Quantitative Trait Locus Mapping of Genes That Regulate Phospholipid Transfer Activity in SM/J and NZB/BlNJ Inbred Mice

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Objective—Phospholipid transfer protein (PLTP), an important protein in the transfer of phospholipids between lipoprotein particles and in the remodeling of HDL, is regulated at both the transcriptional and the protein level. We performed quantitative trait locus (QTL) analysis to identify genomic loci regulating PLTP activity in mice.

Methods and Results—Plasma PLTP activity was measured in 217 male F_2 progeny from a SM/J × NZB/B1NJ intercross. Two QTL for plasma PLTP activity in mice fed chow (*Pltpq1* and *Pltpq2*) were found on chromosomes 3 (34 cM, logarithm of odds [LOD] 3.5) and 10 (66 cM, LOD 4.1); two additional QTL in mice fed atherogenic diet (*Pltpq3* and *Pltpq4*) were found on chromosomes 9 (56 cM, LOD 4.5) and 15 (34 cM, LOD 5.0); and one QTL (*Pltiq1*) for the inducibility of PLTP activity was found on chromosome 4 (70 cM, LOD 3.7). Several candidate genes for these 5 QTL were tested by sequence comparison and expression studies.

Conclusions—We identified five significant loci involved in PLTP activity in the mouse and provided supporting evidence for the candidacy of *Nr1h4* and *Apof* as the genes underlying *Pltpq2*. **(*Arterioscler Thromb Vasc Biol*. 2004; 24:155-160.)**

Key Words: plasma phospholipid transfer protein ■ quantitative trait locus ■ mouse ■ *Nr1h4*, *Apof*

Plasma phospholipid transfer protein (PLTP) is responsible for the transfer of phospholipids from very low-density lipoproteins (VLDL) to high-density lipoproteins (HDL)[1] and remodels HDL into larger and smaller particles, generating prebeta HDL.[2] PLTP is believed to be an important factor in the development of cardiovascular disease, but its precise role is not understood. Recently published reports assigned both atherogenic and antiatherogenic properties to PLTP. For example, mice with a hyperlipidemic background show a decrease in atherosclerosis susceptibility when PLTP is knocked out.[3] On the other hand, PLTP enhances the removal of cellular cholesterol and phospholipids[4] and PLTP overexpression prevents accumulation of intracellular cholesterol in macrophages.[5] Furthermore, PLTP mediates alpha-tocopherol transfer, which provides LDL with antioxidants and preserves the normal function of endothelial cells.[6] More insight is needed to determine the relation between PLTP activity and the atherosclerotic process. Regulation of PLTP activity is an interesting target for future drug development and understanding the mechanism is a first step toward this goal.

Several transcription factor binding sites (sterol regulatory element-binding protein [SREBP], peroxisome proliferator–activated receptor [PPAR], and CCAT/enhancer-binding protein [C/EBP]) have been identified in the promoter region[7] and PPAR alpha (PPARA), liver X receptor (LXR), and farnesoid X receptor (FXR) have been reported to be involved in PLTP transcription.[8–10] But PLTP activity and mass in human plasma do not correlate, suggesting that there may be varying amounts of a catalytically inactive form of PLTP.[11] Not much is known about the regulation at the protein level or the factors that are involved. The lipoprotein profile in plasma seems to influence the ratio between "active" and "inactive" PLTP,[12] but the molecular basis and the functional differences between the two forms remains unclear.

To identify key loci that are involved in regulation of PLTP activity, we performed a quantitative trait locus (QTL) analysis on male F_2 intercross mice between the SM/J and NZB/B1NJ inbred strains. These two strains differ significantly for PLTP activity,[13] HDL levels,[14] and atherosclerosis susceptibility.[15] We report two QTL for PLTP activity in mice fed chow, two different QTL for mice fed atherogenic diet, and a QTL for inducibility using the log transformation of the ratio of the activities for both diets.

Methods

Mice and Diets

SM/J and NZB/BlNJ inbred mice were obtained from the Jackson Laboratory (Bar Harbor, Me). SM females were mated to NZB males

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to produce the F_1 progeny; F_1 mice were intercrossed to produce 217 male F_2 progeny. Mice were housed in a climate-controlled facility with a 14-hour:10-hour light-dark cycle with free access to food and water throughout the experiment. After weaning, mice were maintained on a chow diet (Old Guilford 234A, Guilford, Conn) until 8 weeks of age and then fed an atherogenic diet for 6 weeks containing (w/w) 15% dairy fat, 50% sucrose, 20% casein, 0.5% cholic acid, 1.0% cholesterol, as well as cellulose, vitamins, and minerals. The source of chemicals and the diet have been described previously.[16] All experiments were approved by the Jackson Laboratory's Animal Care and Use Committee.

Phospholipid Transfer Protein Activity

At 0 and 6 weeks of diet consumption, mice were fasted for 4 hours, blood was collected by retro-orbital bleeding into EDTA-coated tubes, and plasma was separated by centrifugation at 1500 rpm for 5 minutes at 4°C. The plasma phospholipid transfer activity mediated by PLTP was determined by measuring the transfer of [^{14}C]phosphatidylcholine from phospholipid liposomes to HDL_3 as described previously.[17] As phospholipid transfer activity in the mouse plasma of these two particular strains is considerably higher than in human plasma,[13] only half the amount of plasma (0.5 μL) was assayed compared with human plasma to keep the assay in the linear range. We included in each assay a 50-μL aliquot of three different 1:50 diluted, human control plasma samples in quadruplicate. The amount of phospholipid transferred by plasma PLTP was calculated as percent of total radioactivity per assay tube transferred to HDL minus background transfer (tubes without PLTP source).

Genotyping and Real-Time Polymerase Chain Reaction

DNA was isolated and genotyping performed as described previously.[14] Total RNA was extracted from livers of two groups of NZB and SM male mice: eight 8-week old mice fed a chow diet, and eight 12-week old mice fed the atherogenic diet for 4 weeks. Total RNA was obtained using the Trizol method (Invitrogen, Carlsbad, Calif) according to manufacturer's recommendations and was converted to cDNA using the Omniscript RT kit (Qiagen, Valencia, Calif). Gene-specific primers (Table I, available online at http://atvb.ahajournals.org) were designed and tested for specificity by sequencing the polymerase chain reaction (PCR) product. To amplify and detect the target gene and the reference gene, we used the Quantitect SYBR green PCR kit (Qiagen) according to the manufacturer's recommendations. Real-time PCR on cDNA was performed using an ABI Prism 7000 (Applied Biosystems, Foster City, Calif). Expression was calculated as the number of molecules per 1000 molecules of beta-2-microglobin (B2m) and the results are shown as the mean±SE.

Sequencing of Candidate Genes

To sequence the coding regions of the candidate genes *Apoa1bp*, *Nr1h4*, *Apof*, *Scap*, *Ppara*, and *Nr0b2*, primers were designed to span each of the exons for amplification using NZB/BlNJ, SM/J, 129S1/SvImJ, C57BL/6J, CAST/Ei, DBA2/J, C3H/HeJ, A/J, SJL/J, and SWR/J genomic DNA (Mouse DNA Resource, the Jackson Laboratory). Direct sequencing was performed on the PCR products using Big Dye Terminator Cycle Sequencing Chemistry and the ABI 3700 Sequence Detection System (Applied Biosystems). Results were analyzed using the Wisconsin Package (Accelrys Inc, San Diego, Calif).

Statistical Analysis

We performed genome-wide scans for QTL using the method of Sen and Churchill;[18] significance was determined by permutation testing for each trait.[19] The software package Pseudomarker (release 9.1, Sen and Churchill, http://www.jax.org/staff/churchill/labsite) was used. Significant and suggestive QTL meet or exceed the 95% and 90% genome-wide thresholds, respectively. First, we performed one-dimensional genome scans on a single-QTL basis to detect QTL with main effects. Then, simultaneous genome scans for all pairs of markers were performed to detect epistatic interactions. Finally, all the detected main effect and interacting QTL were used to fit multiple regression models. The type III sum of squares of each marker or marker pair to the total sum of squares is the percentage of variance explained by each marker or marker pair. Other statistical analyses were done using Prism V3.02 (GraphPad Software, Inc). Between-group comparisons were analyzed by one-way ANOVA followed by Tukey HSD test to determine statistical significance. Correlation between measurements was tested using simple linear regression analysis.

Results

PLTP Activity in Parental Strains, F_1 and F_2

The plasma PLTP activity in the parental strains and the F_1 and F_2 populations are summarized in Figure 1. PLTP activity in NZB mice was significantly higher than in SM mice. In F_1 animals on chow, the F_1 values were closer to NZB mice, whereas on atherogenic diet, F_1 values more resembled SM mice. Mean PLTP activity in F_2 progeny did not significantly differ from those of the F_1 animals and the values were distributed normally around the mean on both diets (Figure 1).

Expression of *Pltp* in the liver was measured in the parental strains by quantifying *Pltp* mRNA using real-time PCR. NZB had significantly higher levels than SM ($P<0.05$) on both diets, consistent with the higher PLTP activity in plasma. No significant increase in *Pltp* expression occurred in mice fed the atherogenic diet (Figure 2A).

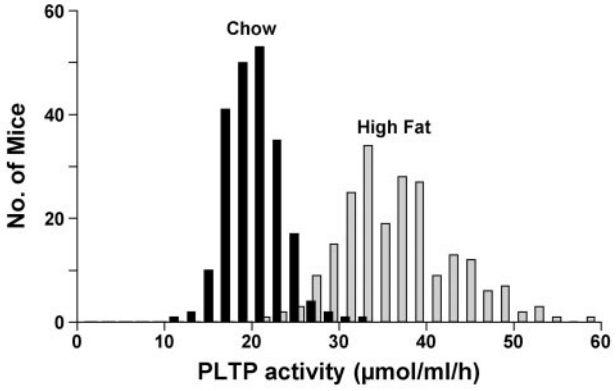
Identification of QTL for PLTP Activity

The genome-wide scan indicated significant QTL for PLTP activity in chow-fed mice on chromosomes 3 and 10 (Figure 3A) and were named *Pltpq1* (LOD 3.5, Figure 4A) and

QTL for PLTP Activity Identified in the NZB× SM Intercross

Traits	Name	Chr	Location (cM)	95% CI(cM)	Max LOD	Peak Marker	High Allele(inheritance)	Candidate Genes (cM)
PLTP (chow)	*Pltpq1*	3	34	30–45	3.5	*D3Mit22*	NZB (additive)	*Apo1bp* (40)
	Pltpq2	10	66	55–70	4.1	*D10Mit271*	Heterozygotes	*Nr1h4* (50), *Apof* (73)
PLTP (fat)	*Pltpq3*	9	56	50–60	4.5	*D9Mit15*	NZB (recessive)	*Scap* (61)
	Pltpq4	15	34	30–45	5.0	*D15Mit90*	SM (additive)	*Ppara* (50)
PLTP inducibility	*Pltiq1*	4	70	60–80	3.7	*D4Mit312*	NZB (dominant)	*Nr0b2* (60)

For each of the three analyzed phenotypes (PLTP on chow, PLTP on high fat diet, and the log of the ratio between the two diets) the significant QTL are shown with their chromosomal position, maximum LOD score, 95% confidence interval (CI), and the allele effect. The allele effect shows the high allele (allele with the highest PLTP activity) and the mode of inheritance (dominant, additive, or recessive).



	n	Chow	High Fat	% Change
NZB	14	20.2 ± 0.6[a]	42.3 ± 0.8[d,e,g]	111.2 ± 4.8
SM	17	12.8 ± 0.7[a,b,c]	28.7 ± 0.8[d,f]	131.4 ± 9.9[h,i]
(SM x NZB) F_1	13	18.2 ± 0.7[b]	32.6 ± 1.7[e]	82.6 ± 11.3[i]
F_2	217	19.3 ± 0.2[c]	35.6 ± 0.5[f,g]	87.3 ± 2.6[h]

Common superscript characters indicate pairs of significantly different groups. [a,b,c,d,e,f,h] $p<0.001$, [g,i] $p<0.01$

Figure 1. PLTP activity (mean±SEM in mol/mL/h) in chow and high-fat diet-fed male mice and distribution of the PLTP activity in the F_2 population. The black bars represent the population on chow, the gray bars show the distribution of the population on high fat diet.

Pltpq2 (LOD 4.1, Figure 4B), respectively (for *P*hospho*l*ipid *t*ransfer *p*rotein *Q*TL). The *Pltpq1* allele for high PLTP activity came from NZB and is additive. For *Pltpq2*, the heterozygous F_2 animals had a significantly ($P<0.05$) higher PLTP activity than the animals that were homozygous for either SM or NZB. Interestingly, QTL for HDL cholesterol levels overlap with these two QTL in the same cross (Korstanje et al, in preparation).

Pltpq1 and *Pltpq2* were not found when PLTP activity was measured after a 6-week atherogenic diet. Instead, two other significant QTL were found, *Pltpq3* (LOD 4.5) on chromosome 9 and *Pltpq4* (LOD 5.0) on chromosome 15 (Figures 3B, 4C, and 4D). For *Pltpq3*, animals that were homozygous for the NZB allele had high PLTP activity, whereas heterozygous animals and animals homozygous for the SM allele had lower PLTP activity. For *Pltpq4*, animals that were homozygous for the SM allele had higher PLTP activity than animals that were homozygous for the NZB allele. Heterozygous animals were intermediate.

PLTP inducibility, defined as the logarithm of the ratio between the PLTP activities on atherogenic diet and chow diet, was affected by one significant QTL (LOD 3.7) on chromosome 4 (Figures 3C and 4E); we named this *Pltiq1* (for *P*hospho*l*ipid *t*ransfer protein *i*nducibility *Q*TL). The NZB allele for high inducibility was dominant over the SM allele. The QTL are summarized in Table 1.

The QTL on chromosome 10 was fitted with models comprising one, two, or three QTL, and a maximum LOD score calculated for each. Permutation testing was used to determine significance thresholds. Increases of 2.2, 1.6, and 1.4 or greater in the LOD score were the thresholds used to

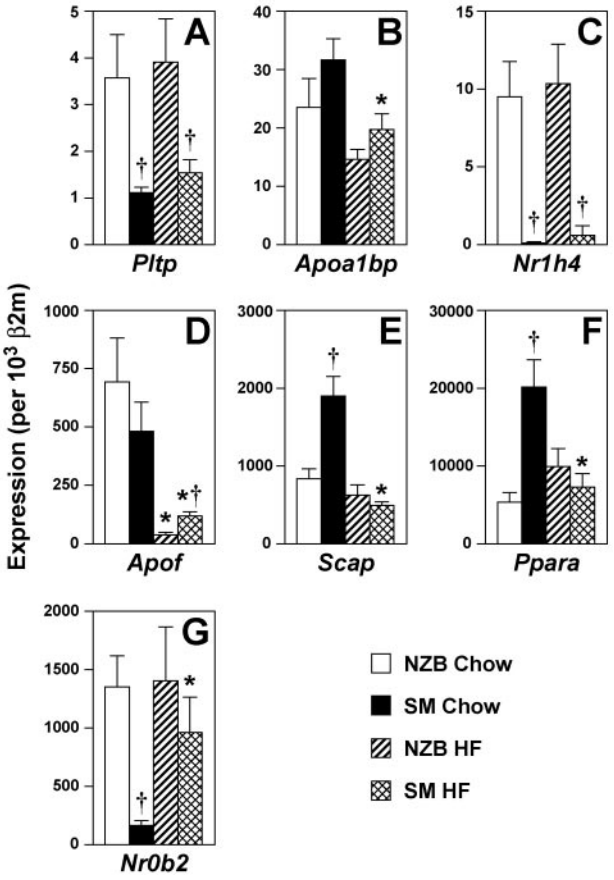


Figure 2. Hepatic mRNA expression analysis determined by real-time PCR. Data are reported as the number of target molecules per 1000 *B2m* molecules (mean±SEM; n=8 animals per group). *$P<0.05$ between diets for the same strain. †$P<0.05$ between strains on the same diet.

declare multiple QTL at the 95%, 90%, and 80% confidence levels, respectively. We observed an increase of 1.5 LOD (3.8 versus 5.2) for the one-QTL versus the two-QTL model, but an increase of only 0.5 LOD for the three-QTL model. Therefore, it is likely, but not conclusive, that two QTL on chromosome 10 contribute to PLTP activity. No evidence was found for multiple QTL for the other chromosomes. Tests for interaction between loci did not show significant interactions for any of the three phenotypes. The QTL found in this analysis explained 11.2%, 12.9%, and 7.9% of the variance of PLTP activity in chow-fed mice, atherogenic diet-fed mice, and the inducibility, respectively (multiple regression models for the QTL analyses [Table III, available online at http://atvb.ahajournals.org]).

Testing of Candidate Genes

We examined each QTL interval and selected several candidates for additional study (Table 1). Because either expression or functional differences in a gene can give rise to a QTL, we analyzed both the mRNA expression levels and the coding sequences of these candidate genes.

The candidate gene for *Pltpq1*, apolipoprotein AI binding protein (*Apoa1bp*), had no sequence differences in the coding region and no significant difference in expression in the liver

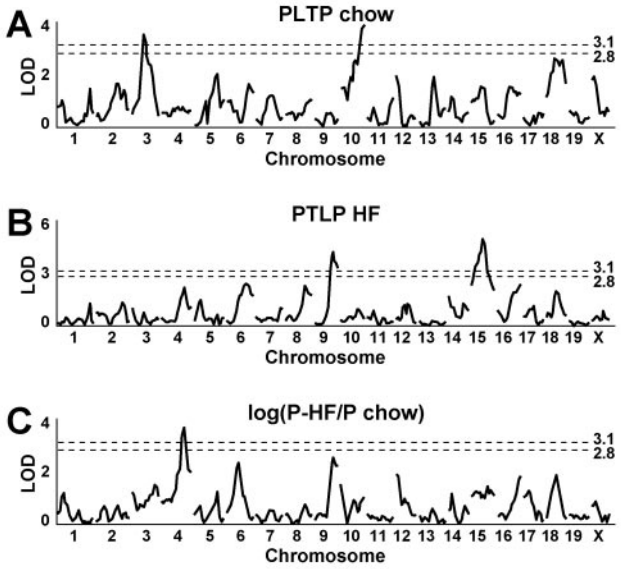


Figure 3. A, Genome-wide scans for PLTP activity in mice fed chow; B, high fat diet; and C, the log of the ratio of PLTP activity in mice fed high fat to chow diet. The upper and lower horizontal lines in each scan represent significant ($P=0.05$) and suggestive ($P=0.10$) LOD thresholds (3.1 and 2.8), respectively, as determined by permutation testing using 1000 permutations.

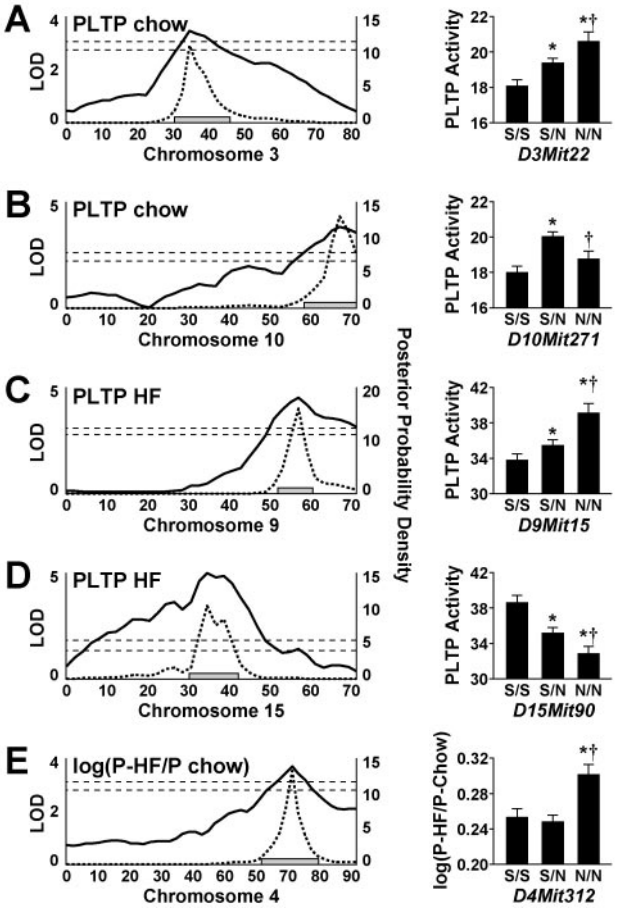


Figure 4. Genome scans for the chromosomes containing significant QTL. A, *Pltpq1* on chromosome 3; B, *Pltpq2* on chromosome 10; C, *Pltpq3* on chromosome 9; D, *Pltpq4* on chromosome 15; and E, *Pltiq1* on chromosome 4. For each scan, the posterior probability density shown is a likelihood statistic that gives the 95% confidence interval[18] (indicated by the black bar under the QTL). Allele effects for the peak marker are shown. Homozygous SM alleles are represented by S/S, homozygous NZB alleles by N/N, and heterozygous alleles by S/N. A, An additive NZB allele at *Pltpq1*, linked to *D3Mit22*, was associated with higher PLTP activity on a chow diet. For *Pltpq2*, linked to *D10Mit271*, the heterozygous alleles are associated with higher PLTP activity. B, Two genes with opposite allele effects could be responsible for this observation. C, A recessive NZB allele at *Pltpq3*, linked to *D9Mit15*, was associated with higher PLTP activity on a high-fat diet. D, An additive SM allele at *Pltpq4*, linked to *D15Mit90*, was associated with higher PLTP activity on a high-fat diet. For *Pltiq1*, linked to *D4Mit312*, a dominant NZB allele was associated with higher inducibility of PLTP. Data represents mean±SEM. *$P<0.05$ compared with S/S. †$P<0.05$ compared with S/N.

between SM and NZB mice fed chow (Figure 2B). Therefore, this gene is unlikely to underlie the QTL.

NR1H4 (FXR), a candidate for *Pltpq2*, is a known transcription factor for *Pltp*.[7] We identified 6 SNPs in the coding region of *Nr1h4*, three of them leading to conserved amino acid changes (Table II, available online at http://atvb.ahajournals.org). *Nr1h4* showed a 10-fold increase in hepatic expression in NZB compared with SM mice fed either diet and a slight increase in SM fed the atherogenic diet compared with the chow diet (Figure 2C). Thus, the candidacy of *Nr1h4* is supported by the expression difference between the two strains.

Another candidate gene for *Pltpq2* is *Apof*, a lipid transfer inhibitor protein.[20] We did not observe a difference in expression of *Apof* between NZB and SM, however, both strains showed a significant decrease in *Apof* expression on the atherogenic diet (Figure 2D). Two SNPs were found in the coding region of *Apof*. One difference, guanine in SM and cytosine in NZB at position 801, gives rise to a nonconservative amino acid difference at position 267 (on the basis of the first ATG as +1), a glycine to a histidine, in the structural part of the protein. Sequencing in other inbred strains shows that guanine is present in SM, C57BL/6J, DBA/2J, C3H/HeJ, MOLF/Ei, and CAST/Ei, and cytosine is present in NZB and 129SV/J. Thus, if *Apof* is the gene for *Pltpq2*, we suggest that the amino acid change may cause a functional difference in the protein.

The gene-encoding SREBP cleavage activating protein (*Scap*) is a candidate gene for *Pltpq3*, a QTL found only in atherogenic diet-fed mice. Expression analysis in the liver showed a two-fold difference in chow fed mice, but no difference in the atherogenic diet fed mice (Figure 2E). A sequence difference in the coding region of *Scap* leads to a conservative amino acid change. Thus, the lack of a difference in expression and the conservative amino acid change do not support the candidacy of *Scap*.

The gene encoding PPARA, another known transcription factor for *Pltp*,[8] is a candidate gene for *Pltpq4*. Sequence differences were found in the 5′ and 3′ UTR of the gene, but not in the coding region, and there was no significant difference of *Ppara* expression in the liver between NZB and SM on the atherogenic diet. Interestingly, the expression level of *Ppara* in SM on chow was significantly higher than in NZB on chow ($P<0.05$) and higher than in SM on the atherogenic diet (Figure 2F).

Small heterodimeric partner (SHP) is a transcription factor involved in cholesterol metabolism and the gene (*Nr0b2*) is a candidate for *Plti1*. We found a significant difference between NZB and SM on the chow diet, but not on the atherogenic diet (Figure 2G). We also found a significant difference between SM on chow and on the atherogenic diet ($P<0.05$). Sequencing of the coding region revealed no differences between SM and NZB.

Discussion

Urizar et al.[10] found a significant increase in hepatic *Pltp* mRNA expression when feeding C57BL/6 male mice a diet containing 1% cholic acid. In the present study, SM and NZB male mice have no increased hepatic *Pltp* mRNA expression on a diet containing 0.5% cholic acid. One explanation why these results differ could be the difference in the amount of cholic acid in the diet. A better explanation is the reference gene used; we observed that hepatic beta-actin mRNA levels, which is the reference used by Urizar et al.,[10] decreases because of a cholic acid diet (data not shown), causing an apparent increase in *Pltp* expression. This decrease of beta-actin made the gene, in our view, unfit as a reference. Therefore, we used a gene, *B2m*, whose expression levels are not affected by the diet change.

Because no QTL was found at the location of the *Pltp* gene (chromosome 2, 93 cM), we conclude that the difference in PLTP activity and the differential gene expression of hepatic *Pltp* between SM and NZB is because of differences in transacting elements that regulate PLTP activity and *Pltp* expression.

A locus associated with PLTP activity on the chow diet is *Plptq1* on chromosome 3. A candidate gene, the ApoA-I binding protein (*Apoa1bp*), is located within the 95% confidence interval of this QTL. Because PLTP interacts with Apo A-I[21], APOA1bp might be involved in this interaction. However, there is no evidence to support the candidacy of this gene; we found no sequence differences in the coding region and no difference in expression in the liver (Figure 2B).

Pltp can be regulated by the farnesoid X-activated receptor (FXR),[10] which is encoded by *Nr1h4*, a candidate gene for *Pltpq2*. Higher expression levels of FXR lead to higher expression of PLTP, and NZB mice had a 10-fold higher expression of *Nr1h4* in liver compared with SM mice (Figure 2C). We observed a small increase in expression in SM mice fed atherogenic diet; this might be enough to reach the maximum threshold for FXR regulation of PLTP, which could explain why we do not observe a QTL for *Pltpq2* on the atherogenic diet. Sequencing of *Nr1h4* found six base pair differences, leading to amino acid difference in FXR (Table II). Another candidate gene for this same QTL is the gene-encoding Apo F (*Apof*). Apo F is known to inhibit the phospholipid transfer activity of cholesterol ester transfer protein (CETP) in human[20] and inhibits CETP in humans; it might also inhibit PLTP, another member of the lipopolysaccharide-lipid transfer protein gene family.[22] We found two SNPs within the coding region of the gene resulting in nonconserved amino acid differences. One (AA128) is in the pre-protein, whereas the other (AA267), is in the part of the protein that is bound to HDL after cleavage. If Apo F is an inhibitor of PLTP, this latter substitution might be responsible for differential inhibition. *Nr1h4* and *Apof* are located at 50 and 73 cM, on either side of the 95% confidence interval of *Pltpq2*. Our current hypothesis is that both genes may be involved and that we have two closely linked independent QTL in this region as suggested by the test for multiple QTL. If two QTL exist, this would also explain the seemingly strange allele effect of the QTL, where heterozygous animals for the peak marker *D10Mit271* have a significantly higher PLTP activity than homozygous animals for both parental genotypes. If both genes are involved in PLTP activity, we might expect opposite allele effects for these genes, which could lead to a net allele effect at *D10Mit271*, as depicted in Figure 4B.

After 6 weeks of the atherogenic diet, we found different QTL associated with PLTP activity. We did not find the expected increase in *Pltp* mRNA expression, which might be because of compensatory mechanisms in gene regulation. We did not observe *Pltpq1* and *Pltpq2* on the atherogenic diet and speculate that the atherogenic diet causes the factors underlying these QTL to reach maximum levels for PLTP regulation both in NZB and SM, and therefore eliminates differences between the two strains. Instead, we found two novel QTL, *Pltpq3* and *Pltpq4* on chromosomes 9 and 15, respectively. Because the promotor sequence of *Pltp* contains sterol regulatory element-binding protein-1 (SREBP-1) binding sites,[7,8] we consider the gene for SREBP cleavage-activating protein (*Scap*) as a candidate gene for *Pltpq4*. However, we found no evidence to support the candidacy of this gene because expression did not differ and the coding region contained only one basepair difference, leading to a conservative amino acid change. *Ppara*, whose gene product is involved in *Pltp* regulation might be a candidate gene for *Pltpq4*. Measuring the relative expression of *Ppara* in liver showed no significant difference between NZB and SM on the atherogenic diet. Interestingly, we do see a significant ($P=0.0034$) difference between NZB and SM on the chow diet, but this apparently does not influence the PLTP activity on chow enough to be detected as a QTL. Two sequence differences, one in the 5′UTR and one in the 3′UTR of the gene, were found between SM and NZB. These SNPs might influence the translation efficiency of the mRNA.

The gene for an orphan nuclear receptor SHP, *Nr0b2*, is a candidate for *Pltiq1*. Although a direct relation between SHP and PLTP has not been found, *Nr0b2* is regulated by FXR[23] and is known to be involved in regulation of phospholipid metabolism. We found no sequence differences in the coding sequence between the two strains. The results of the expression analysis for SHP show little difference between diets for NZB but an increase in atherogenic diet fed SM ($P=0.015$). If SHP regulates PLTP expression, inducibility would be smaller for NZB than for SM. However, the allele effect for *D4Mit312* shows the opposite (Figure 4E). Therefore, we must conclude that *Nr0b2* is probably not the gene responsible for *Pltiq1* and the differences in expression are probably a direct effect of FXR levels.

In conclusion, we have identified 5 QTL that regulate PLTP activity between the two inbred mouse strains NZB and

SM. Considering the relation between PLTP activity and expression levels of PLTP in the liver, it is likely that some of the QTL are transcription factors that have an expressional or functional difference between the two inbred strains. *Nr1h4* is a good candidate gene for *Pltpq2*, the QTL on chromosome 10. The difference of both inbred strains between PLTP activity on chow and atherogenic diet cannot be explained by expression differences of *Pltp*. Therefore, factors other than transcription factors might underlay some of the QTL. Apo F, which is involved in CETP inhibition and has an amino acid difference between SM and NZB, is a good candidate for *Pltpq2*. Thus, we have found evidence for two candidate genes for *Pltpq2* on chromosome 10, *Nr1h4* and *Apof*, and some evidence that two closely linked QTL exist at this locus. Further study will be necessary to identify the genes underlying these QTL.

Acknowledgments

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Arteriosclerosis, Thrombosis, and Vascular Biology

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Quantitative Trait Locus Mapping of Genes That Regulate Phospholipid Transfer Activity in SM/J and NZB/BlNJ Inbred Mice

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Gene-specific oligonucleotide primers employed for real-time PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Pltp	GATCTGCCCTGTGCTCTACC	TCCTTCAGAGGGAAGAATGC
Apoa1bp	AGCCAGCAGAGTGTGTGT	TCGTTAAAAAGCTCTTGGTC
Nr1h4 (*Fxr*)	CTCCCCATACAACAATGTCC	TCGTCCTCATTCACTGTCTG
Apof	AGAGGCAACCAGTGTCTCTA	GGTAAAGCTGAAGCTGAAGA
Scap	AGATGTTACCTCCCTCACCT	TTCTTGCCCAAGTAGACTGT
Ppara	GCGGCCCCATACAGGAGAGCAG	CTAACCTTGGGCCACACCTTGACT
Nr0b2 (*Shp*)	CCTAACCAACAGCTTATGCC	GGCGGAAGAAGAGATCTACC
B2m	GACCGGCCTGTATGCTATCC	TTTTTTCCCGTTCTTCAGCATT

Sequence differences found between SM/J and NZB/B1NJ. Exons as shown in Ensembl (and Celera for *Nr1h4*) were sequenced for *Apoa1bp*, *Apof*, *Ppara*, *Scap*, and *Shp*.

Gene	Basepair*[1]	Region	NZB	SM	AA[†]	NZB	SM
Nr1h4	200	Transl	C	A	23	Asp	Glu
	292	Transl	G	A	54	Glu	Lys
	804	Transl	G	A	225	Arg	Lys
	991	Transl	A	G	287	Asp	Asp
	1273	Transl	A	T	381	Gly	Gly
	1525	Transl	A	G	465	Pro	Pro
Apof	400	Transl	G	C	128	Arg	Pro
	818	Transl	G	C	267	Gln	His
Ppara	210	5' UTR	C	T	-	-	-
	1868	3' UTR	G	A	-	-	-
Scap	1402	Transl	A	G	468	Met	Val

*basepair numbering based upon the start of transcription.

†amino acid numbering based upon the first ATG as +1.

Multiple regression models for loci involved in PLTP activity.

Trait	Source	df	Adjusted SS	*F*	*P*
PLTP (chow)	*D3Mit22*	2	102.6	5.79	0.0036
	D10Mit271	2	124.2	7.01	0.0011
	Total	203	2026.0		
	% var.explained		11.2		
PLTP (fat)	*D15Mit90*	2	747.6	9.61	0.0001
	D9Mit15	2	502.1	6.46	0.0019
	Total	215	9652.9		
	% var.explained		12.9		
PLTP inducibility	*D4Mit312*	2	0.1	8.83	0.0002
	Total	203	1.5		
	% var.explained		7.9		

P values are based on the *F* distribution with 1 and 203 (215 for PLTP (fat)) degrees of freedom (df). *F* statistics from the multiple regression models are based on sums of squares (SS).