The Expression of Intact and Mutant Human \textit{apoAI/CIII/AIV/AV} Gene Cluster in Transgenic Mice*

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The \textit{apoAI/CIII/AIV} gene cluster is involved in lipid metabolism and has a complex pattern of gene expression modulated by a common regulatory element, the \textit{apoCIII} enhancer. A new member of this cluster, apolipoprotein (apo) AV, has recently been discovered as a novel modifier in triglyceride metabolism. To determine the expression of all four apo genes in combination and, most importantly, whether the transcription of \textit{apoAI} is coregulated by the \textit{apoCIII} enhancer in the cluster, we generated an intact transgenic line carrying the 116-kb human \textit{apoAI/CIII/AIV/AV} gene cluster and a mutant transgenic line in which the \textit{apoCIII} enhancer was deleted from the 116-kb structure. We demonstrated that the \textit{apoCIII} enhancer regulated hepatic and intestinal \textit{apoAI}, \textit{apoCIII}, and \textit{apoAIV} expression; however, it did not direct the newly identified \textit{apoAV} in the cluster. Furthermore, human apo genes displayed integrated position-independent expression and a closer approximation of copy number-dependent expression in the intact transgenic mice. Because \textit{apoCIII} and \textit{apoAV} play opposite roles in triglyceride homeostasis, we analyzed the lipid profiles in our transgenic mice to assess the effects of human \textit{apoAI} gene cluster expression on lipid metabolism. The triglyceride level was elevated in intact transgenic mice but decreased in mutant ones compared with nontransgenic mice. In addition, the expression of human \textit{apoAI} and \textit{apoAIV} elevated high density lipoprotein cholesterol in transgenic mice fed an atherogenic diet. In conclusion, our studies with human \textit{apoAI/CIII/AIV/AV} gene cluster transgenic models showed that the \textit{apoCIII} enhancer regulated expression of \textit{apoAI}, \textit{apoCIII}, and \textit{apoAIV} but not \textit{apoAV} in vivo and showed the influences of expression of the entire cluster on lipid metabolism.

The genes coding \textit{apoAI}, \textit{apoCIII}, and \textit{apoAIV} are clustered within a 17-kb DNA segment on the long arm of human chromosome 11 (1). Comparative sequence analysis recently disclosed a new apolipoprotein (apo) family member, apoAV, located about 30 kb proximal to the \textit{apoAI/CIII/AIV} gene cluster (2) (Fig. 1).

\textit{apoAI} is the major protein component of HDL. \textit{apoAI} levels correlate positively with HDL cholesterol and negatively with atherosclerotic cardiovascular diseases (3). Transgenic mice overexpressing human \textit{apoAI} are protected from diet-related or \textit{apoE} deficiency-related atherosclerotic lesions (4, 5). \textit{apoCIII} is the major component of VLDL and a minor component of HDL, and mice carrying human \textit{apoCIII} develop severe hypertriglyceridemia (6, 7). HDL is a major carrier of plasma \textit{apoAIV}, and overexpression of human \textit{apoAIV} decreases aortic lesions in transgenic mice (8, 9). \textit{apoAIV} is found mainly in HDL and VLDL, and human \textit{apoAIV} transgenic mice display ~30% triglyceride level compared with wild-type mice (2). In humans, several important single-nucleotide polymorphisms within the \textit{apoAI} cluster genes have been shown to be strongly associated with dyslipidemia and to increase susceptibility to atherosclerosis (10, 11). Recently, such research has focused on association analysis of these single-nucleotide polymorphisms in the whole cluster from general population (12, 13) and familial combined hyperlipidemia (14, 15).

Besides its important functions, the \textit{apoAI} cluster is a useful model with which to study the transcriptional regulation and tissue-specific expression of clustered genes. The apo genes in this cluster are expressed at different levels in the liver and intestine: \textit{apoAI} is expressed at high levels in the liver and intestine, \textit{apoCIII} synthesis occurs predominantly in the liver and to a much lesser extent in the intestine, and \textit{apoAIV} is expressed mainly in the intestine and to a lesser extent in the liver (16), whereas \textit{apoAV} is expressed only in the liver (2). Previous transgenic mice carrying one or two apo genes displayed different expression levels, and these short genome fragments may lack much of the genetic information contained in the large flanking sequence for proper expression of the transgenes (16). With the increasing interest in research on the gene cluster, more and more important cis-acting elements have been identified to be involved in the transcriptional regulation of the gene cluster in the long distance, and this mechanism differs from that in a single gene (17). In the present study, we generated transgenic mice carrying a 116-kb intact genomic fragment containing the entire human \textit{apoAI}, \textit{apoCIII}, \textit{apoAIV}, and \textit{apoAV} to investigate the expression pattern of these genes in the cluster containing complete flanking sequences in vivo.

Previous studies have shown that a common regulatory element, the \textit{apoCIII} enhancer, located 490–890 bp upstream of \textit{apoCIII}, regulates tissue-specific expression of \textit{apoAI}, \textit{apoCIII},...
and apoAIV in vitro and in vivo (16) (Fig. 1). Although the proximal promoter of apoAIV has been studied, transcriptional regulation of this newly discovered gene is largely unknown (18, 19). Whether apoAIV transcription is also regulated by the apoCIII enhancer or by some other element is an interesting question, the answer to which will deepen understanding of the mechanism underlying transcriptional regulation of the apoAI gene cluster. To study these issues, we created a mutant transgenic mouse line containing the 116-kb human apoAI gene cluster in which the apoCIII enhancer core region was deleted.

Transgenic lines containing a 33-kbhuman apoAI/CIII/AIV gene gene cluster construct were recently generated, in which the expression of transgenes induced hyperlipidemia and reduced atherosclerosis (20). This model carried only a relatively short genomic fragment and did not contain the newly identified apoAIV. Because apoCIII and apoAV have predominant but opposite roles in triglyceride homeostasis, to explore the relationship between these two genes and altered triglyceride, and to observe the action of apoCIII and apoAV in triglyceride metabolism via our intact and mutant mice (21). Here we investigate the action of apoCIII and apoAV in triglyceride concentrations between the transgenic and control mice (21). We used 4–6-week-old mice for RNAse protection analysis (RPA) of human apoAI, apoCIII, apoAIV, and apoAV mRNA. In brief, 10 μg of total RNA was hybridized with [α-32P]UTP-labeled probes (1 × 106 cpm) in hybridization solution at 55 °C overnight, followed by RNase digestion (8 μg/ml) at 37 °C for 15 min and proteinase K treatment. The protected fragments were purified by water-saturated phenol/chloroform and electrophoresed on a 6% polyacrylamide-7 M urea gels. The gel was exposed to an x-ray film for 24–48 h, and the band intensity was quantified by a scanning densitometer (Amersham Biosciences).

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The apoAI; human apoCIII, pT7h-apoAIV, pSP6h-apoAV; and human apoAIV, pT7h-apoAV. All constructed plasmids were cloned by our laboratory.

Animals and Diets—Transgenic mice containing the intact and mutant 116-kb apoAI gene cluster were used. Nontransgenic littermates were used as controls. Five-week-old transgenic mice and nontransgenic littermates were housed in a temperature-controlled room with alternating 12-h light (7 a.m. to 7 p.m.) and dark (7 p.m. to 7 a.m.) periods. Animals were kept on a regular mouse chow. Male and female mice were used in approximately equal ratio in all experiments. For blood sampling, mice were fasted overnight and bled the next morning from the retro-orbital plexus while under anesthesia. Blood was collected into a tube containing 6 μl of EDTA (0.5 μl), separated by centrifu- nation at 2000 × g for 15 min, and kept at 4 °C until analysis.

In an atherogenic food experiment, the positive Fv mice of intact apoAI gene cluster transgenic line 4, mutant transgenic line 4 mice, and nontransgenic littermates had free access to an atherogenic diet (40% basic food, 15% fat, 1.25% cholesterol, 0.5% sodium chloride) or regular mouse chow for 20 weeks, and then plasma lipids were analyzed.
HDL, $d = 1.063-1.21$ g/ml were isolated from pooled serum of at least six mice of intact transgenic line 4 and six mice of mutant transgenic line 4, respectively. Human and wild-type mouse serum was used as a positive and negative control. Isolation was achieved by sequential ultracentrifugation at the respective densities at 42,000 rpm in a CP 70 MX ultracentrifuge equipped with a P42AT 0313 rotor (Hitachi) for 3, 12, and 16 h at 10 °C. Each lipoprotein fraction was analyzed for human apolipoprotein composition by 3–15% sequential SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Schleicher & Schuell) followed by incubation with polyclonal goat antibody against human apoAI, apoCIII, apoAIV (Santa Cruz Biotechnology), and apoAV (a gift from Dr. Robert O. Ryan and Michael N. Oda), respectively. With regard to apoCIII, the total plasma (5 μl) of transgenic mice was also used for Western blot. The antibodies directed against human apolipoproteins were highly specific and did not show any cross-species reactivity. Rabbit anti-goat IgG (Amersham Biosciences) was used as a secondary antibody.

The positive PCR mice of intact transgenic mouse line 4 and mutant line 4 were selected to analyze lipid profile after consumption of atherogenic and chow diets. Triglyceride concentrations were determined by enzymatic glycerol oxidase and cholestan enzymatic method; total and HDL cholesterol concentrations were determined by enzymatic cholestan oxidase method; total and HDL cholesterol concentrations were determined by enzymatic cholesterol oxidase method.

**RESULTS**

**Screening and Identification of BAC 286I4 and Mutant Clone**—After SnaBI digestion and pulse field gel electrophoresis of BAC DNA, the approximate sizes of the selected clones were estimated by comparison with size markers of multimers of bacteriophage (New England Biolabs) (data not shown). Clone 286I4 is 116-kb long and contains all of the human apoAI, apoCIII, apoAIV, and apoAV genes. Two terminal sequencing further proved that 286I4 was the fragment from lane 4 were selected to analyze lipid profile after consumption of atherogenic and chow diets. Triglyceride concentrations were determined by enzymatic glycerol oxidase and cholestan enzymatic method; total and HDL cholesterol concentrations were determined by enzymatic cholestan oxidase method; total and HDL cholesterol concentrations were determined by enzymatic cholesterol oxidase method.

Using temperature-sensitive shuttle vector-mediated homologous recombination, we identified a mutant 286I4 clone. Southern blotting results indicated that a ~300-bp core sequence (~491/793 to apoCIII) of the apoCIII enhancer was deleted in the mutant clone (Fig. 2, lane 1). Further sequencing results of the PCR product verified that the core sequence of the apoCIII enhancer was deleted.

**Creation of Intact Human apoAI/CIII/AIV/AV Transgenic Mice**—Four founder mice were identified by PCR and further verified by Southern blot. They all had the same hybrid bands with the human genomic DNA control (Fig. 4), which confirmed the structural integration of the human apoAI gene cluster. Compared with human genomic DNA, the copy numbers of transgenes in mice lines T1, T2, T3, and T4 were 1, 2, 3, and 2, respectively (Fig. 4).

**Tissue-specific Expression of apoAI, apoCIII, apoAIV, and apoAV in Intact apoAI Gene Cluster Transgenic Mice**—Human apo gene expression in the transgenic mouse lines was determined by RPA. The human apoAI clustered genes were primarily expressed in the liver and intestine of transgenic mice and were hardly detected in the kidney, brain, heart, lung, stomach, spleen, and skeletal muscle (data not shown). Human apoAI is expressed mainly in the liver and to a much lesser extent in the intestine of transgenic mice. Human apoCIII expression was high in the liver and moderate in the intestine. apoAIV is expressed mainly in the intestine and to a lesser extent in the liver. Consistent with previous findings, apoAV expression was detected only in the liver (shown as T1, T2, T3, and T4 in Figs. 5 and 6) (2). The expression pattern of the four apo genes in transgenic mice is similar to that of the human in vivo (2, 16).

Besides the expression of apoAI and apoAIV in transgenic mouse lines T1 (T1 in Fig. 9a), each transgene in the apoAI cluster transgenic mice showed <3-fold variation in expression per transgene copy (T1, T2, T3 and T4 in Fig. 9). Normalized
apoAI, apoCIII, apoAIV, and apoAV specific values were plotted against the gene copy number of the transgene for each animal. This indicated that the expression of apoAI clustered genes showed a closer approximation of copy number dependence (Fig. 7).

Creation of Mutant Human apoAI/CIII/AIV/AV Transgenic Mice—PCR and Southern blot verified that five stable mutant transgenic mouse lines were generated by linear mutant 286I4 BAC DNA microinjection. All mutant transgenic mouse lines had the same hybrid bands as the human genomic DNA control. Compared with human genomic DNA, the copy numbers for each strain of the five founders were 3, 20, 1, 2, and 6, respectively (Fig. 8).

Tissue-specific Expression of apoAI, apoCIII, apoAIV, and apoAV in Mutant apoAI Gene Cluster Transgenic Mice—No apoAI clustered gene expression was detected in tissues other than the liver and intestine in mutant transgenic mice (data not shown). Hepatic expression of human apoAI was abolished in mutant transgenic mouse lines 3 and 4 and was reduced significantly in lines 1, 2, and 5. The expression of human apoCIII and apoAIV was also reduced remarkably in the liver of mutant transgenic mice (M1–M5 in Figs. 5, 6, and 9). However, hepatic expression of human apoAV in mutant mice was not different from that in the intact apoAI cluster transgenic mice (Figs. 5, 6, and 9). This suggested that deletion of the apoCIII enhancer did not influence hepatic apoAV expression in the cluster.

Plasma Apolipoproteins and Lipids in the Transgenic Mice—The distribution of human apolipoproteins among various lipoprotein classes in the plasma of transgenic mice was analyzed by Western blot. As positive control (human plasma), human apoAI and apoAIV were present mainly in HDL (d =
1.063–1.21 g/ml) particles, and human apoAV was present in HDL and VLDL particles of intact and mutant transgenic mice (Fig. 10a). Human apoCIII was detected in the whole plasma of transgenic mice. All four human apolipoproteins were not detected in the lipoproteins of the negative control, wild-type mice plasma (Fig. 10).

Table I presents plasma lipid profiles in the intact human apoAI cluster transgenic mice (line 4), mutant apoAI cluster transgenic mice (line 4), and nontransgenic littermates. Plasma triglyceride levels in the intact apoAI cluster transgenic mice increased 3-fold compared with control animals (296 ± 127 versus 81 ± 15, p < 0.05), whereas in the mutant transgenic mice, the level decreased to 70% compared with control mice (57 ± 12 versus 81 ± 15, p < 0.05). Total cholesterol in the intact transgenic mice increased about 60% (141 ± 31 versus 87 ± 14, p < 0.05), mainly due to an increase in the non-HDL subclass, whereas no significant change was observed in mutant transgenic mice compared with control animals (90 ± 15 versus 87 ± 14) (Table I).

**Effects of Atherogenic Diets on apo Gene Expression and Lipid Profile in Transgenic Mice**—Total cholesterol was elevated by 36% in the intact human apoAI gene cluster transgenic mice (from 141 ± 31 to 192 ± 84, p < 0.05), 66% in the mutant transgenic mice (from 90 ± 15 to 149 ± 24, p < 0.05), and 44% (from 87 ± 14 to 125 ± 32, p < 0.05) in control littermates when these three groups of mice were fed the atherogenic diet for 20 weeks, respectively. The atherogenic diet significantly increased the concentration of HDL cholesterol (from 73 ± 21 to 104 ± 31, p < 0.05) in the intact transgenic mice and mutant transgenic mice (from 67 ± 16 to 92 ± 14, p < 0.05), whereas no significant change was observed in HDL cholesterol level in control littermates (Table I).

The expression of human apo genes in the liver and intestine of intact transgenic mice was determined by RPA after these animals were fed the atherogenic diet. The atherogenic diet did not seem to influence hepatic expression of human apoAI, apoCIII, apoAIV, and apoAV, whereas up-regulated intestinal expression of human apoCIII and apoAV was seen in transgenic mice fed the atherogenic diet as compared with those fed the chow diet (Fig. 11).

**DISCUSSION**

Transgenic animals carrying large genomic fragments provide more complete and accurate information than animals harboring short constructs, and this is especially important for studying clustered genes. In this study, we screened the human BAC library and obtained a 116-kb clone containing the whole human apoAI/CIII/AIV/AV gene cluster. BAC DNA was suc-
studies on transgenic mice carrying a single apo gene or short pattern in our intact apoAI cluster transgenic mice. Moreover, gene expression exhibited an integrated position-independent transgenic mice, wild-type mice, and human.

### TABLE I

**Lipid concentration in human apoAI cluster transgenic and control mice fed with atherogenic and chow diets**

| Analyte               | Control mice | ApoAI cluster Tg mice | Mutant cluster Tg mice |
|-----------------------|--------------|-----------------------|------------------------|
|                       | Chow diet    | Atherogenic diet      | Chow diet              | Atherogenic diet      |
|                       | (n = 12)     | (n = 12)              | (n = 14)               | (n = 8)               |
| Triglyceride          | mg/dl        | mg/dl                 | mg/dl                  | mg/dl                 |
|                       | 81 ± 15      | 61 ± 13\(^a\)         | 296 ± 127\(^b\)        | 116 ± 58\(^a\)        |
| Total cholesterol     | 87 ± 14      | 125 ± 32\(^a\)        | 141 ± 31\(^b\)         | 192 ± 84\(^a\)        |
| HDL cholesterol       | 61 ± 14      | 77 ± 21               | 73 ± 21                | 104 ± 31\(^a\)        |
| Non-HDL cholesterol   | 28 ± 14      | 48 ± 23               | 68 ± 21\(^b\)          | 88 ± 30               |

\(^a\) p < 0.05 versus mice fed a chow diet.  
\(^b\) p < 0.05 versus control mice fed chow diet.

The tissue-specific expression patterns of apoAI, apoCIII, apoAIV, and apoAV in our intact apoAI cluster transgenic mice were similar to those of humans, but the intestinal apoAI expression level was very low in the intact transgenic mice compared with the relatively high apoAI mRNA levels in human and mouse intestine (2, 16, 28, 29). This apoAI expression level is similar to those in the report of transgenic mice carrying a 33-kb human apoAI/CIII/AIV cluster in which intestinal apoAI mRNA did not exceed 20% of liver apoAI mRNA (20). However, in apoAI/CIII transgenic mice carrying only the apoCIII enhancer, 2.1-kb 5' region and 1.6-kb 3' region of the human apoAI gene, the apoAI expression level in the intestine was almost as high as that seen in the liver (23). From the above-mentioned results, we hypothesize that this discrepancy may be caused by a different reaction to the 5'-flanking sequence up to 2.1 kb or the 3'-flanking sequence up to 1.6 kb of apoAI in humans and mice. Moreover, overexpression of other apo genes in the cluster and subsequent lipid profile alterations might another possible reason. In addition, human apoAIV was ex-

**FIG. 10.** Western blot analysis of four human apolipoproteins in the plasma lipoprotein of intact and mutant apoAI cluster transgenic mice, wild-type mice, and human. a, the distribution of human apoAI, apoAIV, and apoAV among the various lipoprotein classes in plasma. Approximately 40 μl from each corresponding fraction were run on 3–15% gradient SDS-PAGE and then blotted onto nitrocellulose. Blots were treated with the indicated anti-human antibody as described under “Experimental Procedures.” b, overexpression of human apoCIII in plasma. h, wild-type mice; t, intact human apoAI gene cluster transgenic mice; m, mutant human apoAI gene cluster transgenic mice; p, plasma; H, HDL; L, IDL + LDL; V, VLDL.

**Fig. 11.** The influence of atherogenic diets on transcription of the human apo genes in intact human apoAI gene cluster transgenic mice. a, liver; b, intestine.
pressed mainly in the intestine and to a lesser extent in the liver of transgenic mice. This observation is consistent with the tissue-specific expression of apoAIV in humans and in human apoAI gene transgenic mice (30). In contrast, apoAV was expressed mainly in the liver and at relatively low levels in the intestine in 33-kb human apoAI/CIII/AIV gene cluster transgenic mice that had the same 3’-flanking sequence as the human apoAI gene transgenic mouse (20). Excluding possible differences in methods used to measure human apoAV mRNA and the physiological variability of apoAV mRNA levels, we think this discrepancy is worth noting, and it suggests that our large fragment used to establish the transgenic mice recapitulates the endogenous expression pattern more authentically.

Previous in vitro and in vivo studies have proved that the apoCIII enhancer, as a common regulatory element, regulates tissue-specific expression of apoAI, apoCIII, and apoAV (16). In order to gain further insight into the role of this common enhancer in the whole cluster, we engineered five stable mutant transgenic mouse lines that carried the whole human apoAI cluster construct but in which the apoCIII enhancer was deleted. We found that deleting the apoCIII enhancer almost abolished intestinal expression of human apoAI and apoCIII, as compared with the expression level of genes in intact gene cluster transgenic mice. The enhancer-deleted mice also showed a significant decrease in hepatic expression of human apoAI, apoCIII, and apoAV and intestinal expression of human apoAIV, respectively (Figs. 5, 6, and 9). These results are in accord with studies using transgenic lines for short mutant human apoAI, apoCIII, and apoAV constructs (23–25). In contrast with other apo genes in the cluster, hepatic expression of human apoAV in all mutant transgenic mice was not influenced by deletion of the apoCIII enhancer, providing evidence that the newly identified apoAV is not regulated by the apoCIII enhancer in the cluster (Figs. 5 and 9). This conclusion was further supported by the results from lipid profile analysis in the transgenic mice: plasma triglyceride in our mutant apoAI cluster transgenic mice decreased to 70% compared with nontransgenic mice (57 ± 12 versus 81 ± 15, p < 0.05) (Table I). apoCIII and apoAV have predominant but opposite roles in plasma triglyceride: compared with nontransgenic controls, the triglyceride level in human apoCIII transgenic mice increases to 200–2000% but decreases to 30% in human apoAV transgenic mice (2, 6). In our mutant transgenic mouse, human apoAV overexpression was not influenced, whereas human apoCIII expression was nearly abolished after deletion of the apoCIII enhancer, which led to the fall of triglyceride level (apoAI and apoAV were not considered here because these genes do not seem to influence the plasma triglyceride in transgenic mice, respectively) (29, 32). In contrast, in our intact apoAI gene cluster transgenic mice, the overexpression of both apoCIII and apoAV induced—2-fold increase of plasma triglyceride (Table I). Our results revealed that the apoCIII enhancer was not a common enhancer for all four apo genes in the cluster and thus raised the following issue: is there any other element that performs coregulation between apoAV and the other three apo genes in the cluster?

To investigate the relationship of the apoCIII and apoAV genes, Baroukh et al. (21) recently generated independent transgenic lines that either overexpressed or completely lacked both these genes and proposed that apoAV and apoCIII independently influence plasma triglyceride concentrations but in an opposing manner. In our intact apoAI gene cluster transgenic lines, plasma triglyceride level increased—3-fold compared with control mice. This value is higher than that from the apoAI/CIII/AIV/AV transgenic mouse generated by Baroukh et al. (21) but is less than that in single human apoCIII transgenic mice (5–20-fold) (6) and in the 33-kb human apoAI/CIII/AIV/AV transgenic mice (4–10-fold) (20). In addition, in our mutant transgenic mouse, deletion of the apoCIII enhancer did not affect human apoAV expression but significantly reduced human apoCIII expression, thus leading to a lower triglyceride level compared with control mice (Table I). Therefore, triglyceride levels in our intact and mutant transgenic mice further indicated that apoCIII and apoAV independently influence plasma triglyceride homeostasis in an opposing manner, which was supported by Baroukh et al. (21).

The total cholesterol concentration of the intact transgenic mice was significantly elevated, due mostly to an increase in non-HDL cholesterol, a result similar to those seen in the study of the 33-kb human apoAI/CIII/AIV gene cluster transgenic mice (20) but different from those of studies of the human apoAI transgenic mice, which showed a significant increase in HDL cholesterol but non-HDL cholesterol (6, 29). Cholesterol level in our transgenic mice indicates the complex action of apolipoproteins in the cluster because expression of human apoCIII raised the total cholesterol, mainly due to an increase in non-HDL cholesterol (7, 33). Total cholesterol did not change in human apoAV or apoAIV transgenic mice (2, 8).

The atherogenic diet elevated total cholesterol levels in control mice as a result of an increase in non-HDL cholesterol fractions, whereas in intact transgenic mice, HDL cholesterol was significantly increased as a result of atherogenic diet feeding, and this change was similar to that seen in the single human apoAI transgenic line (29) and the human 33-kb apoAI/CIII/AIV gene cluster transgenic mice (20) but different from that in another line of human apoAI transgenic mice (6) and human apoAIV transgenic mice (8). Human apoAI and apoAIV were detected mainly in the HDL particle of transgenic mice plasma (Fig. 10a), and these two well-known agents in promoting reverse cholesterol transport led to the elevation of HDL cholesterol after atherogenic diet feeding (31). This primarily suggested the anti-atherosclerosis property of our transgenic mice because the inverse association between HDL cholesterol and the risk of coronary heart disease is well known (3).

The atherogenic diet increased both HDL cholesterol and non-HDL cholesterol in mutant mice (Table I). This was different from the effect seen in intact transgenic mice and nontransgenic mice. We think that a low level of apoAI and apoAIV in the plasma of mutant transgenic mice may lead to these differences (Fig. 10).

The atherogenic diet did not seem to influence hepatic expression of human apoAI, apoCIII, and apoAV, whereas upregulated intestinal expression of human apoCIII and apoAV was seen in transgenic mice fed the atherogenic diet as compared with those fed the chow diet (Fig. 11). These expression changes were similar to the results of studies of the 33-kb apoAI/CIII/AIV gene cluster transgenic mice (20). Moreover, our results firstly indicated that human apoAIV expression did not seem to be influenced by the atherogenic diet (Fig. 11).

In conclusion, we generated an intact transgenic line carrying the 116-kb human apoAI/CIII/AIVAV gene cluster and a mutant transgenic line in which the apoCIII enhancer was deleted from the 116-kb structure. Comparing expression of the apo genes in the cluster, we demonstrated that the apoCIII enhancer directed the hepatic and intestinal expression of apoAI, apoCIII, and apoAV in vivo but did not regulate the transcription of apoAV. The triglyceride levels in the plasma of our transgenic mice indicated apoCIII and apoAV independently play opposite roles in plasma triglyceride homeostasis. Moreover, expression of human apoAI and apoAIV led to the elevation of HDL cholesterol in apoAI cluster transgenic mice fed with atherogenic diet. Thus we have generated useful mod-
els with which to study apoAI, apoCIII, apoAV, and apoAV and their effects on lipid metabolism as well as to explore mechanisms underlying transcriptional regulation of these genes in combination.

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