* I digests of PCR-amplified exon 6 DNA from the hypercholesterolemic animals: a 183-bp fragment derived from the normal allele and fragments of 124 and 59 bp, resulting from the *Spe* I digestion of the mutant allele. Two animals with moderate hypercholesterolemia, 7587 and 7436 (Table 1), also had the nonsense mutation in exon 6.

We attempted to confirm the presence of the nonsense mutation in exon 6 by Southern blot analysis of genomic DNAs digested with *Spe* I and hybridized to a human LDLR cDNA probe encoding the region of exons 1–7. However, we were unable to resolve the large *Spe* I fragments derived from the normal and mutant alleles. Upon double digestion with *Spe* I and *Bam*HI, fragments of 13 kb and 10 kb were clearly resolved in DNAs from the hypercholesterolemic monkeys 766I, B1000, 7643, 7587, 7436, and 7069. A single fragment of 13 kb was identified in DNAs from the normocholesterolemic monkeys 8204, 8806, 6229, 6234, and 7700 (data not shown). DNAs from the other monkeys were not analyzed. Thus, the presence of the *Spe* I site, which is a marker for the nonsense mutation in exon 6, has been confirmed by Southern blot analysis of genomic DNA.

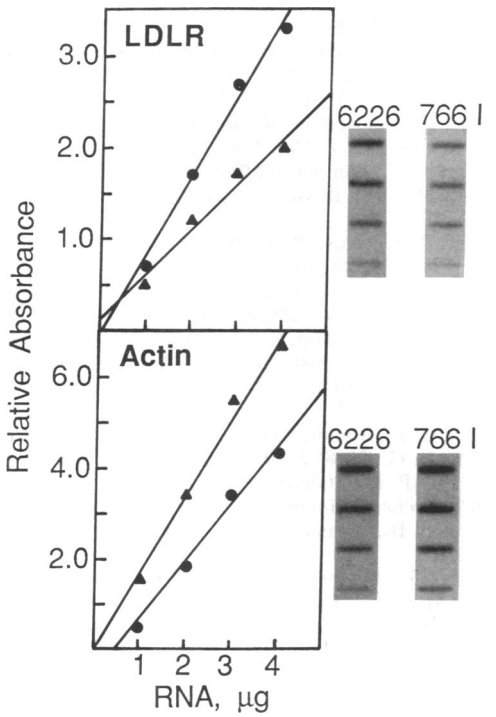

FIG. 3. Slot blot analysis of total cell RNA isolated from 766I, a hypercholesterolemic monkey, and 6226, a normocholesterolemic control. RNA (1, 2, 3, or 4 μg) from each monkey was applied to Zeta-Probe filter in triplicate. (*A*) The filter was hybridized to a human LDLR cDNA probe and scanned by laser densitometry. The average value for each point was calculated and the slope was determined by linear regression. One set of these data is shown at the right. The slopes are 0.50 (r = 0.98) and 0.88 (r = 0.99) for 766I and 6226, respectively. (*B*) The filter was stripped and rehybridized with a mouse β-actin cDNA probe. Slopes of 1.77 (r = 0.99) and 1.29 (r = 0.99) were calculated for 766I and 6226, respectively. ●, 6226; ▲, 766I.

Analysis of LDLR RNA Expression. Immunoblot and ligand blot analyses (3, 4) indicated that the abundance of the LDLR protein in cells from the hypercholesterolemic monkey was approximately 50% of that in cells from the normocholesterolemic animals. We used Northern blot and slot blot analyses to determine whether the expression of the LDLR RNA was also affected. Polyadenylylated RNA was isolated from fibroblast cell lines established from individuals B1000, a hypercholesterolemic monkey, and 8806, a normocholesterolemic sibling. The RNA was then separated on a formaldehyde/agarose gel, transferred to nitrocellulose, and hybridized to LDLR and actin cDNA probes (data not shown). LDLR mRNAs of 5.3 kb were detected in both cell lines with no aberrantly sized species. However, the abundance of the LDLR RNA isolated from B1000 cells was approximately half that of 8806. Northern blot analyses of total cell RNA isolated from liver biopsies of all the animals of the pedigree were consistent with this result. To further quantitate the LDLR RNA, slot blot analyses were performed on total cell RNA isolated from liver biopsies from monkey 766I, a hypercholesterolemic monkey, and monkey 6226, a normocholesterolemic control. Various amounts of RNA were applied to a Zeta-Probe filter in triplicate. The filter was hybridized to a human LDLR cDNA probe containing exons 1–7 and the film was then scanned by laser densitometry. The slope of the linear regression calculated by averaging triplicate values (Fig. 3) confirmed that the abundance of the LDLR RNA was reduced by approximately 50% in the cells from the hypercholesterolemic monkeys. The filter was subsequently stripped and rehybridized with a β-actin probe (Fig. 3). The actin hybridization was approximately 1.4 times stronger in the samples from the hypercholesterolemic monkey 766I, indicating that the lower LDLR hybridization signal in the 766I cells was due to a specific reduction in the abundance of the LDLR RNA. These results suggest that the truncated protein might be expressed at very low levels, or perhaps not at all.

In conclusion, the results of the genomic DNA and liver RNA analyses indicate that all of the animals that were previously found to have an elevation of total plasma cholesterol and LDL cholesterol as well as LDLR deficiency by studies on cultured skin fibroblasts are heterozygous for a nonsense mutation in exon 6 leading to a truncated protein (Fig. 4). However, it should be noted that two of the animals presenting with a mild hypercholesterolemia (e.g., 7587 and 7436), found to be defective by genomic analyses, could not be unequivocally phenotyped as LDLR-deficient based on the binding studies in cultured skin fibroblasts (3, 4).

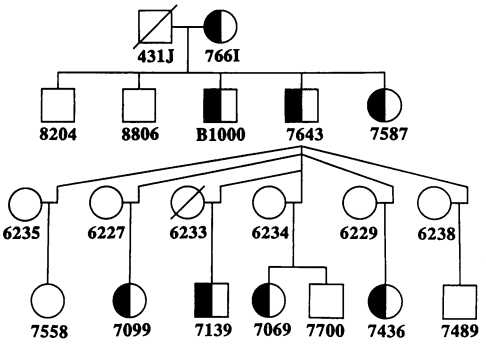

FIG. 4. Pedigree of the rhesus monkey family. Half-filled symbols indicate monkeys heterozygous for the mutation in exon 6 in the LDLR gene as determined by the presence of an *Spe* I restriction site. Circles, females; squares, males; slashed lines, deceased animals.

DISCUSSION

In this report we have shown that animals of a rhesus monkey pedigree with a spontaneous elevation of plasma total and LDL cholesterol and LDLR deficiency (3, 4) are heterozygous for a nonsense mutation in exon 6 of the LDLR gene. This mutation is predicted to result in the expression of a protein truncated at a position corresponding to amino acid 284 of the human LDLR and is likely to be the primary cause of the abnormal phenotype in these animals. The mutation creates a new *Spe* I site in exon 6, providing a convenient marker for the mutation which we have shown to segregate with the abnormal phenotype through three generations. The product of the mutant allele, if expressed, would be a 30-kDa polypeptide which, since it lacks the transmembrane anchoring sequence, would probably be secreted. However, it is unlikely that such a polypeptide is produced, since the abundance of the RNA expressed from the mutant gene is severely reduced. The mechanism by which premature termination codons affect mRNA abundance are unknown, but similar results have been described for mutations of the genes for β-globin (18, 19), apolipoprotein CII (20), the immunoglobulin heavy chain (21), dihydrofolate reductase (22), and triosephosphate isomerase (23). It should be noted that two of the animals studied, 7587 and 7436, both exhibiting mild hypercholesterolemia had their LDLR deficiency defined only on the basis of genomic analyses. The difficulty of verifying heterozygous subjects for LDLR deficiency is well known and our animals provide further evidence for it.

Ours is an example of a nonhuman primate model of FH. The only other animal model at present is the Watanabe rabbit (2). We believe, however, that the rhesus monkey model has several advantages over the Watanabe rabbit. (*i*) The close phylogenetic relationship between monkeys and humans allows a more ready application of the results obtained in the rhesus monkey to man. (*ii*) Because of its relatively larger size, the rhesus monkey offers the opportunity of studying individual animals, thus eliminating possible interindividual heterogeneity of plasma lipoproteins. (*iii*) The life span of rhesus monkey is considerably longer than that of the rabbit, thus permitting experiments on a long range basis. (*iv*) Unlike rabbits, rhesus monkeys express lipoprotein (a) [Lp(a)], a genetically determined LDL variant which is considered to be an independent risk factor for atherosclerosis (24). In this respect, the monkeys of our pedigree have recently permitted us to carry out studies on the possible interaction between LDLR function and Lp(a), which suggests that the LDLR may not be involved in a major way in Lp(a) metabolism (4, 25). Overall, the animals of our pedigree represent a unique and valuable model for studying the relationship between LDLR and lipoprotein metabolism and for assessing the efficacy of diets and drugs considered for the treatment of human heterozygous FH.

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