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METABOLIC AND GENETIC DETERMINANTS OF HDL IN FAMILIES WITH LOW HDL CHOLESTEROL AND PREMATURE CORONARY HEART DISEASE

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ACADEMIC DISSERTATION

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals

I Aino Soro*, Päivi Pajukanta*, Heidi E. Lilja, Kati Ylitalo, Markus Perola, Tero Hiekkalinna, Jorma S. A. Viikari, Marja-Riitta Taskinen, Leena Peltonen. Genome scans provide evidence for low HDL loci on 8q23, 16q24.1-24.2 and 20q13.11 in Finnish families. *American Journal of Human Genetics* 2002; 70(5):1333-4012.

II Corradina Alagona*, Aino Soro*, Kati Ylitalo, Riitta Salonen, Jukka T. Salonen, Marja-Riitta Taskinen. Low high density lipoprotein (HDL) level is associated with carotid artery intima-media thickness in asymptomatic members of low HDL families. *Atherosclerosis* 2002; 165(2):309-316.

III Aino Soro, Matti Jauhiainen, Christian Ehnholm, Marja-Riitta Taskinen. Determinants of low HDL levels in familial combined hyperlipidemia. *Journal of Lipid Research* 2003; 44(8):1536-1544

IV Aino Soro-Paavonen, Jukka Westerbacka, Christian Ehnholm, Marja-Riitta Taskinen. Inflammation and endothelial activation coexist with the metabolic syndrome in subjects with familial low HDL. *Submitted for publication*.

*)Equal contribution

Abbreviations

θ	recombination fraction
ABCA1	ATP-binding cassette transporter A1
ANOVA	analysis of variance
Apo	apolipoprotein
ARIC	Atherosclerosis Risk in Communities
ASP	affected sibpair
ATP III	Adult Treatment Panel III
ATP	adenosine tri-phosphate
BP	blood pressure
CAM	cellular adhesion molecule
CB	carotid bulb
CCA	common carotid artery
CETP	cholesterol-ester transfer protein
CHD	coronary heart disease
CK	creatine kinase
cM	centiMorgan
CM	chylomicron
CRP	C-reactive protein
d	density
DBP	diastolic blood pressure
eNOS	endothelial nitric oxide synthase
EUFAM	European Multicenter Study on Familial Dyslipidemias in Patients with Premature Coronary Heart Disease
FCHL	familial combined hyperlipidemia
FED	fish-eye disease
FH	familial hypercholesterolemia
FHA	familial hypoalphalipoproteinemia
FW	far wall
FXR	farnesoid X-activated receptor
HA	hypoalphalipoproteinemia
HDL	high-density lipoprotein
HL	hepatic lipase
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HOMA IR	homeostasis model assessment for insulin resistance
HRT	hormone replacement therapy
IBD	identical-by-descent
ICAM-1	intracellular adhesion molecule-1
IDL	intermediate density lipoprotein
IL-1	interleukin-1
IMT	intima media thickness
km	kilometer
LCAT	lecithin:cholesterol -acyltransferase
LDL	low-density lipoprotein
LDL-r	low-density lipoprotein receptor
LpA-I	lipoprotein A-I
LpAI-AII	lipoprotein A-I/A-II
LPL	lipoprotein lipase
LRP	low-density lipoprotein receptor related protein
LXRα	liver-X-receptor alpha
m	men
MI	myocardial infarction
NEFA	non-esterified free fatty acids

NO	nitric oxide
NPL	non-parametric linkage
n.s.	not significant
NW	near wall
PAF-AH	platelet activating factor - acetylhydrolase
PCR	polymerase chain reaction
PLTP	phospholipid transfer protein
PON	paraoxonase
PPAR	peroxisome proliferator-activated receptor
PRIME	etude PRospective de l'Infarctus du MyocardE
PWA	pulse wave analysis
QTL	quantitative trait loci
r	correlation coefficient
RCT	reverse cholesterol transport
RXRα	retinoid-X-receptor alpha
SAA	serum amyloid A
SBP	systolic blood pressure
SD	standard deviation
SNP	single nucleotide polymorphism
SOLAR	The Sequential Oligogenic Linkage Analysis Routines
$sPLA_2$	Group IIA secretory secretory phospholipase A_2
SR-B1	scavenger receptor B1
SREBP	sterol regulatory binding protein
TD	Tangier disease
TG	triglyceride
TNF	tumor necrosis factor
TRL	triglyceride-rich lipoprotein
VA-HIT	Veterans Affairs HDL Intervention Trial
VCAM-1	vascular cell adhesion molecule-1
VLDL	very-low density lipoprotein
w	women
w/h	waist/hip

1. ABSTRACT

Aims
The main objective of the current thesis is to elucidate the metabolic and genetic defects underlying low levels of high-density lipoprotein (HDL) and premature coronary heart disease (CHD) in the Finnish population. Low HDL occurs as a heterogeneous disorder and in most cases is associated with other lipoprotein abnormalities, such as hypertriglyceridemia or familial combined hyperlipidemia (FCHL). Numerous population and intervention studies have revealed low HDL as a major independent risk factor for CHD. Despite intensive research, many unanswered questions remain regarding the role of various HDL actions with respect to its cardioprotective activity.

Methods and results
This thesis involved the investigation of the metabolic and genetic defects underlying low HDL cholesterol (HDL-C) in Finnish families with low HDL and premature CHD. A genome-wide scan in 25 low HDL families was performed to identify novel low HDL loci. The significance of the observed loci was further tested in an independent study sample of 29 Finnish FCHL families. The use of the low HDL trait as a disease phenotype (HDL-C <10th age- and sex-specific Finnish population percentile) in both low HDL and FCHL families provided evidence for linkage to three loci, on chromosomes 8q23, 16q24.1-24.2 and 20q13.11. Region on 8q23 produced the best linkage evidence, a two-point lod score of 4.7. Carotid ultrasonography with intima-media thickness (IMT) measurement was performed in 89 low HDL family members to define the parameters of HDL metabolism that predict thickening of the arterial wall. The affected family members in low HDL families had higher mean IMT than did the unaffected family members. They had markedly reduced levels of the large HDL particles, HDL_2 and LpA-I particles. Mean IMT was inversely associated with HDL-C and HDL_2-C. Metabolic studies were conducted in FCHL family members to examine which of the lipolytic enzymes and lipid transfer proteins contribute to the decreased HDL levels observed in FCHL dyslipidemia. Postheparin plasma activities of hepatic lipase (HL) and lipoprotein lipase (LPL), and activities of phospholipid transfer protein (PLTP) and cholesterol-ester transfer protein (CETP) were measured. Subjects affected by FCHL had a distinct reduction in the larger HDL_2–particles and a small low-density lipoprotein (LDL) particle size. Increased HL activity was found as a significant independent predictor of decreased HDL-C and HDL_2-C levels in FCHL family members. Finally, the levels of soluble cellular adhesion molecules (sCAMs) and C-reactive protein (CRP) were measured in subjects with familial low HDL and in matched normolipidemic controls. The low HDL subjects had significantly increased levels of sCAMs and CRP, and this increase was strongly related to the accumulation of the features of the metabolic syndrome.

Significance of the study
The present thesis showed that familial low HDL has partially shared genetic background with the FCHL. Chromosomal regions on 8q23, 16q24.1-24.2 and 20q13.11 may harbor genes important for HDL-C level regulation. 8q23 has provided evidence for linkage to HDL-C regulation in Mexican-American families. Thus far, no candidate genes contributing to HDL metabolism have been identified in 8q23. According to previous linkage studies, in turn, chromosome 20q harbors genes contributing to body adiposity, fasting insulin levels, and type 2 diabetes. This region may conceal loci involved in the regulation of HDL metabolism, particularly in the presence of decreased insulin sensitivity. Also the region on 16q has provided linkage evidence for the regulation of HDL and triglyceride (TG) levels in other study samples. The affected FCHL family members have enhanced HL activity, together with a higher degree of insulin resistance, decreased HDL-C and smaller HDL and LDL particles. Both in FCHL and in low HDL families, the affected family members have reduced concentrations of HDL_2-C and LpA-I, suggesting that the same regulatory pathways are disturbed in these lipoprotein abnormalities. Subjects with familial low HDL have early atherosclerotic changes as indicated by the increased carotid artery IMT. Finally, the familial low HDL trait associates with the metabolic syndrome. Low HDL-C, together with other features of the metabolic syndrome, predisposes the affected subjects to increased endothelial activation; increased inflammatory cytokine expression, and thereby, to increased risk for atherosclerotic disease.

2. REVIEW OF THE LITERATURE

2.1 DEVELOPMENT OF ATHEROSCLEROTIC VASCULAR DISEASE

Atherosclerotic vascular diseases are the leading causes of death in the Western world. The classic risk factors for atherosclerosis include increased total and LDL cholesterol, cigarette smoking, hypertension, male gender, and a family history of myocardial infarction (MI) (Kannel et al. 1979; (NCEP) 2001). Low level of HDL is a well-known cardiovascular risk factor (Brewer 2004). Evidence from epidemiological and clinical studies has established that inflammation has a role in the development of atherosclerosis (Ross 1999). The earliest changes in atherogenesis are increased endothelial permeability, migration of monocytes, and transcytosis of lipoproteins into the artery wall. LDL particles bind to the extracellular matrix within the intima, where they are predisposed to oxidative modification (Yla-Herttuala 1991). The oxidized LDL activates the endothelial cells, which attract more monocytes that transform into macrophages. As the macrophages accumulate the extracellular cholesterol they are transformed into foam cells that are the earliest sign of lipid retention in the artery wall (Stary 1989). Subsequently, smooth muscle cells join the foam cells and a fatty streak starts to develop. As the fatty streak expands, it forms an atheroma that has a core containing leukocytes, lipid, and necrotic cell debris, and a fibrous cap facing the arterial lumen. Any endothelial injury may weaken this fibrous cap, leading to an acute cap rupture, local thrombosis, and eventually, to the occlusion of the vessel lumen. For the effective treatment and prevention of atherosclerotic vascular events, we should identify the complex processes predisposing to the vascular injury and to the accumulation of lipids within the arterial wall.

2.2 OVERVIEW OF LIPOPROTEIN METABOLISM

Lipids are carried in the circulation in water-soluble lipoprotein particles that consist of a hydrophobic core of esterified cholesterol and triglycerides (TGs), and a hydrophilic surface of free cholesterol, phospholipids and apolipoproteins. Apolipoproteins maintain the structural integrity of lipoproteins and direct their metabolic interactions with cell-surface receptors, hydrolytic enzymes, and lipid transport proteins. Lipoproteins are classified on the basis of particle size, electrophoretic mobility, apolipoprotein content or hydrated density in ultracentrifugation. Traditionally, five classes of lipoproteins are separated according to the hydrated density (d) in ultracentrifugation as follows: chylomicrons (CMs, d<0.96 g/ml), very-low density lipoproteins (VLDL, d<1.006g/ml), intermediate density lipoproteins (IDL, d=1.006-1.019 g/ml), low-density lipoproteins (LDL, d=1.063-1.21 mg/dl) and high density lipoproteins (HDL, d=1.125-1.21 mg/dl)(Gotto et al. 1986).

The absorbed dietary lipids are packed into CMs in the gut epithelium. CMs are first secreted into the lymph, from where they enter the general circulation. CMs have a TG-rich core and apolipoproteins (apo) B48, apoA-I, apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III and apoE as their protein constituents. Endogenously produced TGs and cholesterol are secreted from the liver in VLDL particles. The major protein in VLDL is apoB100, but they also contain apoC-I, apoC-II, apoC-III and apoE. CMs and VLDLs release fatty acids, which either serve as energy in muscles, or are stored in the adipose tissue. Lipoprotein lipase (LPL) hydrolyzes the TGs in the CM and VLDL particles, creating CM and VLDL remnants (Santamarina-Fojo and Dugi 1994). The apoC-II on the surface of CM and VLDL activates the hydrolytic activity of LPL.

The remnant particles are removed from the circulation to the liver, by hepatic LDL receptor or the LDL-receptor related protein (LRP) (Herz et al. 1988). VLDL remnants can also be transformed into IDL particles. IDL is further hydrolyzed by hepatic lipase (HL) and LPL into LDL particles (Demant et al. 1988). LDLs are cholesterol-rich particles that serve as the major cholesterol carriers

to the peripheral cells. Hepatocytes and peripheral cells have LDL receptors on their surface (Brown and Goldstein 1986). After binding to the LDL receptor on the cell membrane, LDL is taken into the cell, which results in the suppression of cellular cholesterol synthesis by the suppression of microsomal enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. The intracellular cholesterol content down-regulates the LDL receptor expression, with the intention to prevent excess cholesterol accumulation within the cells (Brown and Goldstein 1986). Thus, the LDL receptor system coordinates the cellular cholesterol homeostasis. ApoB-100 and apoE proteins on LDL surface act as ligands to the LDL receptor. Macrophages, however, express also scavenger receptors that are not down-regulated by intracellular cholesterol level (Steinberg et al. 1989).

HDL particles originate *de novo* from liver and intestine, or they are synthesized from excess surface components during lipolysis of CMs and VLDL particles (Eisenberg 1984). Lipid-free, nascent HDLs (also called pre-$ß_1$-HDL particles) acquire also intracellular cholesterol from peripheral cells. Maturation of discoidal HDL particles includes esterification of non-esterified cholesterol on HDLs by lecithin:cholesterol-acyltransferase (LCAT) (Rye et al. 1999). Subsequently, cholesterol esters on mature HDLs are transferred back to the liver or are taken up by steroidogenic cells that express scavenger receptors on their surface. In addition, cholesterol esters in mature HDLs can be transferred to apoB-containing particles by cholesterol-ester transfer protein (CETP) and replaced for TGs.

2.2.1 Structure of the HDL particle

HDLs are the smallest of the five lipoprotein classes. HDLs are rich in protein, containing approximately 50% of lipid and 50% of protein by weight (Gotto et al. 1986). HDL contains a hydrophobic lipid core of cholesteryl esters and TGs surrounded by phospholipids, unesterified cholesterol and apolipoproteins. ApoA-I and apoA-II are the main structural proteins in HDL, accounting for approximately 70% and 20%, respectively, of total HDL protein mass. ApoA-I is found in all HDL subclasses, but apoA-II is present only with apoA-I. ApoA-IV, apoA-V, apoC-I, apoC-II, apoC-III, apoIV, apoD, apoE, and apoJ are found in lower amounts in mature HDLs (Barter et al. 2003). Some HDL-associated proteins are enzymatically active, e.g. LCAT, paraoxonase 1 (PON1), and platelet activating factor - acetylhydrolase (PAF-AH), or serve as lipid transfer proteins, e.g. CETP and phospholipid transfer protein (PLTP).

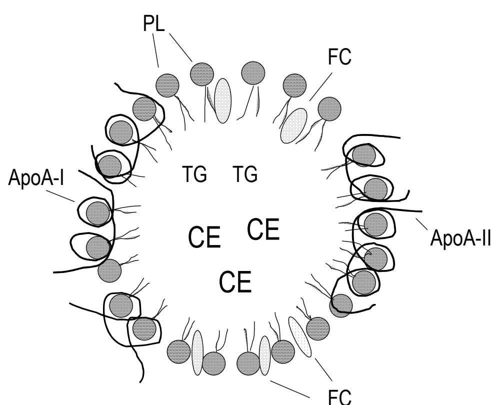


Figure 1. Structure of the HDL particle. PL, phospholipid; FC, free cholesterol; CE, cholesterol ester; TG, triglyceride; apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II

2.2.2 HDL subclasses and apolipoproteins

HDLs are a heterogeneous group of particles, which can be separated according to particle size, protein composition, or hydrated density in ultracentrifugation. The two main fractions according to the density in ultracentrifugation are HDL_2 and HDL_3. HDL_2 particles are less dense, having a higher relative amount of cholesterol and phospholipids than HDL_3 particles (Gotto et al. 1986). Non-denaturating gradient gel electrophoresis separates HDLs according to particle size into five subclasses: HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, and HDL_{3c} subclasses. HDLs can be divided into subclasses having either an α- or a β-electrophoretic mobility as follows: preα-HDL, α-HDL, and preβ-HDL (Davidson et al. 1994). Furthermore, as many as 12 distinct HDL subpopulations have been identified using two-dimensional electrophoresis (Asztalos et al. 1993).

HDLs can also be classified on the basis of their apolipoprotein composition into A-I -containing lipoproteins without A-II; lipoprotein A-I (LpA-I), and A-I -containing lipoproteins with A-II (LpAI-AII) (Cheung and Albers 1984). LpA-I particles are denser and have higher cholesterol content than LpAI-AII particles. Overall, subclasses based on apolipoprotein content do not exactly correspond to those separated by ultracentrifugation, and they widely overlap. LpA-I predominates in HDL_2 density range, while LpAI-AII predominates in HDL_3 density range.

2.2.3 Metabolism of HDL particles

Nascent HDL particles originate from excess surface components during lipolysis of CMs and VLDL particles, or from apoAI-containing particles secreted by the liver and intestine (Eisenberg 1984). ApoA-I is produced both in the liver by hepatocytes, and by the intestinal mucosa, while apoA-II is produced in the liver only. Peroxisome proliferator-activated receptor alpha (PPAR-α), a nuclear transcription factor, upregulates the hepatic synthesis of apoA-I and apoA-II (Staels et al. 1998). The nascent, lipid-free apoA-I-phospholipid complexes are termed as pre-$ß_1$-HDL particles due to their electrophoretic mobility (Davidson et al. 1994). The pre-$ß_1$-HDLs receive cellular cholesterol by the ATP-binding cassette transporter A1 (ABCA1) mediated cholesterol efflux (Lawn et al. 1999). Thereby, lipid-poor pre-$ß_1$-HDL transforms into discoid pre-$ß_2$-HDL complexes with a small amount of unesterified cholesterol. ApoA-I serves as a ligand for HDL in the binding to ABCA1 on the cell membrane.

LCAT esterifies the cholesterol on HDL, transforming the discoid particles into small, spherical HDL_3 particles (Rye et al. 1999). The apolipoproteins in HDL, including apoA-I, apoA-II, apoA-IV and apoC-I, activate the esterification by LCAT. LPL hydrolyzes circulating TGs in CMs and VLDL particles (Santamarina-Fojo and Dugi 1994). As a result, more phospholipids and apoA-I are transferred into pre-$ß_1$-HDLs. PLTP transfers the surface lipids from the post-lipolytic VLDL and CM particles to HDL (Tall 1995). CETP, in turn, transfers the cholesterol esters from HDL to VLDL and LDL. TG is transferred in reverse direction, resulting in TG-enrichment of HDL. TG-enriched-HDL is a preferred substrate for HL, which then hydrolyses TGs and phospholipids in HDL. The combined action of CETP and HL reduces the core of large HDL_2 particles converting these to smaller HDL_3 particles (Clay et al. 1992). The endothelial lipase hydrolyses HDL phospholipids generating free fatty acids, which are taken up by the endothelial cells (Jaye et al. 1999).

Eventually, by the action of CETP, HL and PLTP, lipid-free apoA-I, or pre-β1-HDL, is released. These particles may either serve again as acceptors for cellular lipids and phospholipids, or they may be re-incorporated into pre-existing HDL particles. Scavenger Receptor B1 (SR-B1), defined as the selective HDL receptor, regulates the selective uptake of HDL lipids to the liver and

steroidogenic tissues, namely the adrenal gland, the ovaries, and the testes (Krieger 1999). HDL-bound apolipoproteins, including apoE, apoA-I, apoA-II, and apoC-III, serve as ligands in the binding to the SR-B1 (Xu et al. 1997; Bultel-Brienne et al. 2002). The catabolism of the HDL protein components is less well understood than the removal of HDL-associated lipids. HDL particles are secreted into glomerular filtrate in the kidney, from where they are either excreted or reabsorbed. HDL particles, apolipoproteins, or other HDL constituents may be reabsorbed from the tubular lumen. The endocytic receptors, cubilin and megalin, mediate this reuptake in the proximal tubulus lumen (Barth and Argraves 2001). Cubilin deficiency has first been found in patients with selective vitamin B_{12} malabsorption and increased proteinuria. It serves as the receptor for intrinsic factor/vitamin B_{12} complex, and as an albumin-binding protein for normal proximal tubule reabsorption (Kozyraki et al. 1999; Birn et al. 2000). However, the importance of cubilin with respect to determining the rate of apoA-I removal or regulating the circulating levels of HDL is somewhat unclear.

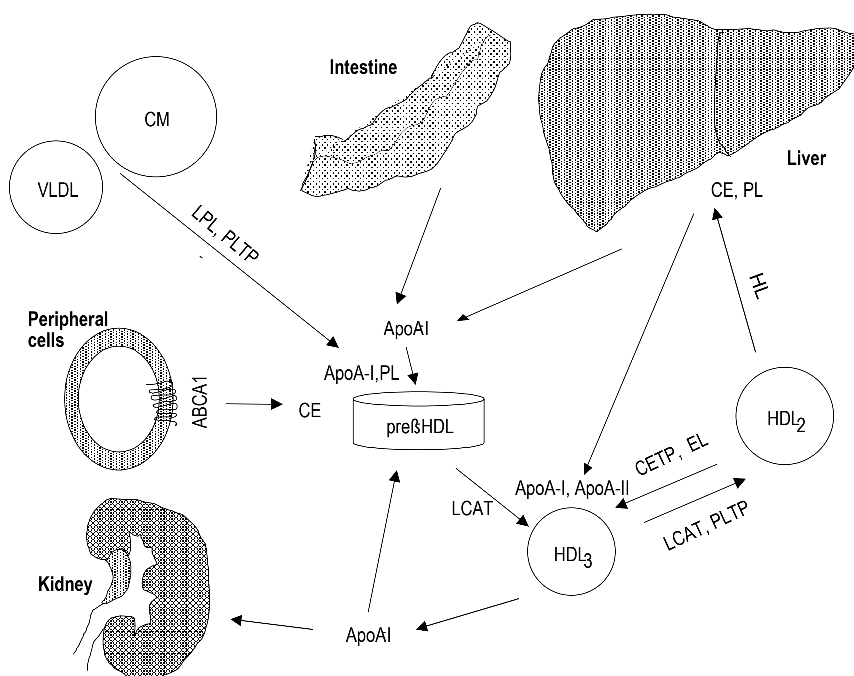


Figure 2. Simple scheme of the HDL metabolism. CM, chylomicron; LPL, lipoprotein lipase; PLTP, phospholipid transfer protein; ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II, PL, phospholipid; ABCA1, ATP-binding cassette transporter A1; HDL_2, high density lipoprotein 2; HDL_3, high density lipoprotein 3; LCAT, lecithin:cholesterol acyltransferase; HL, hepatic lipase; CETP, cholesterol ester transfer protein; CE, cholesterol ester; TG, triglyceride; VLDL, very low density lipoprotein

2.3 REGULATION OF HDL LEVELS

HDL levels are regulated by both environmental and genetic factors; approximately half of the variation is attributable to genetic factors (Breslow and Dammerman 1995; Almasy et al. 1999; De Oliveira e Silva et al. 1999). There exist certain genetic defects that cause structural changes in HDL particle, or prevent the secretion of either HDL apolipoproteins or the lipid-transfer enzymes involved in HDL formation (listed in Table 1). Such candidate genes have been linked to HDL levels in various study samples. However, these do not necessarily increase the risk for CHD. The genetic regulation of HDL levels is still largely unknown.

Table 1. Molecular defects leading to increased or decreased level of HDL

Protein	Syndrome	HDL	CHD	Locus	Site of synthesis	References
ABCA1	TD, Familial hypoalphalipoproteinemia	↓	↑	9q31	Liver, intestine, lung, kidney, tissues containing inflammatory cells	(Bodzioch et al. 1999; Brooks-Wilson et al. 1999; Rust et al. 1999; Lawn et al. 2001)
LCAT	LCAT deficiency, FED	↓	↔	16q22	Liver, epidermis, testes, central nervous system	(Smith et al. 1990; Funke et al. 1991a; Kuivenhoven et al. 1997)
ApoA-I	ApoA-I mutations (listed below)			11q13-qter	Liver, intestine	(Assmann et al. 1993)
	ApoA-$I_{Zavalla}$	↓	(↑)			(Miller et al. 1998)
	ApoA-$I_{Milano,}$ ApoA-I_{Paris}	↓	↓			(Franceschini et al. 1980; Perez-Mendez et al. 2000)
	ApoA-$I_{Helsinki}$	↓	↔			(Tilly-Kiesi et al. 1995)
	ApoA-$I_{Marburg}$	↓	↔			(Utermann et al. 1982)
	ApoA-I_{Fin}	↓	↔			(Miettinen et al. 1997)
	Familial hypoalphalipoproteinemia	↓	↑			(Ordovas et al. 1986; Yamakawa-Kobayashi et al. 1999)
	ApoA-I deficiency with corneal opacities	↓	↔			(Funke et al. 1991b)
	ApoA-I deficiency with periorbital xanthelasmas	↓	↔			(Romling et al. 1994)
	Familial hyperalphalipoproteinemia	↑	↓			(Jeenah et al. 1990; Rader et al. 1993b)
ApoA-I, C-III	ApoA-I/C-III deficiency	↓	↑			(Norum et al. 1982; Karathanasis et al. 1983; Forte et al. 1984)
ApoA-I, C-III, A-IV	ApoA-I/C-III/A-IV deficiency	↓	↑			(Schaefer et al. 1985; Ordovas et al. 1989)
ApoA-II	ApoA-$II_{Hiroshima}$	↔	↔	1q21-q23	Liver	(Deeb et al. 1990)
HL	Familial HL deficiency	↑	↑	15q21	Liver	(Breckenridge et al. 1982; Knudsen et al. 1997; Connelly and Hegele 1998)
LPL	Familial LPL deficiency*	↓	↔ ↑	8p22	Muscle, adipose tissue, macrophages	(Brunzell 1989; Reymer et al. 1995a)
PLTP	No described mutations in human	↓†	↓†	20q12-q13.1	Liver, endothelium	(Whitmore et al. 1995; Jiang et al. 2001)
CETP	CETP deficiency	↑	↑↓	16q21	Liver, small intestine, macrophages, spleen	(Brown et al. 1989; Zhong et al. 1996; Barzilai et al. 2003)
SR-B1	No described mutations in human	↑†	↑†	12q24.2-qter	Liver, adrenal, testes, ovary, macrophages	(Rigotti et al. 1997; Acton et al. 1999)
Cubilin	Megaloblastic anaemia	↓	↔	10p12-p14	Kidney, intestine, placenta	(Aminoff et al. 1999)

*) More than 50 different mutations have been described; †) In mice with a targeted null mutation in the gene

2.3.1 Monogenic HDL deficiency syndromes

2.3.1.1 ATP-binding cassette transporter A1 and Tangier disease

Tangier disease (TD) is a rare autosomal recessive disorder, characterized by enlarged yellow tonsils, hepatosplenomegaly, peripheral neuropathy, a virtual absence of HDL-C, apoA-I and apoA-II from plasma, and frequently premature CHD (Assmann et al. 1995). It is caused by a homozygous mutation in the ABCA1 gene (*ABCA1)* [MIM 205400, 600046] located in chromosome 9q31 (Bodzioch et al. 1999; Brooks-Wilson et al. 1999; Rust et al. 1999). The cholesterol efflux from cells is blocked in TD patients, which results in cellular accumulation of cholesterol esters. This prevents the lipid transfer to apoA-I, which leads to a rapid clearance of apoA-I from the plasma, and an abnormally low level of circulating HDL.

In subjects heterozygous for a defective allele in the *ABCA1* gene, the cholesterol efflux is decreased to approximately one-half of those in normal individuals (Clee et al. 2000). The heterozygotes also have increased carotid artery intima-media thickness (IMT), and thus an increased risk for the development for early atherogenesis (van Dam et al. 2002). The identification of ABCA1 as the gatekeeper in the cellular cholesterol efflux has been considered as an important step in the HDL research. In the general population, however, the data on the association between the allelic variants in *ABCA1* and HDL-C level regulation is inconsistent. Some studies have reported associations between *ABCA1* and HDL level regulation (Brooks-Wilson et al. 1999; Wang et al. 2000), while in others no such association existed (Brousseau et al. 2001; Kakko et al. 2003). The substitution of a lysine for arginine at amino acid 219 of the ABCA1 protein (R219K), a common variant with a carrier frequency of 46% in Europeans, has been linked to increased levels of HDL, lower TGs and reduced progression of atherosclerosis (Clee et al. 2001). In transgenic mice, the upregulation of hepatic and macrophage *ABCA1* expression raises the levels of HDL-C and apoA-I, enhances the reverse cholesterol transport (Singaraja et al. 2001), and reduces diet-induced atherosclerosis (Joyce et al. 2002). Novel ATP-binding cassette transporters, which belong to the ABCG subfamily, have been recently identified, including ABCG1, ABCG5 and ABCG8; all these contribute to cholesterol-trafficking in macrophages, hepatocytes, and intestine (Schmitz et al. 2001).

2.3.1.2 Familial LCAT deficiency and Fish-Eye Disease

LCAT plays a central role in the maturation of the HDL particles, by esterifying the non-esterified cholesterol on HDLs (Rye et al. 1999). Gene coding for LCAT is located in chromosome 16q22 (Azoulay et al. 1987). Several LCAT mutations have been described, as reviewed by Kuivenhoven et al. (Kuivenhoven et al. 1997). In patients homotsygous for mutated LCAT, serum HDL is nearly absent whereas in heterozygotes, HDL-C is reduced 30% to 50%. However, premature CHD is uncommon in LCAT deficient patients. They have accumulation of lecithin, free cholesterol and nascent type of HDLs in the plasma, and a triad of diffuse corneal opacities, hemolytic anemia, and proteinuria (Norum and Gjone 1967). In the classic LCAT deficiency, cholesterol esterification in plasma is reduced, whereas in fish-eye disease (FED), there is a specific inability of LCAT to esterify the free cholesterol on HDL particles (Funke et al. 1991a). The FED patients have normal serum TC but raised TG, VLDL, strikingly high LDL, and a 90% reduction in the level of esterified cholesterol in HDL.

2.3.2 Mutations in apolipoprotein genes

2.3.2.1 ApoA-I

The apoA-I gene [(MIM 107680)] is located in chromosome 11, in ApoAI/CIII/AIV gene (*APOA1C3A4*) cluster (Law et al. 1984). Several mutations that affect apoA-I production have been described (Assmann et al. 1993; Romling et al. 1994; Tilly-Kiesi et al. 1995; Miettinen et al. 1997). Variants in the *APOA1C3A4* that affect the synthesis of either apoA-I (Miller et al. 1998), apoA-I and apoC-III (Norum et al. 1982; Karathanasis et al. 1983), or apoAI, apoC-III, and apoA-IV (Ordovas et al. 1989), have been associated with premature atherosclerosis. Certain variants appear to protect from CHD (Franceschini et al. 1980; von Eckardstein et al. 1989; Rader et al. 1993a). The apoA-I$_{Milano}$ and apoA-I$_{Paris}$ increase the antioxidant capacity of apoA-I particles (Bielicki and Oda 2002). Moreover, studies in mice and rabbits with experimental atherosclerosis have demonstrated that intravenous administration of reconstituted apoA-I$_{Milano}$/phospholipid complexes decreases atherosclerotic plaque lipid and macrophage content (Shah et al. 2001). Recently, Nissen et al. confirmed that an intravenous treatment with recombinant apoA-I$_{Milano}$/phospholipid complex (ETC-216, Esperion Therapeutics, Ann Arbor, Michigan) causes significant regression in the volume of coronary atheromas in patients with acute coronary syndromes (Nissen et al. 2003). Also such variants exist that cause increased levels of HDL-C, apoA-I, and LpA-I, potentially due to the selective upregulation of apoA-I production (Jeenah et al. 1990; Rader et al. 1993b).

2.3.2.2 ApoC-III, apoA-IV and apoA-V

The *in vivo* role of apoC-III, an apolipoprotein found on both apoB-containing lipoproteins and HDL, is poorly understood. Both apoC-III and apoC-I inhibit LPL activity (Brown and Baginsky 1972; Ginsberg et al. 1986). ApoC-III also inhibits the lipolysis of VLDLs and reduces the remnant lipoprotein clearance. In mice overexpressing human apoC-III, the tissue uptake of triglyceride-rich lipoproteins (TRLs) from the circulation is reduced, due to increased apoC-III and decreased apoE on VLDL particles (Aalto-Setälä et al. 1992). The specific physiological function of apoA-IV, a minor protein component of HDL, has also remained unclear (Aalto-Setälä et al. 1994). It is synthesized mainly in the intestine, and associates with CM and HDL particles. Together with apoA-I, apoA-IV activates LCAT (Steinmetz and Utermann 1985). ApoA-IV is involved in intestinal cholesterol absorption and in the postprandial clearance of TRLs (Hockey et al. 2001). Furthermore, plasma level of apoA-IV appears to correlate inversely with the presence of CAD (Kronenberg et al. 2000). ApoA-V, a recently characterized apolipoprotein, was first found to be expressed in liver in studies with rats (van der Vliet et al. 2001). ApoA-V is suggested to have a role in the metabolism of TRLs, and single nucleotide polymorphisms (SNPs) across the *APOAV* locus have been linked to FCHL and TG level regulation in separate study samples (Pennacchio et al. 2002; Ribalta et al. 2002; Talmud et al. 2002; Mar et al. 2004). The locus for the apoA-V gene (*APOA5)* locates near to the *APOA1C3A4*, which in fact, could be termed as the *APOA1C3A4A5* cluster (Pennacchio et al. 2001). PPAR-α increases the hepatic expression of apoA-V (Prieur et al. 2003).

2.3.2.3 ApoA-II

ApoA-II is the second most abundant protein component of HDL, having several potential roles in lipid metabolism. ApoA-II stabilizes the structure and function of apoA-I and modulates the reaction of HDL with LCAT (Durbin and Jonas 1997). ApoA-II expression in the liver is upregulated by nuclear transcription factors, including PPAR-α, sterol regulatory binding protein (SREBP), and retinoid-X-receptor (RXR) (Tailleux et al. 2002). The apoA-II locus [APOA2 (MIM *107670)] is mapped to chromosome 1q21-q23 (Middleton-Price et al. 1988). The first familial apoA-II deficiency trait was discovered in Hiroshima, Japan, and designated apoA-II$_{Hiroshima}$ (Deeb et al. 1990). However, the carriers of this variation do not have either dyslipidemia or increased risk for CHD. So far, no significant association has been reported between common apoA-II variants

and CHD. *In vitro*, human apoA-II reduces the PLTP-mediated HDL interconversion (Pussinen et al. 1997).

Studies in transgenic mice overexpressing apoA-II have not resulted in a clear conclusion on the atherogenic properties of apoA-II. Mice transgenic for both human apoA-I and apoA-II are more susceptible for atherogenesis than mice transgenic for human apoA-I only (Schultz et al. 1993). Moreover, mice that overexpress mouse apoA-II exhibit increased atherosclerotic lesion development as compared to normal mice, despite elevated HDL-C concentrations (Warden et al. 1993). ApoA-II inhibits the LPL and HL mediated hydrolysis of VLDL-triglycerides, and thereby affects the clearance of TRLs (Boisfer et al. 1999). Sauvaget et. al (Sauvaget et al. 2004) recently showed that glucose induces the *APOA2* expression in mice, and that the mice overexpressing human apoA-II are glucose intolerant. Moreover, they suggested that increased apoA-II causes hypertriglyceridemia, low HDL, and contributes to the development of type II diabetes.

2.3.3 Effect of lipolytic enzymes and lipid transfer proteins on the regulation of HDL levels

2.3.3.1 Hepatic lipase (HL)

HL is an endothelial enzyme, found in the luminal surface of endothelium of hepatic sinusoids and in capillaries lining the tissues that synthesize steroid hormones (Kuusi et al. 1979). HL can hydrolyze TG and phospholipid in all lipoprotein classes, though the most preferable substrates are TG-rich HDL particles (Clay et al. 1991). The HL-mediated hydrolysis of HDL increases in hypertriglyceridemia, and in fact, data from *in vitro* and animal studies suggest that HL activity in hypertriglyceridemic states leads to increased catabolic rate of HDL (Rashid et al. 2003). HL is also involved in the SR-B1 mediated selective uptake of HDL cholesteryl esters in liver and in steroidogenic tissues. It serves as a cofactor for the interaction between HDL and SR-B1, and remodels HDL by hydrolyzing its phopholipids (Ji et al. 1997c; Lambert et al. 1999). In addition, HL acts as a ligand in the binding of LDL to the LDL receptor (Choi et al. 1998).

HL gene resides in chromosome 15q21 (Sparkes et al. 1987b). Some data exist supporting the role of HL locus in the regulation of HDL levels (Guerra et al. 1997; Almasy et al. 1999). Familial HL deficiency is a rare, single point mutation disorder, characterized by increased HDL-C, enlarged, TG-rich HDL particles (Knudsen et al. 1997) and frequently, premature CHD (Connelly and Hegele 1998). The HL activity level is regulated by hormonal, environmental and genetic factors. Exercise training decreases HL activity, whereas chronic alcohol abuse enhances the hepatic expression of HL (Kuusi et al. 1982; Taskinen et al. 1982). Androgen steroids markedly increase, and estrogens decrease HL activity (Ehnholm et al. 1975; Tikkanen et al. 1982). Consequently, estrogens have a moderate HDL_2-increasing effect.

2.3.3.2 Lipoprotein lipase (LPL)

LPL is located on the luminal surface of the capillary endothelium in several tissues, being abundant in skeletal muscle, myocardium and adipose tissue. The primary function of LPL is to catalyze the hydrolysis of TGs and phospholipids within CM and VLDL, converting these into remnant particles (Santamarina-Fojo and Dugi 1994). The resulting phospholipid and apolipoprotein remnants are transferred to HDL. Apo-CII serves as a cofactor in the LPL-mediated hydrolysis. LPL enhances the clearance of CM and CM surface remnants by the liver, and promotes cellular catabolism of VLDL via LRP (Mann et al. 1995). Macrophages are the primary source of LPL in the subendothelial space of the capillary vessel wall. Indeed, LPL may exert proatherogenic effects when produced by macrophages: It facilitates the macrophage lipoprotein uptake, and increases lipoprotein retention within the subendothelial space (Lindqvist et al. 1983; Rumsey et al. 1992; Saxena et al. 1992).

Increased LPL activity reflects increased lipolysis and availability of surface compounds for HDL formation; therefore, it decreases TGs and increases HDL levels (Nikkila et al. 1982). Reduced LPL activity results in an atherogenic plasma lipid profile, i.e. elevated levels of TC and TGs, and low HDL. Thus, the LPL gene has been extensively studied as a potential candidate gene responsible for familial dyslipidemic syndromes, namely FCHL or low HDL (Babirak et al. 1992; Reymer et al. 1995a; Reymer et al. 1995b; Hoffer et al. 1996; Fisher et al. 1997). The LPL gene is located on chromosome 8p22 (Sparkes et al. 1987b). Patients homozygous for a mutation in the LPL gene typically have fasting chylomicronemia, elevated TGs and decreased HDL. Over 50 mutations within the LPL gene have been identified (Santamarina-Fojo 1992). Reymer et al (Reymer et al. 1995a) first identified an LPL variant A291S in patients with CHD and low HDL. This relatively common variant has a carrier frequency of approximately 4% in the European population. The heterozygosity for A291S predisposes to lower LPL activity, and preponderance for small, dense LDL particles, hyperinsulinemia and hypertriglyceridemia. In addition, carriers of the A291S variant have prolonged postprandial lipaemia (Mero et al. 1999). In contrast, no linkage evidence has been found between the LPL locus and the low HDL or FCHL traits in Finnish families (Pajukanta et al. 1997; Lilja et al. 2002).

2.3.3.3 Phospholipid transfer protein (PLTP)

PLTP is a plasma glycoprotein that transfers surface phospholipids and unesterified cholesterol from the post-lipolytic VLDL and chylomicron particles into HDL and other plasma lipoproteins (Jauhiainen et al. 1993). Thereby, PLTP contributes to the fusion of HDLs into large HDL_2 particles and small preβ-HDL–precursors. The TG-enrichment of HDL enhances this particle conversion (Settasatian et al. 2001). Interestingly, the majority of PLTP in the circulation is inactive, while the metabolic role of the inactive form remains to be established (Karkkainen et al. 2002).

The gene encoding PLTP is located on chromosome 20q12-q13.1 (Whitmore et al. 1995). Genetic PLTP deficiency has not been described in humans. Studies in mice and in humans have provided evidence for the contribution of the chromosome 20q locus to body adiposity, fasting insulin levels and to the development of type 2 diabetes (Lembertas et al. 1997; Zouali et al. 1997; Frayling et al. 1999; Ghosh et al. 2000; Mehrabian et al. 2000). In mice, the farnesoid X-activated receptor (FXR) and PPAR-α are involved in the regulation of the transcriptional activity of the PLTP gene (Tu and Albers 2001). Mice treated with fenofibrate, a fibric acid derivative, have an increased activity level of PLTP as well as an increased HDL particle size through a PPAR-α-dependent increase in PLTP expression (Bouly et al. 2001). PLTP may also be involved in the intracellular lipidation of apoB containing lipoproteins. A recent study in PLTP deficient mice showed that the absence of PLTP causes decreased hepatic secretion of apoB-containing lipoproteins and decreased atherosclerosis (Jiang et al. 2001). Consequently, high PLTP activity, observed in hyperinsulinemia or obesity, might enhance hepatic production of apoB-containing lipoproteins.

2.3.3.4 Cholesterol ester transfer protein (CETP)

CETP is a hydrophobic plasma glycoprotein that redistributes cholesteryl esters and TG between HDL and apoB-containing lipoproteins (Tall 1995). It is most abundantly expressed in the liver, spleen and adipose tissue (Jiang et al. 1991). CETP plays a major role in the catabolism of HDL, as it transfers HDL cholesteryl esters to TG-rich lipoproteins. As a result, the lipid content in the HDL core is reduced, and large HDL_2-particles transform into smaller HDL_3-particles. The resulting HDL particles are TG–enriched, which in turn, are preferred substrates for HL. CETP also exchanges cholesteryl esters between LDL and VLDL. CETP activity levels are increased in type 2 diabetes, FCHL, hypercholesterolemia, and combined hyperlipidemia, all these conditions characterized by low HDL and high TGs (Tato et al. 1995; Riemens et al. 1998). A high-fat diet

enhances the CETP activity, whereas the activity decreases during fasting or physical exercise (Son and Zilversmit 1986; Seip et al. 1993).

The gene coding for CETP [OMIM118470] is located in chromosome 16q21 (Sparkes et al. 1987a). Most reports on the mutations resulting in CETP deficiency are based on findings in Japanese population (Brown et al. 1989), while some mutations have been identified in Caucasian populations (Miettinen et al. 1998; Teh et al. 1998). In CETP deficiency, the catabolism of apoA-I and ApoA-II is delayed, whereas the production rate remains unaffected (Ikewaki et al. 1993). Therefore, patients with a hereditary CETP deficiency have elevated levels of serum HDL-C and apoA-I, and retention of cholesteryl esters within the HDL pool (Brown et al. 1989; Inazu et al. 1990). They also have large, TG-poor HDL particles that are enriched with apoE (Yamashita et al. 1990). Serum TG, total cholesterol, and apoB levels are slightly elevated, while serum apoA-I, apoC-III and apoE levels are markedly increased.

The functional polymorphisms in *CETP* locus and in *CETP* promoter region have been extensively studied as potential candidates for the HDL regulation. The common polymorphisms Taq1B in intron 1 and isoleucine 405 to valine (I405V) have been associated with decreased CETP activity, increased HDL-C and increased apoA-I concentration (Gudnason et al. 1999). In the Veterans Affairs HDL-C Intervention Trial (VA-HIT) and in the Framingham Offspring Study, the rare B2 allele in TaqIB associated with higher HDL-C levels and decreased risk for CHD (Ordovas et al. 2000; Brousseau et al. 2002). In Finnish subjects, the I405V polymorphism was significantly associated with HDL-C levels, explaining about 8% of the HDL-C variability in women (Kakko et al. 2001). In men, the I405V and arginine 451 to glutamine (R451Q) polymorphisms associated significantly with carotid IMT, explaining 9.7% of the variability. Promoter polymorphism C to A -629 relative to the first transcribed nucleotide in the CETP gene (CETP/-629), and the I405V and R451Q genotypes explained about 7% of the variability in carotid IMT in women (Kakko et al. 2001). Alcohol consumption and smoking influence the effects of the CETP polymorphisms on the HDL level regulation (Hannuksela et al. 1994; Fumeron et al. 1995; Corbex et al. 2000; Kakko et al. 2000).

A recent interesting finding was that the VV allelic variant in the I405V polymorphism associates with exceptional longevity (Barzilai et al. 2003). The VV genotype appeared to be linked with significantly larger HDL and LDL particle sizes, lower serum CETP concentrations, and lower prevalence of hypertension and cardiovascular disease. In turn, Agerholm-Larsen showed that alanine 373 for proline substitution (A373P) and R451Q polymorphisms in *CETP* predispose to a decreased HDL-C concentration, but paradoxically, decreased risk of CHD in women not receiving hormone replacement therapy (HRT) (Agerholm-Larsen et al. 2000b). However, an opposite trend was found in men and in women using HRT. In some studies, the variants causing low CETP activity have shown association with increased CHD risk (Zhong et al. 1996; Agerholm-Larsen et al. 2000a). In Danish women, both the VV and IV variants in the I405V increased the risk of ischemic heart disease, despite the elevated levels of HDL-C (Agerholm-Larsen et al. 2000a).

2.3.4 Low HDL-C as a complex disorder – previous linkage findings

Several loci for the low HDL trait have been reported in previous genome-wide scans and candidate gene studies. Allelic variants in the *ABCA1* locus in chromosome 9 have been linked to the common low HDL trait in French-Canadian study samples and in subjects from other origin (Brooks-Wilson et al. 1999; Wang et al. 2000). Kort et al. (Kort et al. 2000) found evidence for linkage for low HDL on chromosome 11q23.3, approximately 10 cM distal to the *APOA1C3A4* cluster. Almasy et al. (Almasy et al. 1999) identified two suggestive loci for HDL level on chromosomes 8 and 15, in a population-based sample of Mexican-Americans. They applied the pedigree-based quantitative-trait

linkage (QTL) analysis, with the intention to avoid the dichotomization of a quantitative phenotype, the level of HDL-C. A linkage evidence for HDL-C and TG on chromosome 8p was found in a sample of 172 twin pairs with no lipid abnormalities (Knoblauch et al. 1997). In the genome-wide scan in families from the Framingham Heart Study, suggestive evidence for linkage for TG/HDL–ratio was obtained on chromosome 7 (Shearman et al. 2000). The NHLBI Family Heart Study, a multicenter study incorporating altogether 4 population-based cohorts from the Framingham Heart Study, Utah Health Family Tree Study, and two centers from the Atherosclerosis Risk in Communities (ARIC) Study reported a QTL for HDL-C on chromosome 5p, and a suggestive linkage evidence for chromosome 13 (Peacock et al. 2001). In the Hypertension Genetic Epidemiology Network study, a locus on chromosome 5 produced suggestive evidence for linkage for HDL-C in variance component analysis (Coon et al. 2001). In a subsequent study also utilizing variance components method in Mexican American families, a QTL locus for HDL-C was found on chromosome 9p (Arya et al. 2002). Recently, chromosome 16q exhibited evidence for linkage for HDL-C in Mexican Americans from the San Antonio Family Heart Study (Mahaney et al. 2003). This locus was placed within a 15 cM region, near the structural loci for the LCAT and CETP genes. In turn, a locus on chromosome 6 has been linked to elevated HDL-C levels in a family with familial hypercholesterolemia (FH) (Canizales-Quinteros et al. 2003).

2.3.5 Environmental factors regulating HDL levels

Several life-style effects contribute to the HDL level regulation (listed in Table 2.). In general, smokers have lower HDL level than non-smokers and the negative association between cigarette smoking and HDL is dose-dependent (Craig et al. 1989). Some medications affect the levels of HDL, including beta-adrenergic blockers, androgenic steroids, and androgenic progestins. Hypertriglyceridemia of any cause is associated with low HDL (Despres et al. 2000). Obesity, total adiposity and hyperglycemia have a lowering effect on HDL, decreasing especially the amount of the larger HDL_2 particles (Williams et al. 1995). HDL increases about 0.03mmol/l (1 mg/dl) for every 3 kg of weight loss in both men and women, provided that the weight reduction is maintained (Dattilo and Kris-Etherton 1992). On the other hand, very-low-fat diets, for example, a vegetarian diet, may also decrease HDL (Knopp et al. 1997). Exogenous estrogen therapy has an HDL-raising effect (Sacks et al. 1995).

Table 2. Factors affecting HDL-C levels

HDL- lowering effect	HDL-raising effect
Obesity	Weight reduction
Smoking	Exercise
Hypertriglyceridemia	Alcohol consumption
Beta-blocker therapy	Estrogen therapy
Androgenic sex hormones	Statins
Chronic and acute infections	Fibrates
	Niacin
	Cholestyramine
	Probucol
	CETP inhibitors

2.3.5.1 Effects of alcohol consumption on HDL

Moderate alcohol consumption protects from atheroclerotic diseases. In a meta-analysis of 42 studies, Rimm et al. (Rimm et al. 1999) concluded that an alcohol intake of 30 g (2.5 servings) per day reduces the CHD risk by 24.7%, through changes in lipids, lipoproteins and fibrinogen. Up to 50% of this protective action was explained by the increase in HDL level. In fact, alcohol may increase the plasma levels of HDL-C even 18% (De Oliveira et al. 2000). The beneficial effect is not dependent on the type of the beverage (wine, beer, or spirit) (van der Gaag et al. 2001). Alcohol increases the production rates of both apoA-I and apoA-II, without affecting their fractional catabolic rate (De Oliveira et al. 2000). Alcohol consumption also decreases the activities of HL and CETP, and increases LPL activity (Taskinen et al. 1985; Savolainen et al. 1990; Hannuksela et al. 1992). It induces the RCT by increasing the cellular cholesterol efflux and cholesterol esterification (van der Gaag et al. 2001). Interestingly, Fumeron et al. showed that alcohol consumption contributes significantly to the effect of CETP/TaqIB polymorphism on HDL-C and CHD risk (Fumeron et al. 1995). They found that the B2 allele associates with increased HDL-C level and decreased CHD risk only in subjects drinking over 25 g/d of alcohol per day. Still, the epidemiological evidence proposes a J- or U-shaped relationship between alcohol consumption and CHD mortality (Agarwal 2002).

2.3.5.2 Effects of exercise on HDL

Physical activity induces beneficial changes in the lipoprotein levels. A positive dose-response effect exists between the kilometers (km) run per week, and an increase in HDL-C. A study in healthy, middle-aged men showed that those who ran 11.3 to 22.6 km per week at moderate intensity achieved the best changes in their lipoproteins (Kokkinos et al. 1995). However, it has also been shown that a threshold of 11.3 to 12.9 km per week should be exceeded in order to attain any signicant increase in HDL-C. Interestingly, recent studies have suggested that genetic regulation, namely CETP and HL genotypes contribute to the effect of exercise on HDL level regulation (Wilund et al. 2002; Halverstadt et al. 2003).

2.3.5.3 Effects of lipid-lowering drugs on HDL

The lowering of LDL-C is considered the primary goal in the prevention of CHD. In the Adult Treatment Panel III (ATP III) guidelines, the LDL-C threshold for the start of drug therapy varied from 3,3 mmol/l to 4.1 mmol/l (130 to 160 mg/dl), depending on the presence of other CHD risk factors ((NCEP) 2001). HMG-CoA inhibitors (statins) and fibric acid derivatives (fibrates) are widely used hypolipidemic drugs in the primary and secondary prevention of CHD. Both of these have a moderate HDL raising effect. In the Helsinki Heart Study and in the Veterans Affairs HDL Intervention Trial (VA-HIT), the HDL-C elevation with gemfibrozil treatment was significantly associated with a reduced number of the CHD events (Manninen et al. 1992; Rubins et al. 1999). However, no such associations have been observed in any of the large-scale secondary or primary prevention trials with statins. Generally, the conclusions on the beneficial HDL-increasing effects by statins are obtained from results of *post hoc* analyses of large intervention trials.

2.3.5.3.1 Statins

The primary target of statins is to decrease LDL-C by inhibiting the HMG-CoA reductase, the rate-limiting enzyme in cellular cholesterol synthesis. Statins also increase the expression of LDL receptors and inhibit the entry of LDL into the circulation (Arad et al. 1992). Their effects on HDL and TGs are moderate. Statins increase HDL-C levels by approximately 4% to 10% (Gotto 2002). Data from *in vitro* studies indicate that certain statins influence both the production and the catabolism of apoA-I, by PPAR-α-mediated up-regulation of *APOA1* gene transcription (Martin et al. 2001). Statins increase the catabolism of HDLs by the up-regulation of SR-B1, which results in

increased selective uptake of HDL lipids (Tsuruoka et al. 2002). Statins also reduce the CETP-mediated cholesteryl ester transfer from HDL to apoB-containing lipoproteins, which results in increased HDL-C (Guerin et al. 2000).

2.3.5.3.2 Fibric acid derivatives (fibrates)

Fibrates are hypolipidemic drugs that primarily lower TG levels and also raise HDL-C. Fibrates stimulate the fatty-acid oxidation in muscle and liver. In the liver, the oxidative metabolism of fatty acids increases, the synthesis of TGs decreases, and the secretion of VLDL decreases (Staels et al. 1998). In the muscle, fibrates increase the LPL activity and enhance the uptake of fatty acids. On the molecular level, fibrates act through the activation of the PPAR-α (Fruchart et al. 2001). PPARs are nuclear hormone receptors, consisting of three distinct classes termed alpha (α), delta (δ) and gamma (γ), each encoded by a separate gene (Schoonjans et al. 1996). Activated PPAR-α effects the transcription of several genes involved in lipid and energy metabolism, including the induction of apoA-I, apoA-II, SR-B1, LPL, ABCA1, and apoA-V expression, and down-regulation of the apoC-III expression (Staels and Auwerx 1998; Chinetti et al. 2001; Prieur et al. 2003). The PPAR-α activation by fibrates enhances the lipolysis of TRLs and increases the production surface remnants for the formation of HDL particles. Consequenly, TGs decrease and HDL-C rises.

2.3.5.3.3 Cholestyramine, nicotinic acid (niacin) and probucol

Cholestyramine (Shepherd et al. 1979a) and niacin (Shepherd et al. 1979b) are hypolipidemic drugs that raise HDL levels. Cholestyramine stimulates the synthesis of apoA-I whereas niacin increases the synthesis of both apoA-I and apoA-II. The effect of cholestyramine was examined in the Lipid Research Clinics Coronary Primary Prevention Trial, in which the cholestyramine treatment reduced the CHD risk, with a subsequent small increase in HDL-C (1984).

The use of niacin at traditional doses is often limited by side effects. However, the combination therapy with statin and low-dose niacin increases HDL-C levels effectively (Wink et al. 2002). Probucol, an antioxidative and hypolipidemic agent, has been postulated to increase reverse cholesterol transport by enhancing CETP activity, while it causes a marked decrease in HDL-C (McPherson et al. 1991). Though probucol has been shown to reduce the rate of coronary artery restenosis after balloon angioplasty in humans (Tardif et al. 1997), its use in clinical practice is not common.

2.3.5.3.4 CETP inhibitors

The potentially anti-atherogenic effect of reduced CETP activity has raised interest towards developing CETP inhibitors as therapeutic agents to the treatment of hyperlipidemia and low HDL. In rabbits, CETP suppression reduces the levels of LDL and VLDL, increases HDL-C, and increases the expression of LDL receptor (Sugano et al. 1998). Moreover, the CETP suppression decreases the aortic atherosclerotic lesions in experimental rabit models (Okamoto et al. 2000). De Grooth et al. (de Grooth et al. 2002) showed that a 4 week treatment with the CETP inhibitor JJT-705 resulted in significantly elevated levels of HDL-C (34% increase), reduced CETP activity, and minor changes in LDL levels in humans. Similar results were obtained in healthy subjects, in a recent phase-1 multidose study with CETP inhibitor torcetrapib (Clark et al. 2004). However, in some studies the patients with a low CETP activity due to polymorphisms or mutations within the CETP gene have had an increased susceptibility for atheosclerotic disease (Agerholm-Larsen et al. 2000a). Though the function of CETP can be thought to be atherogenic due to its ability to decrease plasma levels of HDL-C, it may also have an antiatherogenic role in the reverse cholesterol transport system. Therefore, more information is needed on the efficacy of chemical CETP inhibitors in humans.

2.4. CARDIOPROTECTIVE EFFECTS OF HDL

2.4.1 Epidemiological studies

Low HDL was recognized as one of the main CHD risk factors already in the early 1950's (Nikkila 1953). To date, numerous epidemiological and interventional studies have confirmed the inverse relationship between HDL levels and CHD event rates (Assmann et al. 1996; Goldbourt et al. 1997; Despres et al. 2000). The estimated reduction of CHD risk by each 0.026 mmol/l (1 mg/dl) increase in HDL-C is 2% in men and 3% in women (Gordon et al. 1989). The ATP III has defined the risk threshold value of low HDL-C level as <1.04 mmol/l (40 mg/dl)((NCEP) 2001). Low HDL-C is an important prognostic risk factor for a new coronary event also in patients with a previously diagnosed CHD (Bolibar et al. 2000). The prognostic value of different HDL subpopulations or apolipoproteins has been investigated in some prospective studies. In "etude PRospective de l'Infarctus du MyocardE" (PRIME) Study, apoA-I was found to be a better CHD risk indicator than total HDL-C (Luc et al. 2002), whereas in the ARIC Study and in the Physicians Health Study, no such differences existed (Stampfer et al. 1991; Sharrett et al. 2001).

2.4.2 Reverse cholesterol transport

The main anti-atherogenic function of HDL is traditionally thought to be the ability to promote the efflux of free cholesterol from cells and to transport it to the liver, a process termed reverse cholesterol transport (RCT) (Figure 3) (Stein and Stein 1999). The ABCA1 plays a key role in the RCT, by mediating the efflux of unesterified cholesterol and phospholipids from the cells into HDL particles (Lawn et al. 1999). Phospholipid is the primary substrate for ABCA1, and the apoA-I-phospholipid complex, the pre-$ß_1$-HDL particle, is the most effective cholesterol acceptor (Fielding et al. 2000). The esterification of cholesterol by LCAT increases the net efflux of cellular cholesterol to HDL by preventing the re-diffusion of cholesterol back to the cells (Czarnecka and Yokoyama 1995). The cholesterol esters on HDL are eventually transferred to the liver or steroidogenic cells via SR-BI, or to apoB-containing lipoproteins by CETP. The HDL-derived cholesterol in apoB-containing particles can be internalized to the liver by the LDL receptor (Brown and Goldstein 1986). The selective cholesterol uptake by SR-B1 is enhanced by LCAT (Temel et al. 2003). In contrast to the action in hepatocytes or steroidogenic cells, where SR-B1 mediates the lipid uptake, the SR-B1 expression on macrophages facilitates the cholesterol efflux from the cells into HDL (Ji et al. 1997b). Thus, SR-B1 has a dual enhancing effect on the RCT system. PLTP transfers the surface phospholipids and unesterified cholesterol from apoB-containing lipoproteins to HDL. It also mediates the fusion of HDL particles, thus re-generating both pre-$ß_1$-HDL and HDL_2 particles (Jauhiainen et al. 1993).

Pertubation of the RCT impairs body cholesterol homeostasis; it leads to the deposition of cholesterol in the arterial wall, which initiates the development of arteriosclerosis. Therefore, the modulation of HDL metabolism and the RCT system might be important targets for anti-atherosclerotic drug therapy. The ABCA1 expression is regulated by transcription factors, including the sterol regulatory binding protein (SREBP) and the liver-X-receptor alpha / retinoid-X-receptor alpha (LXRα/RXRα) (Repa et al. 2000; Santamarina-Fojo et al. 2001). The SREBP and LXRα/RXRα are up regulated by cellular cholesterol level, oxysterols, and retinoids, which therefore have a positive effect on the ABCA1 expression and the lipid efflux (Costet et al. 2000). PPAR-α induces the *ABCA1*-promoter transcription by enhancing the expression of LXRα (Chinetti et al. 2001). The excess of free fatty acids, in turn, increases the degradation of ABCA1 (Wang and Oram 2002). Inflammatory cytokines, including interferon-γ and lipopolysaccharides, suppress the ABCA1 expression (Baranova et al. 2002; Wang et al. 2002).

HDL can also accept cellular cholesterol from macrophages by aqueous diffusion and microsolubilization (Rothblat et al. 1999). These slow processes are not dependent on cellular proteins. LCAT-mediated cholesterol esterification enhances the passive diffusional efflux of unesterified cholesterol from cellular plasma membrane to HDL. During the microsolubilization, phopsholipids on the cellular plasma membrane associate with lipid-free apolipoproteins, which sequester them to the extracellular compartment together with some cholesterol efflux. Nonetheless, passive diffusion and microsolubilization are rather slow and inefficient ways to remove the excess cholesterol from macrophages.

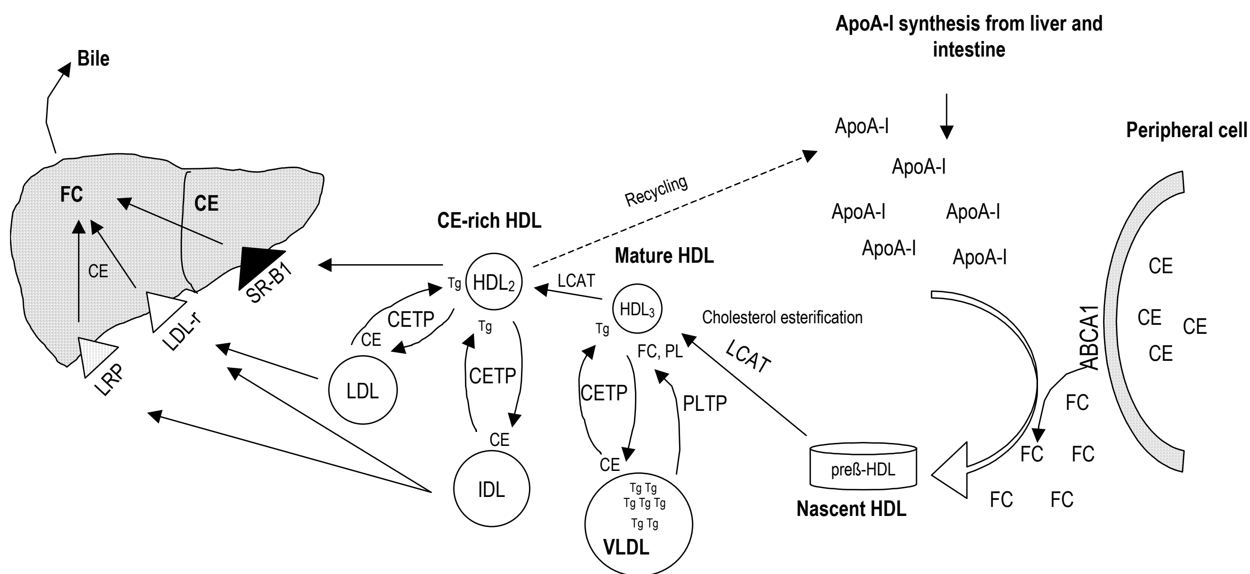


Figure 3. Pathways involved in the reverse cholesterol transport. CE, cholesterol ester; FC, free cholesterol; SR-B1, scavenger receptor B1; HDL_2, high density lipoprotein 2; HDL_3, high density lipoprotein 3; ApoA-I, apolipoprotein A-I; PLTP, phospholipid transfer protein; PL, phospholipid; TG, triglyceride; VLDL, very low density lipoprotein; ApoB, apolipoprotein B; LCAT, lecithin:cholesterol acyltransferase; ABCA1, ATP-binding cassette transporter A1; CETP cholesterol ester transfer protein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LDL-r, low density lipoprotein receptor; LRP, low density lipoprotein receptor related protein

2.4.3 Pleiotropic effects of HDL

2.4.3.1 HDL-bound Paraoxonase-1

The beneficial effects by which HDL protects against arteriosclerosis and thrombosis extend beyond reverse cholesterol transport (Table 3). HDL protects the arterial wall against oxidative damage by preventing the oxidation of LDL. Paraoxonase 1 (PON1) is an HDL-bound esterase enzyme that is responsible for the prevention of LDL oxidation (Mackness et al. 2002). The PON content of HDL is considered as the main determinant of its anti-inflammatory capacity. It blocks the accumulation of lipid peroxides in LDL, and it prevents the activation of monocytes by oxidised LDL (Watson et al. 1995; Mackness et al. 2000). An increased serum PON1 activity reduces the peroxide and aldehyde formation in HDLs and reduces the susceptibility of HDL to oxidation. *In vitro* studies have shown that this anti-oxidant activity does not differ between particles containing either apoAI or apoAII (Garner et al. 1998). PON1 knock-out mice express increased oxidation of HDLs and LDLs (Shih et al. 1998). These mice are also more susceptible to the development of atherosclerosis than wild-type mice. The PON1 gene locus is mapped to chromosome 7q21.3 (Humbert et al. 1993). It contains several polymorphisms that modify the serum PON activity and mass concentration (Leviev and James 2000). The association between the common PON1 polymorphisms and the risk of CHD has been proven in a number of cohorts (Serrato and Marian 1995; Sanghera et al. 1997; James et al. 2000). However, contradictory findings have been obtained in some study samples (Herrmann et al. 1996; Rice et al. 1997). Neither the arginine-to-glutamine variant at the position 192 (Q192R) or the leucine-to-methionine variant at the position 55 (L55M) showed association with CHD risk in Finnish or German individuals (Antikainen et al. 1996; Gardemann et al. 2000).

Table 3. Anti-atherogenic actions of HDL

Reverse cholesterol transport
Anti-oxidant activity
Inhibition of cellular adhesion molecule expression
Enhanced endothelium-dependent vasodilatation
Pro-fibrinolytic activity
Anti-thrombotic activity

2.4.3.2 Platelet activating factor (PAF) and Platelet activating factor acetylhydrolase (PAF-AH)

Platelet activating factor (PAF) is a pro-inflammatory phospholipid that activates platelets and inflammatory cells. It has multiple effects including increased vascular permeability, hypotension, and leukocyte adhesion to endothelial cells (Venable et al. 1993; Zimmerman et al. 1996). Endothelial cells produce PAF in response to inflammation and anaphylaxis. HDL has a concentration-dependent inhibitory effect on the endothelial production of PAF (Sugatani et al. 1996). PAF-acetylhydrolase (PAF-AH), also termed as lipoprotein-associated phospholipase A_2, is responsible for the PAF level regulation (Tjoelker and Stafforini 2000). PAF-AH is secreted mainly by macrophages, and in the circulation, on average 70% of this protein binds to LDL, and the remainder to HDL (Tselepis et al. 1995). Plasma levels of PAF-AH are increased in CHD patients (Caslake et al. 2000). In the West of Scotland Coronary Prevention Study and in the ARIC Study, the level of PAF-AH had a strong positive association with the risk of coronary events (Packard et al. 2000; Ballantyne et al. 2004).

The HDL-bound LCAT has anti-oxidant properties, as it inactivates PAF by hydrolyzing it into lyso-PAF (Liu and Subbaiah 1994). LCAT also catalyzes the hydrolysis of oxidized phospholipids. In LCAT deficient mice, the activities of PON1 and PAF-AH are markedly decreased (Forte et al. 1999).

2.4.3.3 Inhibition of leukocyte adhesion molecule expression by HDL

The earliest changes in atherogenesis include the adhesion of mononuclear leukocytes to the endothelium, mediated predominantly by leukocyte cellular adhesion molecules (CAMs) including vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1), and E-selectin (Ross 1999). VCAM-1, ICAM-1 and E-selectin originate from endothelial cells, vascular smooth muscle cells and macrophages (O'Brien et al. 1996). Selectins mediate the initiation of the rolling of leukocytes on the surface of endothelial cells at sites of inflammation or endothelial injury, prior to the firm adhesion and transmigration (Price and Loscalzo 1999). VCAM-1 and ICAM-1 mediate the adhesion of monocytes and lymphocytes to the endothelium. The CAM expression is induced by inflammatory cytokines such as tumor necrosis factor -alpha (TNF-α) or interleukin-1 (IL-1) (Blankenberg et al. 2003). These up-regulate the CAM expression in various conditions related to increased CHD risk, such as smoking, hypertension, hypercholesterolemia, hypertriglyceridemia and insulin resistance (DeSouza et al. 1997; Abe et al. 1998; Mazzone et al. 2001). Injured endothelial surface releases CAMs to the circulation, and the serum concentrations of these soluble forms are considered as markers of their endothelial expression. In hypertensive patients, shear stress force is suggested to increase the endothelial CAM expression. In hypertriglyceridemia, the expression of inflammatory cytokines increases due to the elevated circulating free fatty acids and the decreased endothelial nitric oxide activity (Steinberg et al. 1997; Bauersachs et al. 1999).

HDL particles inhibit the cytokine-induced expression of CAMs in cultured endothelial cells in a concentration dependent manner (Cockerill et al. 2001). Thereby, HDL prevents the recruitment of circulating leukocytes to sites of atherogenesis. The inhibitory effect of HDL is dependent on the phospholipid composition of HDL particles (Baker et al. 2000). HDL inhibits the TNF-α -stimulated sphingosine kinase activity in endothelial cells, which causes the inhibition of CAM expression (Xia et al. 1998; Xia et al. 1999). Furthermore, HDL-associated lysosphingolipids, including sphingosyl-phosphoryl-choline and lysosulfatide, reduce the TNF-α-induced CAM expression (Nofer et al. 2003). Interestingly, fibric acids may have a direct inhibitory effect on the expression of VCAM-1 and ICAM-1 in endothelial cells, through the activation of PPAR-α (Pasceri et al. 2000).

2.4.3.4 HDL’s effect on the endothelial vasodilatiation and coagulation

HDL enhances the vascular endothelium-dependent vasodilatation by increasing the availability of the nitric oxide (NO). HDL decreases the degradation of the endothelial nitric oxide synthase (eNOS) protein in cultured endothelial cells (Rämet et al. 2003). Recently, Nofer at al. (Nofer et al. 2004) showed that HDL-associated lysophospholipids also activate eNOS both in vitro and in isolated mouse and rat aortae. ApoA-I in HDL inhibits the procoagulant activity of human erythrocytes (Epand et al. 1994). HDLs may also prevent the accumulation of platelet thrombi at sites of vascular damage by stimulating prostacyclin synthesis by endothelial cells (Yui et al. 1988).

2.5 CLINICAL CONDITIONS RELATED TO LOW HDL

2.5.1 Familial combined hyperlipidemia (FCHL)

FCHL is a genetic disorder with a population prevalence of 0.3 to 2.0% (Goldstein et al. 1973; Grundy et al. 1987). It is the most frequent familial dyslipidemia among the survivors of myocardial infarction below the age of 60 (Nikkilä and Aro 1973). In fact, subjects affected with FCHL have a 10-fold higher risk for premature CHD (Goldstein et al. 1973; Genest et al. 1992). The FCHL patients typically have elevated fasting TGs, low HDL and elevated apoB.

The FCHL diagnosis is based on the presence of multiple types of hyperlipidemia in affected family members: elevated serum TC levels (Fredrickson's lipid phenotype IIa), elevated TG levels (type IV), or both (type IIb), and a positive family history of premature CHD (Goldstein et al. 1973; Nikkilä and Aro 1973; Grundy et al. 1987). The definition of the lipid phenotypes IIa, IV and IIb for the FCHL diagnosis is based on age- and sex-specific cut-off points (the 90th or 95th percentiles) for TC and TG levels. The metabolic abnormalities in FCHL include high production and slow clearance of apoB-containing lipoproteins (de Graaf and Stalenhoef 1998). In addition, FCHL patients have prolonged postprandial lipemia, due to impaired CMR clearance (Castro Cabezas et al. 1994). Consequently, the residence time for triglyceride rich lipoproteins (TRLs) in the circulation is increased. The FCHL patients have small and dense LDL particles, especially the individuals with lipid phenotypes IIb and IV (Vakkilainen et al. 2002). Moreover, FCHL patients are frequently insulin resistant (Bredie et al. 1997; Pihlajamäki et al. 2000). They also have disturbed postprandial metabolism of non-esterified free fatty acids (NEFA), so that the uptake of NEFA by fibroblasts and adipocytes is impaired, and the NEFA flux to the liver is enhanced (Sniderman et al. 1998; Meijssen et al. 2000).

The molecular basis for the FCHL trait was recently clarified; Pajukanta et al. showed an association between FCHL and the upstream transcription factor 1 (*USF1*) gene on chromosome 1q21 in 60 Finnish FCHL families (Pajukanta et al. 2004). This region was previously linked to FCHL in this family sample (Pajukanta et al. 1998) and in FCHL families from other origin (Coon et al. 2000; Pei et al. 2000; Allayee et al. 2002). In fact, *USF1* regulates the transcriptional activation of several genes involved in the glucose and lipid metabolism (Ribeiro et al. 1999; Portois et al. 2002; Yang et al. 2002).

2.5.2 Low HDL trait as a component of the metabolic syndrome, obesity and type 2 diabetes

The most frequent causes of low HDL-C concentration are the metabolic syndrome, abdominal obesity and type 2 diabetes. These patients share a unique lipid profile characterized by elevated levels of TG and apoB, small, dense LDL particles, and low HDL-C, particularly HDL_2-C. The coincidence of low HDL with many additional risk factors greatly increases the risk for coronary events in these patients. The metabolic abnormalities observed in type 2 diabetes and in the metabolic syndrome become even more intense as the ratio of visceral-to-subcutaneous fat increases (Imbeault et al. 1999).

2.5.2.1 Type 2 diabetes

Type 2 diabetic patients have an excess risk of CHD (Laakso 2001). The same lipid abnormalities are present in individuals with the metabolic syndrome and type 2 diabetes, but the dyslipidemic phenotype tends to be more severe in the patients with type 2 diabetes (Taskinen 2003). HDL-C,

specifically HDL_2-C level is low in the majority of these patients. Patients with type I diabetes, in turn, usually have normal or slightly elevated levels of HDL and apoA-I (Kahri et al. 1993).

2.5.2.2 The metabolic syndrome

The metabolic syndrome comprises a cluster of CHD risk factors: hypertension, hypertriglyceridemia, small, dense LDL particles, low HDL-C, elevated plasma glucose, insulin resistance and visceral fat accumulation (Grundy 1999). Recently, the Adult treatment Panel (ATP) III stated criteria for the clinical diagnosis of the metabolic syndrome as follows: abdominal obesity (waist circumference >102 cm in men and >88cm in women), TG >1.7mmol/l in both genders, HDL-C <1.03 mmol/l in men and <1.29 mmol/l in women, blood pressure (BP) ≥130/85 mmHg and fasting glucose ≥6.1 mmol/l in both genders ((NCEP) 2001). Subjects with 3 or more of these risk factors have the metabolic syndrome. If these guidelines are applied, the prevalence of the metabolic syndrome in US is over 20% (Park et al. 2003). The corresponding Finnish prevalence was 8.8% in a population sample of middle-aged men (Lakka et al. 2002). However, the prevalence has a relatively wide range variation, depending on the study sample and the definition criteria used, being up to 35.3% in Finnish hypertensive men (Rantala et al. 1999).

Patients with the metabolic syndrome, type 2 diabetes, or abdominal obesity are insulin resistant and hypertriglyceridemic. The lowering of HDL-C is largely a consequence of the elevated TGs, both in the fasting state and postprandially (Rashid et al. 2003). The mass transfer of TGs from TRLs to HDL particles is enhanced, with simultaneous exchange of cholesteryl esters from HDL, via the action of CETP (Tall 1995). TG-rich HDL particles are rapidly hydrolyzed by HL, transforming HDL into smaller, more dense HDL_3-particles with a subsequent release of lipid-poor apoA-I (Rashid et al. 2003). Data from *in vitro* and clinical studies show that the TG-enrichment of HDL enhances the fractional catabolic rate of apoA-I (Clay et al. 1991; Lamarche et al. 1999; Rashid et al. 2002). The increased catabolic rate of apoA-I in insulin resistant individuals has been observed in a kinetic study utilizing stable isotopes (Pont et al. 2002).

2.5.3 Effects of inflammation on HDL

Epidemiological studies have established a link between infection, inflammation and atherosclerosis (Pearson et al. 2003). Measurement of the inflammatory biomarkers can add to the predictive value achieved by the assessment of plasma lipid levels alone (Ridker 2001; Ridker et al. 2003). A similar systemic acute phase response takes place in trauma, myocardial infarction, inflammation, and in acute and chronic infections. The hepatic production of various acute phase reactants increases radically, including C-reactive protein (CRP), secretory phospholipase A_2 ($sPLA_2$), serum amyloid A (SAA), and ceruloplasmin (Van Lenten et al. 2001). Inflammation affects HDL-C levels. The apolipoprotein composition and anti-oxidant properties of HDL may change during inflammation in such a way that HDL may lose its protective effects (Ivandic et al. 1999; Rye and Duong 2000; Tietge et al. 2000).

As a result of the acute phase response, serum amyloid A (SAA) is released to the circulation where it associates with HDL, with a subsequent displacement of apoA-I from the HDL (Malle et al. 1993; Van Lenten et al. 1995). The SAA-enriched HDL may have reduced capacity to promote reverse cholesterol transport or to prevent LDL from oxidation. The SAA-enriched HDL has reduced activities of LCAT, PON1 and PAF-AH (Van Lenten et al. 2001). Ceruloplasmin, a copper-containing acute phase reactant, is also a constituent of acute phase HDL (Gutteridge and Stocks 1981; Van Lenten et al. 2001). Ceruloplasmin participates in the oxidation of LDL by monocytic cells (Ehrenwald and Fox 1996). The enrichment of HDL with ceruloplasmin may diminish the ability of HDL to inhibit LDL modification, and in fact, it may convert HDL into a pro-inflammatory particle.

Group IIA secretory phospholipase A_2 ($sPLA_2$) is an acute-phase protein, expressed together with SAA. Plasma levels of $sPLA_2$ are increased in systemic inflammatory conditions, including rheumatoid arthiritis, sepsis and cardiovascular disease (Hurt-Camejo et al. 2001). Increased $sPLA_2$ activity within the arterial wall and in plasma may be involved in atherogenesis (Kovanen and Pentikainen 2000; Kovanen and Pentikainen 2003). The over-expression of this protein leads to the accumulation of toxic oxygenated fatty acids in lipoproteins (Pruzanski et al. 1998). $sPLA_2$ hydrolyses the phospholipids in LDL and HDL particles. The $sPLA_2$-modified LDL particles are more susceptible to enzymatic modifications. Furthermore, $sPLA_2$ may induce the accumulation of intracellular lipids in macrophages and cause the retention of LDL particles within the arterial wall (Hakala et al. 2001). *In vitro*, the $sPLA_2$ -induced phospholipid depletion of HDLs decreases the HDL particle size and decreases their remodeling by CETP (Rye and Duong 2000). The over-expression of $sPLA_2$ in transgenic mice results in an increased catabolic rate of apoA-I, a reduced amount of larger HDL particles, and a simultaneous increase in the small HDL particles (Tietge et al. 2000; Tietge et al. 2002). Thus, increased $sPLA_2$ activity during inflammation affects HDL levels.

As a response to the stimulation by cytokines, the cholesterol-loaded macrophages and smooth muscle cells (SMCs) produce matrix metalloproteinases (MMPs) (Galis et al. 1994). MMPs are a family of enzymes that degrade extracellular matrix in atherosclerotic plaques. Besides acting in the processes concerning matrix turnover and repair, the MMPs can induce the degradation of apoA-I in HDL particles (Lindstedt et al. 1999). The active transport of cholesterol from cells requires a structurally intact cholesterol acceptor. Therefore, this action of MMPs on apoA-I may dramatically decrease the cholesterol efflux from cells and impair the HDL function in the RCT process.

3. AIMS OF THE STUDY

A growing interest has focused on HDL as a primary and logic target to prevent CHD. The main objective of this study was to understand the regulation of serum HDL levels. Our rationale focused on the characterization of the low HDL phenotype in Finnish families with low HDL and premature CHD.

The present study aimed to answer the following questions:

1. What are the predisposing loci for low HDL-C in Finnish families with low HDL and premature CHD, and does the *ABCA1* gene contribute to the HDL level regulation in Finnish low HDL families?

2. Are there specific changes in the HDL subclass distribution in subjects affected by familial low HDL, as compared to unaffected family members or normolipidemic control subjects?

3. Which parameters of HDL metabolism best predict the thickening of the arterial wall in the asymptomatic members of low HDL families?

4. Are there precise metabolic alterations responsible for the decreased HDL level in FCHL patients, and what are the roles of HL, LPL, CETP, or PLTP in the HDL level regulation?

5. Does the familial low HDL associate with increased endothelial activation and with increased levels of inflammatory parameters, such as CRP, sICAM-1, sVCAM-1 and sE-selectin?

4 SUBJECTS

4.1 Family collection

The participants for the studies presented in this thesis were selected from multigenerational, carefully characterized low HDL and FCHL pedigrees. The families were collected in Helsinki and Turku University Central Hospitals in Finland, as a part of the European Multicenter Study on Familial Dyslipidemias in Patients with Premature Coronary Heart Disease (EUFAM) (Porkka et al. 1997). The EUFAM study aimed at resolving the genetic and metabolic defects underlying familial dyslipidemias predisposing to early CHD. The family collection consisted of three phases. In the first phase, the probands for the low HDL and FCHL families were selected from patients undergoing elective coronary angiography in Helsinki and Turku University Central Hospitals, and from a registry of patients with confirmed myocardial infarction. To become selected, the proband was required to have at least three accessible first-degree relatives. In the second phase, the probands and all first-degree relatives of the probands were studied to identify low HDL and FCHL families. The third phase comprised extensive metabolic and genetic studies, and all accessible relatives and spouses were contacted and examined. The data collection, laboratory measurements and phenotype determination for both the low HDL and FCHL study samples were performed in the same study center. Also 112 normolipidemic control subjects were studied, including spouses and healthy volunteers with no lipid abnormalities, CHD, diabetes mellitus or lipid lowering medication.

Each study subject gave a written informed consent prior to participating in the study. All samples were collected in accordance with the Helsinki declaration and the ethics committees of the participating centers approved the design of the studies presented in this thesis.

4.2 Low HDL families

Inclusion criteria for the low HDL probands were as follows:

1) Age of 30-60 years
2) HDL-C level below the 10th age-sex specific Finnish population percentile (≤0.9 mmol/l in men and ≤1.1 mmol/l in women)
3) CHD verified by either coronary angiography (>50% stenosis in one or more coronary arteries) or myocardial infarction (MI). The diagnostic criteria for MI were the following: 1. Typical clinical symptoms; 2. Definite electrocardiography findings, according to the Minnesota coding (World Health Organization criteria) (Rose et al. 1982); and 3. Elevated levels of the creatine kinase (CK) enzyme and its cardiac isoenzyme, CK-MB.
4) Additional lipid criteria: 1. Total cholesterol (TC) <6.3 mmol/l in men and <6.0 mmol/l in women, 2. triglycerides (TG) <2.3 mmol/l in both genders.

Exclusion criteria for the probands were type I or II diabetes, significant hepatic or renal diseases, untreated hypothyroidism, familial hypercholesterolemia (FH) or body mass index (BMI) >30 kg/m^2. The family members were coded as affected if their HDL-C levels were below the 10th Finnish age-sex specific population percentile. Families with at least two affected subjects were included in the third phase.

4.3 Familial combined hyperlipidemia families

Low levels of HDL-C characterize the FCHL lipid profile, and the clinical phenotypes of the affected subjects in both FCHL and low HDL widely overlap. Therefore, we extended our studies on the genetic and metabolic regulation of HDL-C levels to the FCHL family material.

Inclusion criteria for the FCHL probands were as follows:

1) Age 30-60 years
2) CHD
3) Serum TC ≥ 90th age and gender-specific Finnish population percentile, (Fredrickson´s lipid phenotype IIA), or TG ≥ 90th percentile (Fredrikson´s lipid phenotype IV) or both TC and TG ≥ 90th percentile (combined lipid phenotype IIB).

Exclusion criteria for the probands were type 1 diabetes mellitus, hepatic or renal diseases, untreated hypothyroidism and FH. The lymphocyte culture method was used in each proband to exclude FH (Cuthbert et al. 1986). Families with at least two affected (serum TC and/or TG ≥ 90th percentile) family members were included in the third phase of the study. Families presenting only the lipid phenotype IIA were excluded. As an example, in the age group of 50 to 54 years, the 90th percentile cut-off level for TC was 7.5 mmol/l in men and 7.1 mmol/l in women. For TG, the corresponding cut-off levels were 3.5 mmol/l and 2.1 mmol/l, respectively.

Altogether 25 low HDL and 29 FCHL families were included in Study I. In Study II, 27 low HDL families were included, and in in Study III, 49 FCHL families were included. Study IV included 43 low HDL families and 112 normolidemic control subjects.

4.4 Population percentiles

The lipid cut-off points used to define the phenotypes in low HDL and FCHL families were Finnish age-sex specific lipid percentiles. For study subjects older than 25 years, percentile criteria were based on the population survey FINRISK (Vartiainen et al. 1994; Vartiainen et al. 2000) For subjects younger than 25 years, the corresponding percentiles were obtained from the Cardiovascular Risk Factors in Young Finns study (Porkka et al. 1994).

5. METHODS

5.1 Lipid, lipoprotein, and other analytical methods

For each participant over 5 years of age, venous blood was drawn for lipid phenotype determination and DNA extraction after a 12 hours overnight fast. The laboratory measurements were performed at the Helsinki University Hospital, Division of Cardiology, and at the National Public Health Institute. Serum TC and TG were determined with an automated Cobas Mira analyser (Hoffman-La Roche, Basel, Switzerland) by enzymatic methods (Hoffman-La Roche kits 0722138 and 0715166 respectively). LDL was calculated using the Friedewald formula in studies I and II, and measured by ultracentrifugation in studies III and IV (Friedewald et al. 1972). In studies I and II, HDL-C was quantified by phosphotungastic acid/magnesium chloride precipitation procedures (Hoffman-La Roche kit 0720674), and in studies III and IV, HDL was quantified by sequential flotation by ultracentrifugation (Taskinen et al. 1988). HDL_2-C and HDL_3–C were separated by sequential flotation in an ultracentrifuge (Taskinen et al. 1988). Concentrations of apoA-I, apoA-II and apoB were measured by immunoturbidometric methods with commercial kits (Boehringer-Mannheim, Mannheim, Germany) and LpA-I particles by differential electroimmunoassay (Sebia, Issy-les-Moulienaux, France) (Parra et al. 1990). The concentration of LpAI-AII -particles was calculated by subtracting the concentration of LpA-I from the total concentration of apoA-I in serum. Plasma glucose concentrations were analyzed by the glucose dehydrogenase method (Precision-G Blood Glucose Testing System, Medisense, Abbott, Illinois, USA). Serum free insulin concentrations were measured by radioimmunoassay (Phadeseph INSULIN RIA, Pharmacia & Upjohn, Uppsala, Sweden) and by fluoroimmunoassay (Wallac AutoDELFIA Insulin, Wallac Oy, Turku, Finland). The homeostasis model assessment for insulin resistance (HOMA IR) was calculated from the fasting plasma glucose and serum insulin concentrations as follows: fasting insulin (µU/ml) x fasting glucose (mmol/l) / 22.5 (Matthews et al. 1985). Any lipid lowering medication was discontinued 4 weeks prior to the blood sampling.

Postheparin lipoprotein HL and LPL activities were measured after an intravenous bolus of heparin (100 IU/kg body weight) that released LPL and HL into the circulation. Blood was drawn 15 minutes after the bolus into heparinized tubes that were kept on ice. LPL activity was measured using an immunochemical assay after inhibition of HL by a specific antiserum, and HL was measured in a medium containing 1.0 M NaCl but no additional serum (Huttunen et al. 1975). CETP activity was determined using a substrate-independent isotope assay that measured radiolabeled CE transfer from exogenous LDL to exogenous HDL, as described by Groener et al (Groener et al. 1986). PLTP activity was measured using a rapid transfer assay, based on the selective precipitation of liposomes by heparin and $MnCl_2$ (Damen et al. 1982). LDL peak particle sizes were determined using native gradient gel electrophoresis on linear 1 mm thick 2% to 10% gradient gels (Vakkilainen et al. 2002). Two isolated LDL samples, isolated by ultracentrifugation, were used as a size reference on each gel. The particle sizes of the two standard samples were measured by electron microscopy (Forte and Nordhausen 1986). The major peak diameter of LDL particles (LDL size) was determined by comparing the mobility of the sample to the mobility of the reference LDL preparations run on each gel. In addition, a control LDL sample was run on each gel. The levels of serum sICAM-1, sVCAM-1, and sE-selectin 1 levels were determined by using commercially available monoclonal antibody-based ELISAs (R&D Systems, Minneapolis, USA). Ultra-sensitive CRP was determined by a commercial kit by Medix Biochemica, Kauniainen, Finland.

Subjects completed standard questionnaires to provide data on previous medical history, medication, smoking and alcohol consumption. Body weight and height were measured and BMI calculated as weight/height^2 (kg/m^2). Waist and hip circumference and waist/hip (w/h)-ratio were recorded. Blood pressure was measured in the supine position and hypertension was defined as

systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure (DBP) ≥90 mmHg, or self-reported use of antihypertensive medications. The metabolic syndrome score was calculated for each study subject in Studies III and IV, using the following determinants: Abdominal obesity (waist circumference >102 cm in men and >88cm in women), TG >1.7mmol/l in both genders, HDL-C <1.03 mmol/l in men and <1.29 mmol/l in women, blood pressure ≥130/85 mmHg and fasting glucose ≥6.1 mmol/l in both genders ((NCEP) 2001).

5.2 B-mode ultrasonography and intima media thickness measurement

B-mode ultrasound imaging was used to measure the intima-media complex of carotid artery wall in a selected group of low HDL family members (Study II). The low HDL probands and other family members known to have clinical cardiovascular disease were not included in the Study II, because they would most likely have a high degree of atherosclerosis and thickened carotid artery walls. Also subjects who had lipid lowering treatment or diabetes were excluded. Ultrasound scans were performed with a Hewlett Packard Image Point M2410A ultrasound system (Hewlett Packard, Andover, MA) equipped with a 10 MHz linear array transducer. Scannings were videotaped with a Panasonic AG-MD830E PAL S-VHS VCR. Subjects were examined in the supine position. Longitudinal images from three angles of interrogation (anterolateral, lateral, and posterolateral) were displayed bilaterally for the common carotid artery (CCA) and the carotid bulb (CB). The carotid artery intima media thickness (IMT) was measured at 24 sites: 3 projections for far wall (FW, the carotid wall farthest from the probe) and near wall (NW, the carotid wall closest to the probe) of 4 arterial segments: Right and left CCA and CB (Figure 5).

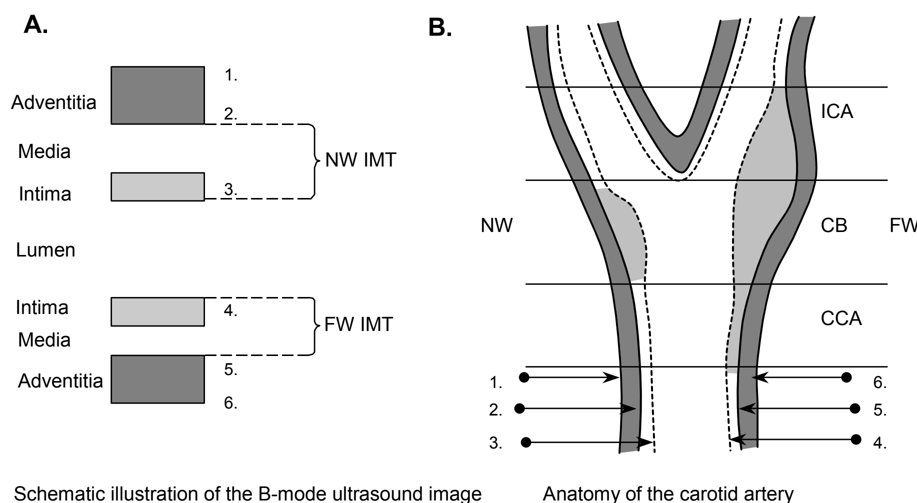


Figure 5. Images in the B-mode ultrasound measurement. Numbers in the schematic (A) and anatomic (B) illustrations indicate the tissue interfaces as follows: 1. periadventitia-adventitia, 2. adventitia-media, 3. intima-lumen, 4. lumen-intima, 5. media-adventitia, and 6. adventitia-periadventitia. The leading edges of the lumen-intima and the media-adventitia interfaces are visualized to quantify the intima-media thickness in the near and far walls of the carotid bulb and common carotid artery. NW; near wall, FW; far wall, ICA; internal carotid artery, CB; carotid bulb, CCA; common carotid artery, IMT; intima-media thickness. Modified from Kati Ylitalo, Doctoral Thesis 2001.

Two observers at the Research Institute of Public Health, Kuopio, Finland, made IMT measurements from the videotaped scannings. Computer analysis of the images was performed with a PC computer with a video frame grabber interfaced to S-VHS VCR. The Prosound software (Caltech, Pasadena, CA) with an automated boundary detection (Selzer et al. 1994) was used to measure the IMTs at a total of 24 sites corresponding to the 24 sites where the scanning was focused. This software digitizes the video ultrasound image, locates the interfaces, and computes

the IMTs. All measurements were taken in the diastole, measured in the phase when the lumen diameter is at its smallest and IMT at its largest. Three variables were derived from each measurement: the minimum, the mean, and the maximum IMT. For each subject the mean IMT was calculated as the average of all mean IMT measurements over 24 sites (or fewer, if the measurement could not be performed at all 24 sites). Likewise, the mean FW-IMT was calculated to be the average of the mean IMT of FWs of all the segments examined. The maximum IMT was calculated as the average of the thickest points recorded in all the segments studied. The maximum IMTs of CCA and CB were calculated as the average of the thickest points recorded in each segment. Mean IMT was selected as the primary outcome variable. To estimate the intra-sonographer variability in the scannings, the sonographer scanned 10 subjects (12.5% of all subjects in Study II) twice on two different occasions. The coefficient of variation between the mean IMTs of the paired scannings was 3.0%, and the absolute difference 0.027±0.022 mm (mean±SD). Intra-reader variability was assessed by reading the scannings of 10 study subjects twice. The coefficient of variation between the mean IMT of the paired scans was 1.0%, and the absolute difference 0.014 ± 0.018mm, respectively. The readers were blinded for the identities of the study subjects.

5.3 Genome-wide scan

A two-stage strategy was employed in the low HDL genome-wide scan (Figure 6). In stage 1, all chromosomes were analyzed with 358 microsatellite markers from a modified Weber's screening set 9.0 with an average intermarker spacing of 10 cM (Sheffield et al. 1995). A total of 176 individuals from 20 low HDL families were included and genotyped in stage 1. In stage 2, 11 chromosomal regions that resulted in pairwise lod scores >1.0 in the linkage analyses in stage 1, were covered with 67 additional markers. The significance of these loci was further tested by analyzing the identified loci in an independent study sample of 29 Finnish FCHL families. Data collection, laboratory measurements and phenotype determinations for the low HDL and FCHL families were performed in the same center. Therefore, the clinical and biochemical data in these two samples were fully compatible. Parametric linkage analyses using both dominant and recessive mode of inheritance, and nonparametric ASP analyses were carried out for all the markers.

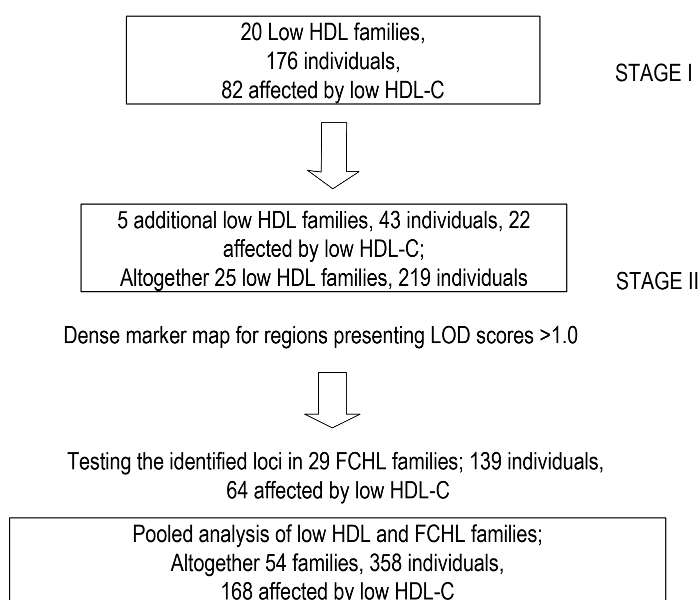


Figure 6. Flow chart of the study design in the two-stage genome wide scan.

5.3.1 Genotyping

Genotyping was performed at the Department of Human Genetics, University of California, Los Angeles, USA. DNA was isolated according to procedures described earlier (Vandenplas et al. 1984). Pipetting of the polymerase chain reactions (PCR) was performed using two robotic systems: Robbins Hydra Microdispenser, (Robbins Scientific, Sunnyvale, CA, USA), and a Packard MultiPROBE II (Packard Instrument Company, Meriden, CT, USA). The PCR amplification of the samples was performed in microtiter well plates using MJ Research Quadra thermal cyclers (MJ Research Incorporation, Waltham, MA, USA). The genotyping of the fluorescently labeled PCR products was performed using LI-COR DNA 4200 Genetic Analyzer. Raw gel data produced by the LI-COR machines was interpreted into genotypes using SAGA program version 5.1, a software package developed at the University of Washington and licensed to LI-COR. The checking for Mendelian errors was performed by the PedCheck program (Ott 1992; O'Connell and Weeks 1998).

5.3.2 Marker maps

Selected markers from the modified Weber's screening set 9.0 were genotyped in stage 1 of the genome scan. The average intermarker spacing was 10 cM. The markers that failed were replaced with additional microsatellite markers from the Human Genome Database (http://www.gdb.org/), the Cooperative Human Linkage Center map (http://lpg.nci.nih.gov/CHLC/), the Marshfield Medical Research Foundation Genome Database (www.marshfieldclinic.org/research/genetics/), and from the Genetic Location Database (http://cedar.genetics.soton.ac.uk/public_html/). The markers for denser maps in stage 2 were selected and mapped based on the maps of the Human Genome Database, Cooperative Human Linkage Center, the Marshfield Medical Research Foundation Genome Database, Genetic Location Database, and Genethon.

5.4 Sequence analysis of the ATP-binding cassette transporter –A1 gene

Forward and reverse PCR primers of all 49 exons of the *ABCA1* gene (GenBank accession number AAF98175) were used to sequence *ABCA1* in selected low HDL family members. Generally, primers were located within 50–70 basepairs of the splice junction. The primers were designed at Xenon Bioresearch Inc, NRC Innovation Centre, Vancouver, Canada as described in detail by Marcil et al (Marcil et al. 1999). Cycle sequencing was done on the ABI 373 with Big Dye terminators and appropriate exon-flanking primers. Sequences were assembled by means of the Sequencher program (Genecodes corporation).

5.5 Statistical methods

5.5.1 Statistical analyses of continuous and categorical variables

Statistical comparisons of clinical and biomedical parameters were performed with SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Data are expressed as mean± Standard Deviation (SD), as frequencies, or as percentages. Variables with a skewed distribution were $\log_{10}$ transformed before the analyses, but the values in the text, tables and figures of this thesis are presented as non-transformed. Continuous variables were compared between the groups by univariate analysis of variance (ANOVA). $P<0.050$ was considered significant (two-tailed). The frequency distribution of the categorical variables was compared between the groups by the χ^2-test. The correlation coefficients (r) for the associations between the biochemical and clinical characteristics were

examined either by Pearson's correlation for continuous variables, partial Pearson's correlation or the Spearman correlation analysis, when appropriate. In study III, stepwise regression analysis was performed on correlations and partial correlations. In study IV, Bonferroni posthoc test was used for multiple comparisons among the three groups. Because the studies were based on a family material, the study subjects did not fully accomplish the assumption of independence. In order to correct for the non-independence of the study subjects, a dummy variable indicating belonging to a certain family was created for each subject in studies II and III (Glantz and Slinker 1990). The dummy variables were included in the univariate analysis and multiple regression analysis as covariates, having always a value of 1, 0, or –1.

5.5.2 Linkage and haplotype analysis

The qualitative statistical methods used in the genome-wide scan included both parametric linkage and non-parametric linkage (NPL) analysis. The MLINK program of the linkage package (Lathrop et al. 1984) version FASTLINK 4.1P (Cottingham et al. 1993; Schaffer et al. 1994) was used to perform the parametric linkage analyses. We used the affecteds-only strategy, in which the subjects were coded either as affected or as unknown based on their HDL-C level below or above the 10th percentile level. The unaffected individuals were genotyped to increase phase information and were treated as if their phenotype were unknown in statistical analyses. The parametric linkage analyses were carried out with both a dominant and a recessive mode of inheritance. Gene frequencies of 0.4% and 8% were used, reflecting an estimated population prevalence of about 1%, respectively. In addition, linkage analyses were performed on the component nuclear families. For each marker, the allele frequencies were estimated from all individuals included in the analysis with the DOWNFREQ program (Terwilliger and Göring 2000).

The affected sib-pair (ASP) analysis was also applied in the linkage calculations. The ASP analysis is a non-parametric method requiring no knowledge of the mode of inheritance of the disease; therefore, it is less dependent on the estimated inheritance parameters. The ASP is based on the fact that two sibs share zero, one or two copies of any locus with a 25%-50%-25% distribution expected under a random Mendelian segregation. Excess allele sharing of disease associate locus can be monitored with a simple χ^2-test. If it is known from which parent the affected sibs inherited the shared allele, the allele is called identical by descent (IBD). If the alleles are only two copies of the same allele, they are called identical by state (IBS). The IBD status of affected sib-pairs was assessed with an allele sharing analysis on nuclear families with the SIBPAIR program of the ANALYZE package (Kuokkanen et al. 1996; Terwilliger and Göring 2000). The genetic heterogeneity between the families was examined using the HOMOG program (Ott 1986).

The two-point analyses were performed with the AUTOSCAN program, a helper program which enables the analysis of a whole genome-wide scan in a single computer analysis (www.helsinki.fi/~tsjuntun/autoscan/). For regions that showed suggestive evidence of linkage in the two-point analysis, multipoint analyses were performed with the Simwalk2 program, version 2.80 (www.genetics.ucla.edu/software/simwalk2.html) (Sobel and Lange 1996). The clustering analysis option and the parametric location score analysis option were selected for the multipoint analyses. Simwalk 2.80 was utilized to construct haplotypes spanning a 21.8 cM region over the *ABCA1* locus (Brooks-Wilson et al. 1999). The segregation of the haplotype with the low HDL affection status was monitored. Families that exposed potential co-segregation were subjected to the sequencing of the coding region of *ABCA1*.

5.5.3 Quantitative trait analysis by SOLAR program

We used a multipoint variance–components linkage method to test for linkage between marker loci and two quantitative traits, HDL-C and TG. The Sequential Oligogenic linkage Analysis Routines (SOLAR) package, version 1.6.7 was employed for the QTL analysis (Almasy and Blangero 1998). Environmental covariates thought to affect HDL-C and TG (sex, age and BMI), the family ascertainment (HDL or FCHL family), and the proband status were incorporated into the QTL analysis. Both a two-point analysis and a multipoint analysis were performed with the quantitative traits.

6 RESULTS

6.1 Identification of low HDL-C loci in the genome-wide scan (Study I)

6.1.1 Linkage results in a two-stage genome-wide scan

The clinical characteristics of the low HDL family sample in the genome-wide scan are presented in Table 4. The results of the two-point linkage analyses in stage 1 of the genome scan, using either the recessive mode of inheritance or the ASP analysis, are presented in Figure 7. In stage 1, fifteen chromosomal regions on chromosomes 1, 2, 3, 6, 7, 8, 9, 16, 17, 18, 11, 19, and 20 yielded a lod score >1.0 in the two-point linkage analyses. In addition, a region on chromosome 19 gave a lod score >1.0 in the ASP analysis. The highest lod score of stage 1, 2.9 with a recombination fraction (θ)=0.10, was observed on chromosome 3, using the dominant mode of inheritance, with marker D3S4545. Using either the dominant or recessive mode of inheritance, the markers on chromosomes 1, 2, 3, 8, 17 and 18 showed pairwise lod scores >2.0. On chromosomes 1 and 9, two separate regions produced lod scores >1.0. These two regions were located 72 cM and 10.7 cM apart from each other, respectively.

In stage 2, five additional low HDL families were included in the study. The regions showing two-point lod scores >1.0 in stage 1 were further studied by genotyping 67 additional markers. The fine mapping produced further support for linkage for chromosomes 2, 3, 8, 16 and 20. Chromosome 2p produced a lod score of 2.1 (θ=0.10). Chromosome 3p produced a lod score of 2.1 (θ=0.06). Locus on chromosome 8q23 resulted in lod score of 2.3 (θ=0.10). The 16q24.1-24.2 region produced a lod score of 2.2 (θ=0.08). Chromosome 20q13.11 yielded a lod score of 1.4 (θ=0.16). For the remaining eight chromosomal regions, no further support for linkage was obtained in stage 2.

Table 4. Clinical characteristics of the low HDL family members (Study I)

	Affected family members (HDL-C <10th percentile)	Unaffected family members
	n=104	n=115
Age (years)	49.3 ± 15.7	43.7 ± 17.2
Sex (% males)	47	50
BMI (kg/m^2)	26.8 ± 4.1	24.7 ± 4.1
HDL-C (mmol/l)	0.86 ± 0.16	1.37 ± 0.34
TG (mmol/l)	1.69 ± 0.81	1.22 ± 0.59
TC (mmol/l)	5.27 ± 0.97	5.46 ± 1.11
apoA-I (mg/dl)	120 ± 16	147 ± 25
apoA-II (mg/dl)	34 ± 6	38 ± 6
HDL_2-C (mmol/l)	0.43 ± 0.19	0.65 ± 0.29
HDL_3-C (mmol/l)	0.59 ± 0.11	0.73 ± 0.14

Values are expressed as mean ± SD.

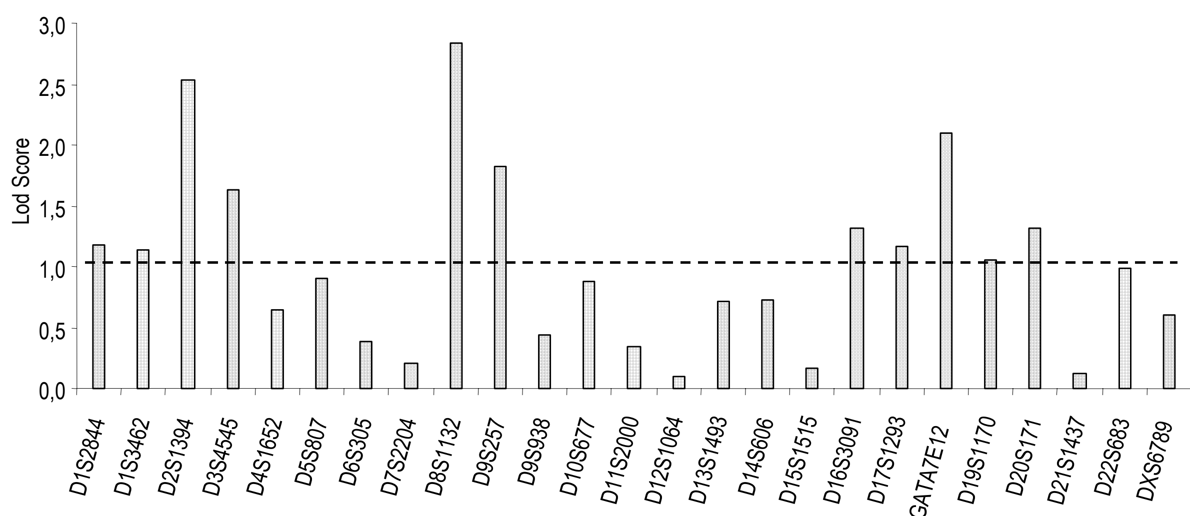


Figure 7. The highest two-point lod scores of the low HDL study sample for each chromosome in stage 1, using either a recessive mode of inheritance or ASP analysis. On chromosome 1 there was two separate regions producing lod scores >1.0, as indicated by the marker names.

6.1.2 Testing the identified loci in familial combined hyperlipidemia family sample

Low HDL-C is a consistent metabolic finding in FCHL, together with hypertriglyceridemia and increased levels of apoB. Consequently, we used the low HDL trait (HDL-C <10th age-sex specific Finnish population percentile) as the disease phenotype in an independent sample of 29 Finnish FCHL families, and tested the significance of the suggestive low HDL loci identified in the stage 1. Characteristics of the 29 FCHL families are shown in Table 5.

Table 5. Clinical characteristics of the FCHL family sample (Study I)

	Affected family members (HDL-C <10th percentile)	Unaffected family members
	n= 64	n = 75
Age (years)	49.0 ± 14.1	45.0 ± 16.8
Sex (% males)	48	39
BMI (kg/m2)	28.3 ± 4.1	25.5 ± 3.6
HDL-C (mmol/l)	0.94 ± 0.25	1.57 ± 0.42
TG (mmol/l)	3.24 ± 2.52	2.02 ± 1.24
TC (mmol/l)	6.78 ± 1.32	6.85 ± 1.27
apoA-I (mg/dl)	125 ± 21	163 ± 25
apoA-II (mg/dl)	38 ± 9	43 ± 7
HDL_2-C (mmol/l)	0.54 ± 0.30	0.79 ± 0.29
HDL_3-C (mmol/l)	0.60 ± 0.11	0.67 ± 0.16

Data are presented as mean ± S.D.

In a pooled data analysis of low HDL and FCHL families, we found evidence for linkage on three loci, and in FCHL families separately, on one locus. The most significant linkage results of the pooled data on chromosomes 8, 16, and 20 are given in Tables 6, 7, and 8.

Table 6. Two-point and multipoint chromosome 8q23 linkage evidence (Study I)

Marker	Position cM[a]	Pairwise Lod Score[b]	ASP[c]	Simwalk Statistics A[d]
GAAT1A4*	0.0	0.0 (0.50) / 0.3 (0.26)	0.2 / 0.5	1.3
D8S1132*	8.9	2.3 (0.10) / 4.7 (0.06)	1.4 / 3.1	3.3
D8S592*	14.9	0.1 (0.32) / 1.0 (0.18)	0.2 / 0.9	2.0

Table 7. Two-point and multipoint chromosome 16q24.1-24.2 linkage evidence (Study I)

Marker	Position cM[a]	Pairwise Lod Score[b]	ASP[c]	Simwalk Statistics C[d]
D16S3040	0.0	0.1 (0.32)	0.1	1.1
D16S505*	4.5	1.5 (0.12) / 1.3 (0.18)	1.8 / 1.5	1.2
D16S3091*	6.7	1.9 (0.08) / 2.2 (0.12)	1.8 / 1.9	1.2
D16S402	9.1	0.2 (0.34)	0.6	0.9
D16S3061	17.0	0.0 (0.50)	0.0	0.5

Table 8. Two-point and multipoint chromosome 20q13.11 linkage evidence (Study I)

Marker	Position cM[a]	Pairwise Lod Score[b]	ASP[c]	Simwalk Statistics A[d]
D20S120	0.0	0.8 (0.20)	0.9	1.2
D20S102	3.5	0.4 (0.20)	0.4	1.1
D20S171*	8.7	1.3 (0.16) / 1.9 (0.14)	1.3 / 1.4	1.7
D20S94	11.0	0.4 (0.26)	0.3	1.4
D20S173	11.1	0.6 (0.20)	0.2	1.4

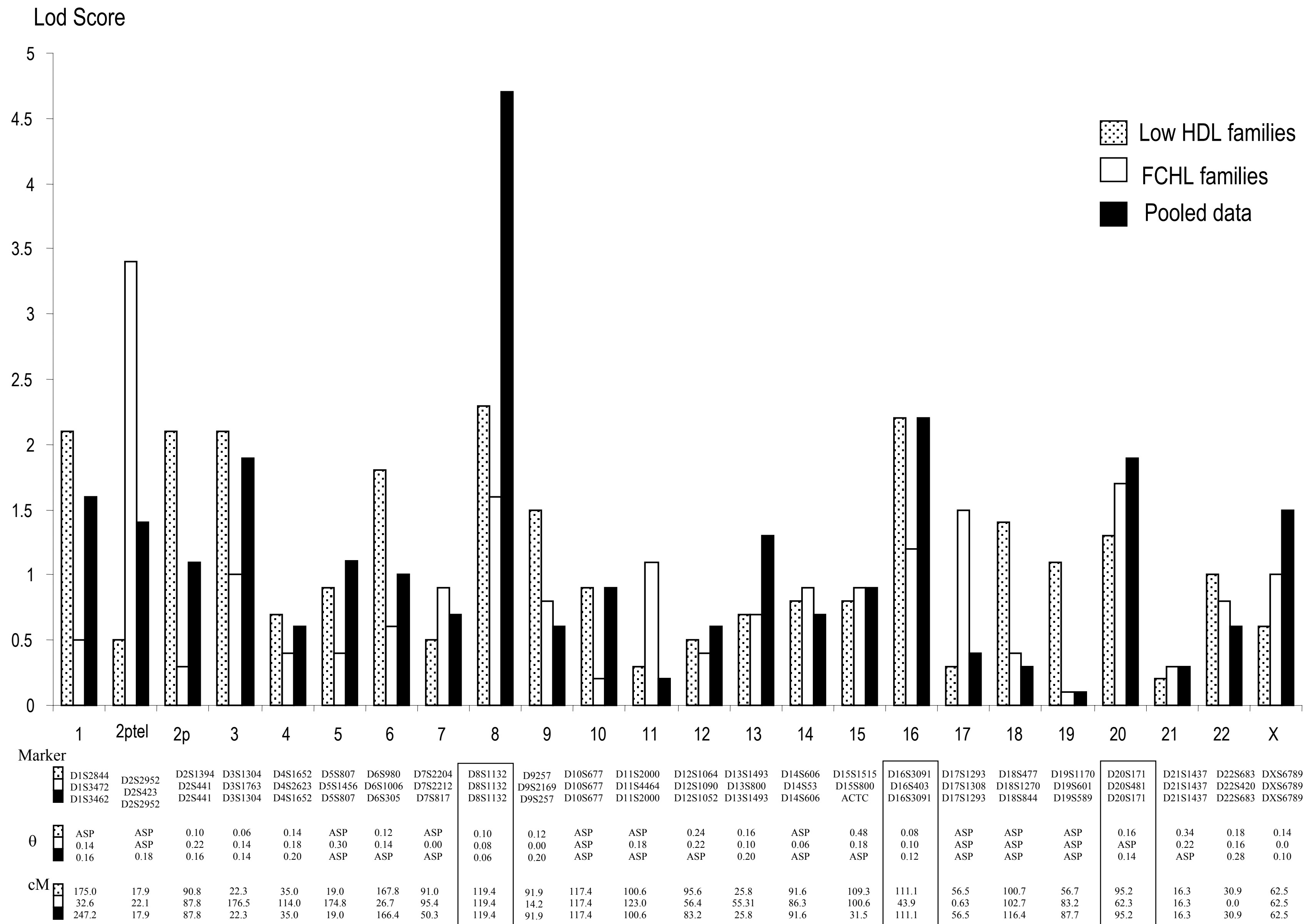
Tables 6, 7, and 8: *Markers that are also genotyped in the sample group with FCHL. a)Distance (in cM) from the first marker; b)Maximum lod scores for the two-point linkage analysis. The recombination fractions (θ) are given in parentheses. The first LOD score is that for the low HDL family sample and the second (i.e., after the slash mark) is that for the pooled sample; c) Lod scores of the ASP analysis; d) Results of the NPL analysis from Simwalk, version 2.80, for the pooled sample group. Statistic A is most powerful at detecting linkage to a recessive trait, statistic B is most powerful at detecting linkage to a dominant trait, and statistic C is a more general statistic that indicates if a few founder-alleles are overly represented among affecteds

The highest two-point lod scores of each chromosome in low HDL and FCHL families and in the pooled data set in stage 2 are presented in Figure 8. The highest statistical significance was obtained on 8q23, with a two-point lod score of 4.7 (θ=0.06). The multipoint analysis for a 15 cM region on this locus supported the result, producing NPL scores >3.0. We also found evidence for linkage of low HDL to chromosomes 16q24.1-24.2 [a lod score of 2.2 (θ=0.12)] and 20q13.11 [a lod score of 1.9 (θ=0.14)] in the pooled data analysis. In the FCHL family sample, we observed a promising linkage finding for low HDL on chromosome 2ptel, resulting in an ASP lod score of 3.4 (θ=0.0). This region did not gain further support in pooled analysis of low HDL and FCHL families. Thus, three regions on chromosomes 8, 16 and 20 remained interesting for low HDL trait. In all the statistical analyses, the recessive mode of inheritance gave systematically higher lod scores than the dominant model.

6.1.3 Contribution of the ATP-binding cassette transporter A1 – gene

As *ABCA1* was a promising candidate for the HDL level regulation, we decided to explore the genetic variations in that particular region more precisely than what the density of the genome scan marker map would have provided. Before conducting these detailed analyses, we found it likely that a part of our families would have been linked to the *ABCA1*, in which case the identification of those linked families and their exclusion of the linkage analyses would have helped to decrease genetic heterogeneity in our genome scan data. Two markers located 19 cM and 5.4 cM telomeric to the *ABCA1* produced lod scores of 1.8 (θ=0.06) and 1.3 (θ=0.06) in stage 1 of the genome scan. This region did not gain any further evidence for linkage in the low HDL families in stage 2. Yet, five low HDL families showed potential co-segregation with the *ABCA1* in the haplotype analysis. Genomic DNA of these families was subjected to the sequencing of the coding region of *ABCA1* with the intention to monitor for segregating variants. Sequence analyses identified four previously characterized polymorphisms: The sequence variants corresponding R219K in exon 6, V825I in exon 16, I883M in exon 17, and R1587K in exon 34. None of the nucleotide changes showed co-segregation with the low HDL trait.

Figure 8. The highest two-point lod scores of the three sample sets for each chromosome in stage 2. The numbers below the horizontal axis indicate the chromosome numbers. On chromosome 2, there were two separate regions producing lod scores >2.0, indicated as 2ptel and 2p. The markers producing the highest lod scores, and the recombination fractions (θ) of the two-point maximum lod score in the parametric linkage analysis are presented separately for each study sample. The distance from pter for each marker is presented in centiMorgans (cM).



Chromosome	Marker (Low HDL)	Marker (FCHL)	Marker (Pooled)	θ (Low HDL)	θ (FCHL)	θ (Pooled)	cM (Low HDL)	cM (FCHL)	cM (Pooled)
1	D1S2844	D1S3472	D1S3462	ASP	0.14	0.16	175.0	32.6	247.2
2ptel	D2S2952	D2S423	D2S2952	ASP	ASP	0.18	17.9	22.1	17.9
2p	D2S1394	D2S441	D2S441	0.10	0.22	0.16	90.8	87.8	87.8
3	D3S1304	D3S1763	D3S1304	0.06	0.14	0.14	22.3	176.5	22.3
4	D4S1652	D4S2623	D4S1652	0.14	0.18	0.20	35.0	114.0	35.0
5	D5S807	D5S1456	D5S807	ASP	0.30	ASP	19.0	174.8	19.0
6	D6S980	D6S1006	D6S305	0.12	0.14	ASP	167.8	26.7	166.4
7	D7S2204	D7S2212	D7S817	ASP	0.00	ASP	91.0	95.4	50.3
8	D8S1132	D8S1132	D8S1132	0.10	0.08	0.06	119.4	119.4	119.4
9	D9257	D9S2169	D9S257	0.12	0.00	0.20	91.9	14.2	91.9
10	D10S677	D10S677	D10S677	ASP	ASP	ASP	117.4	117.4	117.4
11	D11S2000	D11S4464	D11S2000	ASP	0.18	ASP	100.6	123.0	100.6
12	D12S1064	D12S1090	D12S1052	0.24	0.22	ASP	95.6	56.4	83.2
13	D13S1493	D13S800	D13S1493	0.16	0.10	0.20	25.8	55.31	25.8
14	D14S606	D14S53	D14S606	ASP	0.06	ASP	91.6	86.3	91.6
15	D15S1515	D15S800	ACTC	0.48	0.18	ASP	109.3	100.6	31.5
16	D16S3091	D16S403	D16S3091	0.08	0.10	0.12	111.1	43.9	111.1
17	D17S1293	D17S1308	D17S1293	ASP	ASP	ASP	56.5	0.63	56.5
18	D18S477	D18S1270	D18S844	ASP	ASP	ASP	100.7	102.7	116.4
19	D19S1170	D19S601	D19S589	ASP	ASP	ASP	56.7	83.2	87.7
20	D20S171	D20S481	D20S171	0.16	ASP	0.14	95.2	62.3	95.2
21	D21S1437	D21S1437	D21S1437	0.34	0.22	ASP	16.3	16.3	16.3
22	D22S683	D22S420	D22S683	0.18	0.16	0.28	30.9	0.0	30.9
X	DXS6789	DXS6789	DXS6789	0.14	0.0	0.10	62.5	62.5	62.5

6.2 HDL particle composition and apolipoprotein distribution in familial low HDL family members in the carotid intima media thickness (IMT) study (Study II)

All accessible family members in the low HDL families, between 18 to 70 years of age and living at a reasonable distance from Helsinki, were invited into the carotid IMT study. Altogether 89 individuals, from 27 low HDL families were included (Table 9). Of these, 41 had HDL-C level below the 10th age-sex percentile and were termed as affected, and the remaining 48 were unaffected family members, i.e. they had normal HDL-C. Gender distribution was equal among the groups. Affected family members were significantly older, and they had higher BMI, fasting insulin and pulse pressure than unaffected family members. They were also more insulin resistant, as measured by the homeostasis model assessment for insulin resistance (HOMA IR).

Table 9. Characteristics of the low HDL family members (Study II)

Variable		Affected family members (n = 41)	Unaffected family members (n = 48)	P-value*
Age (years)		44.0±10.9	41.2±11.5	0.033
Men/Women (n)		24 / 17	19 / 29	n.s.
Cigarette pack years		5.8±8.4	2.1±6.3	n.s.
Smokers (n)	Current	11	7	n.s.
	Former	8	4	n.s.
	Never	22	37	n.s.
BMI (kg/m^2)		27.3±4.3	23.9±3.8	<0.001
Waist-hip ratio		0.89±0.08	0.82±0.10	0.005
SBP (mmHg)		128±16	125±14	n.s.
DBP (mmHg)		79±9	79±8	n.s.
Pulse pressure (mmHg)		49±12	46±9	0.022
Hypertension (n)		7	2	0.044
TG (mmol/l)		1.51±0.77	1.13±0.44	n.s.
LDL-C (mmol/l)		3.29±0.84	3.06±0.62	n.s.
TC (mmol/l)		5.00±0.96	5.10±0.73	n.s.
ApoB (mg/dl)		108±31	94±22	n.s.
Glucose (mmol/l)		5.0±0.6	4.7±0.5	0.016
Insulin (mU/l)		8.9±6.5	5.1±2.9	0.002
HOMA IR		2.00±1.43	1.09±0.62	0.001

Data are presented as mean±SD, frequencies, or percentages. *) P-value using the univariate ANOVA, with an adjustment for the family number indicator. HOMA IR; The homeostasis model assessment for insulin resistance. Cigarrette pack years, numer of cigarettes smoked daily x years of continued smoking / 20, n.s., not significant.

The affected low HDL family members had reduced levels of apoA-I, apoA-II, LpA-I, LpAI-AII, HDL_2-Cand HDL_3-C as compared to the unaffected (Table 10). The most marked decrease was observed in HDL_2 subclass (109% difference between the groups). The affected subjects also had reduced levels of LpAI-AII particles and apoA-II, but the reduction in these particles was less marked than in HDL_2, LpA-I, and apoA-I. Thus, in Study II, the between-group difference in the HDL subclasses was mainly explained by the reduction of the particles in the larger HDL_2 density range.

Table 10. Concentrations of HDL-C and HDL subclasses in the low HDL family members (Study II)

	Affected family members	Unaffected family members	P-value*,†,††	Percentage difference
Variable	n=41	n=48		
HDL-C (mmol/l)	1.00±0.22	1.60±0.43		60.0%
Apo A-I (mg/dl)	117±15	149±23	<0.001*,†,††	27.4%
Apo A-II (mg/dl)	34±5	40±6	<0.001*,†,††	17.6%
LpA-I (mg/dl)	35.0±10.9	53.2±16.4	<0.001*,†,††	52.0%
LpA-I/A-II (mg/dl)	81.8±12.7	96.3±14.3	<0.001*,†,††	17.7%
HDL_2-chol (mmol/l)	0.35±0.14	0.73±0.34	<0.001*,†,††	109%
HDL_3-chol (mmol/l)	0.73±0.15	0.91±0.14	<0.001*,†,††	21.3%

Data are presented as mean±SD. $^{*)}$P-value when adjusted for the family number indicator, $^{\dagger)}$P-value when adjusted for the family number indicator and age and $^{\dagger\dagger)}$ P- value when adjusted for the family number indicator and gender

6.3 Carotid artery intima-media thickness in subjects with familial low HDL (Study II)

The affected subjects had a significantly higher mean IMT (0.79±0.14 mm) than the unaffected subjects (0.75±0.13 mm, *P*=0.021, shown in Table 11). The difference between the affected and unaffected in mean-IMT, CCA-IMT, and Maximum IMT remained significant after adjustment for gender but became non-significant after the adjustment for age. The mean-IMT was higher in males than in females (0.80±0.16 mm vs. 0.74±0.11 mm, *P*=0.018, when adjusted for age).

Table 11. IMT in affected and unaffected low HDL family members (Study II)

	Affected family members, n=41	Unaffected family members, n=48	*P*-value*	*P*-value†
Mean IMT (mm)	0.79±0.14	0.75±0.13	0.021	0.038
CCA IMT (mm)	0.91±0.15	0.86±0.12	0.021	0.038
CB IMT (mm)	1.13±0.25	1.05±0.28	0.037	n.s.
FW IMT (mm)	0.78±0.15	0.74±0.15	n.s.	n.s.
Maximum-IMT	1.04±0.19	0.95±0.18	0.028	0.045

* *P*-value for the univariate analysis of variance, with adjustment for the family number indicators; † *P*-value with adjustment for the family number indicators and gender

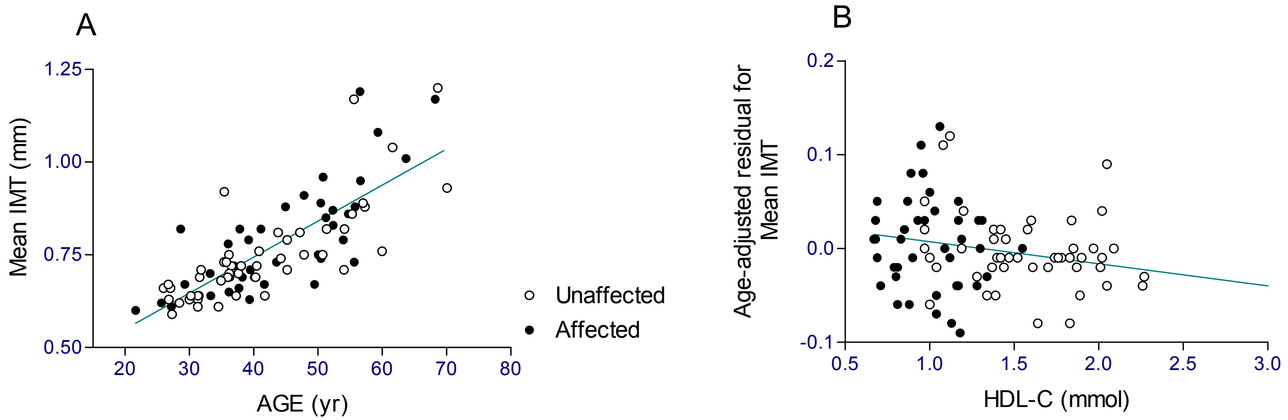


Figure 9 A and B. Relationships between carotid mean IMT and age (9A), and HDL-C and age-adjusted mean IMT (9B) in unaffected (white circles) and affected (black circles) low HDL-C family members in Study II. Significant correlation exists between mean IMT and age (r=0.880, P<0.001) and between age-adjusted mean IMT and HDL-C (r=-0.186, P=0.043). The correlation coefficients in the Figure are calculated without adjustment for family number indicator.

Mean-IMT showed strong positive association with age (r=0.880, P<0.001; Figure 9 A). With adjustment for age and family number indicators, the mean IMT correlated significantly with HDL-C (r=-0.186, P=0.043; Figure 9 B), HDL_2-C (r=-0.208, P=0.029), gender (r=-0.225, P=0.008), and w/h –ratio (r=0.193, P=0.033). Correlation between HDL-C and mean IMT remained statistically significant also after adjustment for gender, but not with adjustment for both gender and age in the same model.

Taken together, in Study II, we found that asymptomatic individuals with familial low HDL have increased carotid artery IMT as compared to their family members with normal HDL-C. A significant inverse association existed between the HDL-C level and mean IMT in these subjects.

6.4 Determinants of low HDL-C in familial combined hyperlipidemia families (Study III)

In Study III, we focused on the underlying metabolic determinants of low HDL-C levels in 49 FCHL families. Affected subjects had higher blood pressure, BMI, fasting insulin level and HOMA IR than unaffected subjects or spouses (Table 12). As expected, the concentrations of TC, TG, LDL-C and apoB were significantly increased and the LDL particle size was decreased in affected family members. The levels of HDL-C, HDL_2-C and HDL_3-C were significantly lower in the affected family members than in the unaffected family members or spouses. The activity levels of HL activity and PLTP were higher in the affected family members activity as compared to the unaffected family members (Table 13).

Table 12. Clinical and biochemical characteristics of study subjects (Study III)

	FCHL Affected n=88, 48m/40f	FCHL Unaffected n=88, 48m/40f	P value[a]	Spouses n=52, 31m/21f	P-value[b,c]
Age, years	40.1 ± 11.4	37.7 ± 9.8	n.s.	51.1 ± 7.90	<0.001[b,c]
Hypertensive	17 %	6 %	0.018	21 %	n.s.[b,c]
Smokers (%)	45 %	36 %	n.s.	37 %	n.s.[b,c]
BMI, kg/m^2	27.0 ± 4.0	25.4 ± 4.0	0.004	26.4 ± 4.5	n.s.[b,c]
TC (mmol/l)	6.27 ± 1.02	5.09 ± 0.79	<0.00	5.48 ± 0.90	<0.001[b], n.s.[c]
TG (mmol/l)	2.32 ± 1.39	1.05 ± 0.39	<0.00	1.21 ± 0.43	<0.001[b], n.s.[c]
HDL-C	1.26 ± 0.35	1.48 ± 0.36	0.001	1.38 ± 0.29	0.004[b], n.s.[c]
LDL (mmol/l)	4.00 ± 0.93	3.18 ± 0.78	<0.00	3.59 ± 0.79	<0.001[b], n.s.[c]
ApoB (g/l)	123 ± 27	86 ± 19	<0.00	98 ± 20	<0.001[b], n.s.[c]
LDL size, (nm)	25.7 ± 1.6	26.9 ± 1.2	<0.00	26.7 ± 1.2	<0.001[b], n.s.[c]
Glucose	4.8 ± 0.7	4.7 ± 0.7	n.s.	5.0 ± 0.8	n.s.[b,c]
Insulin (mU/l)	10.1 ± 4.7	6.7 ± 3.6	<0.00	7.1 ± 2.9	<0.001[b], n.s.[c]
HOMA IR	2.22 ± 1.19	1.46 ± 0.92	<0.00	1.64 ± 0.82	<0.001[b], n.s.[c]
Waist/hip –ratio	0.89 ± 0.10	0.87 ± 0.09	n.s.	0.91 ± 0.08	n.s.[b,c]
Diastolic BP	84 ± 14	77 ± 11	0.001	81 ± 9	0.003[b], n.s.[c]
Systolic BP	134 ± 18	125 ± 16	<0.00	129 ± 16	<0.001[b], n.s.[c]
Pulse pressure	50 ± 14	48 ± 10	n.s.	47 ± 12	n.s.[b,c]
Glucose	4.8 ± 0.7	4.7 ± 0.7	n.s.	5.0 ± 0.8	n.s.[b,c]
HDL_2–C	0.63 ± 0.31	0.79 ± 0.33	<0.00	0.72 ± 0.26	0.035[b], n.s.[c]
HDL_3–C	0.63 ± 0.12	0.69 ± 0.13	0.002	0.66 ± 0.12	n.s.[b,c]
ApoA-I (g/l)	140 ± 26	146 ± 26	0.033	143 ± 23	n.s.[b,c]
ApoA-II (g/l)	39.9 ± 6.8	40.3 ± 8.4	n.s.	37.9 ± 6.8	n.s.[b,c]
LpA-I (mg/dl)	52.0 ± 14.7	54.6 ± 16.6	n.s.	57.4 ± 15.2	n.s.[b,c]
LpAI-AII	87.9 ± 17.0	91.3 ± 18.1	n.s.	86.0 ± 15.3	n.s.[b,c]

Data are presented as mean ± SD, frequencies, or percentages. [a)]ANOVA for the comparisons between affected and unaffected family members, with adjustment for family number, as well as between spouses and [b)]affected and [c)]unaffected family members, with adjustment for family number, age, and gender. M, males; f, females.

Table 13. Activities of lipolytic enzymes and lipid transfer proteins (Study III)

	FCHL Affected n=88	FCHL Unaffected n=88	P value[a]	Spouses n=52	P value[b,c]
HL activity (mU/ml)	357 ± 172	292 ± 138	0.001	282 ± 121	0.013[b], n.s.[c]
PLTP activity (µmol/ml/h)	4.66 ± 1.36	4.29 ± 1.34	0.025	4.49 ± 1.23	n.s.[b,c]
LPL activity (mU/ml)	212 ± 64	216 ± 69	n.s.	228 ± 67	n.s.[b,c]
CETP activity (nmol/ml/h)	21.1 ± 5.7	22.0 ± 6.8	n.s.	21.9 ± 5.7	n.s.[b,c]

Data are presented as mean ± SD. *P*-values are presented for the ANOVA between [a)]affected and unaffected family members, with a family number adjustment, and [b)]affected family members and spouses, and [c)]unaffected family members and spouses, with adjustment for family number, age and gender.

A significant inverse correlation existed between gender-adjusted HDL_2-C and HL activity (*r*=-0.339, *P*<0.001, in pooled study group) (Figure 10). These correlations were significant also when the two groups were analyzed separately (in the affected family members *r*=-0.394, *P*<0.001, with adjustment for gender, and in the unaffected family members *r*=-0.416, *P*<0.001, with adjustment for gender). LPL activity correlated positively with HDL_2-C (*r*=0.142, *P*=0.030), whereas PLTP or

CETP activities did not show significant correlations. In multivariate analysis for HDL_2-C, the final model explained 54.8% of the variation (adjusted multiple R^2=0.548). The variables that remained in the final model were gender, HL activity, TG, BMI, HOMA IR, and LPL activity. Gender had by far the highest standardized coefficient (r=0.246, P<0.001), followed by HL activity (r=-0.213, P=0.008), TG (r=-0.194, P=0.010) and BMI (r=-0.208, P=0.016). HOMA IR and LPL activity did not have an independent contribution to HDL_2-C level in the multivariate analysis.

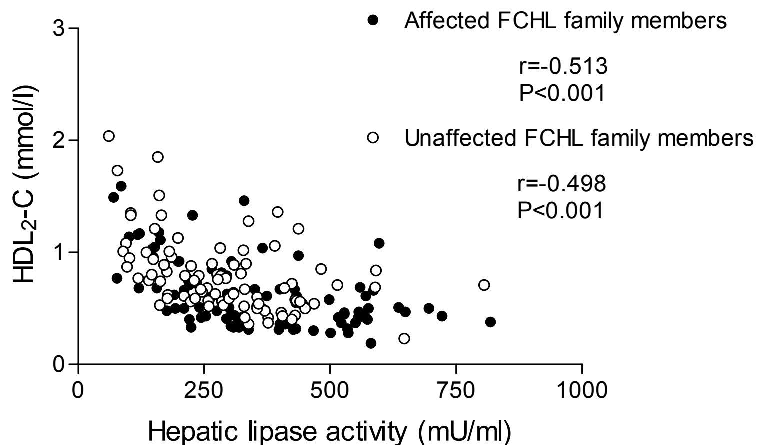


Figure 10. Correlation between HL activity and HDL_2-C (non-adjusted), separately in affected and unaffected FCHL family members.

HOMA IR showed a strong correlation with HL activity (r=0.309, P=0.002, in the combined study group), (Figure 11). HOMA IR correlated negatively with LDL size (r=-0.274, P=0.003), LpA-I (r=-0.268, P=0.001), apoA-I (r=-0.271, P=0.001), HDL-C (r=-0.357, P<0.001), HDL_2-C (r=-0.293, P=0.002), HDL_3-C(r=-0.232, P=0.007), and LPL activity (r=-0.247, P=0.001). Age-adjusted LDL particle size was associated with HL activity, both in affected subjects analyzed in a separate group (r=-0.320, P=0.002) as well as in the whole group (r=-0.294, P=0.001). The strongest predictor of the age-adjusted LDL particle size were TGs; in the affected FCHL family members (r=-0.652, P<0.001) and in the whole group (r=-0.537, P<0.001). LPL, CETP or PLTP activities did not have significant effects on LDL particle size.

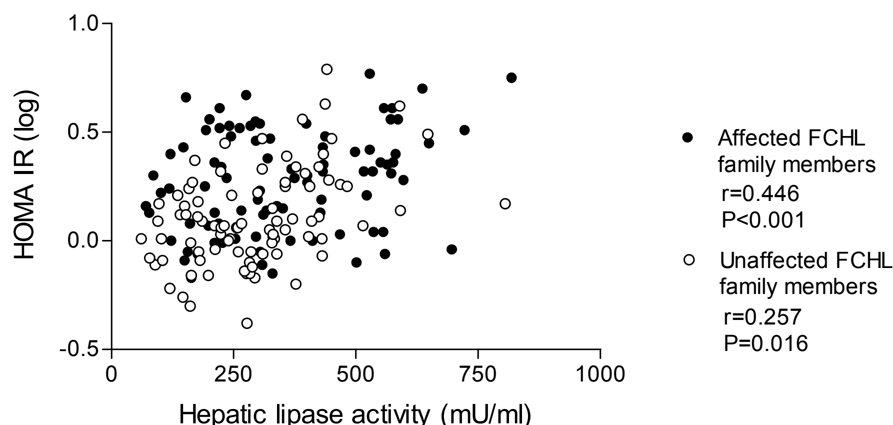


Figure 11. Correlation between HL activity and HOMA IR, separately in affected and unaffected FCHL family members.

To summarize, affected FCHL family members had decreased HDL-C, especially the HDL_2-subclass. Increased HL contributed to the changes in the HDL pool. The marker for insulin sensitivity, HOMA IR, associated positively with HL activity, inversely with HDL_2-C, and inversely with LDL particle size FCHL family members.

6.5 Soluble cellular adhesion molecules in subjects with familial low HDL (Study IV)

In Study IV, the levels of soluble cellular adhesion molecules (sCAMs) were measured in 91 subjects with low HDL from 43 low HDL families, and in 112 control subjects with no lipid abnormalities. Age and gender distribution between the groups were comparable (Table 14). Low HDL subjects had increased BMI, w/h-ratio, TG, apoB, LDL-C, fasting insulin level, and HOMA IR (Tables 14 and 15).

Table 14. Clinical characteristics of the study subjects (Study IV)

	Low HDL group n=91 (65m/26f)	Control group n=112 (73m/39f)
Age, years	48.3 ± 12.8	49.1 ± 13.6
CHD, n (%)	29 (32%)	0**
Hypertensive subjects, n (%)	45 (49%)	19 (17%)*
Smokers, n (%)	52 (57%)	30 (27%)*
BMI, kg/m^2	27.5 ± 4.1	24.6 ± 3.1**
Waist/hip –ratio	0.92 ± 0.08	0.86 ± 0.09**
SBP, mmHg	128 ± 16	126 ± 17
Glucose (mmol/l)	5.2 ± 0.9	5.0 ± 0.6
Insulin (mU/l)	9.7 ± 5.9	5.6 ± 2.6**
HOMA IR	2.29 ± 1.63	1.26 ± 0.69**

Data are presented as mean±S.D. m; men, w; women. *)Significantly different from the low HDL subjects (*P*-value <0.05); **)Significantly different from the low HDL subjects (*P*-value <0.01)

Table 15. Biochemical characteristics of the study subjects (Study IV)

	Low HDL group n=91	Controls n=112
TC (mmol/l)	5.16 ± 0.94	4.95 ± 0.77
TG (mmol/l)	1.70 ± 0.77	0.95 ± 0.35*†
LDL (mmol/l)	3.50 ± 0.84	2.99 ± 0.73*†
ApoB (g/l)	112 ± 27	89 ± 19*†
HDL-C (mmol/l)	1.01 ± 0.17	1.63 ± 0.37*†
HDL_2–C (mmol/l)	0.35 ± 0.15	0.75 ± 0.31*†
HDL_3–C (mmol/l)	0.65 ± 0.14	0.85 ± 0.15*†
ApoA-I (g/l)	115 ± 13	147 ± 24*†
ApoA-II (g/l)	33 ± 5	37 ± 6*†
LpA-I (mg/dl)	36 ± 10	56 ± 18*†
LpAI-AII (mg/dl)	79 ± 11	91 ± 14*†

Data are presented as mean±S.D. *)Significantly different from low HDL family members (*P*-value <0.010); †)Significantly different from low HDL family members with adjustment for age, gender, BMI and smoking (*P*-value <0.010)

Low HDL subjects had significantly higher concentrations of sVCAM-1, sICAM-1, sE-selectin and CRP than the controls (Table 16). The between group differences remained statistically significant for sICAM-1 and sVCAM-1 also after adjustment for age, gender, BMI and smoking. HDL-C had an inverse relation with the levels of sICAM-1 (*r*=-0.263, *P*<0.010), sVCAM-1 (*r*=-0.187, *P*<0.010), sE-selectin (*r*=-0.231, *P*<0.010), and CRP (*r*=-0.250, *P*<0.010) in the combined study group (Figure 12).

Table 16. Serum concentrations of soluble cell adhesion molecules (Study IV)

	Low HDL subjects, N=91	Controls, N=112
SVCAM-1 (ng/ml)	560±147	496±95**†
SICAM-1 (ng/ml)	247±60	215±47**†
sE-selectin (ng/ml)	52±20	44±16*
CRP (mg/l)	1.73±2.05	0.85±1.10**

Data are presented as mean ± S.D. *)Significantly different from low HDL subjects (*P*-value <0.05) **)Significantly different from low HDL subjects (*P*-value <0.01); †)Significantly different from low HDL subjects with adjustment for age, gender, BMI and smoking (*P*-value <0.01)

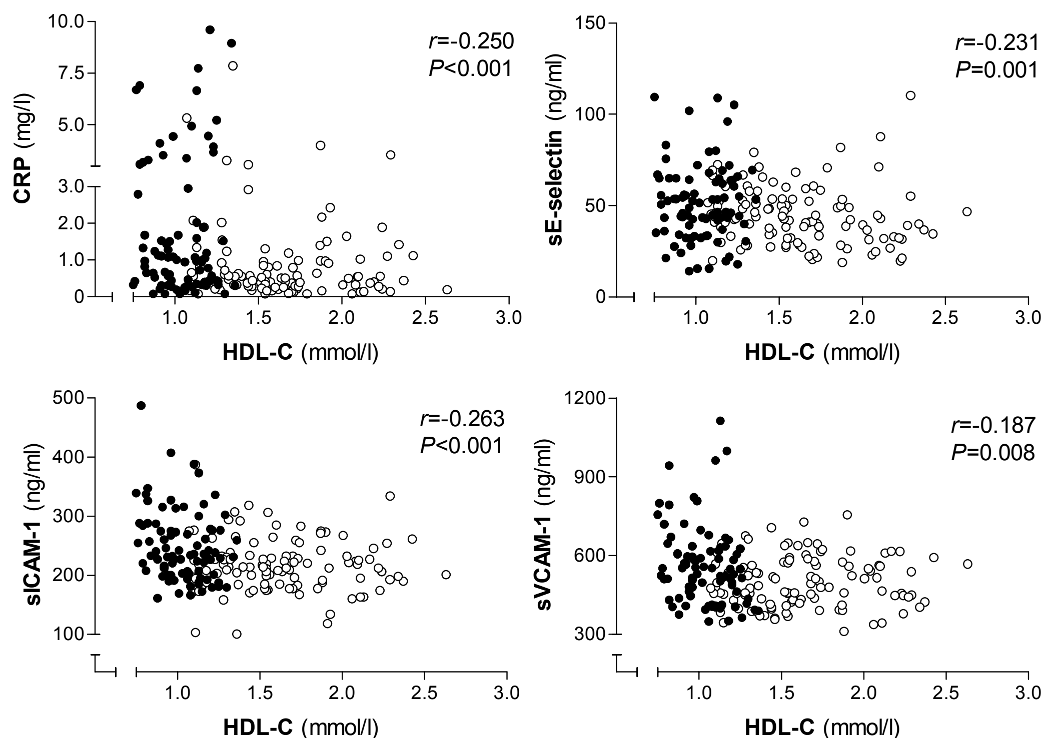


Figure 12. Correlation results between HDL-C and the levels of CRP, sE-selectin, sICAM-1, and sVCAM-1, in the combined study group. Black circles indicate the low HDL subjects (n=91), and white circles indicate the control subjects (n=112). Correlations and *P*-values are calculated for the combined study sample, with $\log_{10}$-transformed HDL-C, CRP, sE-selectin, sICAM-1, and sVCAM-1. These correlations were not statistically significant in separate analysis of the two groups.

Next, we categorized the study subjects into three groups according to the features of the metabolic syndrome (Table 17). All low HDL subjects had metabolic syndrome features; 47 low HDL subjects (52%) had 1 to 2 metabolic syndrome features and the remaining 44 low HDL subjects (48%) had 3 or more features. Among the control subjects, 64 (57%) had no features of the metabolic syndrome, 46 (41%) had 1 to 2, and 2 (2%) had 3 or more features of the metabolic syndrome. The levels of sICAM-1, sVCAM-1, sE-selectin and CRP were significantly increased in the subjects having the highest number of the metabolic syndrome features. After adjustment for age, gender, BMI and smoking, the between-group differences remained significant for sVCAM-1 and CRP. Within the low HDL subjects, the level of CRP was significantly higher in those 44 subjects that had 3 or more metabolic syndrome features, as compared to those with 1-2 metabolic syndrome features (2.29±2.25 mg/l vs. 1.21±1.71 mg/l, *P*<0.001).

Table 17. Serum levels of CRP, sE-selectin, sVCAM-1 and sICAM-1 in the combined study sample, categorized according to the presence of the features of the metabolic syndrome (Study IV)

	No metabolic syndrome features, n=64 (38m/26f)	1-2 metabolic syndrome features n=93 (66m/27f)	3-5 metabolic syndrome features n=46 (34/12)
controls / Low HDL (n)	64/0	46/47	2/44
Age, years	45.0±14.0	50.2±13.1	51.0±11.5*
sVCAM-1 (ng/ml)	484±95	537±142*	560±107**†
sICAM-1 (ng/ml)	211±48	230±53	253±62**
sE-selectin (ng/ml)	40±16	49±18**	55±19**
CRP (mg/l)	0.80±1.22	1.02±1.31	2.32±2.26**†
HDL_2-C	0.79±0.33	0.53±0.27**†	0.32±0.16**†
HDL_3-C	0.87±0.13	0.75±0.18**†	0.63±0.15**†
ApoAI	150±24	129±24**†	115±13**†
ApoAII	37.3±6.8	34.4±6.1*†	33.5±4.5**†
LpAI	57.9±18.9	45.6±16.9**†	35.3±10.2**†
LpAI-AII	91.9±14.3	83.7±13.7**†	79.8±11.1**†

Data are presented as mean ± S.D. *Significantly different from subjects with no features of the metabolic syndrome (*P*-value <0.05); **Significantly different from subjects with no features of the metabolic syndrome (*P*-value <0.01); †Significantly different from subjects with no features of the metabolic syndrome (*P*-value <0.05), with adjustment for age, gender, BMI and smoking

In summary, subjects with familial low HDL had significantly higher levels of CRP, sICAM-1, sVCAM-1 and sE-selectin as compared to the controls with normal lipid values. The observed increases in the levels of sCAMs were closely associated to the accumulation of the features of the metabolic syndrome.

7 DISCUSSION

The genetic studies of single gene mutations have revealed important mechanisms and pathways that contribute to HDL level regulation. Yet, the major loci affecting serum HDL-C levels at the population level have remained unclear. The first approach in the genetic studies of the low HDL trait in this unique sample of Finnish low HDL families was a candidate gene study (Lilja et al. 2002). The chosen candidate genes were genes encoding proteins involved in either HDL formation or catabolism, or represented some potential novel functions of HDL in anti-atherogenesis. Two regions on chromosomes 1 and 11, harboring the *APOA2* gene and the *APOA1C3A4* cluster, produced suggestive evidence for linkage in the candidate gene approach. A subset of low HDL families presented potential shared haplotypes on these regions, and these families also produced the observed positive lod scores. In the remaining families, no evidence was obtained to support the role of *APOA2* or *APOA1C3A4*. Worth of note, a newly discovered gene involved in TG metabolism, the *APOA5,* locates in the vicinity of the *APOA1C3A4* cluster, thus making the finding on chromosome 11 particularly interesting (Pennacchio et al. 2002; Talmud et al. 2002).

7.1 Significance of the observed low HDL loci in the genome-wide scan

In our study, the genome-wide scan was performed to search for novel genes for the low HDL trait. Our analyses in low HDL and FCHL families revealed initially six suggestive loci for low HDL, on chromosomes 2ptel, 2p, 3p, 8q23, 16q24.1-24.2 and 20q12-13.1. The pooled data analyses of the low HDL and FCHL families supported three of these loci, on 8q23, 16q24.1-24.2 and 20q12-13.1. Interestingly, these loci have shown linkage to HDL, type 2 diabetes, and obesity in other populations. The region on chromosome 8q23 was the most interesting, with a two-point lod score of 4.7. This region has earlier been linked to HDL regulation in genome scan of Mexican-American families using the QTL mapping strategy (Almasy et al. 1999). The results obtained independently both in the above population-based sample and in our well-defined low HDL families suggest that 8q23 harbors a gene or genes involved in the HDL level regulation. To date, however, no such candidate genes have been identified in the 8q23 region that code for proteins involved in lipid metabolism.

A region on chromosome 16q showed suggestive evidence of linkage in our pooled data analysis. Some evidence for linkage to this region has also been reported in Dutch FCHL families (Aouizerat et al. 1999). In fact, chromosome 16q24.1 resulted in a statistically significant lod score of 3.6 for the low HDL trait in the combined data analysis of our FCHL and low HDL families and Dutch FCHL families (Pajukanta et al. 2003). A potential candidate gene in this 16q region is the *FOXC2* gene, which, in fact, associates with obesity, hypertriglyceridemia, and insulin resistance (Cederberg et al. 2001). In insulin-resistant subjects, the subcutaneous adipose tissue expression the *FOXC2* has been shown to be decreased (Yang et al. 2003). Furthermore, allelic variants on the *FOXC2* gene showed association to the TG level regulation (Yanagisawa et al. 2003), suggesting that *FOXC2* may regulate the TG and/or HDL-C levels. Chromosome 16q was reported to regulate the HDL-C level also in Mexican Americans from San Antonio (Mahaney et al. 2003).

The region on 20q has been linked to obesity and to the development of type 2 diabetes, both of these abnormalities overlapping with low HDL (Bowden et al. 1997; Ji et al. 1997a; Lembertas et al. 1997; Zouali et al. 1997; Ghosh et al. 2000). Taken together, our genome-wide scan identified 3 loci (on 8q23, 16q24.1-24.2 and 20q12-13.1) for low HDL in the pooled data analysis of low HDL and FCHL study samples, suggesting a partially shared genetic background for the low HDL and FCHL phenotypes. However, the density of the pooled genome-scan data did not provide further support for the loci on chromosomes 1 and 11, which initially were observed in the candidate-gene study. Up to 8 loci, different from our findings, have emerged in genome-wide scans for HDL level regulation in other study samples (Almasy et al. 1999; Shearman et al. 2000; Coon et al. 2001;

Peacock et al. 2001; Arya et al. 2002; Canizales-Quinteros et al. 2003; Mahaney et al. 2003). The major genes contributing to the common familial low HDL trait are still unclear.

7.3 Methodological limitations in the linkage analyses in the genome-wide scan

Complex disorders involve complicated interactions between genes and the environment; therefore, several confounding factors limit the mapping of complex diseases (Lander and Schork 1994). First, the inheritance patterns that are applied in the linkage analyses may represent over-simplifications. As an example the familial hypoalphalipoproteinemia (FHA), in which the low HDL patients are heterozygous for the *ABCA1* mutations, is considered as an autosomal dominant disorder (Brooks-Wilson et al. 1999). In the present study, in turn, the low HDL trait appeared to follow the recessive mode of inheritance. Conceivably, the mode of inheritance of the familial low HDL trait remains unsolved.

The complex disorders are genetically heterogeneous. Either several different genes or different allelic variants in the same gene can cause the same disease phenotype. In case of incomplete penetrance, a person carries the predisposing gene, but does not have the disease phenotype. We lacked the genotype and a definite phenotype data on many subjects regarding their CHD status. As the penetrance of CHD is age-dependent, the CHD phenotype of the young family members is unknown. Therefore, we could not calculate linkage for the CHD trait. Phenocopies, in turn, were probably relatively common among low HDL subjects. This means that some individuals represent the disease phenotype (reduced HDL-C) because of some secondary, environmental effects like obesity or a sedentary life style, and not because they had inherited the predisposing gene.

The information concerning the population prevalence of familial low HDL is limited. Therefore, the gene frequencies were estimated from multiple sources of incomplete information, which is often the case when studying complex traits. We were forced to rely on assumptions about the allele frequency in parametric linkage analyses. The allele frequencies of 0.4% for the dominant model and 8% for the recessive model were based on the estimated population prevalence of about 1.0%. This was inferred from the prevalence of FCHL, which represents a more precisely characterized trait. Genest et al. (Genest et al. 1992) estimated the prevalence of FCHL to be 13.7% among the subjects with CHD before the age of 60 years. The prevalences of familial low HDL and familial hypertriglyceridemia with low HDL in this same cohort were 4% and 14.7%, respectively (Genest et al. 1992). The population prevalence of FCHL is considered to be 1-2% (Goldstein et al. 1973; Grundy et al. 1987). We estimated the population prevalence of the common form of familial low HDL to be approximately 1.0%. No other allele frequencies were examined since the hypothesized ones represent the best estimates from the information available, and we wished to avoid the problems of multiple testing.

Finally, varying criteria for the definition of the disease phenotype, especially the use of cut-off levels for quantitative traits such as HDL, may serve as a source of error. The QTL analysis by the variance-components methods is not dependent of arbitrary dichotomization of the disease phenotype (Almasy and Blangero 1998). Thus, we also used the SOLAR program to analyze two quantitative measures, HDL-C and triglycerides. The QTL analyses were adjusted for additional covariates in the model, including sex, BMI and age. No genome-wide significance was found in the QTL analyses, probably reflecting the initial ascertainment strategy, which was aimed for a qualitative trait. This strategy produced a lack of substantial variation in serum HDL-C concentration in the study sample, and thus diminished the power and usefulness of the QTL analysis.

7.4 Studies on the *ABCA1* region

Neither linkage evidence nor functional variants on *ABCA1* were identified, suggesting that *ABCA1* is not the underlying cause for low HDL-C in Finns. In fact, Kakko et al. (Kakko et al. 2003) recently reported that the *ABCA1* locus was not linked to HDL-C regulation, in a separate sample of Finnish families with premature CHD and low HDL-C. The polymorphisms of *ABCA1* associated only with a small variation of HDL-C level. Neither did the common *ABCA1* variants prove significant association with HDL-C concentrations in the VA-HIT or the Framingham Offspring Study study samples (Brousseau et al. 2001). However, also other cellular proteins may affect lipid transport to the plasma membrane of the efflux into apoA-I. Limited data is currently available on the association between HDL-C levels and the cholesterol efflux function in patients with low HDL-C, but *without* mutations in the *ABCA1*.

7.5 Mechanisms of HDL lowering in hypertriglyceridemic states; FCHL as an example

The FCHL patients in our study had markedly decreased levels of HDL-C, especially HDL_2-C. These data are consistent with the concept that in hypertriglyceridemia, HDL particles are TG-enriched, and HDL particle distribution is skewed towards smaller HDL subclasses (Figure 13). The levels of apoA-I, apoA-II, LpA-I or LpAI-AII were also decreased in the affected FCHL family members, but the reduction in these parameters was not as manifest as that observed for HDL_2-C and HDL-C. The HL activity, in turn, was increased in FCHL patients, providing evidence for the role of HL-mediated hydrolysis as a major determinant of HDL_2-C. HL activity also correlated significantly with LDL particle size.

The subjects affected by FCHL have a delayed elimination of atherogenic CMR particles, and therefore, a prolonged residence time of these triglyceride rich particles (TRLs) in the circulation. Consequently, the transfer of TG from the TRLs to HDL increases. The metabolic clearance of the TG-enriched HDL is enhanced due to an increased HL-mediated hydrolysis (Lamarche et al. 1999). This gives rise to the generation of small dense HDL particles as well as lipid-poor apoA-I particles, which are rapidly cleared from circulation mainly via the kidneys (Kee et al. 2002; Rashid et al. 2002). Elevated lipolytic action of HL predominates particularly in insulin resistant states such as obesity and type 2 diabetes. The long residence time of TRLs also favors the core lipid exchange between TRLs and LDL, which results in the conversion of the LDL particles into small and dense form, into LDL subclass pattern B (Vakkilainen et al. 2002). There seems to be symmetry between the mechanisms leading to the generation of small LDL size and to the lowering of HDL in FCHL.

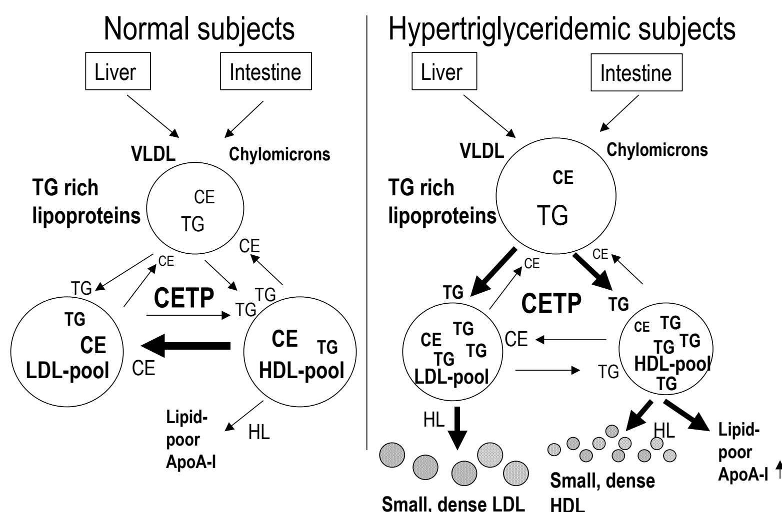


Figure 13. A schematic illustration of the proposed mechanisms of HDL lowering in hypertriglyceridemia. VLDL, very low density lipoprotein; TG, triglyceride; CE, cholesterol ester; LDL, low density lipoprotein; HDL high density lipoprotein; HL, hepatic lipase; CETP, cholesterol ester transfer protein; ApoA-I, apolipoprotein A-I

7.5.1 Contribution of PLTP, CETP and LPL on the regulation of HDL levels in FCHL

The TG-enrichment of HDL enhances the PLTP-mediated conversion of HDL to larger and smaller particles (Rye et al. 1998). A previous study in Finnish men showed that PLTP activity associates with the levels of TG, LDL, and BMI (Tahvanainen et al. 1999). In our study sample, PLTP activity was increased in FCHL patients. However, no association existed between PLTP activity and either HDL parameters or HOMA IR, suggesting that PLTP does not mediate the primary changes in HDL metabolism in FCHL patients.

Reduced LPL activity results in an FCHL-like lipoprotein pattern, i.e. elevated levels of TC and TGs, and decreased HDL-C (Reymer et al. 1995b). Accordingly, a partial LPL deficiency, either genetic or acquired, has been suggested to underlie the phenotype of FCHL in some patients. However, we did not observe significant differences in LPL activity level between the affected and unaffected family members, and in the correlation analyses, LPL activity did not show independent contribution to HDL_2-C level regulation. Neither did CETP activity show independent contribution to the HDL-C level regulation. CETP activity levels were comparable between affected and unaffected family members.

7.6 Carotid artery intima-media thickness in subjects with familial low HDL

Increased carotid IMT has been found to associate with the angiographic presence and extent of CHD (Wofford et al. 1991; Geroulakos et al. 1994; Sonoda et al. 2004), and predict future cardiovascular events (Hodis et al. 1998; van der Meer et al. 2004). The IMT measurement has been suggested to reflect early, diffuse atherosclerosis better than angiography, because it detects the atherosclerotic changes even before the actual narrowing of the arterial lumen (Hodis et al. 1998).

A number of traditional cardiovascular risk factors, including age, smoking, blood pressure, and dyslipidemia have been found to associate with carotid IMT (Prati et al. 1992). Some population-based data exist on the association between IMT and the serum HDL-C concentrations. In a sample of 1013 French middle-aged subjects, an independent association was found between mean IMT and HDL-C in men, but not in women (Ferrieres et al. 1999). In the the Tromsø study, a population-based study of 6408 men and women, HDL-C emerged as an independent predictor of IMT, together with age, SBP, TC, BMI and smoking (Stensland-Bugge et al. 2001). In the Edinburgh Artery Study that comprised 1106 men and women, a significant univariate correlation was found between IMT and HDL/TC-ratio in women (Mowbray et al. 1997). The multivariate analysis showed association between HDL/TC-ratio and IMT in both genders. In contrast, no association was found between carotid IMT and HDL-C level in a population sample of 481 Finnish subjects (Kakko et al. 2001). In the Muscatine Study, age and LDL-C were significant predictors of IMT, whereas HDL-C predicted IMT only in men in a risk factor load model, when added to LDL-C and DBP (Davis et al. 2001).

Some previous data also exist on the carotid IMT in subjects with low HDL-C. Interestingly, individuals with low HDL-C due to the *ABCA1* mutations and a reduced cholesterol efflux have an accelerated increase in IMT with age (van Dam et al. 2002). Also subjects with primary hypoalphalipoproteinemia (HA) had increased mean IMT, as compared to subjects with normal or high levels of HDL-C (Sirtori et al. 2001).

As the carotid artery IMT measurement may provide a surrogate marker to identify the high-risk individuals, we examined the IMT in the low HDL family members, with no symptoms of CHD. Our main finding was that the mean IMT is significantly increased in the subjects with familial low HDL as compared to the normolipidemic family members. HDL-C and HDL_2-C, but not LDL-C,

TG, or fasting glucose showed significant correlations with the age-adjusted mean IMT. Based on these results we suggest that (1) HDL-C and HDL_2-C are more critical predictors for carotid artery IMT than conventional lipid variables in the low HDL families; (2) the asymptomatic individuals with familial low HDL are at increased risk for the development of atherosclerosis.

The two study groups in the carotid IMT study differed in several aspects, all of which could potentially explain the between-group differences in mean IMT. We were not able to match the subjects in terms of age, BMI or all the other lipid values. However, we defend our approach by the fact that this is a family study. Our study sample was not collected from a general lipid clinic population, and therefore, did not represent a general population. In fact, the two groups differed with respect to the components of the metabolic syndrome, but despite this, not in LDL-C, TC or apoB. This finding appears to be an inherent feature of the low HDL family members.

In all of our analyses, age was observed as the single variable that independently contributed to IMT. When adjusted for both gender and age, there were no significant differences between the affected and unaffected family members in any of the IMT measures. This may be due to the relatively low sample size, and due to the strong effect of age on the IMT. However, in a subsequent study in a subgroup of these low HDL family members, we compared the mean IMT between 18 low HDL subjects and normolipidemic control subjects (Alagona et al. 2003). The controls were pair-matched individually for gender, age and BMI with the low HDL subjects. The low HDL subjects had a significantly higher mean carotid IMT than the controls, emphasizing the role of decreased HDL-C as a predictor of early atherosclerotic changes in asymptomatic individuals. The pulse wave analysis (PWA) was also performed to determine the presence of the arterial stiffness. All the blood pressure variables, however, were comparable between the two groups. Likewise, low HDL subjects and controls did not differ from each other with regard to augmentation or the augmentation index.

7.7 Elevated concentrations of circulating cellular adhesion molecules in low HDL subjects

The earliest changes before the occurrence of atherosclerosis are the recruitment of inflammatory cells from the circulation and their transendothelial migration into the arterial intima (Price and Loscalzo 1999). Adhesion molecules, including ICAM-1, VCAM-1, and E-selectin, mediate this process in response to several inflammatory stimuli. As the increased cytokine-induced expression of CAMs is crucial in the development of vascular injury, the capability of HDL to inhibit this process is potentially highly anti-atherogenic. The circulating levels of CAMs, the markers of their endothelial expression, are considered to predict the risk of cardiovascular events (Blankenberg et al. 2003).

Our study in the individuals with familial low HDL showed that (1) the low HDL subjects have significantly higher levels of sVCAM-1, sICAM-1, sE-selectin, and CRP, than controls with similar age- and gender distribution; (2) HDL-C is inversely and significantly associated with the levels of sCAMs and CRP; and (3) the levels of sCAMs and CRP increase according to the number of the features of the metabolic syndrome.

The subjects with low HDL presented a complex phenotype that is commonly observed in FCHL or in the metabolic syndrome, a phenotype characterized by elevated fasting insulin, BMI, TG, and apoB. The growing number of the features of the metabolic syndrome predicted the increase in the levels of CAMs and CRP. This is in line with previous studies in healthy subjects and in patients with type 2 diabetes that established a positive association between the traits related to insulin resistance and obesity and the levels of CAMs and CRP (Chen et al. 1999; Festa et al. 2000; Leinonen et al. 2003). Our data is also complementary to those reported by Calabresi et al., who showed that the levels of sICAM-1 and sE-selectin are elevated in individuals with isolated low

HDL, and in individuals with low HDL combined to other lipid abnormalities (Calabresi et al. 2002).

Taken together, we suggest that the familial low HDL trait associates with low-grade inflammation and increased endothelial activation. Moreover, the accumulation of the features of the metabolic syndrome aggravates the increase in the inflammatory parameters. The individuals with familial low HDL are predisposed to increased inflammatory cytokine expression, and therefore have an increased risk for atherosclerotic disease.

Does the measurement of soluble adhesion molecules add predictive information on CHD risk, in addition to that offered by traditional risk factors? Increased plasma CRP, a marker of systemic inflammation, is an independent risk factor for cardiovascular disease (Ridker 2001; Ridker et al. 2003). The prognostic value of VCAM-1, ICAM-1, E-selectin and CRP as predictors of CHD events has been studied in prospective cohort studies involving healthy subjects and CHD patients (Blankenberg et al. 2003). The Physicians' Health Study and the PRIME study showed that increased sICAM-1 is an independent predictor of future CHD events in healthy men (Ridker et al. 1998; Luc et al. 2003). Contradictory results were obtained from the British Regional Heart Study, which found no strong associations between adhesion molecules and CHD risk when adjusted for smoking, blood pressure, lipid values, inflammation and infection, haemostasis and thrombosis, and indicators of socioeconomic status (Malik et al. 2001). In the ARIC study, the Athero*gene* study, and in the Bezafibrate Infarction Prevention study, increased levels of sICAM-1, sVCAM-1 or sE-selectin predicted the risk of future coronary events in patients with CHD or carotid artery atherosclerosis (Hwang et al. 1997; Blankenberg et al. 2001; Haim et al. 2002). Based on these observations, it has been suggested that ICAM-1 serves as a risk predictor for symtomatic disease in healthy individuals, whereas ICAM-1, VCAM-1 and E-selectin indicate the extent of atherosclerosis in patients with a manifest vascular disease. Together with CRP, the soluble adhesion molecules likely have a role as potential biomarkers for the risk of CHD.

8. SUMMARY

The results of the Studies I-IV can be summarized as follows:

1. The loci on chromosomes 8q23, 16q24.1-24.2 and 20q13.11 may harbor genes essential for the HDL level regulation in Finnish families. In the genome-wide scan, 8q23 produced the strongest statistical evidence for linkage for low HDL, having a lod score of 4.7. This region has been linked to HDL levels in Mexican Americans, but no potential candidate genes have been identified. The loci on 16q24.1-24.2 and 20q13.11 have previously shown association with elevated TGs, insulin resistance, and type 2 diabetes. No genome-wide significance was found in the quantitative trait analyses for HDL-C or TG, probably due to the strategy of ascertainment, aiming for a qualitative trait. No evidence was observed for the involvement of the *ABCA1* in the HDL level regulation in Finnish families.

2. The affected low HDL family members not only had low HDL-C, but also a major rearrangement in the HDL subpopulation profile, when compared to either unaffected family members or normolipidemic matched controls. The affected subjects had markedly decreased HDL_2-C, LpA-I and apoA-I. The reduction in the levels of HDL_3-C, LpAI-AII and apoA-II particles was less marked than the reduction of the particles residing in the HDL_2 density range. Thus, the low levels of HDL-C in the affected low HDL family members were mostly due to the decrease in large particles.

3. The asymptomatic subjects with familial low HDL had a significantly increased carotid artery IMT. Total HDL-C and HDL_2-C were significantly associated with the mean IMT, with adjustment for age. However, no significant association existed between the age-adjusted IMT and LDL-C, TG, fasting glucose, or parameters of BP. HDL-C and HDL_2-C may be important in predicting the cardiovascular risk in asymptomatic members of low HDL families.

4. The affected FCHL family members had strongly reduced HDL-C and HDL_2-C. They also had increased levels of fasting insulin and HOMA IR, and higher HL activity. Increased HL activity, increased TGs, and elevated BMI associated significantly with decreased HDL_2-C. The TG-enrichment of HDL particles and enhanced HL activity skew the HDL particle distribution towards the smaller subclasses, and decrease the concentration of total HDL-C. In FCHL, HL appears to have a more important role in the HDL level regulation, as compared to PLTP, LPL or CETP.

5. Subjects with familial low HDL had increased serum concentrations of the sICAM-1, sVCAM-1 and sE-selectin, and an increased level of the inflammatory acute phase protein CRP. The majority of the individuals with familial low HDL had a complex phenotype that resembles FCHL or the metabolic syndrome, characterized by elevated fasting insulin, BMI, TG, and apoB. The individuals with the highest number of the metabolic syndrome features had increased levels of sCAMs and CRP, and a marked decrease in the levels of HDL_2-C, apoA-I and LpA-I. The presence of the metabolic sydrome predicted an increased CRP level in subjects with familial low HDL.

9. CONCLUSIONS

The present thesis was carried out to better understand the metabolic and genetic factors underlying low HDL levels in Finnish families. Low HDL-C is a frequently observed lipoprotein abnormality in patients with premature CHD. However, data from patients with rare inborn errors of HDL metabolism indicate that for the protection from atherosclerosis, the altered HDL function is more relevant than the actual amount of HDL *per se*. Our study sample, consisting of well-defined Finnish families ascertained for low HDL-C and FCHL, enabled us to study the metabolic and genetic regulation of HDL in each of these disease entities, as well as in the pooled study sample.

In the present study, three loci were identified to be involved in HDL level regulation in low HDL and FCHL families, suggesting that a partially shared genetic background underlies the low HDL and FCHL traits in Finnish population. These data also support the concept that the common familial low HDL results from a dysregulation of several genes, rather than from a single gene defect.

In FCHL, the low HDL level is a consequence of the prolonged hypertriglyceridemia, the TG-enrichment of HDL particles, and the enhanced HL activity. The HDL particle distribution in affected FCHL family members is skewed towards the smaller density range. Most likely, the same regulatory pathways are dysregulated in FCHL and in familial low HDL, resulting in the strongly reduced concentrations of the HDL_2 subclass.

The clinical phenotypes of the familial low HDL, the FCHL, and the metabolic syndrome overlap. The affected family members in low HDL families are characterized by elevated fasting insulin, BMI, TG, and apoB, as well as strongly reduced levels of HDL_2-C, apoA-I and LpA-I. They also have increased levels of leukocyte adhesion molecules and CRP. Besides low HDL-C level, also other factors potentially contribute to the increased inflammatory cytokine expression and endothelial activation, such as a decreased insulin sensitivity and hypertriglyceridemia. The chronic inflammatory process on the vascular endothelium predisposes the low HDL subjects to an increased risk of an atherosclerotic disease. A low HDL level may substitute for a surrogate marker of the pro-atherogenic disturbances. Furthermore, low HDL may precede the manifestations of decreased insulin sensitivity, namely the metabolic syndrome or type 2 diabetes.

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