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Regulation of the expression of the apolipoprotein(a) gene : evidence for a regulatory role of the 5' distal ACR enhancer in YAC transgenic mice

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Objective - The apolipoprotein(a) gene locus is the major determinant of the circulating concentration of the atherothrombogenic lipoprotein, Lp(a). *In vitro* analysis of the intergenic region between the apo(a) and plasminogen genes revealed the presence of a putative apo(a) transcription control region (ACR) ~ 20-kb upstream of the apo(a) gene, that significantly increases the minimal promoter activity of the human apo(a) gene.

Methods and Results - To examine the function of the ACR in its natural genomic context, we used the Cre-*lox*P recombination system to generate two nearly identical apo(a)-YAC transgenic mouse lines that possess a single integration site for the human apo(a) transgene in the mouse genome, but differ by the presence or absence of the ACR enhancer. Analysis of the two groups of animals revealed that the deletion of the ACR was associated with 30% reduction in plasma and mRNA apo(a) levels. Apo(a)-YAC transgenic mice with and without the ACR sequence were similar in all other aspects of apo(a) regulation, including liver specific apo(a) expression and alteration in expression levels in response to sexual maturation and a high-fat diet.

Conclusion - This study provides the first experimental *in vivo* evidence for a functional role of the ACR enhancer in determining levels of apo(a) expression.

Key words : apolipoprotein(a) - gene expression – enhancer – YAC - transgenic mice

Lp(a) is a cholesterol-rich particle that differs from the low density lipoprotein (LDL) by the presence of the highly glycosylated apolipoprotein, apo(a), that is covalently-linked by a disulfide bond to apolipoprotein B100 [1,2]. Clinical interest in lipoprotein Lp(a) lies in its potential role as a proatherogenic and prothrombogenic risk factor [3]. Despite some conflicting results, numerous prospective studies have demonstrated an association between elevated levels of Lp(a) and coronary heart disease [4].

The plasma concentration of Lp(a) is an inherited quantitative trait and remains fairly constant throughout life in a given individual. Nevertheless, Lp(a) levels may differ over a thousand-fold range between individuals. The apo(a) gene is the major determinant of lipoprotein(a) concentration. It has been estimated that the apo(a) gene locus explain from 74% to more than 90% of the intra-individual variability in Lp(a) levels in Caucasians [5,6]. The size polymorphism of apo(a) affects apo(a) protein processing [7] and clearly accounts for a significant portion of the total variability in Lp(a) levels seen in human populations [5,8,9]. However, other cis-sequences which influence apo(a) expression are also believed to contribute to variance in plasma Lp(a) levels [10-12].

In addition to humans, the apo(a) gene is naturally present only in Old World monkeys and the hedgehog. Due to the paucity of model organisms with an apo(a) orthologue, the regulation of apo(a) synthesis has been difficult to assess *in vivo*. Yeast artificial chromosome (YAC) genomic clones carrying human apo(a) alleles with significant 5' and 3' human flanking DNA have been used to generate transgenic mouse lines [13,14]. These transgenic mice, expressing apo(a) in an appropriate liver-specific manner, have provided information regarding the regulation of apo(a) mRNA synthesis most notably by the effect on its expression of ovarian sex steroid hormones [15] and growth hormone [16]. The transcriptional control elements that participate in basal apo(a) expression and in the above regulatory processes are yet to be characterized.

Functional analysis of the plasminogen-apo(a) intergenic 40-kb sequence has revealed the presence of two candidate regions that possess enhancer activities in transient transfection assays [17,18]. These regions coincide with liver-specific Dnase I-hypersensitive sites (DHII and DHIII) characterized previously and suggest that they likely correspond to open chromatin domains accessible to nuclear transcription factors. The DHII region is located approximately 26-kb away from the apo(a) promoter and its activity is repressed *in vitro* by oestrogen, suggesting that it may correspond to the cis-regulatory element mediating oestrogen-dependent repression of apo(a) expression [19]. The DHIII core element, also called the apo(a) transcription control region (ACR), exhibits the highest stimulating activity (10-15 fold) of the two enhancers when ligated to the apo(a) proximal promoter. Of note is the observation that the ACR is located within the 5' untranslated region of a LINE 1 retrotransposon element, nearly ~20 kb upstream of the transcription start site of the apo(a) gene.

To evaluate the functional role of the ACR enhancer in its natural genomic context, we created two mouse lines containing a 270-kb human apo(a) transgene that differs only by the presence or the absence of the ACR element. Analysis of apo(a) expression in these animals suggests that while the targeted deletion of the ACR sequence in apo(a) transgenic mice does not affect various aspects of the regulation of the apo(a) gene, it does impact on basal apo(a) plasma levels. These findings provide the first experimental evidence that the ACR actively participates in apo(a) gene expression *in vivo*.

METHODS

ACR Targeting vector , Replacement of the ACR element by a floxed ACR in the apo(a)-YAC, Analysis of apo(a)-YAC modifications and Apo(a)-YAC integration into mouse ES cells – Please see the expanded Methods section (available online at http://www.ahajournals.org).

Generation of transgenic mice – Two positive ES clones containing the apo(a)-YAC were injected into C57BL/6J blastocysts, which were implanted into recipient females. Chimeric mice were obtained and mated with C57BL/6J mice. Germ line transmission was evaluated in the F1 agouti progeny by PCR analysis. Plasma from PCR-tested positive animals were subjected to immunoblot analysis using a polyclonal anti-apo(a) antibody to evaluate apo(a) production. MMTV-Cre transgenic mice (over 95% FVB background) were bred with apo(a) transgenic mice to produce litter mate mice of the desired genotypes used in this study. Genotyping of the animals for the MMTV-Cre transgene was done by PCR using the primer pair Cre-F (5'-GGTCGATGCAACGAGTGATG) and Cre-R (5'-CAGCATTGCTGTCACTTGGTC) (293 bp). The animals were housed in a conventional animal facility on a 6 a.m.-6 p.m. dark/light cycle. They were weaned at 21 days and fed *ad libitum* a normal mouse chow diet (Purina No.5001). For the high-fat diet experiment, 14-20 weeks old female transgenic mice were placed on a Western-type diet consisting of 1.25% cholesterol, 0.5% cholic acid, and 15% fat for two weeks. Plasma samples were collected prior to and after the diet.

RNA preparation and RT-PCR methods - Please see the expanded Methods section (available online at http://www.ahajournals.org).

Immunoblotting and quantification of plasma apo(a) - Blood samples were collected in heparinized capillary tubes from the retro-orbital sinus at the specified age, centrifuged and plasma stored at -80°C. Samples were electrophoresed on 4.5% SDS-polyacrylamide gel under reducing conditions. Proteins were subsequently transferred onto nitrocellulose membranes (Hybond ECL, Amersham Pharmacia Biotech) for western blot analysis using a peroxydase-conjugated sheep polyclonal anti-apo(a) antibody [20]. The revelation was performed by chemiluminescence using Hyperfilm ECL (Amersham Pharmacia Biotech) for signal capture. Deviation from a linear response of the film to the emitted signal intensity was controlled for by using low film exposures. Films were digitized with a desktop scanner and densitometry was performed with the image processing and analysis program *Scion image*. For normalization and quantification purposes, a pool of plasma collected from several apo(a) transgenic mice was included in all western blots. The apo(a) concentration of the plasma pool was determined by densitometric analysis of a western blot loaded with known quantities of a purified recombinant apo(a) [21] along with the pool. The presence of aliquots of the plasma pool in all western blots allowed the conversion of the measured peak areas into apo(a) quantities. For each plasma sample, at least three independent western blot experiments were performed to calculate a mean value ± SD. This normalization and quantification procedure gave a mean inter-assays CV of 29% calculated on the basis of all western blots performed. The mean CV calculated on intra-assays without normalizing to the pool was 17%. One animal exhibiting an atypical apo(a) plasma level relative to the other members of its group (value above the mean apo(a) level plus 3xSD) was considered as an outlier and excluded from the study. At the indicated time points, apo(a) plasma levels between groups of mice were compared using Student's t test.

RESULTS

Creation of apo(a)-YAC transgenic mice with or without the ACR

The general strategy to modify the apo(a)-YAC by flanking the ACR with *lox*P sequences (floxed ACR) is presented as supplementary data (Figure I, please see http://atvb.ahajournals.org). To generate apo(a) transgenic (tg) mice, ES clones that had integrated the apo(a)-YAC through PEG-mediated spheroplast fusion, were injected into C57BL/6 blastocysts. The introduction of YACs into mammalian cells by spheroplast fusion has been shown in numerous studies to result in the stable integration of single copy, unrearranged YAC transgenes [22-24]. Chimeric mice had variable plasma apo(a) levels, as documented by western blot analysis. When immunodetectable, the size of the apo(a) protein secreted was identical in every animal suggesting that the apo(a)-YAC present in the selected ES clones had not been rearranged. The apparent molecular mass of the apo(a) isoform expressed was consistent with the YAC containing a 12 kringle 4 type-2 repeats apo(a) allele, as originally reported for this human apo(a) YAC [13]. Two out of five chimeric males derived from the same ES clone gave germ line transmission of the apo(a)-YAC when mated with C57BL/6 females and were used to establish transgenic lines. The apo(a)-YAC transgene was maintained in an hemizygous state by repeated breeding with C57BL/6 mice. Males from the F2 generation offspring were mated with MMTV-Cre transgenic females to produce litter mate mice that were either hemizygous for the apo(a) transgene or doubly hemizygous with both transgenes. Cre-mediated recombination of floxed constructs using this specific Cre recombinase expressing line has been shown to occur in every tissue examined and notably the female germ line [25]. We were able to conclude based on PCR analysis that transmission of the cre transgene was systematically accompanied by the deletion of the floxed ACR element in the apo(a)-YAC transgene. Recombination appeared to be total since amplification of the PCR fragment corresponding

to the non-recombined floxed ACR was not observed in any of the tissues analysed (liver, tail) of the double apo(a)/MMTV-cre progeny (fig. 1). These animals were designated apo(a)/ACR^{del} tg mice. Comprehensive PCR analysis covering the entire apo(a)-YAC was run on both apo(a) and apo(a)/ACR^{del} tg mice and showed the presence of all PCR products of the expected size for both genotypes (fig. 1). Taken together, these data are consistent with the presence of the apo(a)-YAC in its entirety in all apo(a)-YAC transgenic mice but differing solely in the apo(a)/ACR^{del} progeny by the absence of the ACR.

Apo(a) transgene tissue expression

While small amounts of apo(a) mRNA were detected in testes and brain of rhesus monkeys, the liver is the major site of apo(a) synthesis in humans and Old World monkeys [26,27]. To investigate whether deletion of the ACR could affect the tissue-specificity of apo(a) mRNA synthesis, we examined the tissue distribution of apo(a) expression in apo(a) and apo(a)/ACR^{del} tg mice by subjecting total RNA preparations from several tissues (adrenal, kidney, heart, spleen, brain, liver) to reverse transcriptase-PCR analysis (fig. 2). Apo(a) mRNA was detected only in the liver for the various lines of mice independent of whether their apo(a) transgene contained the ACR or not.

Apo(a) expression levels

Apo(a) plasma levels in transgenic mice were determined by densitometric analysis of immunoblots performed with a polyclonal anti-apo(a) antibody. Plasmas of apo(a) and apo(a)/ACR^{del} mice were analysed at different time points over an eight weeks period (fig. 3). While male and female mice exhibited similar levels at the 28 day time point (compare band intensities against the "pool" lane in figure 3), apo(a) expression decreased after 4 weeks of age in male mice containing both apo(a) and apo(a)/ACR^{del} transgenes. This decrease associated with the sexual maturation of the animals has been previously reported

in transgenic mice carrying the same apo(a)-YAC transgene clone [13] as well as in another line of transgenic mice containing a different human apo(a) genomic transgene [14]. Such a reduction in plasma apo(a) levels has been previously shown to associate with decreases in expression of the human apo(a) transgene. In contrast, we observed a 2-3 fold increase of plasma levels of apo(a) after 4 weeks in female mice.

To evaluate whether the deletion of the ACR influenced the level of apo(a) synthesis, we compared apo(a) plasma concentrations in apo(a) and apo(a)/ACR^{del} littermate mice (fig. 4A). In young animals (28 day time point), there was a clear inter-individual variability (2-3 fold) in the plasma levels of apo(a) in female and male mice for both genotypes. It is noteworthy that similar variability was also observed in the other apo(a)-YAC transgenic line developed by Acquati *et al* [14]. The mean apo(a) level in female mice containing the apo(a)/ACR^{del} transgene was some 30% ($p=0.01$) lower than that in female mice containing an intact apo(a) transgene (1.4±0.5 vs 2.0±0.8 μg/ml, respectively). After sexual maturity (day 84), apo(a) concentration varied noticeably between individuals among the two groups of female mice. Similarly to day 28, a statistically significant decrease (27%; $p=0.04$) in mean plasma apo(a) level was noted in female mice containing an apo(a)/ACR^{del} transgene as compared to those containing an intact apo(a) transgene (4.0±2.0 vs 5.5±2.0 μg/ml, respectively). Likewise, comparison of the amounts of hepatic apo(a) mRNA between both genotypes using real-time quantitative RT-PCR revealed the same decrease as that seen by comparing plasma apo(a) protein concentrations (fig. 4B). These results provide good support for a role of the ACR in determining the level of apo(a) gene expression and thereby apo(a) plasma levels. Assessing the impact of deleting the ACR from the apo(a) transgene was more complicated in male mice due to diminished expression of apo(a) as the males aged. Because of the extremely low concentrations of apo(a) in post-pubertal males, the comparison of apo(a) levels could not be performed after puberty. In young apo(a) transgenic males, the loss of the ACR element was associated with a 21% reduction

in apo(a) plasma levels, a difference which did not quite achieve statistical significance (fig. 4A).

Changes in apo(a) expression following a high-fat diet

Cholesterol and cholate containing high-fat diet has previously been demonstrated to affect apo(a) mRNA levels in apo(a) transgenic mice [14]. To address the role of the ACR enhancer in this transcriptional regulation of apo(a) expression, adult apo(a) and apo(a)/ACR^{del} female mice (n=4 in each group) were fed a Western-type diet for two weeks. As illustrated in figure 5A, whereas apo(a) plasma concentrations in both groups of mice before the diet were in the same range as those observed at 84 days (fig. 4), a marked decrease (~ 3-fold) in the plasma levels of apo(a) was clearly observed following the Western diet. However, densitometric analyses of the western blots indicated a similar reduction in the apo(a) plasma concentrations in either apo(a) tg or apo(a)/ACR^{del} tg mice. Comparison of hepatic apo(a) mRNA levels from female mice fed a chow or the high-fat diet showed that, as previously reported [14], the pronounced fall in apo(a) plasma levels in mice receiving the high-fat diet was associated with a similar reduction in apo(a) mRNA levels (fig. 5B). Interestingly, despite this important decline in the amount of apo(a) transcript following the high-fat diet, the apo(a)/ACR^{del} tg mice still displayed a ~30% decrease in apo(a) mRNA levels when compared to apo(a) tg mice (fig. 5B).

DISCUSSION

Recent *in vitro* studies which allowed identification of the enhancer element ACR located in a L1 retrotransposon upstream the apo(a) gene have raised the possibility that apo(a) expression is partly determined by this element. Transgenic mice created for this study have enabled us to evaluate *in vivo* as to whether the enhancer is only an inherent element of the L1, or whether it actively influences apo(a) expression. Our findings are consistent with prior *in vitro* characterization of the ACR and add support to the hypothesis that the ACR participates in optimal transcriptional expression of the apo(a) gene. This observation represents a rare example of a L1 element affecting the expression of a nearby gene, and particularly illustrates the manner in which L1s may contribute to genome evolution as potential mobile gene control elements.

To date two apo(a)-YAC clones, including the one used in this study, have successfully been used to create transgenic mice and both constructs exhibit liver-specific expression of the human apo(a) transgene [13,14]. Here, we observed a similar restricted tissue-specificity of apo(a) expression in mice both with and without the ACR element. It is important to note that in the earlier description [13] of the four separate lines of transgenic mice carrying a single copy of the same apo(a)-YAC that we used, a large range of apo(a) plasma concentrations (1-75 mg/dl) was detected; the transgenic line generated in the present study being in the lower expression range. These data suggest that the important cis-acting elements providing liver-specific apo(a) gene transcription are present in the YAC clone; this region of genomic DNA is however likely to some extent to be subject to chromosomal site-integration effect that can influence apo(a) transgene expression. This problem was largely circumvented in the present comparative study since the apo(a) and apo(a)/ACR^{del} transgenes mapped to the exact same genomic location being derived from the same transgenic line.

Several studies have suggested that deletion of individual regulatory elements/enhancers that are part of a locus control region (LCR) may not necessarily result in marked reduction in gene expression since, at least partially, functionally redundant elements may be present and may compensate for the loss of activity [24,28]. Of particular relevance have been studies describing the β-globin LCR that consists of several Dnase I-hypersensitive chromatin domains (HS) spread over a region of 20-30 kb [29]. Targeted deletions of murine 5'HS2 or HS3 sites that demonstrated important LCR activities when tested individually in various expression assays, caused only a 30% reduction of murine globin gene expression [30,31]. Likewise in our study, apo(a) plasma levels were only moderately altered upon deletion of the ACR *in vivo* while *in vitro* the enhancer exhibited robust stimulation of apo(a) promoter activity [17,18]. We cannot exclude however the possibility that integration site-specific silencing effects may have dampened the ACR activity or that the presence of other functional LCR activities contribute along with the ACR element to general transcriptional activation at the apo(a) genomic locus. Notably, in the 40-kb intergenic region that separates the 5' regions of the apo(a) and plasminogen genes, three other DNase I-hypersensitive sites are present [32], including the apo(a) enhancer DHII [19]; these regions represent important candidate regions. Recent findings have also suggested that elements that lie outside the 5' region of the apo(a) gene may also be required for its expression [14].

The ACR region co-localizes with a DNase I-hypersensitive site that was initially identified exclusively in hepatoma cell types *in vitro* [32] and *in vivo* in the liver of apo(a)-YAC transgenic mice [18]. While these observations may indicate that the ACR acts as a hepatic control region of apo(a) expression**,** *in vitro* the enhancer does not exhibit tissue-specificity [17,18]. Besides, ACR enhancer function depends on the Ets and Sp1 transcription factors that are ubiquitously expressed. Taken together, these findings indicate that the ACR is not likely to contribute to the liver-restricted expression of apo(a). The absence of

ectopic expression of the apo(a) transgene in apo(a)/ACR^{del} observed in this study is consistent with the latter hypothesis.

Apo(a)-YAC transgenic mice constitute a valuable model in which to assess the regulation of the human apo(a) gene. However, major differences in apo(a) expression have clearly been observed in this model as compared to that seen in man. For example, marked decrease in apo(a) levels occurs when the animals are fed with a high-fat containing diet [14]; by contrast, variation in the type of dietary fat consumed is associated with none to only moderate variations in Lp(a) levels in man [33,34]. The profound reduction in apo(a) mRNA levels previously reported in male apo(a) tg mice during puberty [13] is equally not observed in man. The results of the present study exclude the possibility that such apo(a) transcriptional regulation in mice is mediated through the ACR element, since apo(a) and apo(a)/ACR^{del} tg behaved similarly with respect to these effects. The understanding of such regulations in apo(a) tg mice may however shed light on metabolic pathways that influence Lp(a) levels in man. Recently, it has been demonstrated that the fall in apo(a) serum levels associated with puberty in apo(a)-YAC transgenic males is determined by the secretory pattern of growth hormone (GH) in mice; this pattern is distinct from that in females which results in a sexually dimorphic regulation of apo(a) expression [16]. Indeed, the latter study offered insights into the mechanisms responsible for the GH-associated increase in plasma levels of Lp(a) observed in humans [35] by revealing that GH regulates apo(a) expression. It is however noteworthy that the gender-specific pattern of apo(a) expression in adult apo(a) tg mouse liver that we presently observed was distinct (apo(a) plasma levels decreased in males during puberty but increased in females) from that previously reported in apo(a)-YAC transgenic lines. Differences in genetic background between transgenic lines in the expression of GH-dependent transcription factors that modulate apo(a) transcription - and which remain to be identified - may account for this finding.

Investigation of the regulation of the expression of genes for which there is no murine orthologue in transgenic mice is beset with problems of relevance, as in the case of apo(a), for which there is a paucity of alternative experimental *in vivo* systems. Nonetheless our present findings have demonstrated the impact of targeted deletion of the ACR upon apo(a) expression levels in an *in vivo* model; moreover, such *in* vivo data are consistent with earlier *in vitro* results. Finally, the large inter-individual variability in Lp(a) concentrations observed in human populations is thought to result in a large part from the combined action of multiple factors that govern apo(a) levels [36]. From the experimental data presented here and from a recent study in which associations between sequence variations in the ACR enhancer and plasma Lp(a) concentrations were reported in human subjects [37], the ACR sequence may appear as one of these functional regulatory elements that warrants further analysis.

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LEGEND TO FIGURES

Figure 1 : **Comprehensive PCR analysis of the apo(a)-YAC transgene in apo(a) and apo(a)/ACR^{del} tg mice.** Genomic DNA prepared from apo(a) transgenic (lane1) or apo(a)/ACR^{del} transgenic (lane2) mice were subjected to PCR amplification using specific primer sets spanning the entire apo(a)-YAC length. DNA prepared from a non-transgenic mouse (lane 3) and from the pLys2-neo retrofitted apo(a)-YAC (lane 4) were included as negative and positive controls, respectively. M is the molecular weight DNA marker. ***Upper part of the panel*** : PCR amplifications for the left YAC arm, apo(a)-like 5' region, apo(a) 3', apo(a) kringle IV type 6, apo(a) 5', apo(a) DHII enhancer, plasminogen (PMG) 5' region, yeast lys2 gene. ***Lower part of the panel*** : PCR amplification of the ACR region. The sizes of the PCR products corresponding to the floxed ACR (apo(a) tg, lane 1), the cre-deleted ACR (apo(a)/ACR^{del} tg, lane 2) and the non-modified ACR region (apo(a)-YAC, lane 4) are 1413 bp, 1207 bp and 1337 bp, respectively.

Figure 2 : **Tissue expression of the apo(a) transgene.** Total RNA isolated from the adrenals (A), kidney (K), heart (H), spleen (S), brain (B) and liver (L) of apo(a) and apo(a)/ACR ^{del} tg mice were subjected to reverse transcription (RT), followed by PCR amplification using apo(a) gene and β-actin gene specific primer pairs. PCR analysis of cDNA prepared from the liver of a non-transgenic (non-tg) mouse and from non-reverse transcribed RNA were used as controls. Molecular weight DNA markers (M) are present at right.

Figure 3 : **Time course of apo(a) plasma levels.** Plasma samples were collected from male and female apo(a) and apo(a)/ACR^{del} transgenic animals at the age of 28, 42, 56 and 84 days and analysed on reducing SDS-PAGE followed by immunoblotting with an anti-apo(a) antibody. A pool of plasma collected from several apo(a) transgenic mice was

included in all western blots for normalization and quantification purposes as described under *Methods*. To clearly reveal the respective increase or decrease in apo(a) plasma levels over time in female and in male mice, a short exposure time for the western blots was used for females and a longer one for males.

Figure 4 : **(A) Apo(a) plasma levels in apo(a) and apo(a)/ACR^{del} tg mice.** Apo(a) levels were determined before (28 days-old) or after (84 days-old) sexual maturity for females and only at pre-pubertal age (28 days) for males. For each group of mice analysed, the number (n) of animals and the calculated mean apo(a) levels ± SD are indicated. Comparison of apo(a) plasma levels between apo(a) and apo(a)/ACR^{del} tg mice in age- and gender-related groups were performed using student's t test (Statistical significance : *, p = 0.01; ¶, p = 0.04). The decrease (%) in plasma levels observed in apo(a)/ACR^{del} tg mice as compared to apo(a) tg mice is indicated for each group. **(B) Apo(a) mRNA expression levels in liver tissue of apo(a) and apo(a)/ACR^{del} tg mice.** Total RNA from the livers of adult female mice (n = four for both genotypes) was pooled, reverse-transcribed and subjected to real-time PCR quantification as described in *Methods*. Beta-glucuronidase (β-GUS) was used as the reference gene to normalize the data. Values (± SD) represent the amount of mRNA relative to that measured in apo(a) tg mice, which was set arbitrarily to1. As an additional control for the normalisation procedure, the expression level of another gene (SR-BI) in both groups is shown.

Figure 5 : **(A) Apo(a) plasma levels after a high-fat diet**. Apo(a) and apo(a)/ACR^{del} female mice were fed with a cholesterol and cholate containing high-fat diet for two weeks. Plasma samples were collected before (day 0) and after the diet (day 14) and apo(a) plasma concentrations compared by western blot densitometric analyses. Plasma pool, same as in

figure 3. **(B) Quantitative real-time PCR of liver apo(a) mRNA from apo(a) and apo(a)/ACR^{del} female mice fed a chow- or a high-fat diet.** Total RNA from the livers of female mice (n = 4 in each group) was pooled and subjected to real-time PCR quantification. β-GUS was used as the reference gene to normalize the data. Apo(a) mRNA levels in each group are presented relative to the amount of apo(a) transcript in the chow-fed apo(a) tg mice, which was set arbitrarily to1.

Figure 1

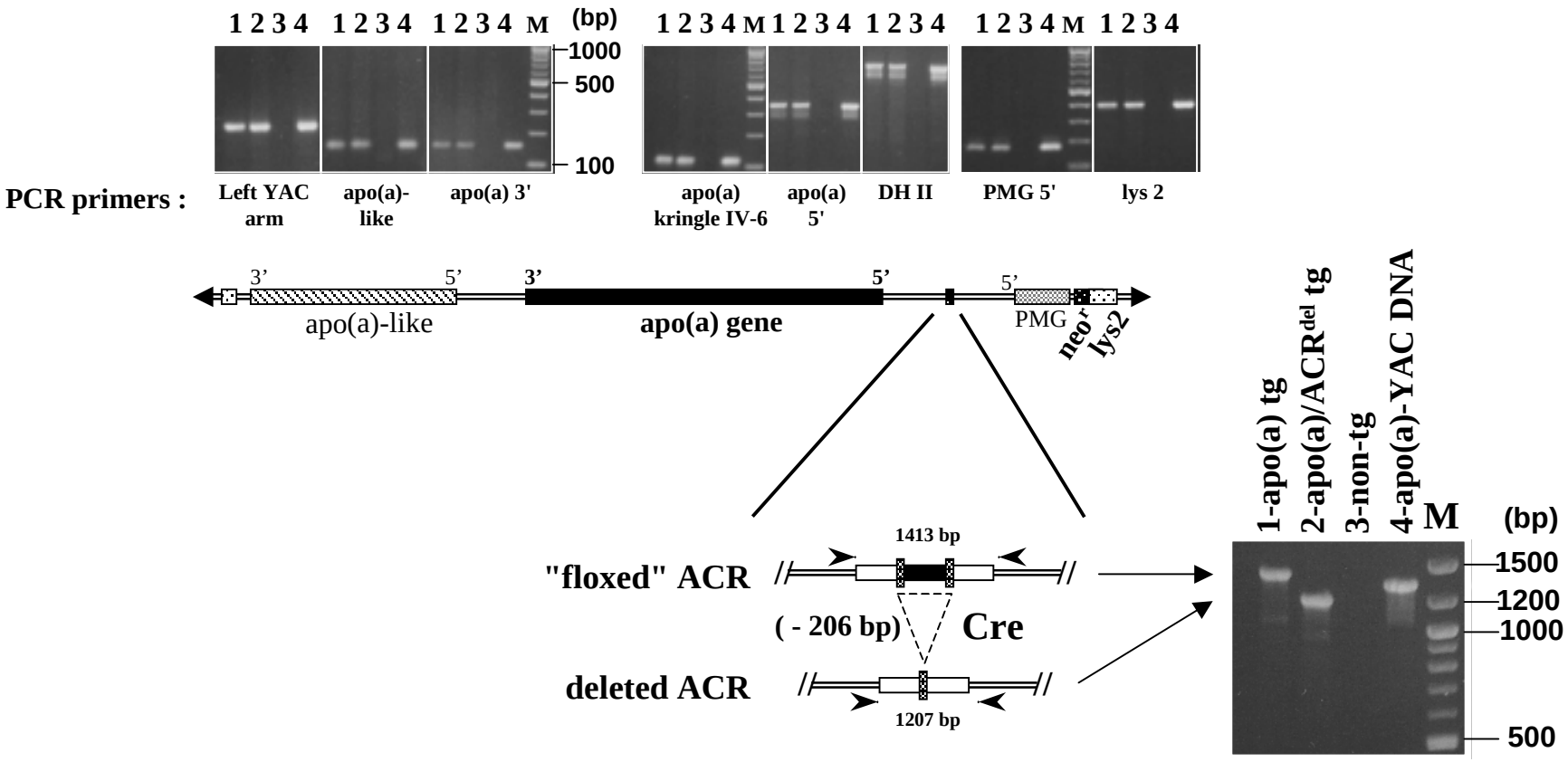

1 2 3 4
1 2 3 4
1 2 3 4 M
(bp)
1000
500
100
1 2 3 4 M 1 2 3 4
1 2 3 4
1 2 3 4 M 1 2 3 4
PCR primers :
Left YAC arm
apo(a)-like
apo(a) 3'
apo(a) kringle IV-6
apo(a) 5'
DH II
PMG 5'
lys 2
3'
5'
3'
5'
5'
apo(a)-like
apo(a) gene
PMG
neo
lys2
1413 bp
"floxed" ACR
(- 206 bp)
Cre
deleted ACR
1207 bp
1-apo(a) tg
2-apo(a)/ACRdel tg
3-non-tg
4-apo(a)-YAC DNA
M
(bp)
1500
1200
1000
500

Figure 2

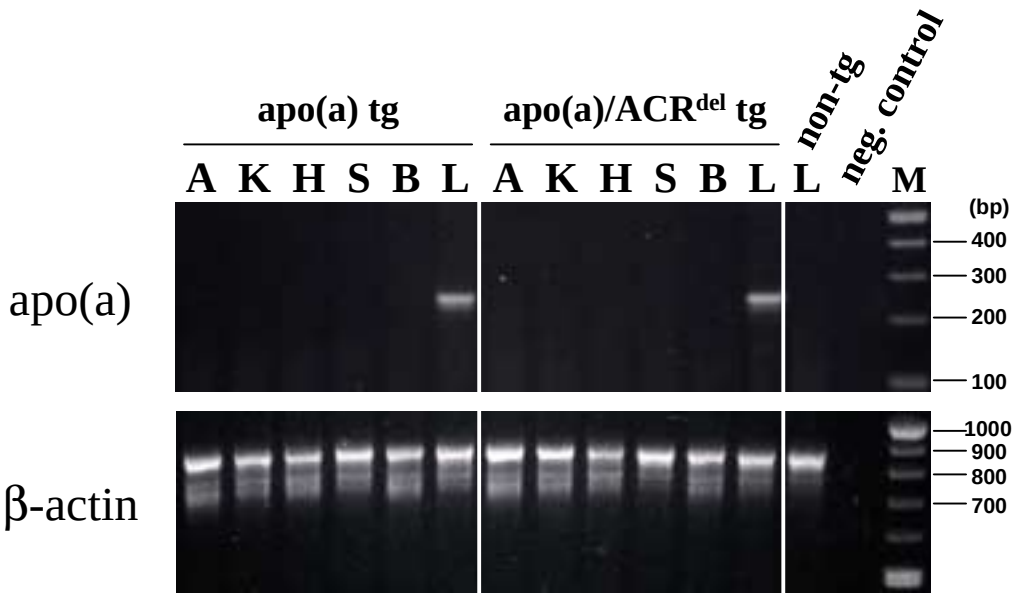

apo(a) tg
apo(a)/ACR^{del} tg
non-tg
neg. control
A K H S B L A K H S B L L M
(bp)
400
300
200
100
1000
900
800
700
apo(a)
β-actin

Figure 3

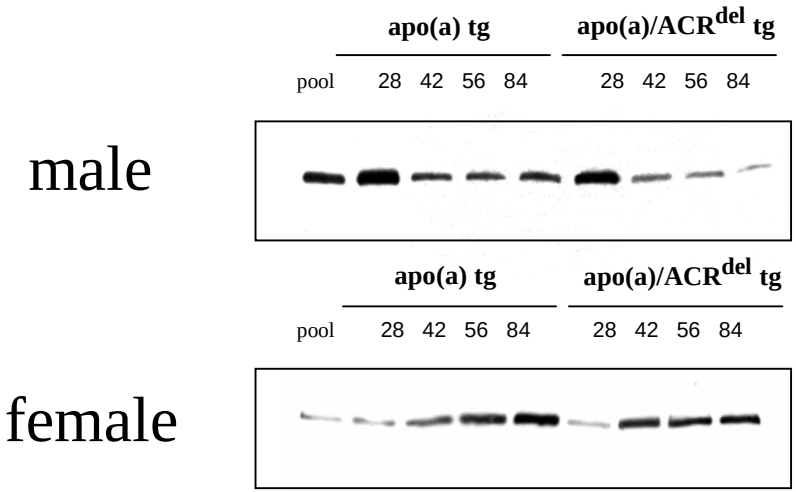

apo(a) tg
apo(a)/ACR^{del} tg
pool
28 42 56 84
28 42 56 84
male
apo(a) tg
apo(a)/ACR^{del} tg
pool
28 42 56 84
28 42 56 84
female

Figure 4

A. Apo(a) plasma levels

	Apo(a) tg	Apo(a)/ACRdel tg	
	plasma apo(a) (µg/ml)		% decrease
28 days-old			
Female	2.0 ± 0.80 (15)	1.4 ± 0.5 (16)	30 %*
Male	1.4 ± 0.6 (8)	1.1 ± 0.5 (11)	21 %
84 days-old			
Female	5.5 ± 2.0 (14)	4.0 ± 2.0 (20)	27 %¶

B. Apo(a) mRNA levels

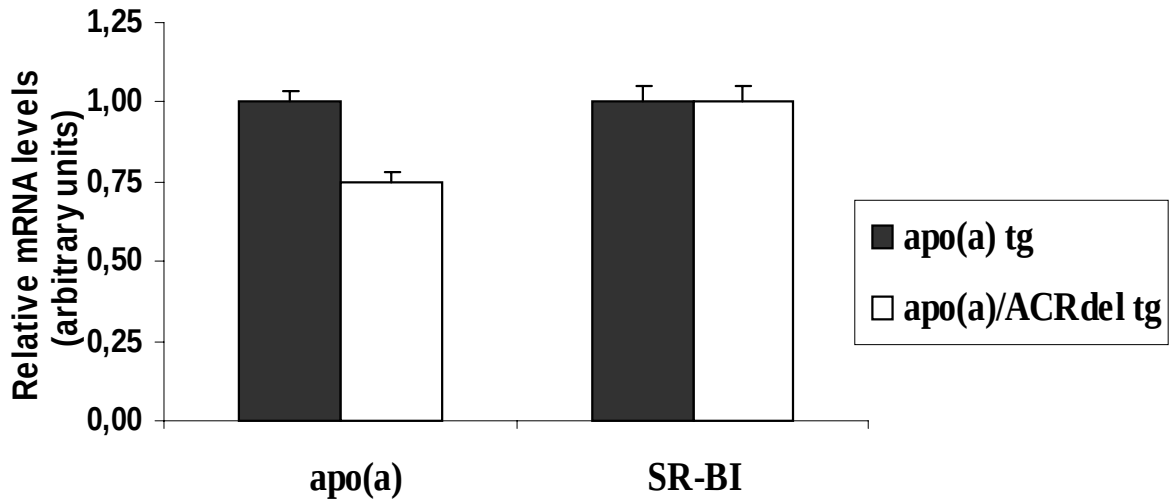
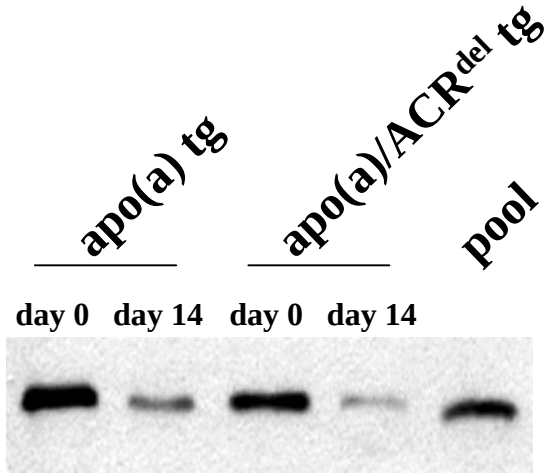
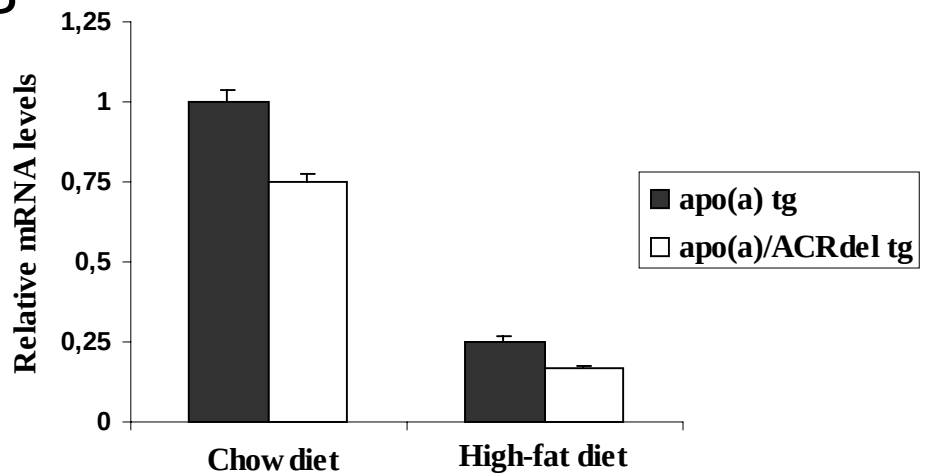


Figure 5

A


apo(a) tg
apo(a)/ACRdel tg
pool
day 0
day 14
day 0
day 14

B


Relative mRNA levels
1,25
1
0,75
0,5
0,25
0
Chow diet
High-fat diet
apo(a) tg
apo(a)/ACRdel tg