Defects of the LDL receptor in WHHL transgenic rabbits lead to a marked accumulation of plasma lipoprotein[a]

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Abstract **In this study, we created LDL receptor (LDLr) defective (WHHL) transgenic rabbits expressing human apo[a] to examine whether LDLr mediates the Lp[a] clearance from the plasma. By crossbreeding WHHL rabbits with human apo[a] transgenic rabbits, we obtained two groups of human apo[a] transgenic rabbits with defective LDLr functions: apo[a]$^{+/0}$ WHHL heterozygous (LDLr$^{+/-}$) and apo[a]$^{+/0}$ WHHL homozygous (LDLr$^{-/-}$) rabbits. The lipid and lipoprotein levels of human apo[a] WHHL rabbits were compared to those of human apo[a] transgenic rabbits with normal LDLr functions (LDLr$^{+/+}$). The apo[a] production rate was evaluated by analyzing apo[a] mRNA expression in the liver, the major site for apo[a] synthesis in transgenic rabbits. We found that pre-β lipoproteins were markedly increased accompanied by a 2-fold increase in the plasma Lp[a] in apo[a]$^{+/0}$/LDLr$^{+/-}$ rabbits and a 4.2-fold increase in apo[a]$^{+/0}$/LDLr$^{-/-}$ rabbits compared with that in apo[a]$^{+/0}$ rabbits with normal LDLr function. In apo[a]$^{+/0}$/LDLr$^{-/-}$ rabbits, there was a marked increase in plasma total cholesterol and triglycerides, as was found in their counterpart non-transgenic WHHL rabbits. Northern blot analysis revealed that hepatic apo[a] expression in WHHL transgenic rabbits was similar to that in LDLr$^{+/+}$ transgenic rabbits, suggesting the accumulation of plasma Lp[a] in WHHL transgenic rabbits was not due to increased apo[a] synthesis. In conclusion, absence of a functional LDLr leads to a marked accumulation of plasma Lp[a] in human apo[a] transgenic WHHL rabbits and LDLr may participate in the catabolism of Lp[a] in rabbits.—**Fan, J., M. Challah, H. Shimoyamada, M. Shiomi, S. Marcovina, and T. Watanabe. **Defects of the LDL receptor in WHHL transgenic rabbits lead to a marked accumulation of plasma lipoprotein[a].** ***J. Lipid Res.*** **2000.** 41: **1004–1012.**

Supplementary key words lipoprotein[a] • WHHL rabbit • apolipoprotein[a] • LDL receptor • transgenic rabbit • metabolism • catabolism • hypercholesterolemia

High lipoprotein[a] (Lp[a]) levels constitute an independent risk factor for the development of atherosclerosis. In many human studies, elevated levels of plasma Lp[a] have been found to be associated with an increased risk of atherosclerotic coronary heart disease, stroke, and restenosis (1–3), although some studies have not detected this association (4–6). The risk of elevated Lp[a] concentrations is significantly increased in patients who also have high levels of LDL cholesterol (7, 8).

The Lp[a] particle closely resembles low density lipoprotein (LDL) in both lipid composition and the presence of apolipoprotein (apo) B-100 (apoB-100). Lp[a] is distinguished from LDL by the presence of an additional protein component designated as apolipoprotein[a] (apo[a]), which is complexed to apoB-100 by disulfide linkage (1). Apo[a] is a large plasma glycoprotein (28% carbohydrate by weight) synthesized primarily in the liver (9). Apo[a] displays genetically determined size heterogeneity, and as many as 34 different isoforms of apo[a] have been identified in human plasma, varying in mass from <300 to >800 kDa (1, 10, 11). Lp[a] concentrations are strongly genetically determined (12) with at least 90% of the variation determined by variation within the gene for apo[a] (13).

Because Lp[a] contains apoB-100 and bears a structural similarity to LDL, it has been proposed that Lp[a] may be removed from the plasma by the LDL receptor (LDLr). Some studies using cultured cells revealed that Lp[a] can bind specifically to the LDLr (14–16) while other studies failed to reproduce those results (17, 18). Utermann et al. (19) first reported that patients with familial hypercholesterolemia (FH) have plasma Lp[a] levels higher than expected for their respective apo[a] phenotypes. This observation was supported by two other clinical studies (20, 21). Furthermore, this notion is further strengthened by a study of transgenic mice overexpressing human LDLr in the

Abbreviations: WHHL, Watanabe heritable hyperlipidemic; apo[a], apolipoprotein[a]; Lp[a], lipoprotein[a]; LDL, low density lipoproteins; LDLr, LDL receptor; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; FH, familial hypercholesterolemia; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody.

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liver, which showed accelerated removal of ^{125}I-labeled human Lp[a] from the plasma (22). However, several clinical studies reported that catabolism of ^{125}I-labeled Lp[a] is not significantly different in patients with homozygous FH who lack functional LDLr compared to their heterozygous parents or individuals with normal levels of functional LDLr (23–25). Therefore, it is still not clear whether or not LDLr plays a physiological role in the removal of Lp[a] from the plasma.

To investigate the functions of Lp[a] in lipoprotein metabolism, we (26) and others (27) recently generated transgenic rabbits expressing human apo[a]. Our study showed that unlike the human apo[a] in the plasma of transgenic mice, expression of human apo[a] resulted in efficient assembly of Lp[a] in transgenic rabbits, which makes this model a potential Lp[a] animal model (26). The current study was designed to examine whether or not LDLr participates in the catabolism of Lp[a]. For this purpose, we crossbred human apo[a] transgenic rabbits with Watanabe heritable hyperlipidemic (WHHL) rabbits (28), an animal model for human FH (28, 29). These rabbits have defective LDL receptor function due to a spontaneously arising deletion in exon 4 of the LDLr gene that encodes a 4-amino acid deletion in the cysteine-rich ligand-binding domain of the protein (30). Homozygous WHHL rabbits are markedly hypercholesterolemic from birth and suffer from tendon xanthoma and atherosclerosis, both of which exhibit remarkable pathological resemblance to those observed in human FH. In this study, we characterized the lipids, lipoproteins, and human apo[a] levels in WHHL transgenic rabbits expressing human apo[a] and we found that there was an apparent accumulation of Lp[a] in the transgenic rabbits in the setting of LDLr defects.

MATERIALS AND METHODS

Animals

The generation of human apo[a] transgenic rabbits has been recently described (26) and, in the current study, hemizygous transgenic rabbits were crossbred with the same colony of homozygous WHHL rabbits as previously described (29). All animal experiments were performed with the approval and according to the guidelines of the Animal Research Committee of the University of Tsukuba. By serial breeding, we obtained two groups of WHHL transgenic rabbits: heterozygous WHHL rabbits expressing human apo[a] (apo[a]$^{+/0}$/LDLr$^{+/-}$) and homozygous WHHL rabbits expressing human apo[a] (apo[a]$^{+/0}$/LDLr$^{-/-}$). The presence of the human apo[a] transgene was confirmed by Southern blotting using a human apo[a] cDNA probe. Genomic DNA (10 μg) from ear biopsies was digested with EcoRI restriction enzyme, subjected to electrophoresis on a 0.8% agarose gel, and transferred to a Nitran nylon membrane using a Turboblotter system (Schleicher & Schuell, Keene, NH). Membranes were hybridized with the ^{32}P-labeled cDNA probe synthesized with a Prime-It II random primer labeling kit (Stratagene, La Jolla, CA). LDL receptor genotype status was examined by the PCR method developed by Brousseau et al. (31) .

Plasma lipids and human apolipoprotein[a] analysis

The plasma lipid and lipoprotein profiles of WHHL transgenic rabbits were compared to those of age-matched apo[a] transgenic rabbits with normal LDLr functions (apo[a]$^{+/0}$/LDLr$^{+/+}$) at the age of 3–4 months. Blood was collected from fasting animals as described (32). The plasma apo[a] concentration was determined by a direct binding double monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) as reported previously (33). The capture monoclonal antibody (a-6) in the assay is directed to an epitope present in apo[a] kringle 4 type 2 and the detection antibody (a-40) is directed to a unique epitope located in apo[a] kringle 4 type 9 (34). The previous study showed that this assay was able to measure human Lp[a] accurately and was not influenced by apo[a] size polymorphism (33). In addition, this assay was successfully used to quantitate apo[a] levels in plasma of transgenic mice expressing either human apo[a] or both human apo[a] and apoB (35). The mean apo[a] levels in the human apo[a] transgenic mice were similar to human apo[a]/apoB double transgenic mice, indicating that these mAbs have the same affinity for both free apo[a] and apoB-bound apo[a] particles (35). The concentration of human apo[a] was also estimated by Western blotting of rabbit plasma with detection by different mouse mAb against human apo[a] (26). Plasma total cholesterol, triglycerides, and HDL-cholesterol were determined using Wako assay kits (Wako Chemicals, Osaka).

To assess apo[a] and apoB interactions, plasma isolated from transgenic rabbits was electrophoresed on a 3.5% nondenaturing polyacrylamide gel without sodium dodecyl sulfate (SDS) (36). This method was shown previously to separate free apo[a] from lipoprotein-bound apo[a]. The lipoproteins were further resolved by 4% SDS polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing (without β-mercaptoethanol) and reducing (with β-mercaptoethanol) conditions (26). Immunoblotting was performed using the anti-human apo[a] mAb and immunocomplexed proteins were identified by reaction with a horseradish peroxidase-conjugated goat Ab to mouse IgG, followed by enhanced chemiluminescent (ECL) detection (ECL kit, Amersham, Arlington Heights, IL). The same immunoblot membranes were rehybridized with goat anti-apoB polyclonal Ab. For the determination of the apolipoprotein distribution in transgenic rabbit lipoproteins, plasma lipoproteins were isolated by sequential ultracentrifugation as described (37, 38). These lipoprotein fractions were subjected to agarose gel electrophoresis and stained with Fat red 7B or transferred to a nitrocellulose membrane for immunoblotting with the anti-human apo[a] mAb and goat anti-apoB, -apoA-I, and -apoE polyclonal Abs (Rockland Inc., Gilbertsville, PA). Cholesterol and triglyceride contents in each density fraction were measured using Wako assay kits.

mRNA analysis

For the investigation of the apo[a] synthesis, the hepatic and renal expression of human apo[a] in WHHL transgenic rabbits was evaluated by Northern blot analysis. Total RNA was isolated using Trizol reagent (Gibco BRL, Life Technology, Frederick, MD) and 10 μg of denatured total RNA was subjected to electrophoresis in a 1.2% agarose gel, transferred to a Nitran nylon membrane and hybridized with the ^{32}P-labeled human apo[a] cDNA probe as described above. The blot was rehybridized with a ^{32}P-labeled human β-actin cDNA probe (Clontech Laboratories, Palo Alto, CA) to ensure that equal amounts of RNA were loaded in each well. The signal bands were scanned using a GS-700 imaging densitometer (Bio-Rad Laboratories, Hercules, CA).

Statistical analysis

Plasma lipids were expressed as mean ± SD and assessed for significance with Student's *t*-test, and in all cases, statistical significance was set at $P < 0.05$.

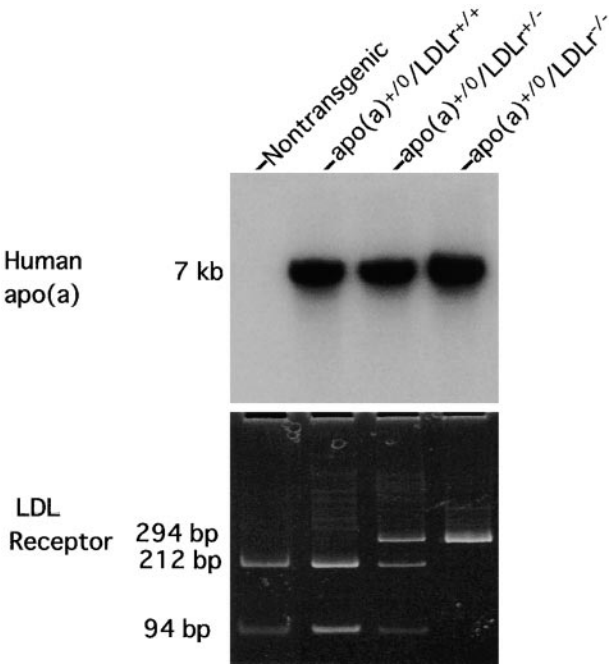


Fig. 1. Detection of human apo[a] transgene and determination of LDLr status in WHHL transgenic rabbits. The presence of human apo[a] transgene was confirmed by Southern blot analysis (upper panel) using a human apo[a] cDNA probe as described in Materials and Methods. Mutation of LDL receptors (LDLr) in WHHL rabbits was analyzed by PCR. Subsequent to gel purification and *Bgl* restriction enzyme digestion of the PCR products, $LDLr^{+/+}$ rabbits generated two bands (212 and 94 bp), whereas $LDLr^{+/-}$ rabbits generated three bands (294, 212, and 94 bp). $LDLr^{-/-}$ animals, however, generated only a single band (294 bp) due to the presence of the 12-bp deletion in both alleles.

RESULTS

Successful crossbreeding between human apo[a] transgenic rabbits and WHHL rabbits was confirmed by the analysis shown in **Fig. 1**. The human apo[a] transgene was detected as a 7-kb band by Southern blot analysis using a human apo[a] cDNA probe. Heterozygosity or homozygosity for the 12-base pair deletion in exon 4 of the rabbit LDLr gene was analyzed by PCR followed by enzymatic digestion. In $LDLr^{+/+}$ rabbits, there were two product bands of 212 and 94 bp, whereas in $LDLr^{+/-}$ rabbits, three product bands were observed after digestion with *Bgl* I restriction enzyme due to the presence of one normal and one mutant allele. In contrast, in $LDLr^{-/-}$ rabbits, only one 294 bp band was evident both before and after digestion with *Bgl* I.

Plasma lipids and lipoprotein composition

Table 1 summarizes the mean plasma lipid and human apo[a] data of WHHL transgenic heterozygotes and homozygotes, as compared with those of normal transgenic rabbits. With a single allele mutation of LDLr, there was a slight increase of total cholesterol and triglycerides, whereas in homozygous WHHL rabbits with two mutant alleles of LDLr, there was a pronounced increase of total cholesterol and triglycerides, accompanied by reduced HDL levels, compared to normal rabbits. In general, the plasma lipid levels of WHHL transgenic rabbits were similar to those of nontransgenic WHHL counterparts, as reported by others (28).

Figure 2 shows that compared with control transgenic rabbits, WHHL heterozygous transgenic rabbits had increased pre-β lipoproteins, while other lipoproteins were unchanged. When the lipoproteins from the same gels were transferred to membranes and incubated with human apo[a] mAb, increased pre-β lipoproteins were identified as human apo[a]. In WHHL homozygous transgenic rabbits, there was a prominent increase of both β and pre-β lipoproteins, but a pronounced reduction of α-lipoproteins. Western blot analysis showed that pre-β lipoproteins contained a large amount of human apo[a] (Fig. 2). Quantitation of plasma Lp[a] by ELISA revealed that there were 2-fold and 4.2-fold increases of Lp[a] in WHHL heterozygous and homozygous transgenic rabbits, respectively, compared to the level in $LDLr^{+/+}$ transgenic rabbits (Table 1).

To investigate whether the elevated apo[a] in the WHHL rabbits was associated with rabbit apoB, the plasma lipoproteins were fractionated on a 3.5% non-denaturing polyacrylamide gel followed by Western blotting analysis. **Figure 3A** shows that the increase of apo[a] in WHHL transgenic rabbits was largely associated with apoB to form Lp[a] complexes, while free apo[a] in $apo[a]^{+/0}/LDLr^{-/-}$ rabbits was also increased to a certain extent. Under nonreducing conditions, the Lp[a] complex in WHHL transgenic rabbit plasma existed as a high molecular weight form (Fig. 3B), suggesting that the apo[a]/apoB complex was linked by disulfide bond(s). Upon reduction, two kinds of apo[a] isoforms were visible (Fig. 3C), as reported previously (26). When the same immunoblot membranes were

TABLE 1. Lipid and lipoprotein levels in WHHL transgenic and control transgenic rabbits

	n	Total Cholesterol	Triglycerides	HDL-cholesterol	Human Lp[a]
		mM/L	mM/L	mM/L	nM/L
$Apo[a]^{+/0}/LDLr^{+/+}$	6	1.22 ± 0.23	0.48 ± 0.05	1.04 ± 0.2	11.30 ± 4.2
$Apo[a]^{+/0}/LDLr^{+/-}$	5	1.46 ± 0.39	0.69 ± 0.15	0.73 ± 0.2	22.21 ± 6.5[a]
$Apo[a]^{+/0}/LDLr^{-/-}$	5	22.4 ± 2.35[a]	4.89 ± 1.02[a]	0.24 ± 0.09[a]	46.98 ± 4.5[a]

Values are represented as mean ± SD. All rabbits in the control and heterozygous WHHL groups were females. In the homozygous WHHL group, two males and three females were studied. Rabbits were at the age of 3–4 months and were on a standard chow diet.

[a] $P < 0.05$ versus $apo[a]^{+/0}/LDLr^{+/+}$ rabbits.

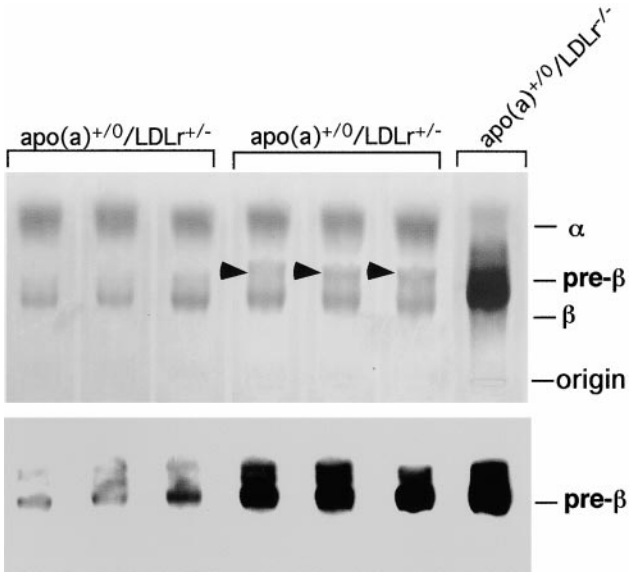


Fig. 2. Agarose gel electrophoresis of the plasma from WHHL transgenic and control transgenic rabbits. Plasma (2 μl) was electrophoresed on a 1% agarose gel; the gel was either stained for neutral lipids with Fat red 7B (upper panel) or used for Western blotting probed with anti-human apo[a] mAb (lower panel). Arrowheads indicate the pre-β lipoproteins. There was more human apo[a] in $LDLr^{+/-}$ and $^{-/-}$ transgenic rabbits than in $LDLr^{+/+}$ transgenic rabbits.

rehybridized with anti-apoB Ab, we found that apoB levels were markedly increased in apo[a]$^{+/0}$/$LDLr^{-/-}$ rabbits but there was no difference between apo[a]$^{+/0}$/$LDLr^{+/+}$ rabbits and apo[a]$^{+/0}$/$LDLr^{+/-}$ rabbits (Figs. 3B and 3C).

Compared to the level in apo[a]$^{+/0}$/$LDLr^{+/-}$ rabbits, the plasma lipoprotein density fractions in apo[a]$^{+/0}$/$LDLr^{-/-}$ rabbits showed a marked increase in lipoproteins with density d $<$ 1.006 g/ml very low density lipoprotein (VLDL), d 1.006–1.02 g/ml intermediate density lipoprotein (IDL), and d 1.02–1.04 g/ml (LDL), whereas lipoproteins with density d 1.06–1.08 and d 1.08–1.10 g/ml (HDL_2), and d 1.10–1.21 g/ml (HDL_3) were markedly reduced (**Fig. 4A**). Quantitation of these apoB-containing lipoproteins revealed that cholesterol and triglyceride contents were remarkably elevated (Fig. 4B). The overall features of lipoproteins in the apo[a]$^{+/0}$/$LDLr^{-/-}$ rabbit were quite similar to those of homozygous WHHL nontransgenic counterparts (39).

As there was a marked increase of human apo[a] in apo[a]$^{+/0}$/$LDLr^{-/-}$ rabbits, we further analyzed the distribution of apolipoproteins (**Fig. 5**). As in apo[a]$^{+/0}$/$LDLr^{+/+}$ and apo[a]$^{+/0}$/$LDLr^{+/-}$ rabbits, apo[a] in apo[a]$^{+/0}$/$LDLr^{-/-}$ rabbit was mainly distributed in a range of density from 1.02 to 1.08 g/ml; however, apo[a] was also found in lighter density fractions, such as in the VLDL(d $\leq$ 1.006 g/ml) and IDL(d 1.006–1.02 g/ml) fractions, indicating that in addition to LDL, apo[a] can bind to other apoB-containing particles. A small amount of free apo[a] was also present in the density range of 1.10–1.21 g/ml. Other changes in apolipoprotein distribution in apo[a]$^{+/0}$/$LDLr^{-/-}$ rabbits included enrichment of apoE in apoB-containing particles and reduction in apoA-I-containing lipoproteins (Fig. 4 and Fig. 5).

Apo[a] mRNA expression

As accumulation of Lp[a] in WHHL transgenic rabbits could result from either delayed catabolism or a relative increase of synthesis, we also examined whether increased apo[a] in WHHL transgenic rabbits was caused by an in-

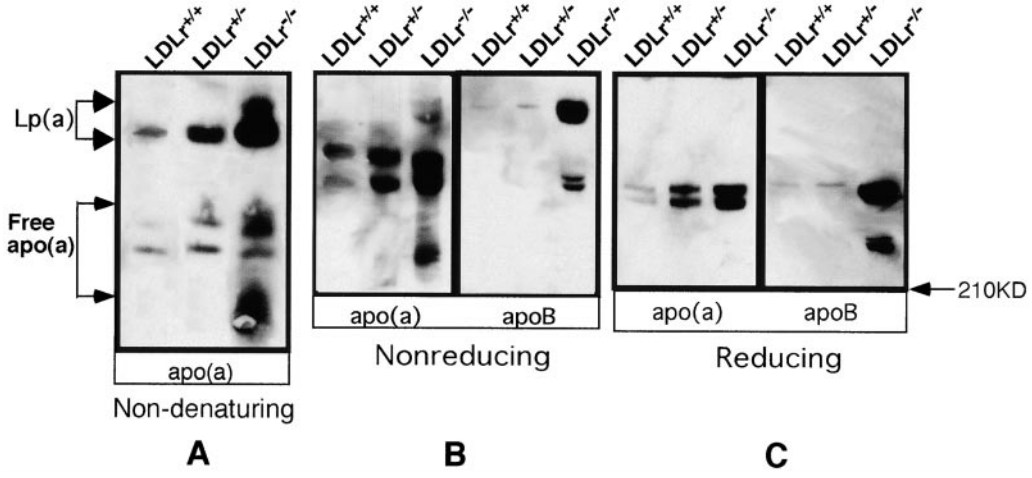
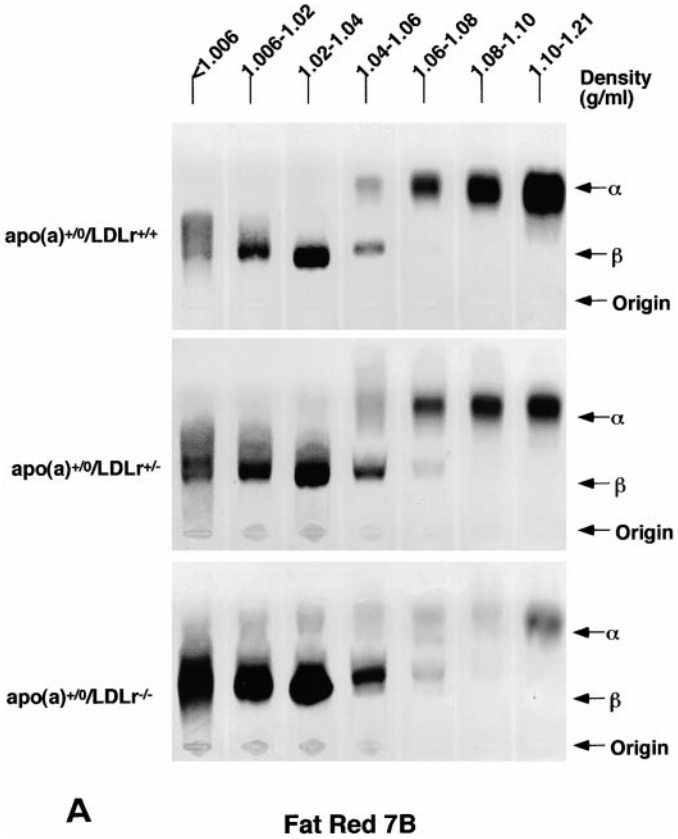


Fig. 3. Immunoblotting analysis of WHHL transgenic rabbit plasma apo[a]. Aliquots of plasma were separated by either 3.5% non-denaturing polyacrylamide gel electrophoresis (A) or 4% SDS-PAGE under non-reducing (B) or reducing (C) conditions. After electrophoretic transfer, the proteins were immunoblotted using anti-human apo[a] mAb as described in Materials and Methods. The same immunoblot membranes (B and C) were stripped in stripping buffer (100 mm 2-mercaptoethanol, 2% SDS, 62.5 mm Tris-HCl, pH 6.7) at 50°C for 30 min and reprobed with anti-apoB polyclonal Ab as described. Of note, there are two small molecular mass forms of apoB in apo[a]$^{+/-}$/$LDLr^{-/-}$ rabbits, presumably, apoB-48. Prestained SDS-PAGE marker (Bio-Rad, Hercules, CA) was loaded in SDS-PAGE under the reducing condition to indicate the molecular mass. The figures are representative of 5 animals in each group.



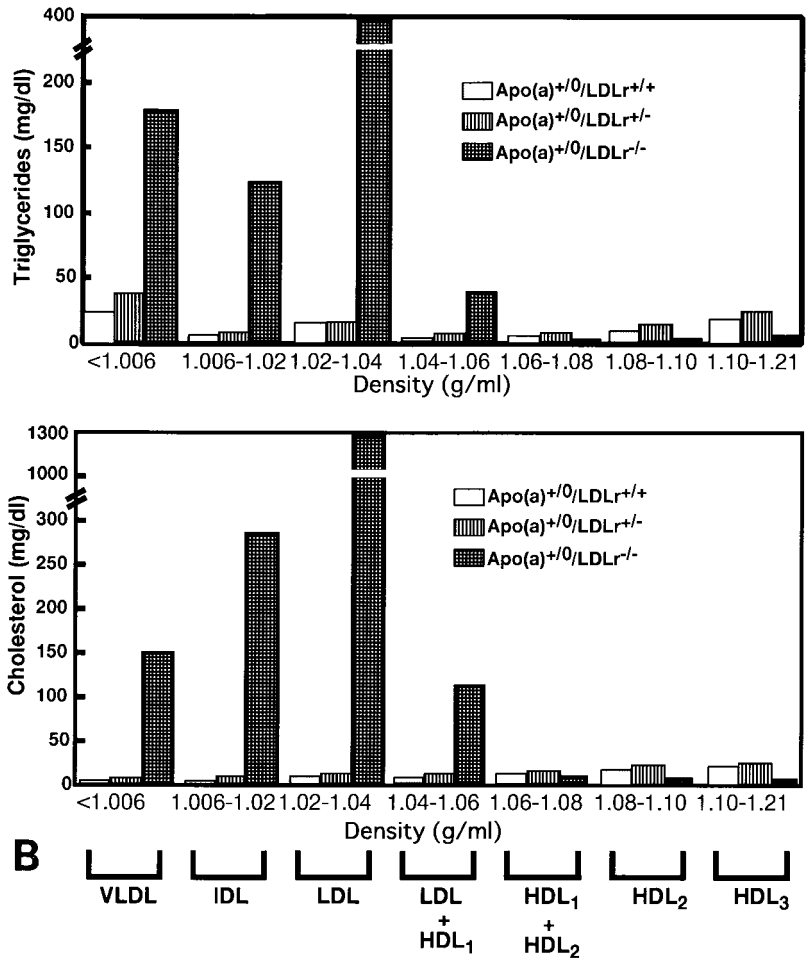


Fig. 4. Lipoprotein and lipid distribution in WHHL transgenic rabbit plasma. A: Lipoprotein profiles in WHHL transgenic rabbits. Plasma lipoproteins from WHHL heterozygotes (upper panel) and homozygotes (lower panel) were separated by sequential density ultracentrifugation and resolved by electrophoresis in a 1% agarose gel. Lipoproteins were stained with Fat red 7B. B: Lipid distribution in plasma lipoproteins in WHHL transgenic rabbits. Density fractions were collected and the cholesterol and triglyceride contents were quantitated as described in Materials and Methods. All rabbits were 4 months old, and were being fed on a chow diet at the time of analysis. The combined recovery for each animal averaged ~80% of total plasma levels.

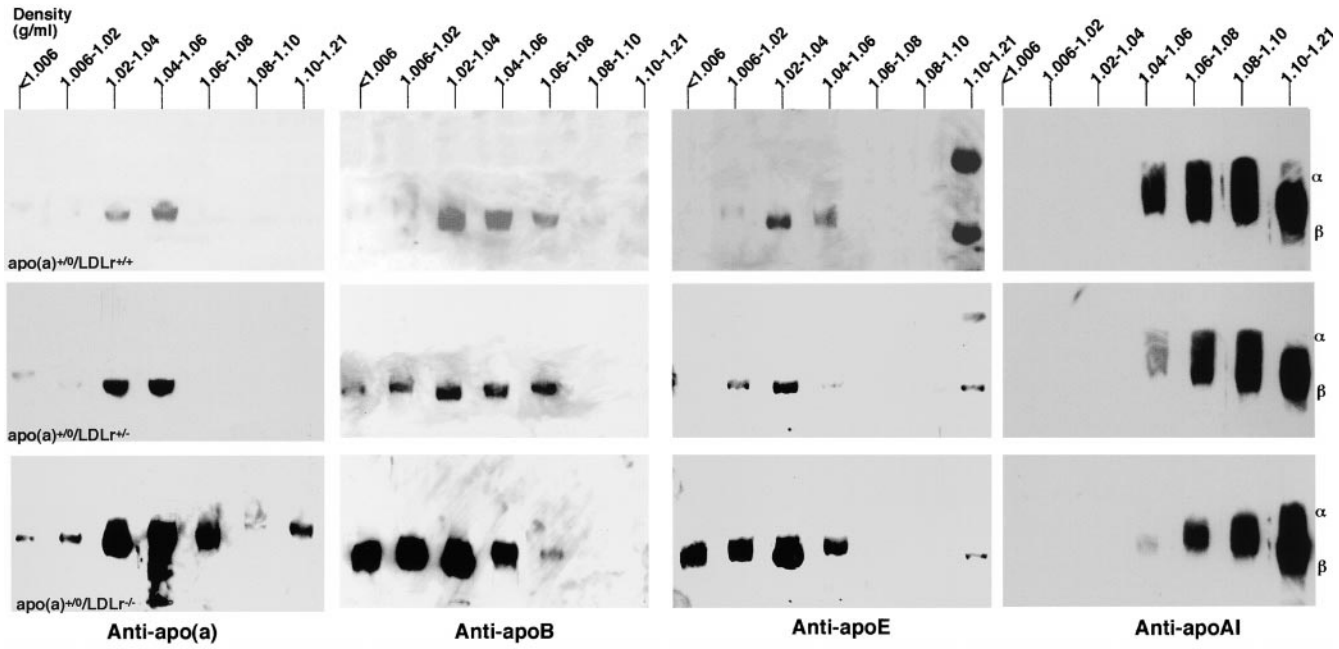


Fig. 5. Western blots showing distribution of human apo[a], rabbit apoB, apoE, and apoA-I in the plasma lipoproteins of WHHL heterozygous (upper panel) and homozygous (lower panel) rabbits. The plasma lipoproteins were isolated by sequential density ultracentrifugation. Two microliters of each lipoprotein fraction was resolved by 1% agarose gel electrophoresis. Western blots were performed by using anti-human apo[a] mAb and goat antisera specific for apoB, apoE, and apoA-I.

creased rate of synthesis. We found that apo[a] mRNA expression was basically not different between apo[a]$^{+/0}$/LDLr$^{-/-}$ and apo[a]$^{+/0}$/LDLr$^{+/+}$ rabbits (**Fig. 6**), suggesting that elevated hepatic synthesis of apo[a] did not account for the accumulation of Lp[a] in WHHL rabbits. Recent studies from other laboratories suggest that the kidney may be an alternative organ for apo[a] catabolism (40). Accordingly, we also investigated apo[a] expression in the kidney. As shown in Fig. 6, although there was a weak expression of apo[a] in the kidneys of transgenic rabbits when the blot membranes were exposed for 72 h, there was no significant difference between apo[a]$^{+/0}$/LDLr$^{-/-}$ and apo[a]$^{+/0}$/LDLr$^{+/+}$ rabbits (Fig. 6). Biochemical and pathological examinations of kidneys of WHHL rabbits did not reveal any abnormal changes (data not shown).

DISCUSSION

The physiological function of Lp[a] is not fully understood; however, elevated plasma levels of Lp[a] have been shown to be associated with an increased risk of coronary and carotid artery disease, stroke, and restenosis (1–3). Several factors have been proposed to influence the plasma levels of Lp[a], including apo[a] size polymorphism, Lp[a] production rate from the liver, and catabolism by different cellular receptors (41). Studies using cultured cells and human subjects have generated conflicting conclusions regarding whether LDL receptor is involved in the catabolism of Lp[a]. To examine whether the LDL receptors may contribute to the catabolism of Lp[a], we generated human apo[a] transgenic WHHL rabbits that have defective LDL receptor functions. We found that there was an accumulation of plasma Lp[a] in transgenic rabbits in the setting of LDLr defective functions, leading to 2-fold and 4.2-fold increases of apo[a] levels in heterozygous and homozygous WHHL transgenic rabbits, respectively, compared to the level in normal transgenic rabbits. The increased apo[a] in WHHL transgenic rabbits was covalently associated with rabbit apoB to form Lp[a] particles, as shown in Fig. 3, and the apo[a] was mainly

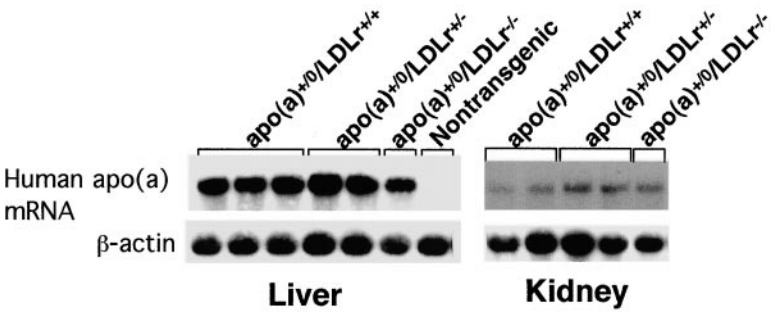


Fig. 6. Northern blot analysis for the determination of human apo[a] expression in transgenic rabbits. Total RNA was extracted from the livers and kidneys. Northern blotting was performed using a human apo[a] cDNA probe. Rehybridization of the membrane with a human β-actin probe showed that similar amounts of RNA had been loaded in each lane. The relative amount of mRNA for human apo[a] was standardized with that of mRNA for β-actin and there was no significant difference between WHHL and normal transgenic rabbits.

distributed in LDL particles, as shown in Fig. 5. It is notable that certain amounts of apo[a] were also found to be associated with VLDL and IDL, suggesting that these particles can bind to apo[a] to a certain extent. It is currently unknown whether these apo[a]-containing VLDL and IDL particles are synthesized in the liver de novo or assembled extracellularly or in circulating plasma. In addition, apo[a]- and apoB-containing particles were rich in apoE in WHHL transgenic rabbits, and associated with enrichment of the triglyceride content, which has been documented in nontransgenic WHHL rabbits (39).

Except for Lp[a] accumulation in the plasma, free apo[a] was also increased to a certain extent in WHHL transgenic rabbits (Fig. 3). We therefore studied whether WHHL transgenic rabbits have enhanced hepatic apo[a] production. This possibility was partly ruled out as the apo[a] mRNA levels in the liver, the major site of apo[a] production in transgenic rabbits, were similar in WHHL and normal transgenic rabbits. The accumulation of both Lp[a] and apo[a] leads us to speculate that both Lp[a] and apo[a] may be removed by LDL receptors. Recent studies indicate that the kidney may function to remove Lp[a] (40); therefore, we also investigated renal functions in WHHL rabbits. Biochemical analysis of rabbit plasma (BUN and creatine) and pathological studies on renal histology did not reveal any abnormalities in WHHL kidneys (data not shown). This result indicates that increased apo[a] levels in WHHL rabbits were not caused by renal dysfunction. Furthermore, we did not find significant apo[a] synthesis in the kidney in either WHHL or normal transgenic rabbits (Fig. 7). In WHHL rabbits, there is a high level of apoB-containing particles (VLDL, IDL, and LDL) due to LDLr defects, leading to marked hypercholesterolemia. One can argue that the increased Lp[a] levels might be caused by accumulation of apoB in the plasma, which might facilitate the binding of apo[a] and apoB to form Lp[a] particles. This may be partly true because cholesterol-fed transgenic rabbits with hypercholesterolemia also showed a 3-fold increase of Lp[a] levels in the plasma (data not shown). It is noteworthy that in cholesterol-fed rabbits, LDL receptor activity was also markedly down-regulated (42). Overall, these results suggest that dysfunction of LDL receptors, either genetic (WHHL rabbits) or as a result of a cholesterol-rich diet (as in cholesterol-fed rabbits), leads to an accumulation of Lp[a] in the plasma. Our results are in agreement with those of a previous study which demonstrated that there was a delayed catabolism of ^{125}I-labeled human Lp[a] (isoforms S1, S2, and S3) in WHHL homozygous rabbits compared to that in normal rabbits, and that the fractional catabolic rates (FCRs) of both Lp[a] and LDL were significantly smaller in WHHL rabbits than in normal rabbits (43). Recently, Sanan and his co-workers (44) generated LDLr-deficient ($LDLr^{-/-}$) mice expressing both human apo[a] and apoB. Compared to human apoB and apo[a] double transgenic mice with normal LDL receptor functions, in which Lp[a] levels were 15 ± 2 mg/dl in males and 12 ± 1 mg/dl in females on a chow diet (35), $LDLr^{-/-}$ transgenic ($apoB^{+/+}/apo[a]^{+/-}$) mice also showed increased levels of plasma Lp[a] (30 ± 4 mg/dl in males and 19 ± 3 mg/dl in females).

In addition to LDLr, several candidate receptors for Lp[a] catabolism have been proposed recently, including VLDL receptors (45), LDL receptor-related protein (LRP) (46), and megalin/pg330 (47), although it is not clear how these receptors contribute to the Lp[a] catabolism in vivo. Bottalico et al. (48) showed that cholesterol loading of macrophages leads to an enhancement of Lp[a] internalization and degradation via induction of a specific receptor that has yet to be identified. With respect to humans, one should be cautious about interpreting the current results found in WHHL transgenic rabbits regarding the role of the LDL receptor in the removal of Lp[a] from the plasma. First, as mentioned above, rabbits do not normally contain Lp[a]; therefore, the site of the Lp[a] removal in rabbits may be different from the normal site used in humans. This is also true for other non-Lp[a]-containing species such as transgenic mice, in which LDLr seems to play a role in Lp[a] catabolism (22). Secondly, the status of other candidate receptors (such as VLDL receptor and megalin/pg330) has not been fully examined in rabbits. Finally, the removal of Lp[a] mediated by LDL receptor may be also dependent upon the isoforms of apo[a] (46); therefore, transgenic rabbits expressing various different-sized apo[a] isoforms must be studied in the future to clarify this issue.

In addition to providing important insights into Lp[a] catabolism, several unique features of WHHL transgenic rabbits may make them a good model for the study of Lp[a] and atherosclerosis. Homozygous WHHL rabbits exhibit hypercholesterolemia from birth on a chow diet; however, Lp[a] is not normally present in rabbits and its significance in atherosclerosis is unknown. This study is currently being extended, and efforts are also now being directed toward breeding more WHHL transgenic rabbits in order to evaluate the effects of Lp[a] on development of atherosclerosis.

In summary, we demonstrated that LDL receptor may participate in the removal of Lp[a] from the plasma in transgenic rabbits. Although the relevance of our observations to human subjects remains uncertain, one may postulate that elevated levels of some, if not all, isoforms of Lp[a] seen in human familial hypercholesterolemia may be partially associated with LDLr defects.

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