



Serum phospholipid transfer protein activity and genetic variation of the PLTP gene

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Abstract

The inverse relationship between serum levels of high density lipoproteins (HDL) and risk of coronary heart disease is well established. The phospholipid transfer protein (PLTP) promotes the transfer of phospholipids between lipoproteins and modulates HDL size and composition. It thus plays a central role in HDL metabolism. Serum PLTP activity was measured in 400 healthy Finnish individuals in order to determine normal PLTP serum values. PLTP activity increased with age ($P < 0.001$), so that the PLTP activity was 3.81 ± 0.84 $\mu\text{mol/ml per h}$ (mean \pm S.D., $n = 52$) for men and 3.97 ± 0.11 $\mu\text{mol/ml per h}$ ($n = 52$) for women in the youngest age group (25–35 years), while it was 6.77 ± 0.17 $\mu\text{mol/ml per h}$ ($n = 45$) for men and 6.68 ± 0.15 $\mu\text{mol/ml per h}$ ($n = 40$) for women in the oldest age group (56–65 years). PLTP activity correlated significantly ($P < 0.001$) with body mass index ($r = 0.22$), serum total cholesterol ($r = 0.17$), the ratio of HDL-cholesterol/total cholesterol ($r = -0.20$), triglycerides ($r = 0.20$), apo A-II ($r = 0.20$), and γ glutamyl transferase ($r = 0.22$) values. Serum PLTP activity correlated negatively ($r = -0.20$, $P < 0.001$) with levels of apolipoprotein A-I in HDL particles that contained only apo A-I [Lp(A-I) particles]. The allelic frequencies of six intragenic polymorphisms, -79G/T , -56G/A , -37T/C , -31A/G , Phe2Leu, Arg121Trp, and two neutral polymorphisms, located in the immediate vicinity of the PLTP gene were determined. There were no significant associations between these polymorphisms and serum PLTP activity. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Epidemiological studies have provided strong evidence for an inverse relationship between serum high density lipoprotein (HDL) levels and coronary heart disease (CHD). The mechanisms by which HDL exerts its antiatherogenic effects have not been conclusively elucidated. An increasing amount of evidence from cell culture and animal studies suggests that the antiatherogenic mechanisms involve HDL-mediated removal of cholesterol from cells of the arterial wall and subsequent transport of the lipid to the liver for excretion [1]. This process, named reverse cholesterol transport

(RTC), limits accumulation of cholesterol in the arterial wall and is one mechanism how HDL lowers CHD risk. HDL in human serum is heterogeneous and consists of several distinct subpopulations. Not all HDL particles are equally effective as acceptors of cell cholesterol. A major initial acceptor of cell-derived cholesterol is a minor subpopulation of HDL that contains apo A-I as its sole apoprotein and has pre- β -electrophoretic mobility [2]. The origin of this subpopulation is not completely elucidated but several lines of evidence support the concept that some serum factors, especially phospholipid transfer protein (PLTP), participate in the generation of these cholesterol acceptor particles. Serum phospholipid transfer protein (PLTP) [3] transfers phospholipids among lipoprotein particles and from liposomes to high density lipoproteins (HDL) [3–5]. There is in vitro and in vivo evidence demon-

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strating that PLTP plays an important role in the remodeling of HDL particles. Specifically, PLTP can cause conversion of HDL₃ to larger (10.9 nm) and smaller (7.8 nm) particles in a time- and concentration-dependent fashion [6–8].

Little is known about the genetic variation of the PLTP gene. Only one case of PLTP deficiency in humans has been reported in an abstract form [9]. The gene is cloned [10] and mapped by fluorescence in situ hybridization [11] to chromosome 20q12-q13.1. The observation that the human protective protein (PPGB) gene partially overlaps the gene coding for PLTP further refined its location [12]. The PLTP protein has a signal peptide of 17 amino acids and the mature protein consists of 476 amino acid residues [10]. The gene is comprised of 16 exons [13]. Most of the mature protein is coded by exons 3–15. The PLTP gene is expressed in several tissues, such as ovaries, testes, thymus, placenta, small intestine, pancreas, lung, and prostate [14–16]. The physiological function of PLTP has been examined by expressing human *PLTP* in mice. With stable low level expression in human PLTP [17] or in human PLTP and human apo A-I [18] transgenic mice, the proportion of HDL cholesterol increased relative to non-HDL cholesterol. Using adenoviral vectors producing transiently very high expression levels of PLTP, the HDL fraction almost disappeared, probably due to increased conversion activity and hepatic uptake of HDL [19,20]. Thus far there are no reports on *PLTP* knockout mice.

The aims of the present study were to establish the serum levels of PLTP activity in the Finnish population and to study genetic variation of the PLTP gene. The results will be useful in future studies assessing the role of PLTP in normal physiology and in pathophysiological states.

2. Materials and methods

2.1. Subjects

Study subjects, $n = 400$, were from a large Finnish cross-sectional study [21] (FINRISK) carried out in 1992. They represent men and women in four age groups from 25 to 65 years in 10-year intervals. The participants of the FINRISK-study were randomly selected from the inhabitants of North Karelia, the Kuopio province, and Southwestern Finland, thus closely representing the total Finnish population. The main objectives of the FINRISK-study were to monitor risk factors of CHD and living habits and conditions in the population. All participants answered a questionnaire regarding their health, diet and environment and gave their informed consent. The study was approved by an ethical committee.

2.2. Lipid and apolipoprotein measurements

The samples were analyzed at the National Public Health Institute, Department of Biochemistry, Helsinki. Total serum cholesterol and triglycerides were measured by automated enzymatic methods using the Olli-C autoanalyzer (Kone Instruments, Espoo, Finland). HDL cholesterol was measured after precipitation of apo B-containing lipoproteins with dextran sulfate and magnesium chloride [22]. LDL-cholesterol was calculated according to the formula of Friedewald [23]. Serum apolipoproteins (apo) A-I, A-II and B were analyzed using immunoturbidometric assays (Hoffman-La Roche, Basle, Switzerland and Orion Diagnostica, Espoo, Finland). The amount of apo A-I in the HDL subfraction containing apo A-I but not apo A-II, LP(A-I) particles, was determined by rocket immunoelectrophoresis (Sebia, Paris, France).

2.3. Measurement of phospholipid transfer activity of serum PLTP

For the radiometric PLTP activity assay, phosphatidylcholine (PC) liposomes were prepared essentially as described [24]. The liposome preparation contained 10 μmol of egg PC, 1 μCi of [¹⁴C]dipalmitoylPC, and 20 nmol of butylated hydroxytoluene. Each assay contained HDL₃ acceptor (250 μg of protein), donor liposomes (150 nmol of labeled PC liposomes), sample (4 μl of 1:10 diluted human serum), and sample buffer (10 mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 1 mM EDTA) in a final assay volume of 400 μl . In each series, samples were analyzed in duplicate. Each series also contained duplicate blank tubes without any sample, and duplicate control serum samples that were stored at -70°C before assay. There have been no detectable differences in PLTP activity of serum or of purified PLTP preparations which have been stored at -70°C over 2 years. For each series, a new control sample vial was thawed. Assay tubes were incubated for 90 min at $+37^{\circ}\text{C}$ after which the reaction was stopped by the addition of 300 μl of a stop-mixture (536 mM NaCl, 363 mM MnCl_2 and 52 U heparin). The tubes were vortexed for 1 min at room temperature and then centrifuged for 10 min at 15 000 rpm. The radioactivity was determined from a 500- μl aliquot of the supernatant. To avoid systematic variation in PLTP activities, samples from all age groups were equally present in each series of assays performed.

2.4. Analyses of genetic variation

To search for genetic variation in the PLTP, gene PCR primers were designed for all exons and for the promoter region up to base -150 , using published sequences of the intron-exon boundaries [13], cDNA

[10] and of the promoter region [25] (Table 1) to be used in single strand conformational polymorphism analysis (SSCP). In the present study nucleotides are numbered according to Tu et al. [25] and the numbering of amino acids begins from the first amino acid (glutamic acid) in the mature protein. The PCR conditions were the following: hot start at 94°C for 5 min, annealing at optimized temperature (Table 1) for 30 s, elongation at 72°C for 30 s and 1 min denaturation at 94°C repeated for 30 cycles. The reaction volume was 20 µl and the reaction mixture contained 30 ng of sample DNA, 5 pmol each primer, 0.5 U polymerase (Dynazyme, Finnzymes, Helsinki, Finland) and 2 nmol of each nucleotide. The validity of reactions was checked by direct sequencing. Samples of 24 individuals were selected for SSCP analysis so that they represented both the highest and the lowest PLTP activity levels in different age groups. Males and females were equally

represented. This strategy was chosen in order to increase the probability of detecting variation in *PLTP* affecting its phospholipid transfer activity. SSCP analyses were performed using the PhastSystem (Pharmacia Biotech, Uppsala, Sweden). Acrylamide gels with gradient (8–25%) were used for fragments below 140 bp and homogeneous 12.5% gels for larger fragments. Both gel types were run with native buffer stripes at 400 V, 10.0 mA and 3.5 W for the Volt hours shown in Table 1. Two running temperatures (+4 and +20°C) were used. In cases where SSCP analysis indicated a mutation the fragment was cloned in the pGEM-T vector (Promega, USA) for sequencing. The sequencing was performed either manually using ³²S-labeling or using an automatic sequencer (ALF, Pharmacia). For the variants which were confirmed by sequencing, we designed extra primers (Table 1), which were used for typing these polymorphisms by the solid-phase minise-

Table 1
PCR primers and conditions used to detect genetic variation in *PLTP*^a

Site	Primer sequences in 5' → 3' direction		Annealing temp. (°C)	Product size (bp)	Volt hours in SSCP	
	Forward	Reverse			+4°C	+20°C
Promoter	CTGTGCAGCCTTTTC- CACTC	TCAGGGGATCCGGGCGAC	62	170	161	108
Exon 1	GTGGCCCGCGTCGCCCCG	CGGATAGGGACGCGCCCC	64	129	128	85
Exon 2	TCCTGCGACCCCACC- CCA	GTCCCTGTCTGCCCCCTGC	64	161	153	102
Exon 3	AGTAAGGGTTTCTCT- GCTGCT	GGCCCCGCCCCCACTTA	62	144	140	93
Exon 4	GATCTGACCCTGAGGC- CTC	TCCCCTGAGGGTGCTGGG	64	179	171	115
Exon 5	GAGTGAATATTAACCC- CCCTG	AGCTGGGGTTGGGGCTGG	62	206	202	140
Exon 6	CATCCGGCCTCTC- CTCTCC	CCCTGCGACCTGTGCGCTG	64	114	115	77
Exon 7	CCTGACTCTGGCTCC- CACC	CCAGACTCACCCGC- CACCA	62	114	115	77
Exon 8	CGCAGTTCTGTGGAC- GAGCT	TCACCCGGAAGTCCAT- GTCC	58	141	139	92
Exon 9	CCTGACTGTGAATGCC- CCAC	ATCCACAAAACAGGC- CATGAC	64	227	244	161
Exon 10	CCTCACTCCTGATTCCC- CTG	TATCCCTGCCCCCGCCAG	64	110	112	75
Exon 11	CCCGCCCCTGCTGCT- CAT	GCAGCCCCACTCTGGGA	62	215	223	150
Exon 12	CATCCTCCTCCCCAT- GTCC	GGTGCAGGGAAGTGCGCC	64	118	120	80
Exon 13	CACAGGGCTC- CTTTCTTTCC	AGCCCACCCACCCTTCCC	66	93	100	67
Exon 14	CTGTTTCCGCC- CTCGCCC	CTCCATCCTCACACCCAG	66	114	115	77
Exon 15	CTGAGCGGGGTGCTC- CCA	CCTTCCCCATCTGCCCC	64	127	124	83
Exon 16	TGAGGCTCCCCTCTC- CCC	GGTGGTGGACGGACTG- TAAT	66	329	500	290

^a Detection primers: -79G/T: CCAGCCCCAAAGGAGGAG; -56G/A: GAGCTGCAGAGAGGAGGAG; -37T/C: GTCGGGGAGGCC-
CGGCT; -31A/G: GGGAGGCCCGGCTTTATAA; 135T/C: GCAGGCGCACATGCAGAG; 492C/T: CCCGGGATCCCGCTGGA.

quencing method [26] in the 400 Finnish study subjects. In this method variable nucleotides are identified by a single nucleotide primer extension reaction catalyzed by DNA polymerase from a PCR product immobilized on a solid support. In addition, we typed in the same study population two polymorphisms Phe2Leu and Arg121Trp (135T/C and 492C/T) (Table 1), which were found by sequencing the entire coding region, splice sites and the proximal promoter sequence of 22 German individuals having serum HDL cholesterol concentration above the 90th percentile. Two neutral polymorphisms, a CTG-trinucleotide repeat [27] and a CA-dinucleotide repeat named *PPGP* [28] were also typed using previously published primers [27,28] in ^{32}S -labeled PCR reactions which were resolved in 6% acrylamide sequencing gels under denaturing conditions and detected by X-ray film. Both these repeats can be found in the immediate vicinity of the PLTP gene in the single PAC-clone 337o18, which has been sequenced in the Sanger Center, UK (<http://www.sanger.ac.uk/>).

2.5. Statistical analyses

The statistical testing was performed using the Statistical Package for Social Sciences (SPSS) version 6.1 (SPSS, Chicago, USA). The dependence of serum PLTP activity on age in four age groups and gender was tested by analysis of variance (ANOVA) controlled for BMI, serum triglycerol and serum cholesterol levels. Partial correlation coefficient controlled for age and gender was calculated between PLTP activity and BMI. Partial correlation coefficients, controlled for age, BMI and gender, were calculated between PLTP activity and serum cholesterol and triglycerides, HDL cholesterol, apo B, apo A-I, apo A-II, apo A-I in Lp(A-I) and Lp(A-I/A-II) particles, and serum γ glutamyl transferase (S-GT). The values of triglycerides were log-transformed to remove positive skewness in distribution. Based on self-filled questionnaires, the relation of PLTP activity to selected clinical conditions, smoking and alcohol usage was tested by analysis of variance controlled for age, gender, BMI, serum cholesterol and triglyceride values.

The genotypic effects of biallelic genetic polymorphisms on serum total lipids and HDL cholesterol, and on the serum PLTP activity were tested using ANOVA controlling for age, gender and BMI. The effects of multiallelic polymorphisms on PLTP activity were analyzed by first dividing each sex-specific age group by levels of PLTP activity into subjects of high and low activity. For these groups allele frequencies were estimated from genotypic data and the non-random distribution of the alleles was tested by a χ^2 -test. Standardized disequilibrium values ($D' = D/D_{\max}$) were determined between any two polymorphisms based on a permutation procedure [29]. The method is included

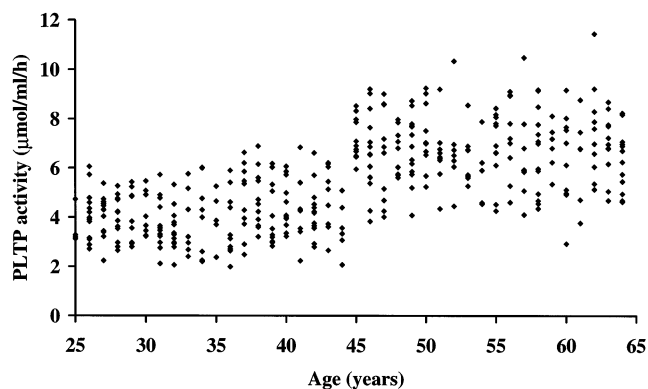


Fig. 1. Correlation of serum PLTP activity with age. There was no statistically significant difference between genders.

in the program package Arlequin [30]. Significance level was set to 0.05 in all analyses.

3. Results

3.1. Serum PLTP activity in the Finnish population

Serum PLTP activity increased with age ($P < 0.001$). PLTP activity in the youngest age group (25–35 years) was 3.82 ± 0.85 $\mu\text{mol/ml}$ per h (mean \pm S.D., $n = 52$) for men and 3.97 ± 0.11 $\mu\text{mol/ml}$ per h ($n = 52$) for women, while it was 6.77 ± 0.17 $\mu\text{mol/ml}$ per h ($n = 45$) for men and 6.68 ± 0.15 $\mu\text{mol/ml}$ per h ($n = 40$) for women in the oldest age group (56–65 years) (Fig. 1 Table 2). The difference in PLTP activity between the age specific groups is not explained by differences in BMI, serum triacylglycerol or serum cholesterol levels. PLTP activity was not significantly different between genders but it was positively correlated with body mass index (BMI) ($r = 0.22$, $P < 0.001$). There were also positive correlations ($P < 0.001$) between serum PLTP activity and serum cholesterol ($r = 0.17$) and triglycerides ($r = 0.20$), and S-GT ($r = 0.22$). The correlation between serum PLTP activity and HDL-C was not significant but the ratio of HDL-C/total cholesterol correlated negatively with PLTP activity ($r = -0.20$, $P < 0.001$) (Table 3). The correlation of PLTP with serum apolipoprotein A-I was not significant but with apo A-II there was a significant positive correlation ($r = 0.20$, $P < 0.001$). When apo A-I concentration was measured in HDL subclasses containing either both apo A-I and apo A-II [Lp(A-I/A-II) particles] or only apo A-I [Lp(A-I) particles], serum PLTP activity showed a negative correlation ($r = -0.21$, $P < 0.01$) with apo A-I in Lp(A-I). The correlation between PLTP activity and apo A-I in Lp(A-I/A-II) was not statistically significant. Based on self-filled questionnaires, the number of individuals having any specific disease was rather low in this study material (Table 4). However, to help further

Table 2
Serum PLTP activity, triacylglycerol, cholesterol and HDL-C values and BMI in different age groups^a

Group	PLTP ($\mu\text{mol}/\text{ml per h}$)	TG (mmol/l)	Cholesterol (mmol/l)	HDL-C (mmol/l)	BMI (kg/m^2)	N
<i>Age 25–35 years</i>						
Men	3.82 ± 0.85	1.07 ± 0.44	4.79 ± 0.89	1.26 ± 0.25	23.89 ± 3.18	52
Women	3.97 ± 1.13	0.86 ± 0.36	4.89 ± 0.68	1.57 ± 0.25	22.79 ± 3.52	52
<i>Age 36–45 years</i>						
Men	4.60 ± 1.54	1.77 ± 1.02	5.81 ± 1.24	1.26 ± 0.33	25.42 ± 3.30	52
Women	4.61 ± 1.43	1.03 ± 0.72	5.20 ± 0.96	1.58 ± 0.36	24.48 ± 4.33	51
<i>Age 46–55 years</i>						
Men	6.71 ± 1.39	1.80 ± 0.91	5.67 ± 1.02	1.21 ± 0.36	26.81 ± 3.62	48
Women	6.57 ± 1.41	1.22 ± 0.73	5.70 ± 0.92	1.53 ± 0.28	26.45 ± 4.75	50
<i>Age 56–65 years</i>						
Men	6.77 ± 1.69	1.87 ± 1.10	5.91 ± 0.90	1.27 ± 0.35	26.62 ± 2.98	45
Women	6.68 ± 1.51	1.49 ± 0.73	6.00 ± 1.03	1.44 ± 0.33	27.52 ± 5.34	40

^a Values are mean \pm S.D.; PLTP activity is expressed as μmol phospholipid transferred/ml per h.

research on PLTP, the relation of PLTP activity to selected clinical conditions, smoking and alcohol consumption are given (Table 4).

3.2. Genetic variation of PLTP

SSCP analyses of all 16 exons and the first 150 base pairs of the promoter region revealed no variation in exons but nucleotide changes at four loci were observed in the promoter region. These changes were $-79\text{G}/\text{T}$, $-56\text{G}/\text{A}$, $-37\text{T}/\text{C}$ and $-31\text{A}/\text{G}$. Based on the results from 400 individuals, the population frequencies were 0.01, 0.02, 0.03, and 0.09 for the rare alleles of the polymorphisms $-79\text{G}/\text{T}$, $-56\text{G}/\text{A}$, $-37\text{T}/\text{C}$, and $-31\text{A}/\text{G}$, respectively. The analysis of the two polymorphisms that change the encoded amino acid sequence, Phe2Leu and Arg121Trp, showed allele frequencies of 0.03 and 0.01. Serum PLTP activity, total triglycerides, cholesterol or HDL-C did not differ significantly between carriers and noncarriers of these polymorphisms in the 400 Finns. The allele frequencies for the di- and trinucleotide repeat polymorphisms were similar as given in the Genome Data Base (hosted by Johns Hopkins University School of Medicine, Baltimore, MD, <http://gdbwww.gdb.org>). The allele frequencies of two nucleotide repeat polymorphisms were not significantly different in individuals with high or low PLTP activity. There was a significant linkage disequilibrium ($P < 0.05$) between the dinucleotide polymorphism and the trinucleotide, the Phe2Leu, Arg121Trp and the $-37\text{T}/\text{C}$ polymorphisms. Also the rare alleles of the polymorphisms $-37\text{T}/\text{C}$ and Phe2Leu ($D' = 0.16$), $-31\text{A}/\text{G}$ and $-56\text{G}/\text{A}$ ($D' = 0.46$) and $-37\text{T}/\text{C}$ and $-56\text{G}/\text{A}$ ($D' = 0.18$) were in linkage disequilibrium.

4. Discussion

Epidemiological studies have provided strong evidence for an inverse relationship between serum HDL cholesterol levels and coronary heart disease (CHD) [31–33]. Cell culture studies [2] and animal models [34] have suggested that the antiatherogenic mechanism of HDL involves HDL-mediated removal of cholesterol from cells of the arterial wall and subsequent transport of cholesterol to the liver for excretion. Functionally the most important HDL subpopulation is believed to be the nascent HDL particles with pre- β -mobility, which take part in cholesterol efflux from peripheral cells and act as transit particles in reverse cholesterol transport (RCT) [35]. The phospholipid transfer protein

Table 3

The mean values of selected characteristics, partial correlation coefficients between them and PLTP activity

Characteristic	Mean value	Correlation coefficient ^a
Body mass index (BMI; kg/m^2)	25.4 ± 4.1	0.22***
Cholesterol (mmol/l)	5.48 ± 1.1	0.17***
Triglycerides (mmol/l)	1.39 ± 0.9	0.20***
γ Glutamyl transferase (U/l)	28.4 ± 32.6	0.22***
HDL-C (mmol/l)	1.40 ± 0.35	-0.08
Apo A-I (g/l)	1.40 ± 0.24	-0.008
Apo A-II (g/l)	0.40 ± 0.11	0.20***
Apo A-I in LP(A-I) (g/l)	0.48 ± 0.11	-0.21**
Apo A-I in LP(A-I/A-II) (g/l)	0.96 ± 0.23	0.08
HDL-C/total-C	0.26 ± 0.08	-0.20***

^a Coefficients adjusted for: BMI, age and gender.

** $P < 0.01$.

*** $P < 0.001$.

Table 4
The effect of selected clinical conditions, smoking and alcohol usage on the PLTP activity^a

	PLTP activity ($\mu\text{mol}/\text{ml}$ per h) in			ANOVA		Number of cases in age specific groups			
	Cases	<i>N</i>	Controls	<i>N</i>	<i>P</i> -value	25–35	36–45	46–55	56–65 years
<i>Clinical condition</i>									
Diabetes	5.69 \pm 1.54	16	5.37 \pm 1.86	374	0.163	2	2	5	7
High blood pressure	6.48 \pm 1.91	56	5.21 \pm 1.78	334	0.468	3	9	20	24
Heart insufficiency	6.92 \pm 2.03	12	5.34 \pm 1.83	378	0.638	1	0	2	9
Angina pectoris	6.62 \pm 2.13	16	5.34 \pm 1.82	374	0.158	2	1	5	8
Myocardial infarction	7.10 \pm 1.32	9	5.34 \pm 1.84	380	0.440	0	0	3	6
Cerebral infarction	6.85 \pm 1.92	10	5.34 \pm 1.83	379	0.947	0	0	2	8
Cancer	3.41 \pm 0.00	1	5.40 \pm 1.85	389	0.257	0	0	1	0
Asthma	5.89 \pm 2.21	7	5.38 \pm 1.84	383	0.118	0	1	0	6
Gallstones	8.49 \pm 0.91	2	5.37 \pm 1.84	388	0.045	0	0	1	1
Rheumatoid arthritis	5.99 \pm 2.20	7	5.38 \pm 1.84	383	0.845	0	1	2	4
<i>Smoking</i>									
Regular smoker	5.32 \pm 1.67	105				27	33	27	18
Occasional smoker	4.78 \pm 2.04	32				13	9	6	4
Non-smoker	5.59 \pm 2.04	104			0.775	28	24	26	26
<i>Alcohol usage</i>									
Highest 6.4%*	5.37 \pm 2.25	25				9	8	5	3
Moderate	5.34 \pm 1.82	338				90	91	88	69
Not at all	6.02 \pm 1.79	27			0.370	5	4	5	13

^a Data are mean \pm S.D. and are based on a self-filled questionnaire.

* The highest 6.4% is based on a question how often study subjects take so much alcohol that they feel intoxicated. The top 6.4% consists of individuals who answered weekly.

(PLTP) has been shown to generate HDL particles of that size and electrophoretic mobility by its HDL conversion activity [6–8]. Thus, PLTP may have physiological and clinical significance in the pathogenesis of coronary heart disease. In addition, it has been suggested that PLTP may be related to glucose metabolism, take part in maintenance of the antioxidant status of cells and be involved in antimicrobial defence [36]. In the present study, how the variation in PLTP activity correlates with the HDL level was investigated in a population and whether there are genetic variations in the PLTP gene.

PLTP activity was shown to be age-dependent and to correlate positively with body mass index. There was no significant difference between men and women in PLTP activities. PLTP activity and serum cholesterol and triglyceride levels were positively correlated but there was no significant correlation with serum HDL-C levels as such. However, the ratio of HDL-C/total cholesterol was negatively correlated with PLTP activity. The correlation of PLTP with BMI, serum triglycerides and cholesterol is in line with previous studies on PLTP. Our recent study clearly indicated that triglyceride enrichment of HDL enhances PLTP-mediated HDL size change [37]. In mice, a high fat diet increased PLTP activity and the level of its mRNA, whereas injection of lipopolysaccharides had opposite effects [18]. Also in human studies it has been shown that PLTP activity is positively correlated with BMI, fasting blood glucose

and serum C-peptide [38], but negatively with smoking (in postprandial state) [39]. The finding that PLTP correlated positively with serum γ glutamyl transferase is in agreement with the previous finding that PLTP activity correlates positively with alcohol usage [40].

BMI, serum triacylglycerol, HDL-C, and total cholesterol concentration are not independent risk factors. They are all associated with insulin resistance syndrome in which serum triacylglycerol concentration is elevated, HDL-C is decreased and BMI increased. Unfortunately we were not able to test the hypothesis whether a major determinant of PLTP activity in FIN-RISK population is insulin resistance as neither glucose nor insulin values were measured in the study. Based on the questionnaires filled by the study subjects some information about the dependence of PLTP activity on some clinical conditions as well as on smoking and alcohol usage can be derived. The two individuals who had a positive record on gallstones had higher PLTP activity than their controls. PLTP activity was not associated with smoking, alcohol consumption or other clinical conditions tested. Because the number of cases was quite low in each group and was not based on a prior power analysis, the results concerning the clinical conditions must be considered as preliminary and are given only to help further research on PLTP.

HDL particles can be classified based on their apolipoprotein content. Some HDL particles contain only apo A-I and are thus named Lp(A-I) and others

both apo A-I and apo A-II and are called Lp(A-I/A-II). In the present study, PLTP correlated negatively with LpA-I, whereas there was no significant correlation between PLTP activity and apo A-I in the Lp(A-I/A-II) particles. The biological basis for the negative correlation between PLTP activity and apo A-I in Lp(A-I) particles cannot be solved on the basis of the present study. A correlation neither proves causality nor gives biological explanation. It is possible, however, that Lp(A-I) particles are preferred substrates for PLTP-mediated HDL conversion in which small pre- β -HDL are generated and that these particles excrete serum to the interstitial space resulting in a concomitant decrease of serum levels of apo A-I. To prove this hypothesis, further research is needed but some earlier findings give support. We have earlier shown that enrichment of HDL with apo A-II causes a reduction in PLTP-mediated HDL conversion, which suggests an inhibitory role for apo A-II [41]. There is also evidence that the early events of reverse cholesterol transport occur extravasally [42] and that small disc-shaped nascent HDL particles similar to per- β -HDL can be found in prenodal lymph of dogs [43].

In the present study, PLTP activity in total human serum was evaluated by measuring the transfer of radiolabeled phosphatidylcholine from [14 C]PC-liposomes to the isolated HDL₃ subfraction [22]. This radiometric assay has been shown to be specific for measurement of PLTP activity since the other serum lipid transfer protein, CETP, although being able to transfer phospholipid, does not transfer phospholipid from liposomes to HDL [44]. Similar assay methods have recently been used to evaluate serum PLTP activity levels [39,40,45]. However, inter-laboratory comparisons are difficult. This is mainly due to the lack of international standardization of the methods. Expression of the serum PLTP activity in different units (i.e. μ mol phospholipid transferred/ml per h or radioactive PC transferred as % of total) further complicates the comparisons. Therefore, a collaborative study among the laboratories performing PLTP assays is needed to establish a standardized method.

Genetic variation was searched from the coding sequences of the *PLTP* as well as from the promoter region of the gene. The functional promoter of the *PLTP* gene consists of a TATA box, a high-GC content region, and several consensus sequences for potential binding of transcription factors. A minimal promoter of 159 bp harboring two transcription factor-binding motifs, SP1 and AP-2 has been reported to be responsible for full activity [25]. Thus, in addition to exons, the effort to find genetic variations affecting the level of serum PLTP activity was focused on the proximal promoter area. After analysing 24 individuals the findings were scarce, no variation was found in the sequences of the exons and only four nucleotide changes

were found in the promoter region studied. The performance of the used SSCP analysis method was tested with four previously known mutations of lipoprotein lipase gene and it detected all three which were synthesized in size below 200 bp and also the last one after using a restriction enzyme to cut it into pieces less than 200 bp (data not shown). We thus believe that we had a good chance of detecting most mutations in the *PLTP* gene which would be common enough to occur in 48 chromosomes.

The population frequencies of four promoter region mutations and of two newly found mutations causing amino acid changes Phe2Leu and Arg121Trp were determined in 400 randomly selected Finnish individuals. In addition, two neutral polymorphisms which were located very close to the *PLTP* were typed to detect possible disequilibrium between them and any mutation in *PLTP*. We could not detect a significant association with any of the polymorphisms and serum PLTP activity. The low number of carriers of *PLTP* polymorphisms in the course of our screening does not allow us to generalize our findings of the allele frequencies to other populations. Especially, although the Phe2Leu and Arg121Trp polymorphisms were initially discovered in German individuals having high serum HDL cholesterol concentration, these polymorphisms have not been detected in our preliminary screens with other German subjects from the general population (data not shown).

In conclusion, the PLTP activity increases with age and is regulated by body weight, levels of serum cholesterol and triglycerides and possibly by apo A-II content in HDL. In the Finnish population, there seems to be very little genetic variation in the *PLTP* gene. The alleles of the eight polymorphisms studied were not associated with serum PLTP activity.

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