The Arg82Cys polymorphism of the protein nepmucin implies a role in HDL metabolism

Sophia Metz, Nikolaj T. Krarup, Thomas Bryrup, Julie Støy, Ehm A. Andersson, Christina Christoffersen, Matt J Neville, Malene R Christiansen, Anna E Jonsson, Daniel R Witte, Ulla Kampmann, Lars B Nielsen, Niklas R. Jørgensen, Fredrik Karpe, Niels Grarup, Oluf Pedersen, Tuomas O. Kilpeläinen, Torben Hansen

*Equal contribution

1. Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
2. Aalborg University Hospital, Department of Cardiology, Aalborg, Denmark
3. Aarhus University Hospital, Steno Diabetes Center Aarhus, Aarhus, Denmark
4. Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark
5. Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
6. Oxford Centre for Diabetes, Endocrinology & Metabolism, Oxford, UK
7. Oxford NIHR Biomedical Research Centre, Churchill Hospital, Oxford, UK
8. Department of Public Health, Section of Epidemiology, Aarhus University, Denmark
9. Faculty of Health, Aarhus University, Aarhus, Denmark
10. Institute of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen Denmark
11. Faculty of Health, University of Southern Denmark, Odense, Denmark

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# Corresponding author and reprint requests

Torben Hansen, Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3B, Room 07-8-26, 2200 København, email: torben.hansen@sund.ku.dk

ORCID: 0000-0001-8748-3831

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Disclosure summary:

TH and OP own stock in Novo Nordisk Inc.. The other authors declare no competing interests.
Abstract

Background

Blood lipid levels are linked to the risk of cardiovascular disease and regulated by genetic factors. A low-frequency polymorphism Arg82Cys (rs72836561) in the membrane protein nepmucin, encoded by CD300LG, is associated with lower fasting concentration of HDL-cholesterol and higher fasting triglycerides. However, whether the variant is linked to postprandial lipids and glycemic status remains elusive. Here, we augment the genetic effect of Arg82Cys on fasting plasma concentrations of HDL subclasses, postprandial lipemia after a standardized high-fat meal, and glycemic status to further untangle its role in HDL-metabolism.

Methods

Therefore, we elucidated fasting associations with HDL subclasses in a population-based cohort study (Oxford Biobank, OBB), including 4,522 healthy men and women. We investigated fasting and postprandial consequences on HDL metabolism in recall-by-genotype (RbG) studies (fasting: 20 carrier/20 non-carrier; postprandial: 7 carrier/17 non-carrier), and shed light on the synergistic interaction with glycemic status.

Results

A lower fasting plasma concentration of cholesterol in large HDL particles was found in healthy male carriers of the Cys82 polymorphism compared to non-carriers, both in the OBB (P=0.004) and RbG studies (P=0.005). Additionally, the Cys82 polymorphism was associated with low fasting plasma concentrations of ApoA1 (P=0.008) in the OBB cohort. On the contrary, we did not find differences in postprandial lipemia or 2 hour plasma glucose levels.
Conclusion

Taken together, our results indicate an association between the Arg82Cys variant and a lower concentration of HDL particles and HDL cholesterol, especially in larger HDL subclasses, suggesting a link between nepmucin and HDL cholesterol metabolism or maturation.

**Keywords:** Lipid Metabolism; Cholesterol Transport; Human Genetics; Apolipoproteins; Triglycerides; Metabolism

**Abbreviations:** Small HDL (S-HDL), medium HDL (M-HDL), large HDL (L-HDL), extra-large HDL (XL-HDL)
Introduction

Dyslipidemia is a major risk factor for cardiovascular disease (CVD) \(^1\text{-}\(^5\)). Higher blood levels of low-density lipoprotein cholesterol (LDLc) and triglycerides (TG) are associated with an increased CVD risk, whereas higher levels of high-density lipoprotein cholesterol (HDLc) are associated with a lower risk. The protective effect of HDLc has been primarily attributed to the beneficial role of HDLc in reverse cholesterol transport \(^6\) where free cholesterol is transported via HDL particles from peripheral tissues to the liver, and cholesterol esters and TGs are exchanged between HDL and very low-density lipoprotein (VLDL), LDL, and other apolipoprotein B containing particles \(^7\text{-}\(^8\)). HDL incorporates a range of particle sizes with different properties for transporting circulating cholesterol \(^9\). The main HDL categories based on diameter include very large (XL) and large (L) particles, also called HDL2 particles, and medium (M) and small (S) particles, also called HDL3 particles. Particle size may affect the cardioprotective potential of HDL, with several studies suggesting that the cardioprotective characteristics are harbored by the smaller HDL3 particles \(^10\text{-}\(^12\)). However, the literature remains controversial in this regard \(^13\).

Genome-wide association studies (GWAS) harbor great potential to identify new targets involved in HDL metabolism. In recent GWAS, the minor Cys82 polymorphism (frequency 3% in European ancestry) of the missense variant Arg82Cys (rs72836561) in \(CD300LG\) has been associated with lower fasting plasma HDLc \(^14\text{-}\(^18\)) and higher plasma triglyceride (TG) levels \(^14\). The Arg82Cys variant is predicted as “damaging” by structural prediction tools (Polyphen-2 and MuPred-2), indicating that the variant hampers \(CD300LG\) function \(^19\text{-}\(^20\)). \(CD300LG\) encodes nepmucin \(^21\), a protein enriched in capillary endothelial cells \(^22\text{-}\(^23\)). The Arg82Cys polymorphism is located in the immunoglobulin (Ig) domain of nepmucin that reaches into the extracellular matrix. Nepmucin has been implicated in lymphocyte trafficking, in particular lymphocyte rolling and binding through its mucin-like and Ig-domain, as well as in trans-endothelial migration \(^24\text{-}\(^26\)). However, a role of nepmucin in lipid homeostasis remains unclear.
To shed light on the role of nepmucin in lipid homeostasis, we carried out population-based genetic association studies to investigate the nepmucin Arg82Cys polymorphism and fasting plasma HDL subclasses. Furthermore, we performed recall-by-genotype (RbG) studies to detect additional subtle abnormalities in lipid metabolism that could not be detected in a fasting state alone, and studied synergistic interactions of Cys82 with glycemic status (Study overview in Figure 1).

Material and methods

**Study I – Nepmucin Arg82Cys variant and fasting plasma HDL subclasses in 4,522 British men and women**

HDL subclasses were measured by nuclear magnetic resonance (NMR)-spectroscopy in 4,522 individuals from the Oxford Biobank (OBB). The OBB is a cohort of healthy, normoglycemic European ancestry men and women aged 30 to 50 years, randomly selected from the Oxfordshire area. A high-throughput proton NMR platform containing ~230 metabolites has been performed on ~7100 OBB plasma samples. The quantified HDL subclasses are defined as XL-HDL (average particle diameter 14.3 nm), L-HDL (12.1 nm), M-HDL (10.9 nm) and S-HDL (8.7 nm). The NMR platform has been used in multiple epidemiological and genetic studies and the details of the method have been described elsewhere.

**Study II – Nepmucin Arg82Cys variant and fasting plasma lipid variables in 20 non-carriers + 20 carriers combined in a meta-analysis with 17 non-carriers and 7 carriers (from Study III)**

Here, we performed a meta-analysis of the associations with fasting plasma HDL subclasses in 20 healthy male heterozygous carriers of the Cys82 variant and 20 matched non-carriers and the baseline/fasting results from study III (7 heterozygous carriers of Cys82, and 17 non-carriers). The 20 Cys82 carriers and matched non-carriers were examined on two separate days as previously described, and biochemical profiling was performed on the first examination day. The study protocol was approved by the Ethical Committee of Central Denmark Region (protocol number 1-10-
Study III – A Recall-by-Genotype study: Lipid meal challenge in 24 Danish men

We recruited 24 middle-aged healthy Danish men by genotype from the population-based Health2006 cohort \(^3\) (n=18) and among staff at the University of Copenhagen (n=6). Of the 24 participants, 7 were heterozygous carriers of the nepmucin Cys82 variant and 17 were non-carriers (Table 1). The carriers and non-carriers were in silico matched by age, BMI, fasting glucose and HbA1c concentrations. The genotypes of the study participants were not blinded to the examining personnel. The study was approved by the regional Ethical Committee of Copenhagen (protocol-number: H-1-12012-136) and conducted in accordance with the principles of the Helsinki Declaration II. Written informed consent was obtained from all study participants.

The participants underwent a six-hour standardized lipid-rich meal challenge after a minimum of eight hours overnight fast. The meal consisted of a lipid-rich soup of water, chicken, leek, butter and cream (80 grams of saturated fat) in a volume of 675 ml. Total energy content was 4459 kJ (1065 kcal). The fat energy percentage was 66%, carbohydrate 16%, and protein 18%. One gram of acetaminophen was added in the meal to estimate gastric emptying time \(^3\). All participants consumed the entire meal within a 15-minute period. The participants were instructed to avoid excessive alcohol intake, smoking and physical activity 48 hours prior to the examination. Height, weight, waist and hip circumferences were measured in light indoor clothes. Waist circumference was measured half-way between the rib cage and the superior iliac spine. Hip circumference was measured at the level of the major femoral trochanter. Blood samples were taken every 30 minutes during the first two hours and subsequently every hour during the last four hours of the meal challenge.
Study IV – Interaction between the Arg82Cys polymorphism and glycemic status on fasting plasma HDL subclasses

The interaction between the Arg82Cys polymorphism and glycemic status on fasting plasma lipid levels was tested in 1330 Danish men and women from the AdditionPro study, of whom 831 were normoglycemic (2-hour plasma glucose < 7.8 mmol/l, fasting blood glucose (FBG) < 6.1 mmol/l) and 499 hyperglycemic (2-hour plasma glucose > 7.8 mmol/l, FBG > 6.1 mmol/l). Fasting plasma lipids were measured using a high-throughput serum NMR platform. The study protocol was approved by the Ethical Committee of Central Denmark Region (no: 20000183) and undertaken in accordance with the principles of the Helsinki declaration II. All participants gave written informed consent.

Biochemical measurements in studies II and III

Plasma glucose in studies II and III was measured by a glucose-oxidase method (Vitros 5600, Ortho Clinical Diagnostics, Parc d’Innovation, Cedex, France). Serum insulin and C-peptide were measured by electrochemistry luminescence-immunoassay (Roche Diagnostics GmbH., Mannheim, Germany). Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR). ApoB48 and apoB100 were measured in EDTA-plasma samples using the enzyme-linked immunosorbent assay-method (ELISA) (Shibayagi Co., Ltd. and Immuno-Biological Laboratories Co., Ltd., respectively). Double-standards were measured for each run. Intra- and inter-assay variation ranged from 3.5% to 5.6% and 2.8% to 8.6%, respectively. Plasma levels of TG, HDLc and total cholesterol (TC) were measured on a Vitros 5.1 Chemistry System (Ortho Clinical Diagnostics, NY, USA). In the fasting state, LDLc levels were calculated according to the Friedewald formula and VLDLc was derived thereof. Ultracentrifugation was performed by adjusting plasma (950 µl) to the density of 1.063 g/l (separation of VLDL/LDL and total HDL) or 1.12 g/l (separation of VLDL/LDL/HDL2 and HDL3) using NaBr. Ultracentrifugation was performed at 50,000 rpm at 4°C for 16 hours using a Beckman Ti 50.3 rotor and a Beckman Optima LE-80K ultracentrifuge (Beckman Coulter, Inc., Fullerton, CA). Cholesterol and TG were measured using enzymatic kits (CHOD-PAP from Roche...
Applied Sciences and GPO-Trinder from Sigma, respectively) in all fractions of plasma samples. The amounts of HDL2-cholesterol and TG were calculated as total HDL (d>1.063) minus HDL3 (d<1.12). The values were corrected for recovery of either cholesterol or triglycerides at each density. Postprandial plasma VLDLc and LDLc were not included as outcome traits due to their high levels of TGs.

Genotyping

The rs72836561 (Arg82Cys) polymorphism of CD300LG was genotyped on the Illumina HumanExome Beadchip 12v1_A and the genotypes were called using GenCall, Genotyping module (version 1.9.4) or GenomeStudio software (version 2011.1, Illumina). Samples were excluded if they showed a low call rate, abnormal mean heterozygosity, high singleton count, non-European ancestry, sex discrepancy, or duplicate discordance. Genetic variants were excluded if they showed a low call rate, deviation from Hardy-Weinberg equilibrium, duplication, chromosome or allele mismatch, GenTrain score <0.6, cluster separation score <0.4, or a deviation in manual cluster checks. Missing genotypes were subsequently re-called using zCall and a second round of QC was performed to exclude poor quality samples and variants.

Statistical analysis

The analyses were performed using R (versions 2.13.2 and 3.4.3) and GraphPad Prism (version 8.3.0). In Study I, the association between Arg82Cys and blood lipids was examined by linear regression using an additive genetic model, adjusting for age, sex and BMI. Meta-analyses of aggregate data of the association of the Arg82Cys variant with fasting HDL subclasses (study II and III) were performed using inverse variance-weighted meta-analysis from linear regression analyses adjusted for age and BMI, using the R package meta and the function forest.meta, where heterogeneity was assessed by $I^2$ and the p-value for Cochran’s Q test 41. If p>0.10 in Cochran’s Q test, we applied a fixed effects model. If p<0.10, a random effects model was employed. In studies II and III, a significance level of a
p-value equal to or below 0.05 was defined as statistically significant. In study III, the associations between Arg82Cys polymorphism and postprandial plasma lipid levels were tested by linear regression analyses of the incremental area-under-the-curve (AUC) and by unpaired t-tests between genotype-groups for specific time-intervals. Incremental AUC was calculated using the trapezoidal method \(^{42,43}\). In Study IV, the interaction between the Arg82Cys variant and glycemic status (hyperglycemic vs. normoglycemic) on lipid levels was tested by incorporating an interaction term in linear regression models. The results in study I and IV were corrected for multiple testing by Bonferroni correction (\(P_{\text{CORRECTED}}=0.05 / 17 = 0.003\)).

Results

**Association of Arg82Cys variant with HDL subclasses in 4,522 British men and women**

To elucidate the association of Arg82Cys with lipids in HDL subclasses in a fasting state, we examined associations with HDL, HDLc, HDL cholesterol-esters, and ApoA1 concentrations measured by NMR spectroscopy in 4,522 men and women from the OBB cohort (study I). The results are given as effect-per-allele from the additive genetic model. We found that the Cys82 variant was associated with 0.037 mg/L lower ApoA1 levels \((p=0.008)\), 0.037 nm lower HDL mean diameter \((p=0.003)\), 0.041 µmol/L lower HDL2 cholesterol \((p=0.004)\), and 0.040 µM lower HDL3 cholesterol \((p=0.024)\) per allele (Table 2). In analyses stratified by HDL particle size, we found that the Cys82 variant was associated with 0.035 nmol/L lower concentration of M-HDL particles \((p=0.01)\), 0.041 nmol/L lower concentration of L-HDL particles \((p=0.003)\), and 0.038 nmol/L lower concentration of XL-HDL particles \((p=0.005)\) per allele. Similarly, the Cys82 variant was associated with 0.034 µmol/L lower concentration of M-HDLc \((p=0.02)\), 0.042 µmol/L lower concentration of L-HDLc \((p=0.001)\), and 0.027 µmol/L lower concentration of XL-HDLc \((p=0.05)\), as well as with 0.034 µmol/L lower concentration of M-HDL cholesterol esters \((p=0.02)\) and 0.051 µmol/L lower concentration of L-HDL cholesterol esters \((p=0.0004)\) per allele. We found no association between Cys82 carriers and concentration of S-HDL particles, suggesting that the Cys82 variant specifically reduces the levels of larger HDL particles.
particles (Figure 2). We did not find an association with HOMA-IR, ApoB, nor LDL cholesterol (Table S1, available from 44).

Association of nepmucin Arg82Cys with fasting plasma lipid levels and HDL subclasses in a meta-analysis of 64 Danish men

Based on results from studies II and baseline results from study III (including 27 heterozygous Cys82 carriers and 37 non-carriers (Table 3 and 4), we performed a meta-analysis to test whether there are differences between Cys82 carriers and non-carriers in relation to fasting TG, TC, LDLc, HDLc, or HDL subclasses measured with ultracentrifugation. We found that HDL2 cholesterol was 0.3 mmol/L lower ($p=0.005$) and the HDL2/HDL3 cholesterol ratio was 0.5 units lower ($p=0.0004$) in Cys82 carriers than in non-carriers (Table 4). Furthermore, the plasma HDL3 TG was 0.01 mmol/L higher in Cys82 carriers than in non-carriers ($p=0.0003$), whereas there was no difference in plasma HDL2 TG content ($p=0.1$). We also found that HDL2/HDL3 TG ratio was 1.25 units lower in Cys82 carriers than in non-carriers ($p=0.002$).

Association of nepmucin Arg82Cys polymorphism with postprandial lipemia

To test whether the Arg82Cys genotype affects postprandial fluctuation of blood lipids in a time-dependent manner, we examined differences between 7 heterozygous Cys82 variant carriers and 17 non-carriers in relation to AUCs for HDLc, TG, total cholesterol (TC), apoB48 and apoB100 (Figure 3A) during a lipid-rich meal challenge (study III). The baseline characteristics were similar between the carriers and non-carriers of the Cys82 variant (Table 1). We found no significant differences in the AUCs or lipid levels at specific time points between Cys82 carriers and non-carriers during the meal challenge (Figure 3B-F and Table 5).
Interaction of Arg82Cys variant with glycemic status on serum lipid levels

Previous studies have suggested an association of the Cys82 variant with abnormal glucose metabolism, which could be mechanistically linked to the association between Arg82Cys and lipid levels. To test whether glycemic status modifies the association between Arg82Cys and HDL subclasses, we tested the interaction with glycemic status among 1330 participants of the Danish Addition-Pro cohort (study IV). We stratified the participants based on 2-hour plasma glucose and FBG concentration into a normoglycemic (normal-GT) group (n=831) and a hyperglycemic (abnormal-GT) group (n=499). No significant interaction between the Cys82 variant and glycemic status was found (p > 0.05 for interaction, data not shown).

Discussion

Nepmucin is a type-I membrane protein expressed in vascular endothelial cells that shows high expression in placental tissue, skeletal muscle and adipose tissue. The Arg82Cys nepmucin polymorphism was originally identified in an exome sequencing study for association with lower fasting plasma HDL cholesterol and higher fasting plasma TG concentration, and the association has been replicated in subsequent fasting and (for HDL) non-fasting studies. Here, we studied association between the Cys82 polymorphism and fasting plasma concentrations of HDL and its subclasses, to draw a more detailed image of its role in lipid metabolism. Furthermore, we assessed postprandial changes after a high-fat stimulus, to detect additional subtle abnormalities in lipid metabolism that could not be detected in a fasting state, and evaluated potential synergistic effects of Cys82 with glucose metabolism on fasting lipid levels.

Our analyses in the OBB cohort, applying an additive genetic model in 4,522 British individuals on HDL subclasses measured by NMR-spectroscopy, showed that Cys82 is associated with a smaller HDL diameter and lower cholesterol concentration in M, L and XL-HDL particles. The associations were strongest in the L-HDL subclass. In a meta-analysis of fasting lipids from two RbG studies, including a total of 27 carriers of the Cys82 polymorphism and 37 non-carriers, we observed lower cholesterol...
levels in plasma HDL2 particles and higher TG levels in plasma HDL3 particles of Cys82 carriers. Our results are concordant with a published GWAS of HDL subclasses measured by NMR spectroscopy in up to 24,925 individuals \(^1\), where the Cys82 variant was associated with lower concentrations of L and M-HDL particles \(^1\) (Table S2, available from \(^4\)). Interestingly, smaller HDL diameter has been associated with adverse cardiometabolic outcomes \(^12,52,53\). In line with this, the Cys82 polymorphism reached nominal significance in the latest GWAS for cardiovascular outcomes (beta: 0.057, P: 2.2E-3) \(^5\) (Table S2, available from \(^4\)), and is linked to increased atherosclerosis (P: 4.18E-6), and peripheral artery disease in FINNGEN (P: 2.73E-5 / unpublished data, URL: https://r5.finngen.fi/variant/17-43848758-C-T). Furthermore, we found the Arg82 polymorphism to be linked to decreased ApoA1 level - a protein component of HDL involved in lipid metabolism (Table 2), which has previously been linked to cardioprotective properties \(^11\).

In our study of 24 Danish men who were challenged with an oral lipid load we did not see a difference in postprandial lipemia between Cys82 carriers and non-carriers. While the study sample size was limited, the RbG design enhanced statistical power by including a relatively large proportion of carriers with the rare allele. We estimated to have 90% statistical power to exclude an allele-dependent reduction in postprandial HDL plasma levels exceeding 0.17 mmol/L in a test of unpaired (α=0.05). This may suggest that the link between Arg82Cys and lipemia is subjected to a fasting state and effects may be obscured in a postprandial state.

A previous RbG study among 42 healthy male carriers and 20 non-carriers of the Cys82 variant showed an association with lower CD300LG mRNA expression in muscle and white adipose tissue, as well as with higher intramyocellular lipid content and forearm glucose uptake \(^34\). Overall, the findings suggested a role for nepmucin in the regulation of glucose and lipid homeostasis. We therefore hypothesized that the association of Arg82Cys with HDL subclasses might be modified by glycemic status. However, we found no epidemiological evidence of such interaction. These findings are
supported by a previous study from 2013, where Arg82Cys was not associated with glycemic traits (fasting glucose, 2h-OGTT, HbA1C) in a linear model 14.

Taken together, the results suggest that nepmucin is involved in lipid transport and lipoprotein maturation. As nepmucin has been shown to adhere to several polar lipids that are known to be present in HDL particles 9,10, we speculate that nepmucin could affect the maturation of HDL molecules from the small (lipid-poor) HDL to the very large spherical HDL particle (e.g. via lecithin cholesterol acyltransferase (LCAT), cholesterol ester transfer protein (CETP) and hepatic lipase (HL)) 7,55. Also, the low levels of ApoA1 in carriers of Arg82Cys may indicate decreased uptake of cholesterol and other lipids in HDL (and thus the general decrease in HDL content) or it may simply reflect smaller HDL particles 56. As ApoA1 is a cofactor for LCAT and thus, is involved in the formation and reverse transport of cholesterol esters, specific binding assays between nepmucin and HDL are needed to confirm the mechanistic basis for the link between nepmucin and HDL metabolism. Considering previous studies, suggesting that a reduction of HDL2 and a shift towards smaller HDL subclasses is linked to obesity 57, it may also be of interest to focus on an obese population in future studies.

Conclusion

Taken together, our results indicate an association between the Arg82Cys variant and a lower concentration of HDL particles and HDL cholesterol, especially in larger HDL subclasses, and lower ApoA1 level, suggesting a link between nepmucin and HDL cholesterol metabolism.
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Data Availability:

Some data generated or analyzed during this study are included in this published article or in the data repositories listed in Reference 44. Table S1 contains associations of Arg82Cys with HOMA-IR, APOB, and LDL in study I. Table S2 contains associations of Arg82Cys from previously published GWAS. Otherwise, restrictions apply to the availability of some data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some of the data may be provided.
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Legends for Tables and Figures:

**TABLE 1: Baseline characteristics of study II and III.** The table comprises mean ± SD for continuous variables and percentages for categorical variables (smoking, treatment status, and lifestyle). *HOMA-IR is calculated from levels of fasting plasma glucose (mmol/L) and fasting serum insulin (µIU/mL). Traits marked with an asterisk (*) show log-transformed effects and SE. β: T-allele effect size; SE: standard error.

**TABLE 2: Association of nepmucin Arg82Cys with HDL subclass in plasma in study I.** Association of nepmucin Arg82Cys with HDL subclass in study I (Oxford Biobank). The data presented are mean ± SD. The analyses were adjusted for age, sex and BMI. No information on HDL3 cholesteryl esters was available.

**TABLE 3: Association of nepmucin Arg82Cys polymorphism with fasting plasma lipid levels in studies II and III.** Meta-analysis of the association of nepmucin Arg82Cys with fasting plasma lipid levels in studies II and III. A total of 37 non-carriers (study II: 20, study III: 17) and 27 carriers (study II: 20, study III: 7) were analyzed. Traits marked with an asterisk (*) show log-transformed effects and SE. No significant heterogeneity between studies II and III was found.

**TABLE 4: Association of nepmucin Arg82Cys with fasting plasma of triglyceride and cholesterol in HDL particles in studies II and III.** Meta-analysis of the association of nepmucin Arg82Cys polymorphism with fasting content of triglyceride and cholesterol in HDL particles in studies II and III. Data from a total of 37 non-carriers (study II: 20, study III: 17) and 27 carriers (study II: 20, study III: 7) were analyzed separately and combined. Data is based on ultracentrifugation estimated fasting levels of cholesterol and triglyceride in HDL2 and HDL3 subclasses. No significant heterogeneity between study group II and III was identified. β: mean effect size; SE: standard error.
TABLE 5: Association of nepmucin Arg82Cys polymorphism with postprandial changes in plasma lipid levels in study III. The presented data are unadjusted. The analyses were done in 24 (17 (Arg82Arg), 7 (Arg82Cys)) individuals from study III. *ΔAUC: incremental area under curve.

FIGURE 1: Study overview of the included studies I – IV. Schematic representation of the included studies and respective analysis. The included number of non-carriers is marked in grey and heterozygous and homozygous risk-allele carriers are combined and presented in orange.

FIGURE 2: Graphical abstract of the main findings of the study. Schematic representation of the associations of the Arg82Cys polymorphism with fasting or postprandial HDL subclasses and HDL composition.

FIGURE 3: Changes in plasma levels of HDL cholesterol, triglycerides, total cholesterol, ApoB48, and ApoB100 in nepmucin Cys82 variant carriers and non-carriers during a lipid rich-meal. The figure shows the dynamic changes of the mean ± SEM of respective traits after a meal-challenge. A) Illustrates the measured values (HDLc, TGS, Tot. cholesterol, ApoB48 (chylomicrons), and ApoB100 (LDL). B) - D) show measurements of HDLc, TGS, and Tot. Cholesterol at 0, 30, 60, 90, 120, 180, 240, 300 and 360 minutes after a meal-challenge. E) and F) show apolipoproteins carried by chylomicrons (ApoB48) and LDL (ApoB100) at 0, 60, 120, 180, 240, 300 and 360 minutes. Grey represents non-carriers, orange represents variant carriers. The analyses were done in 24 (17 (Arg82Arg), 7 (Arg82Cys)) individuals from study III.
|                  | Study II |          | Study III |          |
|------------------|----------|----------|-----------|----------|
|                  | Arg82Arg | Arg82Cys | Arg82Arg  | Arg82Cys |
| N                | 20       | 20       | 17        | 7        |
| Age (years)      | 55.1 ± 9.0 | 55.0 ± 9.1 | 55 ± 12.1 | 52.4 ± 14.6 |
| Weight (kg)      | 79.7 ± 7.2 | 79.1 ± 9.4 | 93.1 ± 12.6 | 85.3 ± 8.7 |
| Body fat (%)     | 21.4 ± 3.8 | 21.5 ± 3.5 | 26.1 ± 5.4 | 23.3 ± 6.6 |
| Basal Metabolic Rate (kcal/day) | 1617 ± 133 | 1675 ± 201 | 2083 ± 263.6 | 2000 ± 175.3 |
| BMI (kg/m²)      | 24.6 ± 1.7 | 24.5 ± 2.4 | 28.5 ± 3.9 | 27.5 ± 3.6 |
| Waist/hip ratio  | 1.00 ± 0.04 | 0.99 ± 0.03 | 0.96 ± 0.06 | 0.99 ± 0.05 |
| HOMA-IR*         | 1.27 ± 0.53 | 1.55 ± 0.99 | 2.54 ± 1.58 | 2.33 ± 1.12 |
| Smoker (%)       | 5 %       | 25 %     | 59 %      | 25 %     |
| Antihypertensive treatment (%) | 5 %   | 10 %     | 24 %      | 25 %     |
| Lipid lowering therapy (%) | 0 %   | 0 %      | 0 %       | 0 %      |
| Alcohol intake > 6 units/week (%) | 50 % | 55 %     | 65 %      | 37 %     |
| Moderate physical activity > 4 hours/week (%) | 10 % | 15 %     | 71 %      | 63 %     |
|                      | Arg82Arg (mean±SD) | Arg82Cys (mean±SD) | Cys82Cys (mean±SD) | Effect per allele (β [95%CI]) | P_{add} |
|----------------------|---------------------|---------------------|---------------------|---------------------------|---------|
| **Total HDL (HDL2 + HDL3)** |                     |                     |                     |                           |         |
| ApoA1 (mg/L)         | 1.44±0.25           | 1.40±0.25           | 1.42±0.14           | -0.037 [-0.064 to 0.011]  | 0.008   |
| HDL Cholesterol (mmol/L) | 1.42±0.33           | 1.36±0.34           | 1.33±0.10           | -0.043 [-0.068 to 0.017]  | 0.001   |
| HDL Mean diameter (nm) | 9.83±0.27           | 9.79±0.28           | 9.66±0.12           | -0.037 [-0.060 to 0.014]  | 0.003   |
| **HDL2 (very large + large HDL)** |                     |                     |                     |                           |         |
| Cholesterol (µmol/L) | 920.2±306.8         | 872±316.2           | 808.9±98.05         | -0.041 [-0.065 to 0.016]  | 0.004   |
| **Very large HDL**    |                     |                     |                     |                           |         |
| Cholesterol (µmol/L) | 170.7±93.15         | 157±94.05           | 165.4±65.05         | -0.027 [-0.055 to 0.0007] | 0.05    |
| Cholesterol esters (µmol/L) | 125.1±65.23         | 115.5±65.16         | 129.8±39.04         | -0.024 [-0.052 to 0.0035] | 0.1     |
| Particle Concentration (nmol/L) | 289.2±175.2         | 265.9±184.3         | 204.3±109           | -0.038 [-0.063 to 0.012]  | 0.005   |
| **Large HDL**         |                     |                     |                     |                           |         |
| Cholesterol (µmol/L) | 315.3±188.3         | 291.9±195.6         | 216.4±112.8         | -0.042 [-0.066 to 0.018]  | 0.001   |
| Cholesterol esters (µmol/L) | 248.6±144.6         | 227.9±151.7         | 178.3±96.68         | -0.051 [-0.075 to 0.027]  | 0.0004  |
| Particle Concentration (nmol/L) | 887.2±441.6         | 821.3±450.3         | 598.8±176.2         | -0.041 [-0.065 to 0.018]  | 0.003   |
| **HDL3 (medium + small HDL)** |                     |                     |                     |                           |         |
|                               | Medium HDL | Small HDL |
|-------------------------------|------------|-----------|
| Cholesterol (µmol/L)          |            |           |
|                               | 523.8±32.29| 1.17±0.13 |
|                               | 495.4±48.96| NA        |
|                               | 505.1±48.22| NA        |
|                               | -0.040 [-0.068 to -0.011] | -0.0088 [-0.037 to 0.020] |
|                               | 0.02       | 0.7       |
| Cholesterol esters (µmol/L)   |            |           |
|                               | 451.2±105.4| NA        |
|                               | 434.3±100.5| NA        |
|                               | 421.2±18.82| NA        |
|                               | -0.034 [-0.062 to -0.0060] | -0.0087 [-0.037 to 0.020] |
|                               | 0.02       | 1         |
| Particle Concentration (nmol/L)| 366.1±83.24| 4.37±0.41 |
|                               | 353.4±80.36| 4.34±0.40 |
|                               | 343.2±10.47| 4.60±0.21 |
|                               | -0.035 [-0.063 to -0.0059] | -0.0087 [-0.037 to 0.020] |
|                               | 0.01       | 1         |
|                                      | Study II (n=40) | Study III (n=24) | Effect                                      |
|--------------------------------------|-----------------|------------------|---------------------------------------------|
|                                      | (β[SE])         | (β[SE])          | (β [95%CI], p-value)                         |
| HDL cholesterol                      | -0.08 (0.12)    | -0.22 (0.17)     | -0.12 [-0.32 to 0.07], 0.2                  |
| Triglycerides *                      | 0.10 (0.15)     | 0.11 (0.18)      | 0.11 [-0.12 to 0.33], 0.3                   |
| Total cholesterol                    | -0.29 (0.21)    | 0.18 (0.57)      | -0.23 [-0.61 to 0.15], 0.2                  |
| LDL cholesterol                      | -0.27 (0.20)    | 0.31 (0.48)      | -0.18 [-0.55 to 0.18], 0.3                  |
|                          | Study II (n=40) | Study III (n=24) | Fixed Effect |
|--------------------------|----------------|-----------------|--------------|
|                          | (β[SE])        | (β[SE])         | (β [95%CI], p-value) |
| **Cholesterol content in HDL2 (nmol/l)** | -0.24 [0.11]  | -0.31 [0.18]    | -0.26 [-0.45 to -0.08], 0.005 |
| **Triglyceride content in HDL2 (nmol/l)** | 0.002 [0.009] | -0.005 [0.003]  | -0.001 [-0.011 to 0.002], 0.1 |
| **Cholesterol content in HDL3 (nmol/l)** | 0.05 [0.04]   | 0.06 [0.05]     | 0.05 [-0.005 to 0.112], 0.07 |
| **Triglyceride content in HDL3 (nmol/l)** | 0.007 [0.005] | 0.008 [0.002]   | 0.01 [0.004 to 0.012], 0.0003 |
| **Cholesterol ratio (HDL2/HDL3)** | -0.74 [0.26]  | -0.45 [0.20]    | -0.57 [-0.88 to -0.25], 0.0004 |
| **Triglyceride ratio (HDL2/HDL3)**     | -0.87 [0.56]  | -1.64 [0.57]    | -1.25 [-2.03 to -0.47], 0.002 |
| Trait                              | Arg82Arg (mean ± SD) | Arg82Cys (mean ± SD) | Effect (β(95%CI)) | P-value |
|-----------------------------------|----------------------|----------------------|--------------------|---------|
| HDL cholesterol iAUC              | -0.7 ± 0.5           | -1.2 ± 1.5           | -0.5 (-1.3 to 0.3) | 0.2     |
| (Δmmol × L⁻¹ × 360 min⁻¹)         |                      |                      |                    |         |
| Triglycerides iAUC                | 6.0 ± 2.8            | 6.1 ± 3.0            | 0.08 (-2.5 to 2.6) | 1       |
| (Δmmol × L⁻¹ × 360 min⁻¹)         |                      |                      |                    |         |
| Total cholesterol iAUC            | -1.9 ± 1.5           | -4.7 ± 5.6           | -2.8 (-5.6 to 0.04)| 0.07    |
| (Δmmol × L⁻¹ × 360 min⁻¹)         |                      |                      |                    |         |
| ApoB48 iAUC                       | 194.7 ± 185.0        | 283.4 ± 82.2         | 88.7 (-55.2 to 232.6) | 0.2    |
| (Δng × ml⁻¹ × 360 min⁻¹)          |                      |                      |                    |         |
| ApoB100 iAUC                      | -0.9 ± 0.5           | -1.3 ± 1.1           | -0.43 (-1.1 to 0.19)| 0.2     |
| (Δµg × ml⁻¹ × 360 min⁻¹)          |                      |                      |                    |         |
FIGURE 1

Study I
Population based cohort study
Oxford Biobank

Study II
Recall-by-Genotype Study

Study III
Recall-by-Genotype Study

Study IV
Longitudinal cohort study
AdditionPRO

Non-carrier
Risk allele carrier (Cys82)

Association study
Arg82Cys and fasting blood lipids

Meta-analysis
Arg82Cys and fasting blood lipids
Baseline samples

Association study
Arg82Cys and postprandial blood lipids

Interaction study
Arg82Cys and glycemic status on fasting blood lipids
FIGURE 3

A) Cholesterol ester, Triglyceride, ApoA1, ApoB100, ApoB48, LDL, HDL, Chylomicron.

B) HDL-C [mmol/L] vs. time [min].

C) Triglyceride [mmol/L] vs. time [min].

D) Total Cholesterol [mmol/L] vs. time [min].

E) ApoB48 [ng/mL] vs. time [min].

F) ApoB100 [microg/mL] vs. time [min].

Symbols:
- non-carrier (Arg82)
- carrier (Cys82)