Apolipoprotein B genetic variants modify the response to fenofibrate: a GOLDN study

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Abstract Hypertriglyceridemia, defined as a triglyceride measurement > 150 mg/dl, occurs in up to 34% of adults. Fenofibrate is a commonly used drug to treat hypertriglyceridemia, but response to fenofibrate varies considerably among individuals. We sought to determine if genetic variation in apolipoprotein B (APOB), an essential core of triglyceride-rich lipoprotein formation, may account for some of the inter-individual differences observed in triglyceride (TG) response to fenofibrate treatment. Participants (N = 958) from the Genetics of Lipid Lowering Drugs and Diet Network study completed a three-week intervention with fenofibrate 160 mg/day. Associations of four APOB gene single nucleotide polymorphisms (SNP) (rs934197, rs693, rs676210, and rs1042031) were tested for association with the TG response to fenofibrate using a mixed growth curve model where the familial structure was modeled as a random effect and cardiovascular risk factors were included as covariates. Three of these four SNPs changed the amino acid sequence of APOB, and the fourth was in the promoter region. TG response to fenofibrate treatment was associated with one APOB SNP, rs676210 (Pro2739Leu), such that participants with the TT genotype of rs676210 had greater TG lowering than those with the CC genotype (additive model, \( P = 0.0017 \)). We conclude the rs676210 variant may identify individuals who respond best to fenofibrate for TG reduction.—Wojczynski, M. K., G. Gao, I. Borecki, P. N. Hopkins, L. Parnell, C-Q. Lai, J. M. Ordovas, B. H. Chung, and D. K. Arnett. Apolipoprotein B genetic variants modify the response to fenofibrate: a GOLDN study. J. Lipid Res. 2010. 51: 3316–3323.

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Hypertriglyceridemia and mixed dyslipidemia [i.e., high levels of low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels combined with decreased levels of high-density lipoprotein cholesterol (HDL-C)] contribute to the development and progression of atherosclerosis (1, 2). Plasma lipid and apolipoprotein levels are influenced by both genetic variation and environmental factors, particularly diet, exercise, and smoking (3, 4). Hypertriglyceridemia, defined as TG > 150 mg/dl, is a common condition, occurring in approximately one-third of adults, and in the United States about 1.3% of the population uses fenofibrate, gemfibrozil, or niacin to lower triglyceride (5). Treatment with fibrates primarily reduces TG (21–35%), but it also reduces LDL-C (9–11%) and increases HDL-C (5–18%) (6). Fenofibrate, a derivative of fibric acid, is an oral pharmacologic agent that is prescribed for mixed dyslipidemia and hypertriglyceridemia in adults that are nonresponsive to lifestyle interventions (7). Fenofibrate activates the nuclear transcription factor peroxisome proliferator-activated receptor (PPARα) in a process that ultimately modulates the expression of genes regulating lipid metabolism. Fenofibrate also enhances β-oxidation of fatty acids.

Abbreviations: APOB, apolipoprotein B; PPARα, peroxisome proliferator-activated receptor α; SNP, single nucleotide polymorphism; TG, triglyceride.

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acids, thereby reducing the amount of fatty acids available for TG synthesis (7).

Apolipoproteins are the structural components of lipoprotein particles and serve as cofactors for lipid-metabolizing enzymes and ligands for lipoprotein receptors (8). Apolipoprotein B (APOB) is a requisite protein constituent of LDL, very low-density lipoprotein (VLDL), and chylomicrons (9), transporting both triglyceride and cholesterol esters during lipoprotein metabolism (10). APOB is necessary for the cellular uptake and catabolism of LDL by the LDL receptor. Thus, APOB is critical for the synthesis, transport, and catabolism of TG-rich and cholesterol-rich lipoproteins in the intestine and liver (11, 12). Polymorphisms in the APOB gene have been associated with the variability of serum cholesterol levels and coronary atherosclerosis in some populations (13) but not all (14). No studies have examined the role of APOB genetic variants in relation to the lipid-lowering effects of fenofibrate.

In this study, we examine the role of four polymorphisms in the APOB gene on the response of triglyceride levels to a three-week fenofibrate intervention of 160 mg/day of micronized fenofibrate among individuals participating in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study to further understand the variability in inter-individual response to fenofibrate treatment.

METHODS

Study design

The analyzed study sample included 958 individuals (453 men and 505 women) participating in the GOLDN Study. The GOLDN Study is a component of the PROgenie Interaction (PROGENI) Network, which is a group of family studies examining gene-environment interactions through the use of controlled interventions. This study is funded by the National Institutes of Health (NIH) through the University of Alabama at Birmingham in collaboration with the University of Utah, Washington University, Tufts University, University of Texas, University of Michigan, University of Minnesota, and Fairview-University of Minnesota Medical Center. Participants in the GOLDN Study were recruited from three-generational pedigrees previously identified in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study to further understand the variability in inter-individual response to fenofibrate treatment.

The main aim of the GOLDN Study is to characterize the genetic basis of individual variability in response to triglycerides in response to a three-week, open label clinical trial of once daily 160 mg micronized fenofibrate (TriCor®, Abbott Laboratories, Chicago, IL). Three weeks was decided as appropriate for our trial for two reasons: first, a steady-state concentration of fenofibrate is reached at three weeks, and second, a shorter duration of treatment will identify those who are most and least responsive to treatment.

Measurements

We obtained clinical and biochemical measurements of all participating individuals before and after response to fenofibrate. For each clinic visit, participants were asked to abstain from using alcohol for at least 24 h and to fast for at least 12 h. We completed anthropometric measurements, assessed current medications, blood pressure, medical history, dietary history, and personal history.

Blood was also collected to obtain DNA for use in genotyping single nucleotide polymorphisms (SNP) for genetic analyses. Additionally, at visits 1–4, blood was collected for biochemical measurements. Centrifugation of blood samples occurred within 20 min of collection at 2000 g for 15 min at 4°C. Collected plasma samples from each participant at each time point were stored at 4°C until study completion. Upon study completion, we analyzed all plasma samples for the same individual at the same time. We made TG measurements using the glycerol-blanked enzymatic method on the Roche COBAS FARA centrifugal analyzer (Roche Diagnostics, Basel, Switzerland). HDL-C measurement used the same procedure after precipitation of nonHDL cholesterol with magnesium/dextran. LDL-C measurement employed a homogeneous direct method (LDL Direct Liquid Select™ Cholesterol Reagent; Equal Diagnostics, Exton, PA) on a Roche Hitachi 911 Automatic Analyzer.

We isolated genomic DNA from peripheral blood leukocytes using the Puregene® reagents following the vendor’s protocol. We genotyped five SNPs in the APOB gene using a TaqMan assay with allele-specific probes using the ABIPrism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) according to standard laboratory protocols (17). Specifically, we analyzed the following SNPs in the APOB gene: -516 C to T (rs934197), XbaI (rs693), EcoRI (rs1042031), Val591Ala (rs679899), and Pro-2739Leu (rs10676210). SNP selection was performed on phase I HapMap data, as that was all that was available at that time. Selection was based on literature reports of established associations, particularly of that by Bentzen et al. (18) for three SNPs (rs679899, rs676210, and rs1042031). Prior results from the lab of Dr. Or dovagas (in the context of dietary lipids, not fenofibrate treatment) for rs934197 and rs693 led to inclusion of these SNPs (19–22).

We performed statistical analyses using SAS 9.1 (SAS Institute, Cary, NC).

Statistical analysis

TG concentrations were log-transformed to normalize the TG distribution. To determine significant differences in percentages between men or women with and without a known cardiovascular disease risk factor, we used the Pearson $\chi^2$ and Fisher’s tests. To compare crude means, we used ANOVA and the Student’s t-test. We performed statistical analyses using SAS 9.1 (SAS Institute, Cary, NC).
We used a growth curve mixed model (24, 25) to assess associations between the APOB polymorphisms and fenofibrate treatment. We fitted a three-level and individual growth mixed model using SAS Proc Mixed. The first level analyzed individual measurements across two time points for the fenofibrate intervention (pre- and post-treatment with fenofibrate). The second level analyzed the individual nested within pedigree, and the third level examined pedigree effects. To model the individual growth curve, we used an unstructured covariance matrix, treating the intercept and time as random effects. The individuals nested within pedigrees were modeled using a generalized linear mixed model (26–28). In the model, SNP genotypes (categorical variables with three levels) were treated as fixed effects, and the dependencies among members within each family were treated as random effects. As pretreatment TG is the best predictor of response to fenofibrate, pretreatment TG level was included as a covariate in all analyses. We adjusted the multivariate growth curve models for potential confounders, including sex, field center, smoking status, diabetes, age, waist circumference, and the baseline TG.

We used growth parameters (slopes) estimated in a growth curve mixed model to characterize TG changes (24–26, 28) because the growth slopes demonstrated higher heritability than the pre- and post-interventional changes. Thus, we calculated the individual slope of the TG change, adjusted using information from all participants, as the phenotype between time points to assess changes in TG and to test gene-treatment interactions. These results were compared with results using the absolute change in TG level as the outcome of interest, and associations were similar between the two methods. Here we report the growth curve results due to higher heritability estimates than the absolute change and better model fit assessed by Aikaike’s Information Criterion (AIC). A log transformation of covariates was performed to obtain normal distributions and meet model assumptions. Overall, the sample contained 3318 Journal of Lipid Research.

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RESULTS

Sample description

Table 1 displays demographic and clinical characteristics of the study sample. Overall, the sample contained more women than men. Most covariates were evenly distributed among men and women with the exception of diabetes (more women were diabetic). While body mass index (BMI) was similar between men and women, the waist-to-hip ratio was larger among men.

Table 2 demonstrates the difference in serum lipid values between men and women pre- and post-fenofibrate treatment. TG and LDL-C levels were lower after fenofibrate treatment, while HDL-C levels increased significantly among men, but not among women, after fenofibrate treatment. For LDL-C, women responded better to treatment than men, as they had higher pre-fenofibrate LDL-C measurements and lower LDL-C measurements post-fenofibrate than men ($P = 0.0353$).

APOB SNP genotyping and characteristics

Table 3 displays the attributes of the five SNPs in the APOB gene, including the accession number (rs #) used by dbSNP, and the justification for choosing each SNP. Three of the four SNPs are located in exons, and all polymorphisms were fairly common, as the minor allele frequencies were all greater than 15%. Only one SNP, rs679899, was not in HWE and was removed from all further analyses. Table 4 shows pair-wise linkage disequilibrium (LD) statistical correlations, $r^2$, between the four SNPs. The $r^2$ values indicate that some of these SNPs were in modest linkage disequilibrium, with $r^2$ values between .058 and .371. All of the SNPs changed the amino acid sequence in exons 14, 26, or 29, or were in the APOB promoter region. The allelic distribution of each SNP, provided in Table 5, demonstrates that 10 of the 12 possible genotypes are common (i.e., frequencies greater than 5%). Table 5 also shows the geometric mean TG level, pre- and post-fenofibrate treatment by genotype.

Table 1. Demographic and social characteristics of study sample

| Characteristic                  | Men $^a$ | Women $^b$ |
|-------------------------------|---------|-----------|
| N = 453 (47.3%)               | N = 505 (52.7%) |
| Age (in years)                | 48.8 (16.5%) | 48.1 (16.3%) |
| Current smoker                | 34 (7.6%) | 39 (7.9%) |
| Currently consumes alcohol    | 211 (47.2%) | 242 (48.7%) |
| Diabetic                      | 29 (6.5%) | 48 (9.7%) |
| Education                     |          |           |
| 1 or more years of high school| 125 (28.0%) | 144 (29.0%) |
| 1 or more years of trade school| 52 (11.6%) | 42 (8.5%) |
| 1 or more years of college    | 213 (47.7%) | 274 (55.1%) |
| 1 or more years post-college  | 57 (12.8%) | 37 (7.4%) |
| Body mass index (kg/m$^2$)    | 28.4 (4.9%) | 28.2 (6.3%) |
| Waist-to-hip ratio            | 0.95 (0.08) | 0.85 (0.08) |

$^a$Continuous characteristics report the mean (standard deviation), and categorical characteristics report the number (%).

$^b$Among men, six individuals were missing information for smoking status, alcohol consumption, and educational level. Seven individuals were missing information for diabetes status.

$^c$Among women, eight individuals were missing information for smoking status, alcohol consumption, diabetes status, and educational level.

$^d$Subjects were classified as having type 2 diabetes when fasting plasma glucose concentration was 126 mg/dl or use of insulin or diabetes medication was reported.

Table 2. Characteristics of unadjusted blood lipids in the study sample

| Measure                | Men       | Women      |
|------------------------|-----------|------------|
|                       | Pre-Fenofibrate | Post-Fenofibrate | Pre-Fenofibrate | Post-Fenofibrate |
|                       | mean (SD) | mean (SD)  | mean (SD) | mean (SD)  |
| Triglycerides (mg/dl)  | 145 (111) | 94 (52)    | 137 (92) | 91 (58)    |
| Total cholesterol (mg/dl) | 190 (40) | 169 (36)  | 189 (38) | 165 (35)  |
| HDL-C (mg/dl)          | 45 (12)   | 47 (13)    | 49 (13)  | 51 (13)    |
| LDL-C (mg/dl)          | 122 (30)  | 108 (32)   | 120 (31) | 101 (31)   |
**APOB SNP association analysis**

Table 5 summarizes the single SNP association analyses examining the response to fenofibrate treatment. Fenofibrate treatment did not demonstrate any significant associations for LDL-C or HDL-C levels with any of the APOB SNPs assessed (supplementary Tables I and II); however, there was a significant treatment effect for one APOB SNP (rs676210) with TG levels. Overall, fenofibrate decreased unadjusted TG measurement by 49 mg/dl (a 35% reduction). For the rs676210 SNP, individuals homozygous for the variant allele (T) demonstrated larger reductions in adjusted TG measurements than those with the CC or CT genotypes.

On the basis of the association of rs676210 with TG response to fenofibrate, we assessed whether demographic or clinical covariates were significantly different by genotype that would explain the association (Table 6). While the majority of clinical variables were not statistically different by rs676210 genotype, waist circumference and waist-to-hip ratio were larger in the TT genotype group, indicating that other components of the metabolic syndrome that are highly correlated with triglyceride levels may mediate the observed finding. However, our results (see Table 5) were adjusted for waist circumference. We performed additional analyses further adjusted for waist-to-hip ratio, but the results remained unchanged (supplementary Table III). Finally, the Minnesota field center yielded 30 of the 46 subjects with the rs676210 TT genotype.

**DISCUSSION**

To our knowledge, the GOLDN Study is one of the largest open-label fenofibrate trials to-date in a general population, and this analysis is the first to examine the role of APOB polymorphisms in response to fenofibrate treatment. Fenofibrate treatment reduces the production of APOB and secretion of VLDL (7, 30). It is likely that genetic variation within the APOB locus explains variable triglyceride lowering with fenofibrate. We examined five SNPs in the APOB gene, three that were previously associated with plasma lipid levels in various contexts (21, 31, 32) and two additional APOB SNPs. One of these SNPs (rs679899) was excluded from our analysis because it was not in HWE. We found a significant association between SNP rs676210 (Pro2739Leu) and TG response to fenofibrate treatment: those homozygous for the rare allele (T) showed a greater TG lowering than did individuals who carried the wild-type allele. These results support the conclusion that APOB gene variants modulate the response of TG concentrations to fenofibrate treatment.

Fenofibrate, a synthetic agonist of PPARα, is one drug in the class of hypolipidemic medications known as fibrates. Fibrates help normalize the dyslipidemia associated with increased triglyceride levels by inducing the lipolysis of triglyceride-rich lipoproteins. The mechanism involves increasing lipoprotein lipase (LPL) gene expression (33) and repressing APOC3 gene expression (34). Fibrates also lower the LDL-cholesterol level by increasing the rate of receptor-mediated removal of LDL particles by favoring the production of larger, more buoyant particles that bind more readily to LDL receptors (35, 36).

Currently, it is unclear how the genetic variation at the rs676210 APOB locus, or an allele in LD, might enhance the triglyceride-lowering effect of fenofibrate. In this study, the TT genotype at this SNP site (Pro2739Leu) was associated with a larger response. A leucine residue at amino acid residue 2739 of APOB is predicted to be damaging to the structure of the APOB100 polypeptide, as judged by SIFT (37) and PolyPhen (38) (supplementary Table IV), and as such, it could reduce either the numbers of VLDL particles assembled, their triglyceride contents, their surface properties, or any combination of these three. Potentially, the VLDL particles made with an APOB100 polypeptide containing a leucine rather than a proline residue at position 2739 may have reduced affinity for the LPL inhibitor, APOC-III, resulting in the production of fewer, small-dense, triglyceride-rich, LDL-particles that are less...

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**TABLE 3.** Description of the APOB SNPs evaluated

| SNP | dbsNP ID | Location | Minor Allele | Minor Allele Frequency | HWE p | Justification |
|-----|----------|----------|--------------|------------------------|-------|---------------|
| −516 C→T | rs934197 | promoter | T | 0.334 | 0.2575 | (20–24) |
| Val591Ala | rs679899 | exon 14 | A | 0.463 | 0.0071 | (19) |
| Xbal | rs693 | exon 26 | G | 0.489 | 0.2708 | (20–23) |
| Pro2739Leu | rs676210 | exon 26 | T | 0.217 | 0.5386 | (19, 20–23, HapMap) |
| EcoRf (Glu1514Lys) | rs1042031 | exon 29 | T | 0.173 | 0.7080 | (19) |

APOB, apolipoprotein B gene; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism.

| SNP ID in the database dbSNP (www.ncbi.nlm.nih.gov/SNP). |
| Hardy-Weinberg equilibrium test. |
| This SNP was excluded from further analysis. |
| HapMap SNP, early phase I. |

**TABLE 4.** Calculated linkage disequilibrium (r²) between APOB SNPs in the study population

| SNP | rs934197 | rs693 | rs676210 | rs1042031 |
|-----|----------|-------|----------|-----------|
| rs934197 | 0.371 |       |          |           |
| rs693  | 0.093   | 0.29  |          |           |
| rs676210 | 0.094 | 0.219 | 0.058    |           |
| rs1042031 | 0.094 | 0.219 | 0.058    |           |

SNP, single nucleotide polymorphism.
TABLE 5. Triglyceride response to fenofibrate by genotype adjusted for pretreatment triglycerides and other covariates

| SNP      | Genotype | Number (%) | Pre-Fenofibrate Geometric Mean (95% CI) | Post-Fenofibrate Geometric Mean (95% CI) | Change in Triglyceride (%) | Change in Triglyceride (mg/dl) |
|----------|----------|------------|-----------------------------------------|------------------------------------------|-----------------------------|--------------------------------|
| rs934197 | CC       | 413 (43.1) | 115.3 (111.3–115.5)                     | 81.3 (79.3–83.4)                        | -25.8 (-21.7 to -30.0)      | 31.8 (29.7–33.9)               |
|          | CT       | 440 (45.9) | 114.2 (112.2–116.2)                     | 81.0 (79.0–83.0)                        | -27.2 (-22.9 to -31.1)      | 30.5 (28.4–32.5)               |
|          | TT       | 105 (11.0) | 112.7 (110.7–114.7)                     | 81.9 (79.8–83.9)                        | -25.3 (-19.6 to -31.0)      | 30.9 (28.7–33.0)               |
| rs693    | AA       | 252 (26.3) | 110.7 (108.7–112.7)                     | 81.0 (79.0–83.0)                        | -23.7 (-19.3 to -28.2)      | 30.4 (28.5–32.6)               |
|          | AG       | 486 (50.4) | 114.5 (112.5–116.5)                     | 81.1 (79.0–83.1)                        | -27.9 (-23.8 to -32.0)      | 31.6 (29.5–33.7)               |
|          | GG       | 220 (23.0) | 115.2 (113.2–117.2)                     | 81.8 (79.8–83.8)                        | -26.3 (-21.7 to -30.9)      | 30.7 (28.6–32.9)               |
| rs676210 | CC       | 593 (61.9) | 113.2 (111.2–115.2)                     | 82.4 (80.4–84.4)                        | -24.7 (-20.8 to -28.5)      | 29.2 (27.1–31.2)               |
|          | CT       | 46 (4.8)   | 115.3 (113.3–117.1)                     | 81.0 (79.0–83.0)                        | -28.3 (-25.9 to -32.6)      | 33.0 (30.9–35.1)               |
|          | TT       | 46 (4.8)   | 114.3 (112.3–116.5)                     | 71.2 (69.1–73.3)                        | -34.5 (-31.7 to -37.1)      | 43.3 (41.2–45.5)               |
| rs1042031| CC       | 654 (68.3) | 115.4 (113.4–115.4)                     | 80.8 (78.8–82.8)                        | -26.8 (-22.9 to -30.8)      | 32.5 (30.4–34.6)               |
|          | CT       | 277 (28.9) | 114.3 (112.3–116.3)                     | 82.7 (80.6–84.7)                        | -25.4 (-20.9 to -29.8)      | 29.5 (27.4–31.6)               |
|          | TT       | 27 (2.8)   | 112.5 (110.4–114.5)                     | 78.8 (76.7–81.0)                        | -25.2 (-14.9 to -35.5)      | 24.3 (21.9–26.7)               |

All values are adjusted for sex, field center, smoking status, diabetes status, age, waist circumference, baseline triglyceride, and family relatedness with the only significant covariate being baseline triglyceride (P < 0.0001).

Additive model.
Genetic response to fibrate

Patient/population samples. In this time of the burgeoning possibilities of personalized medicine, if this association is demonstrated in other studies, APOB polymorphisms, specifically the rs676210 variant, may aid in identifying individuals who will benefit the most from the TG-lowering effects of fenofibrate.

An area of 20,054 bp. Lastly, the association between rs676210 and triglyceride response has not yet been replicated.

In summary, these findings support a role for APOB gene variants in predicting TG response to fenofibrate treatment that warrants further analyses in additional

TABLE 6. Demographic variable distribution based on rs676210 genotype

| Characteristic                        | CC (n = 593) | CT (n = 319) | TT (n = 46) | P   |
|--------------------------------------|--------------|--------------|------------|-----|
| Sex (male)                           | 281 (47.4%)  | 144 (45.1%)  | 28 (60.9%) | 0.1356 |
| Field center (Minnesota)             | 266 (44.9%)  | 166 (52.0%)  | 30 (65.2%) | 0.0072 |
| Current smoker                       | 49 (8.4%)    | 20 (6.3%)    | 4 (8.7%)   | 0.5177 |
| Currently consumes alcohol           | 277 (47.6%)  | 149 (47.2%)  | 27 (58.7%) | 0.3267 |
| Diabetic                             | 48 (8.3%)    | 23 (7.3%)    | 6 (13.0%)  | 0.4069 |
| Age (in years)                       | 48.2 (15.9)  | 48.1 (16.9)  | 54.8 (15.5) | 0.0785 |
| Education                            |              |              |            | 0.6608 |
| 1 or more years of high school       | 168 (28.9%)  | 87 (27.5%)   | 14 (30.4%) |            |
| 1 or more years of trade school      | 55 (9.5%)    | 35 (11.1%)   | 4 (8.7%)   |            |
| 1 or more years of college           | 303 (52.1%)  | 164 (51.9%)  | 20 (43.5%) |            |
| 1 or more years post-college         | 56 (9.6%)    | 30 (9.5%)    | 8 (17.4%)  |            |
| Body mass index (kg/m²)              | 28.4 (5.6)   | 28.0 (5.8)   | 30.0 (6.4) | 0.0517 |
| Waist circumference (cm)             | 96.6 (15.9)  | 95.0 (15.8)  | 103.4 (21.8) | 0.0014 |
| Waist-to-hip ratio                   | 0.89 (0.09)  | 0.89 (0.09)  | 0.95 (0.2) | 0.0003 |

Fig. 1. Evidence-based diagram of known protein-protein interactions between PPARα and APOB from the STRING database. Pink lines demonstrate experimental evidence, blue lines are database evidence, and purple lines are homology evidence. APO, apolipoprotein; CETP, cholesteryl ester transfer protein; CREBBP, CREB-binding protein; HNF, hepatocyte nuclear factor; LCAT, lecithin-cholesterol acyltransferase; LDLR, low density lipoprotein receptor; MTP, microsomal triglyceride transfer protein; NCOA1, nuclear receptor coactivator 1; PCSK9, proprotein convertase subtilisin/kexin type 9; PPAR, peroxisome proliferator activated receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; THRB, thyroid hormone receptor beta.
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