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Short communication

# Associations between common genetic polymorphisms in the liver X receptor alpha and its target genes with the serum HDL-cholesterol concentration in adolescents of the HELENA Study

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ABSTRACT

*Objective:* Genetic variability in the *NR1H3* gene (encoding LXRα) and in several of its target genes is associated with serum HDL-cholesterol (HDL-C) concentrations. We sought to assess if these associations could be detected in adolescents.
*Methods:* Thirty-nine polymorphisms in NR1H3, ABCA1, APOE, CETP, PLTP and LPL were analysed in the HELENA study ($n = 1144$ European adolescents).
*Results:* The minor alleles of rs11039155 in *NR1H3*, rs2575879 in *ABCA1*, rs708272, rs17231506 and rs5882 in *CETP* and rs328 in *LPL* were associated with higher serum HDL-C concentrations ($p \leq 0.0012$). The minor alleles of rs12221497 in *NR1H3*, rs1800978 in *ABCA1* and the *APOE* ε4 allele were associated with lower HDL-C concentrations ($p \leq 0.01$). The combined set of associated polymorphisms accounted for ~6.6% of the variance in HDL-C.
*Conclusion:* We report for the first time that polymorphisms in *NR1H3* and its target genes *ABCA1*, *APOE*, *CETP* and *LPL* contribute to the genetic variance for HDL-C concentrations in adolescence.


## 1. Introduction

Hypercholesterolemia and low serum HDL-cholesterol (HDL-C) concentrations are risk factors for cardiovascular diseases. A low HDL-C concentration is the most common lipoprotein abnormality in men with coronary artery disease (CAD) [1]. Atherosclerosis starts very early in childhood and progresses from fatty streaks to raised lesions in adolescence and young adulthood [2]. Understanding the knowledge of the genetic determinants of HDL-C concentration in childhood may help to target individuals at risk of CAD at an early age.

Liver X receptors (LXRs) are nuclear receptors that act as cholesterol sensors and control cholesterol homeostasis by regulating the expression of numerous target genes (see [3] for review). LXRβ is ubiquitously expressed, whereas LXRα is predominantly expressed in liver, intestine, adipose tissue and macrophages [4]. LXRα is encoded by the *NR1H3* gene located on 11p11.2. Recent studies have shown that single nucleotide polymorphisms (SNPs) in *NR1H3* are associated with serum HDL-C concentrations in adults [5–7]. Recently, a meta-analysis of genome-wide association studies (GWASs) identified 95 loci contributing to inter-individual variations in serum lipid concentrations [8], including the *NR1H3* gene locus which is associated with HDL-C (rs3136441, $p = 3.48 \times 10^{-18}$). Moreover, many SNPs in LXRα's target genes (such as *LPL* (lipoprotein lipase), *ABCA1* (ATP-binding cassette A1), *CETP* (cholesterol ester transfer protein), *APOE* (Apolipoprotein E)

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**Table 1**
Significant associations between SNPs in *CETP*, *APOE*, *NR1H3*, *LPL* or *ABCA1* and HDL-C level (mg/dL).

| Gene | SNP/haplotype | Genotype ($n$), mean ± S.D. | | | $p$ value | $Q$ value |
|---|---|---|---|---|---|---|
| *NR1H3* | rs11039155 | 11 (842), 54.9 ± 10.5 | 12 (266), 56.6 ± 10.9 | 22 (29), 60.4 ± 11.4 | 0.0012 | 0.012 |
| *NR1H3* | rs12221497 | 11 (871), 55.8 ± 10.5 | 12 (249), 54.5 ± 11.0 | 22 (20), 49.6 ± 10.4 | 0.0046 | 0.029 |
| *ABCA1* | rs2575879 | 11 (380), 54.2 ± 9.7 | 12 (554), 55.7 ± 10.9 | 22 (204), 56.8 ± 11.5 | 0.0036 | 0.028 |
| *ABCA1* | rs1800978 | 11 (664), 55.6 ± 10.6 | 12 (238), 54.1 ± 10.5 | 22 (19), 50.3 ± 9.2 | 0.010 | 0.044 |
| *APOE* | $\varepsilon$ | $\varepsilon2^+$ (166), 57.6 ± 10.6 | $\varepsilon3\varepsilon3$ (695), 55.2 ± 11.0 | $\varepsilon3\varepsilon4/\varepsilon4\varepsilon4$ (219), 54.2 ± 9.3 | 4.03E−05 | 5.10E−04 |
| *CETP* | rs17231506 | 11 (517), 53.9 ± 10.1 | 12 (484), 56.2 ± 10.9 | 22 (108), 59.1 ± 10.5 | 1.49E−08 | 5.67E−07 |
| *CETP* | rs708272 | 11 (381), 53.7 ± 10.6 | 12 (554), 55.7 ± 10.6 | 22 (205), 57.8 ± 10.4 | 5.53E−07 | 1.05E−05 |
| *CETP* | rs5882 | 11 (491), 54.4 ± 10.3 | 12,(511), 56.0 ± 10.8 | 22 (138), 56.8 ± 10.9 | 0.0060 | 0.033 |
| *LPL* | rs328 | 11 (878), 55.1 ± 10.4 | 12 (244), 56.1 ± 10.0 | 22 (18), 61.6 ± 10.0 | 0.0063 | 0.030 |

1: frequent allele; 2: minor allele; $\varepsilon2^+$: $\varepsilon2\varepsilon2$ or $\varepsilon2\varepsilon3$ or $\varepsilon2\varepsilon4$.
Additive general linear models were used. $p$ values were adjusted for age, gender, centre and BMI.
Correction for multiple testing was performed using false discovery rate $Q$ values.

and *PLTP* (phospholipid transfer protein)) are also associated with HDL-C concentrations.

In the present study, we investigated associations between SNPs in *NR1H3* and these five target genes (*ABCA1*, *CETP*, *APOE*, *PLTP*, *LPL*) on one hand and the serum HDL-C concentration on the other in adolescents.

## 2. Methods

### 2.1. Subjects

The recruitment and phenotyping of the adolescents in the HELENA cross-sectional study (www.helenastudy.com) have been previously described [9]. Briefly, a total of 3865 adolescents were recruited between 2006 and 2007 in 10 centres in 9 European countries. The protocol was approved by the appropriate ethics review board for each investigating centre. Written, informed consent was obtained from each adolescent and both of his/her parents or legal representatives. Participation in the study was voluntary. One-third of the classes were randomly selected for blood collection ($n$ = 1155). Data on BMI were available for 1144 subjects (i.e. the sample in the present study). Physical activity was assessed with an uni-axial accelerometer (ActigraphTM GT1 M, Pensacola, FL, USA) during 7 days [10]. Moderate-vigorous physical activity was dichotomized into <60 and ≥60 min/day (http://www.health.gov/PAGuidelines). Fasting serum triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol were measured on the Dimension RxL clinical chemistry system (Dade Behring, Schwalbach, Germany) using enzymatic methods [11]. Serum apolipoprotein A1 (ApoA1) concentrations were measured in an immunochemical reaction with a BN II analyzer (Dade Behring). DNA was extracted from white blood cells with the Puregene kit (QIAGEN, Courtaboeuf, France) at the Genomic Analysis Laboratory (Institut Pasteur de Lille, Lille, France).

### 2.2. Single nucleotide polymorphism selection and genotyping

For *NR1H3*, *PLTP* and *LPL*, tagSNPs were selected using the HapMap (Nov08 release) database (with a minor allele frequency (MAF) above 0.10 and tagSNPs with an $r^2$ value cut-off above 0.8). For *CETP*, *APOE* and *ABCA1*, several candidate SNPs were included on the basis of a literature survey. In total, 44 SNPs (14 in *LPL*, 9 in *ABCA1*, 7 in *CETP*, 6 in *PLTP*, 4 in *APOE* and 3 in *NR1H3*) were genotyped using the Illumina GoldenGate or VeraCode technologies (except for rs11039155, typed by restriction length fragment polymorphism, as previously described [6]). The genotyping success rates are shown in Supplementary Table 1.

### 2.3. Statistical analysis

Linkage disequilibrium (LD) between SNPs was assessed by calculating the $D'$ and $r^2$ values using Haploview software [12]. Statistical analyses were performed with SAS software, version 8.02 (SAS Institute Inc., Cary, NC, USA). A chi-squared test was used to compare genotype and allele distributions and to assess deviation from Hardy–Weinberg equilibrium ($p$ < 0.01). Multivariate, additive general linear models (GLMs) were used to study the effects of the SNPs on HDL-C. The homogeneity across centres and by gender was tested using an interaction term in the GLM (all $p$ > 0.05). We used age, gender, centre and BMI as covariates. Correction for multiple testing was performed using false discovery rate Q values. To evaluate the proportion of HDL-C variance explained, we incorporated all the SNPs associated (from Table 1), age, BMI, gender, centre and physical activity level in a stepwise multivariate regression model. Power calculations were performed in an additive model using Quanto software version 1.2.4 [13] (University of Southern California, LA, USA).

## 3. Results

In the study, 48% ($n$ = 549) were boys. The mean age was 14.7 ± 1.4 yrs, the mean BMI was 21.3 ± 3.8 kg/m$^2$ and the mean serum HDL-C concentration was 55.4 ± 10.7 mg/dL.

After genotyping, two SNPs (rs1800775 in *CETP* and rs281 in *LPL*) were eliminated from the analyses, as there was significant deviation from Hardy–Weinberg equilibrium ($p$ = 0.00008 and $p$ = 0.0002, respectively) (Supplementary Table 1). We assessed the linkage disequilibrium (LD) pattern for the SNPs in each gene (Supplementary Figure 1). In *CETP*, rs708272 and rs7205804 were in LD ($r^2$ = 0.88) and rs708272 was used as a proxy for rs7205804 in the statistical analyses. Likewise, rs2246293 and rs2422493 in *ABCA1* were perfect proxies ($r^2$ = 0.98). Hence, we ultimately assessed 39 SNPs for association with HDL-C. As usual, rs429358 and rs7412 in *APOE* define three haplotypes, corresponding to the APOE $\varepsilon2$, $\varepsilon3$ and $\varepsilon4$ isoforms.

We examined associations between the 39 SNPs and the serum HDL-C concentration (Supplementary Figure 2). Only false discovery rate Q values below 0.05 were considered to be significant. None of the *PLTP* SNPs was significantly associated with the HDL-C concentration (Supplementary Table 2). In contrast, two SNPs in *NR1H3* (rs110391555 and rs12221497) were significantly associated with HDL-C concentration (nominal $p$ = 0.012 and 0.0046, respectively) (Table 1). Similarly, seven SNPs in *ABCA1*, *APOE*, *CETP* or *LPL* were significantly associated with HDL-C concentration (Table 1), with the strongest associations being observed for *CETP* (nominal $p = 1.49 \times 10^{-8}$ and $5.53 \times 10^{-7}$) and *APOE* (nominal $p = 4.03 \times 10^{-5}$). As physical activity is an important modulator of HDL-C concentration, at least in adults, we further adjusted the

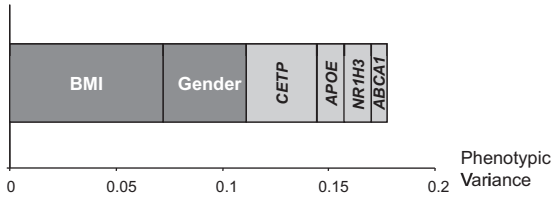


**Fig. 1.** The proportion of adjusted variance (%) in HDL-C explained by the clinical characteristics of subjects (dark grey boxes) and the SNPs (light grey boxes) in the HELENA study. Age, BMI, gender, centre, physical activity level and the nine SNPs significantly associated with the HDL-C concentration (from Table 1) were included in a stepwise regression analysis. In addition to BMI and gender, six SNPs (rs12221497 and rs11039155 in *NR1H3*, rs1800978 in *ABCA1*, $\varepsilon$ in *APOE* and rs17231506 and rs5882 in *CETP*) were retained as significant variables at $p < 0.05$ in the model.

analyses for physical activity level and observed that the associations persisted ($9.30 \times 10^{-7}$ < nominal $p < 0.012$ for these nine SNPs), meaning that they were independent of physical activity level.

We next sought to determine the extent to which these alleles explained the observed inter-individual variability in the HDL-C concentration. BMI and gender explained 11.1% of the variance in HDL-C. Six SNPs (rs12221497 and rs11039155 in *NR1H3*, rs1800978 in *ABCA1*, $\varepsilon$ in *APOE* and rs17231506 and rs5882 in *CETP*) accounted for 6.6% of the variance in HDL-C, with half of that (3.3%) being due to *CETP* alone (Fig. 1).

To note, among the 39 SNPs, only the *APOE* $\varepsilon$ polymorphism was significantly associated with ApoA1 concentrations ($1.54 \pm 0.21$ g/L for $\varepsilon 2^+$ carriers ($n = 166$), $1.51 \pm 0.23$ g/L for $\varepsilon 3\varepsilon 3$ ($n = 669$) and $1.47 \pm 0.20$ g/L for $\varepsilon 3\varepsilon 4/\varepsilon 4\varepsilon 4$ individuals ($n = 215$), nominal $p = 8.41 \times 10^{-5}$ – corrected $Q$ value = 0.0032) (data not shown).

## 4. Discussion

In the present study, we analysed associations between 39 SNPs in *NR1H3* and five LXRα target genes with the serum HDL-C concentration in European adolescents. We detected significant associations between 2 SNPs in *NR1H3* (rs12221497 and rs11039155) and the serum HDL-C concentration. Five studies [5–8] have now reported an impact of *NR1H3* polymorphisms on HDL-C metabolism. We also detected significant associations between SNPs in *ABCA1*, *LPL*, *APOE* and *CETP* and the HDL-C concentration. The strongest effect was observed for *CETP* (~+2 mg/dL). These positive associations have already been described in several GWASs or meta-analyses in adult samples (rs3136441 in *LRP4*/*NR1H3*, rs1883025 in *ABCA1*, rs12678919 in *LPL*, rs4420638 in *APOE* and rs3764261 in *CETP*) [8]. The present results extend these observations to adolescents.

We did not find any association between *PLTP* SNPs and the HDL-C concentration, despite studying rs6065904, a SNP known to modulate serum PLTP activity [14]. The HELENA study has enough statistical power (>80%, at $p < 0.0013$ (0.05 divided by 39 SNPs)) to detect an effect size of 3.0/1.8 mg/dL of HDL-C for a MAF of 0.10/0.49, respectively. In GWASs with adult samples, the effect sizes of the SNPs associated with HDL-C are similar for *PLTP*, *ABCA1* and *APOE* (~1 mg/dL) [8]. Therefore, the absence of a significant association of *PLTP* SNPs with HDL-C in our study could be explained by a lower effect for SNPs in *PLTP* than for the other genes in adolescents – in which case, our study would be underpowered. Concerning ApoA1 concentrations, the *APOE* $\varepsilon$ polymorphism was the only SNP significantly associated in our study. In adults, the effect sizes of the SNPs associated with ApoA1 concentrations range from −1.70 to +4.10 [15]. Again, the SNPs might have a lower effect in adolescents than in adults when considering ApoA1 concentrations.

Our study had several advantages and limitations. We analysed for the first time 39 SNPs in 6 different genes in a relatively large population sample of adolescents. The reverse side is that due to the large number of SNPs tested, we had to correct the data for multiple testing and lost weaker associations. A major limit is that our study lacked a replication sample to confirm the data. However, as other studies conducted in adults do show that these SNPs (or proxies) are associated with HDL-C, we are reasonably confident that our data are not false positives. Still, a larger population sample of adolescents would allow detecting weaker associations and help unravelling the genetic determinants of HDL-C concentration at an early age.

In conclusion, if replicated, our results suggest that genetic variability of *NR1H3* and its target genes modulates HDL-C concentration in adolescents.

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## Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.01.031.

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