Levels of apolipoprotein M are not associated with the risk of coronary heart disease in two independent case-control studies

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Abstract **Apolipoprotein M (apoM), a 25 kDa plasma protein belonging to the lipocalin protein family, is predominantly associated with HDL. Studies in mice have suggested apoM to be important for the formation of pre-β-HDL and to increase cholesterol efflux from macrophage foam cells. Overexpression of human apoM in LDL receptor-deficient mice reduced the atherogenic effect of a cholesterol-rich diet. The aim of the present study was to investigate whether the apoM levels in man predict the risk for coronary heart disease (CHD). ApoM was measured in samples from two separate case-control studies. FINRISK '92 consisted of 255 individuals, of whom 80 developed CHD during follow-up and 175 were controls. The Copenhagen City Heart Study included 1,865 individuals, of whom 921 developed CHD during follow-up and 944 were controls. Correlation studies of apoM concentration with several analytes showed a marked positive correlation with HDL and total cholesterol as well as with apoA-I and apoB. There was no significant difference in mean apoM level between CHD and control subjects in either study. In conditional logistic regression analyses, apoM was not a predictor of CHD events, [odds ratio (95% CI) 0.97 (0.74–1.27) and 0.92 (0.84–1.02), respectively]. In conclusion, no association between apoM and CHD could be found in this study.**—Ahnström, J., O. Axler, M. Jauhiainen, V. Salomaa, A. S. Havulinna, C. Ehnholm, R. Frikke-Schmidt, A. Tybjærg-Hansen, and B. Dahlbäck. **Levels of apolipoprotein M are not associated with the risk of coronary heart disease in two independent case-control studies.** ***J. Lipid Res.*** **2008.** 49: **1912–1917.**

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Coronary heart disease (CHD) is a major cause of mortality and morbidity. A positive relationship between the concentration of LDL-cholesterol and the risk of CHD has been demonstrated (1). Large epidemiological studies have also established an association between an increased risk for CHD and low levels of HDL-cholesterol (2, 3). The anti-atherogenic properties of HDL are believed to be related to the involvement of HDL in reverse cholesterol transport, a process in which cholesterol is transported from peripheral tissues to the liver (4). Apolipoprotein A-I (apoA-I) is the major apolipoprotein of HDL particles, but in addition, HDL also contains several other proteins that have different functions (5–7).

ApoM is a 25 kDa, 188 amino acid residue-containing plasma protein that is mainly expressed in liver and kidney (8). In the circulation, apoM is preferentially associated with HDL and only to a minor extent with other lipoproteins (8, 9). In human plasma, apoM is present on approximately 5% of the HDL particles and in less than 2% of the LDL particles (9). The apoM concentration in human plasma is approximately 0.9 μmol/l (10). A noteworthy feature of apoM is the lack of a signal peptidase cleavage site in the apoM amino acid sequence, explaining why circulating apoM retains its signal peptide (8). This unusual property of an extracellular protein is shared with two other HDL-associated proteins, namely paraoxonase-1

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Abbreviations: apoM, apolipoprotein M; CCHS, Copenhagen City Heart Study; CHD, coronary heart disease; LRH-1, liver receptor homolog-1; SNP, single-nucleotide polymorphism.

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and haptoglobin-related protein (11). The retained signal peptide of apoM serves as a hydrophobic anchor, binding apoM to the phospholipid layer of the lipoproteins (12). Structural analysis and homology modeling have predicted apoM to belong to the lipocalin protein family (13, 14). This is supported by the recent finding that apoM binds retinol and retinoic acid in vitro (13).

ApoM-containing lipoprotein particles have been isolated from human plasma and partially characterized (9). ApoM is associated with a heterogeneous subpopulation of HDL particles. Compared with apoM-free HDL, apoM-containing HDL particles were more efficient in reducing LDL oxidation and in stimulating cholesterol efflux. However, the physiological significance of these findings is uncertain because apoM-containing HDL constitutes only a small portion of total HDL in plasma (9).

The physiological function of apoM is not known, but silencing of apoM expression in mice with small interfering RNA (siRNA) resulted in the accumulation of large, cholesterol-rich HDL particles, due to impaired conversion of HDL to pre-β-HDL (15). However, in a more recent study of apoM knock-out mice (apoM$^{-/-}$), the size of HDL in plasma and the amount of plasma pre-β-HDL were not affected in vivo, although the in vitro formation of pre-β-HDL was slightly decreased in apoM$^{-/-}$ mice and increased in human apoM-transgenic mice, as compared with wild-type mice (16). In both studies, overexpression of apoM in LDL receptor-deficient mice challenged with a cholesterol-rich diet reduced the development of atherosclerosis. These results suggest that apoM plays a role in HDL metabolism and may have anti-atherosclerotic properties (15, 16).

A 2007 study of three single-nucleotide polymorphisms (SNPs), C-1065A, T-855C, and T-778C, all located in the proximal promoter region of the apoM gene, found that the SNP T-778C was associated with increased levels of plasma total cholesterol and fasting plasma glucose and conferred the risk of development of type 2 diabetes (17). A subsequent case-control study showed that CHD patients had an increased frequency of the SNP T-778C allele compared with controls (18). The T-855C allele was also recently linked to CHD when carriers of the C allele were found to have an increased risk of CHD compared with the wild-type TT genotype (19).

To be able to expand apoM research to larger human clinical contexts, we have developed a specific quantitative sandwich ELISA for the measurement of apoM in human plasma and serum (10). Correlation studies between plasma apoM concentration and common clinical-chemical analytes showed a strong correlation of apoM with plasma total cholesterol ($r = 0.52$) (10). The mechanism behind this relationship is not known, but apoM has recently been shown to be a target gene for the orphan nuclear receptor liver receptor homolog-1 (LRH-1) (20). LRH-1 has been implicated as a master regulator of genes that function to decrease cholesterol levels in liver and intestine and is involved in the control of the hepatic inflammatory response, bile acid biosynthesis, and reverse cholesterol transport (20–22). HNF-1α is another transcription factor that might be involved, because it affects both apoM expression and many genes involved in cholesterol metabolism (15).

There is a well-established relationship between total plasma cholesterol and the risk of myocardial infarction (MI) (2, 23, 24). The aim of this study was to investigate whether there is a relationship between the serum level of apoM and the risk for CHD. Therefore, apoM was measured in samples from two different nested frequency matched case-control studies, the FINRISK '92 survey, a cohort study performed in a Finnish population that was followed for 10 years (25–27), and the Copenhagen City Heart Study (CCHS), performed in a Danish population that was followed for 24 years (28, 29).

METHODS

Study design

FINRISK '92. The study was based on the FINRISK '92 survey, which is a population-based CHD risk factor survey. Details have been described previously (25, 26), but in brief, the baseline investigation of the FINRISK '92 survey was carried out during January through March 1992. Random samples of men and women aged 25–64 years were drawn from the national population register for four different geographical areas in Finland. The almost 6,000 participants were advised to fast totally at least 4 h before the scheduled examination and to avoid fatty meals earlier during the day. The participants were followed until December 31, 2001. The follow-up was carried out by a computerized record linkage of the study data with the National Causes of Death Register and the National Hospital Discharge Register using the national social security numbers of the study participants. The cardiovascular diagnoses in these Finnish national registers have recently been validated (30). In the FINRISK '92 survey, a number of clinical-chemical analytes as well as diabetes status and smoking habits were available from the baseline investigation (27, 31).

An additional inclusion criterion for this particular study was that there had to be serum left in the freezer from the FINRISK '92 survey. The serum samples were stored at −20°C, and they had been thawed during follow-up, when aliquots had been taken for other purposes.

In the present study, the cases were participants who had had an MI (ICD-9 code 410 or ICD-10 codes I21–I22) or coronary death (ICD-9 codes 410–414 or IDC-10 codes I20–I25) during the follow-up. Patients with cardiovascular disease at baseline were excluded. Eleven of the participants received lipid-lowering medication during the follow-up. Inclusion of these participants did not alter the results. The control subjects were participants who remained free of MI and stroke events until the end of follow-up. These were frequency matched to cases by age, sex, and area of residence.

The CCHS. The CCHS is a prospective cardiovascular study of the Danish general population initiated in 1976–1978, with follow-up examinations in 1981–1983 and 1991–1994 (28, 29). The Copenhagen and Frederiksberg's ethics committee approved the study. Individuals were randomly selected based on the national Danish Civil Registration System to reflect the adult Danish general population aged 20–80+ years. For the present study, all endpoints were recorded in the follow-up period 1976 through 1999, and 958 participants with a CHD event were age and sex matched with 958 participants who remained

free of CHD and ischemic cerebrovascular disease during the follow-up period. All clinical and laboratory data were available for 921 cases and 944 controls, the numbers used in the present manuscript. Samples were drawn from nonfasting individuals during the follow-up examinations in 1991–1994. Approximately 1% of the participants were treated with lipid-lowering medication but were not excluded, because this did not alter the results. Information on diagnoses of CHD (ICD-8 codes 410–414 or ICD-10 codes I20–I25) was collected and verified by reviewing all hospital admissions and diagnoses entered in the national Danish Patient Registry, all causes of death entered in the national Danish Causes of Death Registry, and medical records from hospitals and general practitioners. CHD was defined as MI or characteristic symptoms of stable angina pectoris (32). A diagnosis of MI required the presence of at least two of the following criteria: characteristic chest pain, elevated cardiac enzymes, and electrocardiographic changes indicative of MI. In 597 cases and 637 controls, citrate plasma samples were available, whereas in the remaining 324 cases and 307 controls, serum samples were available.

Measurements

A sandwich ELISA for apoM based on two monoclonal antibodies, M42 and M58, was used to quantify apoM, as described previously (10). In the present study, the interassay coefficient of variation of the ELISA was 8.7% for the FINRISK '92 samples and 6.1% for the CCHS samples at the 100% level.

Colorimetric and turbidimetric assays had already been used to measure serum levels of total cholesterol, HDL-cholesterol, and triglycerides in both the CCHS samples and FINRISK '92 samples, as well as apoA-I and apoB in the CCHS samples (all Boehringer Mannheim GmbH). HDL-cholesterol had been determined after precipitation of apoB-containing lipoproteins with dextran sulfate and $MgCl_2$. Non-HDL-cholesterol was defined as the difference between total cholesterol and HDL-cholesterol.

Statistical analyses

In the FINRISK study, all samples were serum, whereas the CCHS contained a mixture of serum and plasma samples. Because the level of apoM in serum is higher than in plasma, all plasma values were adjusted to corresponding estimated serum values. A linear regression yielded the following relationship: serum apoM = 1.16 × plasma apoM + 0.13. Data are summarized by means and standard deviations or percentages. Calculating Spearman rank correlation coefficients separately for cases and controls assessed correlation of apoM with other risk factors. The association of apoM concentration with coronary events was evaluated using conditional logistic regression models, and the odds ratios (ORs) are expressed per 1 standard deviation (SD). Age, sex, and, for the FINRISK samples, study area were controlled for by stratification according to the matched case-control design. Hypertension, smoking, and diabetes were included as covariates in second-stage models.

RESULTS

Analysis of apoM concentration

The FINRISK '92-based study included 85 individuals who developed a CHD event during the follow-up period and 177 matched controls. Due to insufficient amounts of sample, apoM levels could be measured in 80 cases and 175 controls. In the final study population, there were 73.8% men among the cases and 70.9% among the controls. The age range was 33–64 years in both groups (**Table 1).**

The CCHS-based study included 958 individuals who developed a CHD event during the follow-up period and 958 controls. ApoM levels and other clinical and laboratory data were available on 921 cases and 944 controls. Among cases, 59.2% were men; among controls, 59.3% were men. The age range was 33–90 years among the cases and 33–94 years among the controls (Table 1).

In both studies, the distribution of apoM concentration in the entire population, as well as among the cases and controls separately, was essentially Gaussian (data not

TABLE 1. Data for the FINRISK '92 and Copenhagen City Heart Study subjects included in the study

	FINRISK '92			CCHS		
	Cases with CHD, n = 80	Controls without CHD, n = 175	*P*	Cases with CHD, n = 921	Controls without CHD, n = 921	*P*
Age (years)	55.7 ± 7.4	55.6 ± 7.3	0.89[a]	69.0 ± 9.35	68.9 ± 9.43	0.90[a]
Men (%)	73.8%	70.9%	0.63[b]	59.2%	59.3%	0.95[b]
Hypertensive (%)	76.3%	65.7%	0.09[b]	74.3%	67.3%	0.001[b]
Smokers (%)	35.0%	26.3%	0.15[b]	49.7%	48.1%	0.48[b]
Diabetic (%)	13.8%	4.0%	0.005[b]	9.0%	3.6%	<0.0001[b]
BMI (kg/m^2)	29.6 ± 4.50	27.7 ± 4.55	0.0009[a]	26.6 ± 4.44	26.2 ± 4.32	0.007[a]
Cholesterol (mmol/l)	6.26 ± 1.19	6.00 ± 1.14	0.008[a]	6.50 ± 1.34	6.35 ± 1.21	0.03[a]
HDL-C (mmol/l)	1.14 ± 0.33	1.34 ± 0.37	<0.0001[a]	1.43 ± 0.48	1.58 ± 0.50	<0.0001[a]
Non-HDL-C (mmol/l)	5.13 ± 1.19	4.67 ± 1.10	0.0002[a]	5.07 ± 1.39	4.77 ± 1.26	<0.0001[a]
Triglyceride (mmol/l)	2.35 ± 1.32	1.73 ± 1.03	<0.0001[a]	2.30 ± 2.36	1.81 ± 0.87	<0.0001[a]
Cholesterol/HDL-C ratio	5.92 ± 2.01	4.78 ± 1.43	<0.0001[a]	5.08 ± 3.61	4.37 ± 1.51	<0.0001[a]
Systolic bp (mm Hg)	146.2 ± 20.8	143.8 ± 20.4	0.32[a]	148.1 ± 22.7	146.7 ± 22.2	0.13[a]
Diastolic bp (mm Hg)	86.9 ± 12.4	85.8 ± 11.4	0.62 [a]	84.8 ± 12.6	85.9 ± 12.1	0.05[a]
ApoA-I (mg/dl)	n.a.	n.a.	n.a.	134.9 ± 28.7	143.1 ± 28.5	<0.0001[a]
ApoB (mg/dl)	n.a.	n.a.	n.a.	95.6 ± 23.1	90.1 ± 22.1	<0.0001[a]
ApoM (μmol/l)	1.31 ± 0.29	1.34 ± 0.30	0.44[a]	1.34 ± 0.32	1.37 ± 0.33	0.12[a]

BMI, body mass index; apoA-I, apolipoprotein A-I; HDL-C, HDL-cholesterol. Data are given as mean ± SD or proportion.
[a] Wilcoxon rank sum test.
[b] χ^2 test.

TABLE 2. Results from correlation studies of apoM (μmol/l) with age and other analytes

Parameter	FINRISK '92		CCHS	
	Cases with CHD, n = 80	Controls without CHD, n = 175	Cases with CHD, n = 921	Controls without CHD, n = 944
Age (years)	−0.13	−0.14	−0.13[c]	−0.11[b]
BMI (kg/m^2)	−0.15	−0.11	−0.14[c]	−0.23[c]
Cholesterol (mmol/l)	0.31[b]	0.53[c]	0.43[c]	0.44[c]
HDL-C (mmol/l)	0.45[c]	0.48[c]	0.33[c]	0.41[c]
Non-HDL-C (mmol/l)	0.11	0.35[c]	0.28[c]	0.26[c]
Triglyceride (mmol/l)	−0.23[a]	−0.09	−0.02	−0.002
Chol/HDL-C-ratio	−0.13	−0.16[a]	−0.07[a]	−0.12[b]
ApoA-I (mg/dl)	n.a.	n.a.	0.38[c]	0.45[c]
ApoB (mg/dl)	n.a.	n.a.	0.29[c]	0.27[c]

CHD, coronary heart disease. ApoM was analyzed by ELISA. Other data are from the FINRISK '92 and Copenhagen City Heart Study (CCHS).
Using the Spearman rank-based correlation,
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.0001$.

shown). There were no significant differences in mean apoM concentration between cases and controls (Table 1).

ApoM levels were associated with HDL and total cholesterol for both cases and controls in both the FINRISK and the CCHS samples (**Table 2**). There were also associations between apoM levels and apoA-I as well as with apoB in the CCHS samples (Table 2). In the CCHS, there was a weak but significant negative correlation between body mass index and apoM (Tables 2 and 3).

In conditional logistic regression analyses, apoM was not a predictor of CHD events [OR (95% CI) 0.97 (0.74–1.27, $P = 0.81$) in the FINRISK samples and 0.92 (0.84–1.01, $P = 0.064$) in the CCHS samples (**Table 3**)]. The ORs are presented per 1 SD. In the second-level model (Model B), where smoking, hypertension, and diabetes had been added as covariates, the OR (95% CI) was 1.01 (0.77–1.33, $P = 0.95$) for the FINRISK samples and 0.93 (0.84–1.02, $P = 0.126$) for the CCHS samples (Table 3). Established CHD risk factors, such as total cholesterol, non-HDL-cholesterol, cholesterol/HDL ratio, and also apoA-I and apoB, behaved as expected, showing significant ORs (Table 3).

DISCUSSION

ApoM is mainly associated with HDL, with smaller amounts present in LDL and VLDL (8, 9). Despite the predominant association with HDL, the concentration of apoM in blood shows a strong association with both HDL- and LDL-cholesterol as well as with total cholesterol (10). Studies of apoM in mice have suggested apoM to be important for the formation of pre-β-HDL and for the efflux of cholesterol from macrophage foam cells to HDL (9, 15, 16). In addition, two of the referred studies showed that apoM-containing HDL was able to protect LDL against oxidation, and the third reported a delay of oxidation of HDL in transgenic mice overexpressing human apoM. Pre-β-HDL is the precursor of mature HDL and is con-

TABLE 3. CHD events during follow-up in subjects from FINRISK '92 and the Copenhagen City Heart Study

Variable	FINRISK '92, n = 255			CCHS, n = 1,865		
	Model A[a] OR (95% CI)	Model B[b] OR (95% CI)	SD	Model A[a] OR (95% CI)	Model B[b] OR (95% CI)	SD
ApoM (μmol/l)	0.97 (0.74–1.27)	1.01 (0.77–1.33)	0.30	0.92 (0.84–1.01)	0.93 (0.84–1.02)	0.32
BMI (kg/m^2)	1.50 (1.13–1.99)[c,d]	1.45 (1.09–.92)[c,d]	4.61	1.12 (1.02–1.23)[d]	1.08 (0.98–1.19)	4.38
Cholesterol (mmol/l)	1.41 (1.07–1.85)[d]	1.39 (1.05–1.83)[d]	1.16	1.13 (1.03–1.25)[d]	1.12 (1.01–1.24)[d]	1.28
HDL-C (mmol/l)	0.61 (0.45–0.82)[e]	0.65 (0.47–0.89)[e]	0.37	0.71 (0.65–0.79)[f]	0.74 (0.67–0.82)[f]	0.50
Non-HDL-C (mmol/l)	1.61 (1.23–2.10)[e]	1.54 (1.18–2.02)[e]	1.14	1.27 (1.15–1.40)[f]	1.25 (1.14–1.37)[f]	1.33
Triglyceride(mmol/l)[c]	1.68 (1.29–2.18)[e]	1.58 (1.20–2.10)[e]	0.54	1.42 (1.29–1.57)[f]	1.36 (1.23–1.51)[f]	0.50
Chol/HDL-C-ratio	1.70 (1.33–2.17)[f]	1.59 (1.23–2.06)[e]	1.71	1.86 (1.58–2.21)[f]	1.78 (1.51–2.12)[f]	2.78
ApoA-I (mg/dl)	n.a.	n.a.	n.a.	0.72 (0.65–0.80)[f]	0.74 (0.67–0.82)[f]	28.87
ApoB (mg/dl)	n.a.	n.a.	n.a.	1.25 (1.17–1.40)[f]	1.25 (1.15–1.39)[f]	22.77

ORs expressed per 1 SD.
[a] Frequency matched by age and sex (in FINRISK '92, also for study area).
[b] Hypertension, smoking and diabetes were included as covariates in addition to Model A in a second-stage model.
[c] Log transformed.
[d] $P < 0.05$.
[e] $P < 0.01$.
[f] $P < 0.0001$.

sidered particularly anti-atherogenic because it mediates ABCA1-dependent efflux of cholesterol from macrophage foam cells. Furthermore, overexpression of apoM in LDL receptor-deficient mice challenged with a cholesterol-rich diet suggested apoM to have anti-atherogenic properties (15, 16). These observations, taken together with the well-established relationship between plasma total cholesterol and the risk of development of MI, prompted the present study of possible association between serum apoM levels and risk of CHD.

The samples of the FINRISK study were serum, whereas the CCHS study included both plasma and serum samples. It was observed that the concentrations of apoM were approximately 30% higher in serum than in plasma. This difference is larger than would be expected from the 10% dilution given by the citrate solution, suggesting that apoM may be released from blood cells during the coagulation process, as was suggested in a recent study (33). The plasma apoM levels in CCHS were similar to the earlier reported levels of apoM in plasma (mean 0.9 μmol/l) in a study (NOBIDA) of healthy individuals from the Nordic countries (10).

Correlations between apoM and total cholesterol, as well as LDL- and HDL-cholesterol, in both cases and controls, are in agreement with previous results (10). ApoM was also found to correlate with apoA-I and apoB, the major structural apolipoproteins of HDL and LDL, in both cases and controls.

In the two nested conditional logistic regression models, traditional risk factors such as cholesterol and triglyceride levels were identified, whereas HDL-cholesterol was associated with decreased risk. This suggests that the study design was appropriate for identification of risk factors as well as protective factors. Despite correlating strongly to cholesterol levels, apoM was not found to be a predictor of CHD, with ORs of 0.97 and 1.01 for the FINRISK samples and 0.92 and 0.93 for the CCHS samples. However, it should be mentioned that even though both studies suggest that the apoM levels are not predictive of CHD, the study design (in particular that of the CCHS study), with a mixture of both prevalent and incident cases, suggests that one cannot fully exclude an association between apoM levels and either prevalent or incident disease, which is a limitation of the study.

In conclusion, in this population-based nested case-control study, although apoM was associated with total and HDL-cholesterol as well as with both apoA-I and apoB, no association between apoM and CHD was found.

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REFERENCES

1. Wilson, P. W., R. J. Garrison, W. P. Castelli, M. Feinleib, P. M. McNamara, and W. B. Kannel. 1980. Prevalence of coronary heart disease in the Framingham Offspring Study: role of lipoprotein cholesterols. *Am. J. Cardiol.* **46:** 649–654.
2. Mensah, G. A., D. W. Brown, J. B. Croft, and K. J. Greenlund. 2005. Major coronary risk factors and death from coronary heart disease: baseline and follow-up mortality data from the Second National Health and Nutrition Examination Survey (NHANES II). *Am. J. Prev. Med.* **29:** 68–74.
3. Goldbourt, U., S. Yaari, and J. H. Medalie. 1997. Isolated low HDL cholesterol as a risk factor for coronary heart disease mortality. A 21-year follow-up of 8000 men. *Arterioscler. Thromb. Vasc. Biol.* **17:** 107–113.
4. von Eckardstein, A., J. R. Nofer, and G. Assmann. 2001. High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport. *Arterioscler. Thromb. Vasc. Biol.* **21:** 13–27.
5. Lewis, G. F., and D. J. Rader. 2005. New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circ. Res.* **96:** 1221–1232.
6. Cheung, M. C., and J. J. Albers. 1982. Distribution of high density lipoprotein particles with different apoprotein composition: particles with A-I and A-II and particles with A-I but no A-II. *J. Lipid Res.* **23:** 747–753.
7. Gotto, A. M., Jr., H. J. Pownall, and R. J. Havel. 1986. Introduction to the plasma lipoproteins. *Methods Enzymol.* **128:** 3–41.
8. Xu, N., and B. Dahlback. 1999. A novel human apolipoprotein (apoM). *J. Biol. Chem.* **274:** 31286–31290.
9. Christoffersen, C., L. B. Nielsen, O. Axler, A. Andersson, A. H. Johnsen, and B. Dahlback. 2006. Isolation and characterization of human apolipoprotein M-containing lipoproteins. *J. Lipid Res.* **47:** 1833–1843.
10. Axler, O., J. Ahnstrom, and B. Dahlback. 2007. An ELISA for apolipoprotein M reveals a strong correlation to total cholesterol in human plasma. *J. Lipid Res.* **48:** 1772–1780.
11. Raper, J., R. Fung, J. Ghiso, V. Nussenzweig, and S. Tomlinson. 1999. Characterization of a novel trypanosome lytic factor from human serum. *Infect. Immun.* **67:** 1910–1916.
12. Axler, O., J. Ahnstrom, and B. Dahlback. 2008. Apolipoprotein M associates to lipoproteins through its retained signal peptide. *FEBS Lett.* **582:** 826–828.
13. Ahnstrom, J., K. Faber, O. Axler, and B. Dahlback. 2007. Hydrophobic ligand binding properties of the human lipocalin apolipoprotein M. *J. Lipid Res.* **48:** 1754–1762.
14. Duan, J., B. Dahlback, and B. O. Villoutreix. 2001. Proposed lipocalin fold for apolipoprotein M based on bioinformatics and site-directed mutagenesis. *FEBS Lett.* **499:** 127–132.
15. Wolfrum, C., M. N. Poy, and M. Stoffel. 2005. Apolipoprotein M is required for prebeta-HDL formation and cholesterol efflux to HDL and protects against atherosclerosis. *Nat. Med.* **11:** 418–422.
16. Christoffersen, C., M. Jauhiainen, M. Moser, B. Porse, C. Ehnholm, M. Boesl, B. Dahlback, and L. B. Nielsen. 2008. Effect of apolipoprotein M on high density lipoprotein metabolism and atherosclerosis in low density lipoprotein receptor knock-out mice. *J. Biol. Chem.* **283:** 1839–1847.
17. Niu, N., X. Zhu, Y. Liu, T. Du, X. Wang, D. Chen, B. Sun, H. F. Gu, and Y. Liu. 2007. Single nucleotide polymorphisms in the proximal promoter region of apolipoprotein M gene (apoM) confer the susceptibility to development of type 2 diabetes in Han Chinese. *Diabetes Metab. Res. Rev.* **23:** 21–25.
18. Jiao, G. Q., Z. X. Yuan, Y. S. Xue, C. J. Yang, C. B. Lu, Z. Q. Lu, and M. D. Xiao. 2007. A prospective evaluation of apolipoprotein M gene T-778C polymorphism in relation to coronary artery disease in Han Chinese. *Clin. Biochem.* **40:** 1108–1112.
19. Xu, W. W., Y. Zhang, Y. B. Tang, Y. L. Xu, H. Z. Zhu, A. Ferro, Y. Ji, Q. Chen, and L. M. Fan. 2007. A genetic variant of apolipoprotein M increases susceptibility to coronary artery disease in a Chinese population. *Clin. Exp. Pharmacol. Physiol.* **35:** 546–551.
20. Venteclef, N., A. Haroniti, J. J. Tousaint, I. Talianidis, and P. Delerive. 2008. Regulation of anti-atherogenic apolipoprotein M gene expression by the orphan nuclear receptor LRH-1. *J. Biol. Chem.* **283:** 3694–3701.
21. Venteclef, N., J. C. Smith, B. Goodwin, and P. Delerive. 2006. Liver receptor homolog 1 is a negative regulator of the hepatic acute-phase response. *Mol. Cell. Biol.* **26:** 6799–6807.
22. Freeman, L. A., A. Kennedy, J. Wu, S. Bark, A. T. Remaley, S. Santamarina-Fojo, and H. B. Brewer, Jr. 2004. The orphan nuclear receptor LRH-1 activates the ABCG5/ABCG8 intergenic promoter. *J. Lipid Res.* **45:** 1197–1206.
23. Kannel, W. B., W. P. Castelli, T. Gordon, and P. M. McNamara. 1971.

Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. *Ann. Intern. Med.* **74:** 1–12.
24. Kannel, W. B., J. D. Neaton, D. Wentworth, H. E. Thomas, J. Stamler, S. B. Hulley, and M. O. Kjelsberg. 1986. Overall and coronary heart disease mortality rates in relation to major risk factors in 325,348 men screened for the MRFIT. Multiple Risk Factor Intervention Trial. *Am. Heart J.* **112:** 825–836.
25. Salomaa, V., V. Rasi, J. Pekkanen, E. Vahtera, M. Jauhiainen, E. Vartiainen, G. Myllyla, and C. Ehnholm. 1994. Haemostatic factors and prevalent coronary heart disease; the FINRISK Haemostasis Study. *Eur. Heart J.* **15:** 1293–1299.
26. Salomaa, V. V., V. P. Rasi, E. M. Vahtera, J. Pekkanen, M. Pursiainen, M. Jauhiainen, E. Vartiainen, C. P. Ehnholm, and G. Myllyla. 1994. Haemostatic factors and lipoprotein (a) in three geographical areas in Finland: the Finrisk Haemostasis Study. *J. Cardiovasc. Risk.* **1:** 241–248.
27. Vartiainen, E., P. Jousilahti, G. Alfthan, J. Sundvall, P. Pietinen, and P. Puska. 2000. Cardiovascular risk factor changes in Finland, 1972–1997. *Int. J. Epidemiol.* **29:** 49–56.
28. Schnohr, P., G. Jensen, P. Lange, H. Scharling, and M. Appleyard. 2001. The Copenhagen City Heart Study, Øesterbroundersoegelsen, tables with data from the third examination 1991–1994. *Eur. Heart J.* **3 (Suppl.):** 1–83.
29. Appleyard, M., A. T. Hansen, G. Jensen, P. Schnorh, and J. Nyboe. 1989. The Copenhagen City Heart Study. Øesterbroundersoegelsen. A book of tables with data from the first examination (1976–78) and a five year follow-up (1981–83). The Copenhagen City Heart Study Group. *Scand. J. Soc. Med. Suppl.* **41:** 1–160.
30. Pajunen, P., H. Koukkunen, M. Ketonen, T. Jerkkola, P. Immonen-Raiha, P. Karja-Koskenkari, M. Mahonen, M. Niemela, K. Kuulasmaa, P. Palomaki, et al. 2005. The validity of the Finnish Hospital Discharge Register and Causes of Death Register data on coronary heart disease. *Eur. J. Cardiovasc. Prev. Rehabil.* **12:** 132–137.
31. Salomaa, V., V. Rasi, S. Kulathinal, E. Vahtera, M. Jauhiainen, C. Ehnholm, and J. Pekkanen. 2002. Hemostatic factors as predictors of coronary events and total mortality: The FINRISK '92 Hemostasis Study. *Arterioscler. Thromb. Vasc. Biol.* **22:** 353–358.
32. Julian, D. G., M. E. Bertrand, Å. Hjalmarson, K. Fox, M. L. Simoons, L. Ceremuzynski, A. Maseri, T. Meinertz, J. Meyer, K. Pyörälä, et al. 1997. Management of stable angina pectoris—recommendations of the task force of the European Society of Cardiology. *Eur. Heart J.* **18:** 394–413.
33. Bengtsson, T., H. Karlsson, P. Gunnarsson, C. Skoglund, C. Elison, P. Leanderson, and M. Lindahl. 2008. The periodontal pathogen Porphyromonas gingivalis cleaves apoB-100 and increases the expression of apoM in LDL in whole blood leading to cell proliferation. *J. Intern. Med.* **263:** 558–571.