Human apoB contributes to increased serum total apo(a) level in LPA transgenic mice

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Abstract

Background: The Lp(a) lipoprotein (Lp(a)) consists of the polymorphic glycoprotein apolipoprotein(a) (apo(a)), which is attached by a disulfide bond to apolipoprotein B (apoB). Apo(a), which has high homology with plasminogen, is present only in primates and hedgehogs. However, transgenic mice and rabbits with high serum apo(a) levels exist. Liver is the main site for apo(a) synthesis, but the site of removal is uncertain. To examine differences between transgenic mice expressing the LPA gene and mice capable of forming Lp(a) particles, LPA-YAC transgenic mice and hAPOB transgenic mice were crossed and their offspring examined.

Results: Comparison of LPA-YAC with LPA-YAC/hAPOB transgenic mice showed that LPA-YAC/hAPOB transgenic mice have higher serum total apo(a) and total cholesterol level than mice lacking the hAPOB gene. However, hepatic apo(a) mRNA level was higher in LPA-YAC transgenic mice than in LPA-YAC/hAPOB transgenic mice. Feeding of a high-cholesterol/high-fat diet to male LPA-YAC transgenic mice with or without the hAPOB gene resulted in reduced serum total apo(a) and hepatic apo(a) mRNA level.

Conclusion: In conclusion, the higher serum total apo(a) level in LPA-YAC/hAPOB transgenic mice than in LPA-YAC transgenic mice is not caused by increased apo(a) synthesis. Lower hepatic apo(a) mRNA level in LPA-YAC/hAPOB than in LPA-YAC transgenic mice may suggest that the increase in total apo(a) level is a result of apo(a) accumulation in serum. Furthermore, observed higher serum total cholesterol level in LPA-YAC/hAPOB transgenic mice than either in wild type or LPA-YAC transgenic mice may further suggest that human APOB transgenicity is a factor that contributes to increased serum total apo(a) and cholesterol levels. Our results on reduced serum total apo(a) and hepatic apo(a) mRNA levels in HCHF fed male LPA-YAC transgenic mice confirm earlier findings in females, and show that there are no sex difference in mechanisms for lowering apo(a) level in response to HCHF feeding.
Background

Association between Lp(a) lipoprotein (Lp(a)) [1], and coronary heart disease was first demonstrated in 1974 [2]. Afterwards, numerous studies have confirmed that high plasma level of Lp(a) is an independent risk factor for atherosclerotic and thrombotic disease [3]. The intact Lp(a) molecule consists of the highly polymorphic glycoprotein apolipoprotein(a) (apo(a)), which is attached through a disulfide bond to apolipoprotein B-100 (apoB-100) of low density lipoprotein (LDL).

Gene encoding for apo(a) (LPA), which expression is mainly confined to the liver, is tightly linked to plasminogen (PLG) gene on chromosome 6 and is homologous to PLG [4,5]. This sequence similarity indicates that the human LPA gene evolved from a duplicated PLG gene during primate evolution [6,7]. Interestingly, in addition to humans, Old World monkeys, great apes and hedgehogs are the only species, which express LPA naturally [8-10]. Varying numbers of KIV structures account for the size variation of apo(a) whose size is inversely related to plasma Lp(a) level [11]. Furthermore, recent studies in transgenic animal models have shown that 50% of human Lp(a) is catabolized by the liver [12,13], but the mechanism for uptake is unclear. Study of WHHL transgenic rabbits expressing human apo(a) showed that defect LDL receptor (LDLR) lead to marked accumulation of plasma Lp(a) [14]. However, despite structural similarity of Lp(a) to LDL, the LDLR does not seem to be involved significantly in Lp(a) removal in humans [15-17].

Studies in female mice transgenic for LPA-yeast artificial chromosome (LPA-YAC), which contains a 40 kilobase (kb) LPA-PLG intergenic region [18], have shown that feeding of a high-fat diet reduces serum apo(a) and hepatic apo(a) mRNA level [19,20]. In contrast, serum apo(a) levels in mice transgenic for human apo(a) cDNA are stable despite high-fat feeding [21], which suggests that the effect of fat-rich diet on apo(a) levels in LPA-YAC transgenic mice is most likely exerted through regulatory elements located within the LPA-PLG intergenic region.

Recent studies on PPARα suggest that PPARα is of major importance for regulation of serum levels of apoB and production of apoB-containing lipoproteins [22,23], but the possible role of PPARα in determining Lp(a) serum level is unclear. The DNase I hypersensitive site (DH) II enhancer part of the LPA-PLG intergenic region contains the gene for peroxisome proliferators-activated receptor (PPAR) α binding site [24,25]. DH II increases the activity of the LPA promoter by over 7-fold in reporter-gene assays in HepG2 cells in vitro [26]. This together with recent cotransfection assays, which showed that PPARα increased the effect of the DHIII enhancer on LPA transcriptional activity may indicate that PPARα plays a role also in determining plasma Lp(a) level [26].

In order to examine differences between transgenic mice only expressing the LPA gene and transgenic mice capable of forming Lp(a) particles, LPA-YAC transgenic and mice transgenic for the human APOB (hAPOB) gene were crossed and their offspring examined. The study revealed that serum total apo(a) levels in LPA-YAC transgenic mice also expressing the hAPOB gene were higher than in LPA-YAC transgenic animals lacking hAPOB. However, hepatic apo(a) mRNA level in LPA-YAC transgenic mice was higher than in LPA-YAC/hAPOB transgenic mice. Furthermore, we showed that feeding of a high-cholesterol/high-fat (HCHF) diet to male LPA-YAC transgenic mice, which on a standard mouse diet exhibit much lower serum total apo(a) level than female mice, reduced serum total apo(a) and hepatic apo(a) mRNA level in a similar manner as previously shown in female mice [19,20]. The lack of significant variation in hepatic PPARα mRNA levels between wild type (WT) and LPA-YAC transgenic mice or between mice fed either a semi-synthetic mouse diet (AIN-76) or the HCHF diet, may indicate that neither LPA transgenicity nor high-fat feeding have a significant impact on expression of PPARα.

Results

Serum apo(a) level

On a regular RM/EL(SQC) mouse diet serum total apo(a) level was significantly higher in LPA-YAC/hAPOB transgenic mice than in LPA-YAC transgenic mice (P < 0.05, figure 1) and serum total apo(a) levels were higher in female transgenic mice than in respective male transgenic mice (P < 0.005, figure 1).

Male LPA-YAC transgenic mice (n= 4) had a mean serum total apo(a) level of 7.6 ± 2.5 mg/dL at the start and of 6.4 ± 3.9 mg/dL at the end of feeding the AIN-76 diet for 7 weeks. Corresponding levels in LPA-YAC/hAPOB transgenic mice (n= 6) were 9.2 ± 1.0 mg/dL and 9.5 ± 2.2 mg/dL, respectively. Serum total apo(a) level in male LPA-YAC/hAPOB transgenic mice was significantly reduced (P < 0.001, figure 2) after two weeks of HCHF feeding and the level remained low during 7 weeks of feeding. Feeding of the HCHF diet to male LPA-YAC transgenic mice resulted in reduced mean serum total apo(a) level from 2.9 ± 1.3 mg/dL to below background level, but because of the small number the difference did not reach statistical significance (P= 0.08).

Serum total cholesterol and triglycerides in male mice

LPA-YAC transgenic mice had similar serum total cholesterol (TC) and serum triglycerides (TG) levels as WT mice, independently of the diets (table 1). LPA-YAC/hAPOB transgenic mice fed the AIN-76 diet had higher serum TG
level than either WT or LPA-YAC transgenic mice ($P < 0.05$). The HCHF fed LPA-YAC/hAPOB transgenic mice had significantly higher serum TC level than the AIN-76 fed mice ($P < 0.005$). In comparison, feeding of the HCHF diet to WT or LPA-YAC transgenic mice had no significant effect on serum TC levels compared to the AIN-76 fed mice. Serum TG levels were lower in the HCHF fed LPA-YAC and LPA-YAC/hAPOB transgenic mice compared to the AIN-76 fed transgenic mice ($P < 0.05$ and $P < 0.005$ respectively, for the two categories of mice). The comparable difference on serum TG level between the AIN-76 and the HCHF fed WT did not reach statistical significance.

**Apo(a), PPARα and LDLR mRNA levels in male mice**

Apo(a) mRNA was mainly detected in liver of LPA-YAC transgenic mice and to a very low degree in kidneys in 3 out of 4 mice (table 2). Apo(a) mRNA was not detected in spleen, heart, brain and intestine.

On the AIN-76 diet hepatic apo(a) mRNA levels were lower in LPA-YAC/hAPOB than in LPA-YAC transgenic mice ($P < 0.05$, figure 3). Furthermore, feeding of the HCHF diet to transgenic mice reduced hepatic apo(a) mRNA level significantly ($P < 0.05$) in both strains.

Hepatic PPARα mRNA levels did not vary significantly between WT and transgenic mice, or between the AIN-76

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**Figure 1**

Serum total apo(a) level in male and female LPA-YAC and LPA-YAC/hAPOB transgenic mice on a regular RMI(E)SQC mouse diet. Values shown are mean ± SEM (mg/dL). Statistically significant difference between LPA-YAC and LPA-YAC/hAPOB transgenic mice is indicated by # ($P < 0.05$) or by ## ($P < 0.01$). Statistically significant difference between respective male and female mice is indicated by * ($P < 0.005$).
Hepatic LDLR mRNA level did not vary significantly between the AIN-76 fed WT, LPA-YAC and LPA-YAC/hAPOB transgenic mice, or between the HCHF fed and the AIN-76 fed LPA-YAC transgenic mice (figure 5). HCHF feeding reduced LDLR mRNA levels in WT and LPA-YAC/hAPOB transgenic mice, compared to the levels in the AIN-76 fed mice ($P < 0.05$).

**Discussion**

Observation of higher serum levels of total apo(a) (figure 1 & 2) in LPA-YAC/hAPOB transgenic mice than in LPA-YAC transgenic mice are in agreement with the results of Callow et al [27]. Our finding of lower hepatic apo(a) mRNA levels (figure 3) in LPA-YAC/hAPOB transgenic mice than in mice having the LPA gene only, suggest that the elevated serum Lp(a) levels in LPA-YAC/hAPOB transgenic mice are not due to increased hepatic apo(a) mRNA transcription rate. Furthermore, analysis of LPA expression in different tissues (table 2) indicates that no other organs than the liver, can produce significant amounts of apo(a).

LDL can be removed from the circulation by the LDL receptor (LDLR), which binds to the apoB-100 part of LDL [28]. The efficient assembly of Lp(a) in mice transgenic for
both LPA and hAPOB and higher efficiency of Lp(a) assembly with the human apoB-100 than with mouse apoB-100 [29,30] may suggest that measured total apo(a) level in LPA-YAC/hAPOB transgenic mice consist mainly of human Lp(a) particles.

Because mouse LDLR has low capacity to bind human apoB-100 containing particles [31], elevated serum total apo(a) level may theoretically be secondary effect of reduced clearance rate of human apoB containing particles via the LDLR dependent pathway in LPA-YAC/hAPOB transgenic mice. Support for this comes from study, which showed that overexpression of LDLR in mice leads to accelerated metabolism of injected human Lp(a) particles [32]. Furthermore, it is notable that serum Lp(a) levels in LDLR+/LPA transgenic mice are similar whether or not the human APOB gene is also present [33]. Since triglyceride-rich apoB particles have increased affinity to apo(a) in man [34,35], it seems likely that the human apoB part of the Lp(a) molecule in mouse serum may associate with TG containing particles, which may explain why hAPOB transgenicity also contributes to increased serum TG levels.

Suggestion for a role of LDLR in Lp(a) clearance comes also from studies in rabbits, where apoB, in contrast to mice, associates with human apo(a) forming Lp(a) particles [36]. A defect in LDLR in LPA transgenic WHHL rabbits is accompanied by accumulation of Lp(a) in plasma [14]. Since mice or rabbits do not normally have apo(a), the mechanism of Lp(a) removal in these animals may well differ from that in man, where the LDLR does not seem to affect Lp(a) metabolism [15-17].

The fall in serum apo(a) and hepatic apo(a) mRNA levels (figure 2 and 3) in male transgenic mice on the HCHF diet agrees with observations by Acquati et al and Huby et al, who showed that hepatic apo(a) mRNA and serum apo(a) levels fell in female LPA transgenic mice after two weeks fat feeding [19,20]. Man, who naturally express LPA, do not have significant variation in plasma Lp(a) levels despite of changes in diet [37]. This indicates that man possesses LPA control mechanisms that are different from

Table 1: Serum triglycerides (TG) and serum total cholesterol (TC) levels in male WT, LPA-YAC transgenic and LPA-YAC/hAPOB transgenic mice

| Mice          | AIN-76 diet |           | HCHF diet |           |
|---------------|-------------|-----------|-----------|-----------|
|               | n       | TG (mmol/L) | n       | TG (mmol/L) |
| WT            | 5       | 1.8 ± 0.3 | 5       | 1.2 ± 0.3 |
| LPA-YAC       | 4       | 1.9 ± 0.2 | 3       | 2.1 ± 0.2 |
| LPA-YAC/hAPOB | 6       | 3.1 ± 0.2 | 8       | 3.4 ± 0.1 |

Concentrations are presented as mean ± SEM (mmol/L). 1): Statistically significant difference (P < 0.05) in serum TG levels as compared to the AIN-76 fed WT or LPA-YAC transgenic mice. 2): Statistically significant difference in serum TG levels as compared to the respective AIN-76 fed mice (P < 0.05 for LPA-YAC, P < 0.005 for LPA-YAC/hAPOB). 3): Statistically significant difference (P < 0.005) in serum TC levels as compared to the AIN-76 fed LPA-YAC/hAPOB transgenic mice.

Table 2: LPA expression level in different tissues of 1-year old male LPA-YAC transgenic mice on a regular RMI(E)SQC mouse diet

| Mice     | Liver | Spleen | Heart | Brain | Intestine | Kidney |
|-----------|-------|--------|-------|-------|-----------|--------|
| LPA-YAC   | High  | -      | -     | -     | -         | Low    |
| WT        | -     | -      | -     | -     | -         | -      |

Table 1: Serum triglycerides (TG) and serum total cholesterol (TC) levels in male WT, LPA-YAC transgenic and LPA-YAC/hAPOB transgenic mice

Table 2: LPA expression level in different tissues of 1-year old male LPA-YAC transgenic mice on a regular RMI(E)SQC mouse diet
those in mice. This agrees with the fact that CYP7A1, which regulates the initial step in the pathway of cholesterol metabolism and bile acid synthesis, is increased during cholesterol feeding in rats and mice, but not in humans [38]. Rodents' capacity to convert cholesterol into bile acids by liver X receptor-α mediated stimulation of CYP7A1 transcription has been suggested to account for some of the differences in response to fat intake between humans and rodents [39]. Furthermore, PPARα, which binds to the DHII enhancer part of LPA-PLG intergenic region [24], and is of importance for production of apoB and apoB-containing lipoproteins [22], was recently shown to be regulated by bile acids in humans but not in rodents [40]. Since cholesterol feeding promotes bile acid synthesis in rodents [41], our results showing similar hepatic PPARα mRNA levels between the AIN-76 and HCHF fed mice may give further support to the notion that bile acids do not increase PPARα mRNA levels in mice. Further studies will be needed to elucidate whether or not the species-specific differences in cholesterol and bile acid metabolism is a key to understanding the mechanism(s), which cause the reduction on apo(a) transcription in HCHF fed transgenic mice.

The reduced hepatic LDLR mRNA level seen in HCHF fed WT mice and LPA-YAC/hAPOB transgenic mice (figure 5) is in agreement with previous results, which showed that hepatic LDLR level in mice is reduced in response to HCHF feeding [42]. This may suggest that the human apoB can interact sufficiently, although less than mouse

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**Figure 3**
Median of normalized hepatic apo(a) mRNA level in male LPA-YAC and LPA-YAC/hAPOB transgenic mice fed the AIN-76 diet or the HCHF diet for 7 weeks. The * indicates statistically significant difference (P < 0.05) in comparison to the level in the AIN-76 fed mice. Statistically significant difference (P < 0.05) between LPA-YAC and LPA-YACHAPOB transgenic mice is indicated by #.
apoB, with mouse LDLR to increase intracellular level of cholesterol and down regulate expression of the LDLR. Because of the small number of HCHF fed LPA-YAC transgenic mice in our study, the absence of an effect of feeding on LDLR mRNA level must be confirmed in further studies.

On the HCHF diet both WT mice and transgenic mice developed fatty livers (not shown). Since hepatic level either of housekeeping genes or PPARα mRNA was not reduced in response to the HCHF feeding, it seems clear that the decreased apo(a) mRNA level detected in the livers of HCHF fed transgenic mice was not due to a general decrease in liver function.

**Conclusions**

In conclusion, we have shown that serum total apo(a) level is higher in LPA-YAC/hAPOB transgenic mice than in mice that are transgenic for the LPA gene alone. The higher level is not caused by increased apo(a) synthesis. Lower hepatic apo(a) mRNA level in LPA-YAC/hAPOB than in LPA-YAC transgenic mice may suggest that the increase in total apo(a) level is a result of apo(a) accumulation in serum. Our results on reduced serum total apo(a) and hepatic apo(a) mRNA level in HCHF fed male LPA-YAC transgenic mice confirm earlier findings in females, and show that there are no sex difference in mechanisms for lowering apo(a) level in response to HCHF feeding.

**Figure 4**

Median of normalized hepatic PPARα mRNA level in male WT, LPA-YAC and LPA-YAC/hAPOB transgenic mice fed the AIN-76 or the HCHF diet for 7 weeks.
Materials and methods

Mice

Mice possessing a 270 kb YAC with a human genomic DNA clone containing the intact \( LPA \) gene and a 70 kb \( LPA \)-like gene with extensive 60 kb flanking regions on both sides and mice possessing the intact \( hAPOB \) gene in FVB genetic background have been described previously [18,27]. Non-transgenic FVB mice were purchased from Harland (Bicester, England). For experiments in Oslo, mice were bred at The Laboratory Animal Unit, which is fully accredited by AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care and Use), at The Norwegian School for Veterinary Science. The Norwegian Animal Research Authority approved the experiments and all animal experiments were performed in accordance with the Norwegian Gene Technology Act of 1994 and the European Community Directive of 24 November 1986.

To develop mice capable of forming Lp(a) particles, \( LPA \)-YAC transgenic mice and \( hAPOB \) transgenic mice were crossed. Sentinel animals were used to run a full felas-style health-monitoring scheme and mice were found to be healthy.

The presence of the \( LPA \) gene and the \( hAPOB \) gene was demonstrated by PCR analysis [43]. For the analysis of \( hAPOB \) transgenicity sense primer TGGAAACGGA-GAAATTATGGA and antisense primer CACITGGCAAT-AACAATTCCCTG were used.

Figure 5

Median of normalized hepatic LDLR mRNA level in male WT, \( LPA \)-YAC or \( LPA \)-YAC/\( hAPOB \) transgenic mice fed the AIN-76 or the HCHF diet for 7 weeks. Statistically significant difference (\( P < 0.05 \)) between the AIN-76 fed and the HCHF fed mice is indicated by *.
Mice were housed in a room with 12 h light/dark cycle and 55% relative humidity at 21 °C. Mice were kept on the regular RM/E(SQ) mouse diet (Special Diet Services, Witham, Essex, England) until the start of the feeding experiment.

Four 1-year old male LPA-YAC transgenic mice and two 1-year old male wild type mice were sacrificed for the study of LPA expression pattern. For the study of serum total apo(a) level prior to the feeding experiment, six female and seven male LPA-YAC transgenic as well as five female and fifteen male LPA-YAC/hAPOB transgenic mice were bled.

Feeding experiment

Ten male WT, seven LPA-YAC transgenic and fourteen LPA-YAC/hAPOB transgenic mice were divided into groups and fed with a semi-synthetic mouse diet (AIN-76, ICN, Asse-Relegem, Belgium) or a high-cholesterol/high-fat diet (Custom high-fat diet, ICN, Asse-Relegem, Belgium) for seven weeks (table 3). The HCHF diet contained 1.25% cholesterol, 18.4% regular butter, corresponding to approximately 9% saturated fat, and 1% corn oil, while the AIN-76 diet contained 5% corn oil. Corn oil contains approximately 1.7% saturated fat. Water and food were given ad libitum. In addition to the blood sampling before feeding, blood samples from the saphenous vein were drawn after two weeks feeding and when the animals were sacrificed after seven weeks feeding.

Table 3: Age at the start and number of mice fed different diets for 7 weeks

| Mice          | Age (weeks) | AIN-76 diet | HCHF diet |
|---------------|-------------|-------------|-----------|
| WT            | 44–47       | 5           | 5         |
| LPA-YAC       | 49–52       | 4           | 3         |
| LPA-YAC/hAPOB | 49–52       | 6           | 8         |

Analysis of serum total apo(a), total cholesterol and triglycerides level

Serum total apo(a) level was measured using a radioimmuno assay (RIA) kit (Mercodia AB, Uppsala, Sweden). This method is based on direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants of apo(a). Results are given in units per liter (U/L). According to the manufacturer, results must be multiplied by 0.07 to obtain values in mg/dL, and levels below 1.2 mg/dL (17 U/L) are considered as background noise. For details of test’s reliability to measure serum apo(a) level see Berg et al [43]. Serum TC and TG level was measured using enzymatic, colorimetric methods (Modular Analytics, Roche Diagnostics, F-Hoffmann-La Roche Ltd, Basel, Switzerland) at Fürst AS, Oslo, Norway.

Tissue preparation for mRNA analysis

For RNA preparations, tissue samples were washed in ice-cold saline, submerged in RNAlater™ buffer (Ambion Diagnostics, Cambridgeshire, England) and stored at -70 °C until analysis.

Quantitative PCR

Level of mRNA was determined by quantitative PCR (qPCR), which was performed using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Primers and probes (table 4) were designed using Primer Express software (Applied Biosystems, Foster City, CA, USA).

Total RNA from 100–300 mg of tissues was isolated after homogenisation in 1 ml Trizol reagent according to the manufacturer’s recommendations (Invitrogen Co, Carlsbad, CA, USA). RNA was treated with Dnase I (Ambion Ltd, Cambridgeshire, England) and 5 µg of total RNA was reverse-transcribed using pd(N)₆ random primers (First strand cDNA synthesis kit, Amersham Biosciences, Little Chalfont Buckinghamshire, England).

Table 4: Primer and probe sequences for cyclophilin, β-actin, LPA, PPARα and LDLR genes used in qPCR

| Gene     | Forward primer | Reverse primer | Probe                      |
|----------|----------------|----------------|----------------------------|
| Cyclophilin | TGGAGAGCACCAGACAGACA | TGCCGAGTCGACAAATGA | CGGGACAGCCACTGAAGGATGT |
| β-actin  | ACAGGGCCAGAGCAAGAG | CGTCCAGGTGTAACATGC | ATGTCTAACTGGGTACCTAGGTCAGGA |
| LPA      | TTGTCGAGCTCTCCAGAATCAG | AGGCAGACT CCAAAGGTACTCT | TGTCACCCGAGCATGTTTACA |
| PPARα    | TGGATGCGGCGGCTGTAC | AAATCTCGGTGGTCTCTGTGATGG | ACGTTGAGGACCTCGGAGGCT |
| LDLR     | GCTCCATAGGCTATCTGCTTCA | CTGCGGCTCAAGGGCTCATC | CAAACGCCCAGAGGTCAGG|

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qPCR was performed using the TaqMan® Universal Master mix (Applied Biosystems, Foster City, CA, USA) containing 2 µl cDNA, 300 nM of each primer and probe in a 25 µl final volume. To evaluate the qPCR performances, cDNA samples, made as described above, from liver of five ten week’s old WT male mice were analyzed for β-actin and cyclophilin mRNA levels. We run triplicates of 1:100 diluted cDNA samples for each mouse. Variance of Ct values at the same threshold for within-run and between-runs is presented in table 5. Analysis of genes of interest was carried out in a duplicate and relative mRNA level was determined by the Standard Curve Method (User Bulletin No. 2, Applied Biosystems). PCR cycle parameters were as follows: 1 cycle of 50°C for 2 min and 95°C for 10 min; 40 cycles of 95°C for 15 sec and 60°C for 1 min. The level of mRNA was normalized to average of cyclophilin and β-actin RNA levels to compensate for variations in input RNA amounts and are presented as median level of normalized mRNA level. In a case of analysis of LPA expression pattern in different tissues, samples were screened qualitatively by qPCR and expression level is presented as high, low or not expressed (-).

LPA primers and probe (Eurogentech, Seraing, Belgium) were designed to bind specifically to the KV part of the LPA gene. Because of the high degree of sequence homology between the LPA and PLG genes, specificity of LPA primers and probes was tested by running PCR reactions with different concentrations of plasmid containing PLG gene or LPA plasmid construct without KV (a kind gift from Dr. S. Frank, University of Graz, Austria). There was no detectable PCR product when these plasmids were used as templates in the PCR reaction. cDNA made from RNA isolated from the liver of WT mouse was used as a negative control when analyzing the transgenes.

Because of poor RNA quality, two samples were excluded from analysis.

### Statistical analyses
The non-parametric Mann-Whitney test was used to analyze differences between groups. All data from serum analysis are presented as mean ± SEM. In the case of mRNA data, results are presented as median value of normalized mRNA level.

### List of abbreviations

- Apo(a) = apolipoprotein(a)
- HCHF = high-cholesterol/high-fat (diet)
- LDL = low density lipoprotein
- LDLR = LDL receptor
- Lp(a) = lipoprotein(a)
- LPA = gene encoding for apolipoprotein(a)
- TG = triglycerides
- TC = total cholesterol
- WT = wild type (mouse)

### Table 5: Variation in A) β-actin and B) cyclophilin mRNA levels in five mouse livers submitted to qPCR. Calculations of variations were based on measured Ct value at the same threshold level.

| A) β-actin | Within-run (SD) | Between-run (SD) | CV% | No. of observations |
|-----------|-----------------|------------------|-----|---------------------|
| Mouse 1   | 0.02            | 0.17             | 0.78 | 9                   |
| Mouse 2   | 0.11            | 0.27             | 1.23 | 9                   |
| Mouse 3   | 0.09            | 0.36             | 1.63 | 9                   |
| Mouse 4   | 0.09            | 0.29             | 1.31 | 9                   |
| Mouse 5   | 0.23            | 0.27             | 1.24 | 9                   |
| All samples | 0.22        | 0.36             | 1.66 | 45                  |

| B) Cyclophilin | Within-run (SD) | Between-run (SD) | CV% | No. of observations |
|---------------|-----------------|------------------|-----|---------------------|
| Mouse 1       | 0.25            | 0.18             | 0.76 | 6                   |
| Mouse 2       | 0.02            | 0.06             | 1.24 | 6                   |
| Mouse 3       | 0.43            | 0.32             | 1.27 | 6                   |
| Mouse 4       | 0.06            | 0.26             | 1.06 | 6                   |
| Mouse 5       | 0.23            | 0.17             | 0.69 | 6                   |
| All samples   | 0.25            | 0.27             | 1.11 | 30                  |
Authors’ contributions
EMR provided transgenic breeder animals. Breeding of transgenic mice in Norway was initiated and overseen by KB, KE and SD. PAT tested transgenic animals used in this study and carried out protein and expression studies, statistical analysis and drafted the manuscript. KB and KE conceived of the study, and participated in its design and coordination. All authors read and approved the final version.

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References
1. Berg K: A New serum type system in man -The Lp system. Acta Pathol Microbiol Scand 1963, 59:369-382.
2. Berg K, Dahlén G, Frick MH: Lip(a) lipoprotein and pre-betalipoprotein in patients with coronary heart disease. Clin Genet 1974, 6:230-235.
3. Djurovic S, Berg K: Epidemiology of Lp(a) lipoprotein: its role in atherosclerotic/thrombotic disease. Clin Genet 1997, 52:281-292.
4. Eaton DL, Fless GM, Kohr WJ, McLean JW, Xu QT, Miller CG, Lawn RM, Scaru AM: Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen. Proc Natl Acad Sci U S A 1987, 84:3224-3228.
5. McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scaru AM, Lawn RM: CDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature 1987, 330:132-137.
6. Lawn RM, Boonmark NW, Schwartz K, Lindahl GE, Wade DP, Byrne CD, Fong KJ, Meer K, Patthy L: A partial amino acid sequence of apolipoprotein(a) from human plasma. J Lipid Res 1988, 29:1157-1170.
7. Lawn R, Pathy L, Pesole G, Saccone C: Apolipoproteins(a): a puzzling evolutionary story. J Mol Evol 1997, 44:234-236.
8. Makino K, Abe A, Mueda S, Noma A, Kawade M, Takenaka O: The recurring evolution of lipoprotein(a). Insights from cloning of hedgehog apolipoprotein(a). J Biol Chem 1995, 270:24004-24009.
9. Lawn R, Pathy L, Pesole G, Saccone C: Apolipoproteins(a): a puzzling evolutionary story. J Mol Evol 1997, 44:234-236.
10. Luyt ER, Afzal V, Doucet C, Lawn RM, Gong EL, Chapman MJ, Thillet J, Rubin EM: Regulation of the expression of the apolipoprotein(a) gene: evidence for a regulatory role of the 5’ distal apolipoprotein(a) transcription control region enhancer in yeast artificial chromosome transgenic mice. Arterioscler Thromb Vasc Biol 2003, 23:1633-1639.
11. Svidland A, Berg K, Eilasssen K, Lawn RM, Djurovic S, Alestriom P, Noren T, Smith A: Human lipoprotein(a) gene expression in LPA transgenic mice on cholesterol-enriched chow. Atherosclerosis 2000, 153:349-354.
12. Linden D, Alsterholm M, Wennebo H, Oscarsson J: PPARalpha deficiency increases secretion and serum levels of apolipoprotein(a)-containing lipoproteins. J Lipid Res 2001, 42:1831-1837.
13. Linden D, Lindberg K, Oscarsson J, Claesson C, Asp L, Li L, Gustafsson M, Boren J, Olofsson SO: Influence of peroxisome proliferator-activated receptor alpha agonists on the intracellular turnover and secretion of apolipoprotein(a) (Apo(a)-100 and Apo(a)-400). J Biol Chem 2002, 277:23044-23052.
14. Wade DP, Buckey LH, Knight BL, Acquisti F, Mihalich A, Taramelli R: Characterization of multiple enhancer regions upstream of the apolipoprotein(a) gene. J Biol Chem 1997, 272:30387-30399.
15. Magnaghi P, Mihalich A, Taramelli R: Several liver specific DNAse hypersensitive sites are present in the intergenic region separating human plasminogen and apolipoprotein(a) genes. Biochem Biophys Res Commun 1994, 205:930-935.
16. Buckey LH, Knight BL: Interaction of oestrogen and peroxisome proliferator-activated receptor alpha activates apolipoprotein(a) gene enhancers. Biochem J 2002, 366:157-163.
17. Callow MJ, Stolzfus LJ, Lawn RM, Rubin EM: Expression of human apolipoprotein B and assembly of lipoprotein(a) in transgenic mice. Proc Natl Acad Sci U S A 1994, 91:2130-2134.
18. Hoffstarkovar AV, Law SW, Lackner K, Brewer HJr: Identification of low density lipoprotein receptor binding domains of human apolipoprotein B-100: a proposed consensus LDL receptor binding sequence of apoB-100. Biochem Biophys Res Commun 1986, 139:1078-1080.
19. Cheesman EJ, Sharp RJ, Slab CH, Liu CY, Taylor S, Marcovina SM, Young SG, McCormick SP: An analysis of the interaction between mouse apolipoprotein B100 and apolipoprotein(a). J Biol Chem 2000, 275:28195-28200.
20. Trieu VN, McConathy WJ: The binding of animal low-density lipoproteins to human apolipoprotein(a). Biochem J 1995, 309 (Pt 3):899-904.
21. Corsini A, Mazzotti M, Villa A, Maggi FM, Bernini F, Romano L, Romano C, Fumagalli R, Catapano AL: Ability of the LDL receptor from several animal species to recognize the human apo B binding domain: studies with LDL from familial defective apo B-100. Atherosclerosis 1992, 93:95-103.
22. Hofmann SL, Eaton DL, Brown MS, McConathy WJ, Goldstein JL, Hammer RE: Overexpression of human low density lipoprotein receptors leads to accelerated catabolism of LDL lipoprotein in transgenic mice. J Clin Invest 1990, 85:1542-1547.
23. Sanan DA, Newland DL, Tao R, Marcovina S, Wang J, Mooser V, Hammer RE, Hobbs HH: Low density lipoprotein receptor-negative mice expressing human apolipoprotein B-100 develop complex atherosclerotic lesions on a chow diet: no accentuation by apolipoprotein(a). Proc Natl Acad Sci U S A 1998, 95:4544-4549.
24. Trieu VN, McConathy WJ: Lipoprotein(a) binding to other apolipoprotein B containing lipoproteins. Biochemistry 1990, 29:5919-5924.
35. Ye SQ, Trieu VN, Stuers DL, McConathy WJ: Interactions of low density lipoprotein2 and other apolipoprotein B-containing lipoproteins with lipoprotein(a). J Biol Chem 1988, 263:6337-6343.

36. Fan J, Araki M, Wu L, Challah M, Shimosayama H, Lawn RM, Kakuta H, Shikama H, Watanabe T: Assembly of lipoprotein (a) in transgenic rabbits expressing human apolipoprotein (a). Biochem Biophys Res Commun 1999, 255:639-644.

37. Puckey L, Knight B: Dietary and genetic interactions in the regulation of plasma lipoprotein(a). Curr Opin Lipidol 1999, 10:35-40.

38. Chen JY, Levy-Wilson B, Goodart S, Cooper AD: Mice expressing the human CYP7A1 gene in the mouse CYP7A1 knock-out background lack induction of CYP7A1 expression by cholesterol feeding and have increased hypercholesterolemia when fed a high fat diet. J Biol Chem 2002, 277:42588-42595.

39. Chiang JY, Kimmel R, Srapou D: Regulation of cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription by the liver orphan receptor (LXRalpha). Gene 2001, 262:257-265.

40. Pineda Torra,I, Claudel T, Duval C, Kosykh V, Fruchart JC, Staels B: Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene via activation of the farnesoid X receptor. Mol Endocrinol 2003, 17:259-272.

41. Yu L, Li-Hawkins J, Hammer RE, Berge KE, Horton JD, Cohen JC, Hobbs HH: Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. J Clin Invest 2002, 110:671-680.

42. Liu J, Zhang YL, Spence MJ, Vestal RE, Wallace PM, Grass DS: Liver LDL receptor mRNA expression is decreased in human ApoB/CETP double transgenic mice and is regulated by diet as well as the cytokine oncostatin M. Arterioscler Thromb Vasc Biol 1997, 17:2948-2954.

43. Berg K, Svindland A, Smith AJ, Lawn RM, Djurovic S, Alestrom A, Alestrom P, Eliassen K: Spontaneous atherosclerosis in the proximal aorta of LPA transgenic mice on a normal diet. Atherosclerosis 2002, 163:99-104.