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Rare LPL gene variants attenuate triglyceride reduction and HDL cholesterol increase in response to fenofibric acid therapy in individuals with mixed dyslipidemia

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Abstract

Objective—Individuals with mixed dyslipidemia have elevated triglycerides (TG), low high-density lipo-protein cholesterol (HDL-C), and increased risk for coronary disease. Fibrate therapy is commonly used to lower TG and increase HDL-C. Common genetic variants are known to affect the response to fibrate therapy. We sought to identify rare genetic variants (frequency ≤ 1%) in genes involved in TG and HDL-C metabolism that affect the response to fenofibric acid (FA) therapy.

Methods—Four genes with a major role in HDL-C and TG metabolism *APOA1, APOC2, APOC-III* and *LPL* were sequenced in 2385 participants with mixed dyslipidemia in a randomized, double-blind, active-controlled study comparing therapy with FA alone, in combination with statins, or statin alone. Rare variants collapsing or SKAT methods were used for the analysis.

Results—Synonymous rare variants in the *LPL* gene were significantly associated with absolute HDL-C change ($P = 9 \times 10^{-4}$) and TG percent change ($P = 6.76 \times 10^{-4}$) in those treated with FA only. Participants with these rare variants had a 2 mg/dL increase in HDL-C and 39 mg/dL decrease in TG as compared to 6.2 mg/dL increase in HDL-C and 100 mg/dL decrease in TG in

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2014.03.010

those without these variants. Rare variants in the *APOC-III* gene were associated with a modest 3 mg/dL less reduction in APOB ($P = 8.72 \times 10^{-4}$) in those receiving FA and statin.

Conclusion—In individuals with mixed dyslipidemia rare synonymous variants within *LPL* gene were associated with attenuated response to FA therapy while *APOCIII* rare variants were associated with a modest effect on APOB response to FA-statin therapy. These results should be replicated in a similar clinical trial for further confirmation.

Keywords

Fenofibric acid; LPL gene variants; High-density lipoprotein; cholesterol Dyslipidemia

1. Introduction

Multiple studies have shown that genetic variants can affect triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) response to fenofibrate. One of the common uses of fibrates including fenofibric acid (FA) is to lower TG and increase HDL-C in the population of mixed dyslipidemia (MD). Individuals with MD have high TG, low HDL-C, with or without high LDL-C, and are at higher risk for coronary heart disease. Understanding the effect of specific genetic variants on FA response can potentially help to predict its efficacy for the individual patient. Multiple common genetic variants have been shown to affect fibrate therapy [1–7], some of which had frequency as much as 20% in the mixed dyslipidemia population [2,4]. However, although these genetic variants are frequent, their effect on drug response is usually modest.

Rare genetic variants, defined as variants with a frequency of 1% or less, have been previously shown to have a strong effect on lipid traits such as TG, HDL-C and low-density lipoprotein cholesterol (LDL-C) [8,9]. We have recently shown that rare variants in the APOA5 gene region have a significant effect on FA response to therapy [10].

By means of pathway approach we sought to examine the association of rare genetic variants in a number of genes involved in TG and HDL-C metabolism pathways with levels of apolipoprotein (APO) AI, TG, APOCIII, and HDL-C in response to FA.

2. Methods

2.1. Study population

Our study population included Europeane–American participants from three separate concurrent prospective, randomized, double-blind, clinical trials that examined the efficacy of FA. A detailed description of the study design has been published previously [11,12]. Individuals with TG ≥ 150 mg/dL, HDL-C <40 mg/dL in men or <50 mg/dL in women, and LDL-C ≥ 130 mg/dL were included. Study participants were randomized into three groups receiving either FA monotherapy, statin monotherapy, or statin-FA combination. Each study used a different statin, rosuvastatin, atorvastatin, or simvastatin. After a 6-week washout period, participants received a 12-week treatment. Lipid measurements were obtained at the beginning and end of the treatment period. The basic characteristics, including sex, age,

body mass index (BMI), diabetes status, and the baseline levels (APOA1, APOB, APOC-III, HDL-C, and TG) of the three treatment groups are shown in Supplementary Table 1.

2.2. Gene selection

Four genes with pivotal roles in HDL-C and TG related pathways–*APOA1*, *APOC2*, *APOC-III*, and *LPL*–were included. Each of these genes has significant impact on either HDL-C or TG metabolism and may be associated with extreme lipid phenotypes such as chylomicronemia, hypertriglyceridemia, hypobetalipoproteinemia, and elevated APOCIII. PPARA, the target gene for FA, which is a peroxisome proliferatoreactivated receptorealpha (PPAR-alpha) agonist, was included as well.

2.3. Sequencing protocol

Bidirectional sequencing was done at the Human Genome Sequencing Center at Baylor College of Medicine using intron-based, exon-specific primers. Polymerase chain reactions (PCR) were performed in 8 ul containing 10 ng of genomic DNA, 0.4 M oligonucleotide primers, and 0.7 × Qiagen® PCR HotStar Taq Master Mix containing buffer and polymerase. Cycling parameters were 95°–15 min, then 95°–45 s, 60°–45 s, and 72°–45 s for 40 cycles followed by a final extension at 72° for 7 min. After thermocycling, 5 ul of a 1:15 dilution of Exo-SAP was added to each well, and reactions were incubated at 37 ° C for 15 min prior to inactivation at 80° for 15 min. Reactions were diluted by 0.6×, and 2 ul were combined with 5 ul of 1/64th Applied Biosystems® (AB) BigDye™ sequencing reaction mix and cycled as above for 25 cycles. Reactions were precipitated with ethanol, resuspended in 0.1 mM EDTA, and loaded on AB 3730XL sequencing instruments using the Rapid36 run module and 3xx base-caller. Single-nucleotide polymorphisms (SNPs) were identified using SNP Detector software [13].

2.4. Association testing of rare variants

Baseline characteristics of the three treatment groups were compared using ANOVA F-test (continuous characteristics, including age, BMI, and all baseline levels) and χ^2 test (binary traits, including sex and diabetes status). Rare variants (frequency ≤ 1%) in the 5 sequenced genes were included in the analyses. Rare variants within a gene were further classified to categories such as intronic, missense, synonymous, promoter, and 5’ or 3’ untranslated region (UTR) variants. A total of 25 genes and gene categories (5 genes, 20 further split gene categories) were used in the analyses. The number of the rare variants within the 25 genes and gene categories, as well as the number of individuals that carry these rare variants, is shown in Supplemental Table 2.

Two complementary statistical approaches were used in this study, the **Sequence Kernel Association Test** (SKAT) and a simple collapsing approach. SKAT is a rare-variant association analysis method for sequencing data, which allows rare variants to influence the phenotype in different directions and with different magnitude of effect and shows better computational efficiency and statistical power over traditional collapsing methods [14]. This method was used to test the association between rare variants and lipid level changes after therapy in our study. The tested pheno-types were the absolute change in lipid levels (the lipid level after therapy subtracting the baseline lipid level before therapy) and percent

change (the absolute lipid level change normalized by the baseline level before therapy) of five lipids or lipid-related proteins (APOA1, APOB, APOC-III, HDL-C and TG). For each analysis, six covariates were included: sex, age, BMI, smoking status, diabetes status, and baseline lipid level. To increase the statistical power of the analyses, each phenotype was tested in the three treatment groups separately. To further increase power and accuracy, all SNPs with missing rate ≥15% were excluded from each analysis, and all individuals with missing phenotype or any missing covariate were also excluded from the analyses. The association test for each phenotype with the 25 genes and gene categories was considered a separate analysis, and a threshold value of 2.0×10^{-3} (corresponding to a nominal *p*-value of 0.05) was considered significant after Bonferroni correction for the 25 gene and gene categories tested.

Collapsing approaches, or “burden tests”, are commonly used for association testing of rare variants [15–17]. In our study, this method was applied to show the dominating direction of the significant associations found by SKAT method, as SKAT allows rare variants to influence the phenotype in different directions and thus does not show the dominating direction of the association. We collapsed the rare variants by counting the number of rare variants in each gene and gene category. Multivariate linear regression was then performed between the number of rare alleles and the tested phenotype using R [18]. The regression coefficient obtained from this method is the magnitude of reduction (if the coefficient is negative) or increase (if the coefficient is positive) in the lipid level change on average when an individual has one more rare variant as compared with the individuals without any rare variant.

3. Results

Baseline characteristics of each of the three treatment groups are presented in Supplemental Table 1. There was no significant difference at baseline between the three treatment groups other than baseline level of APOA1, which was slightly higher in the FA only treatment group. As expected, there were no differences in baseline HDL-C, TG, APOB, or APOCIII levels.

We examined the association of the 25 genes and gene categories with each of the 10 phenotypes (absolute lipid level change and percentage lipid level change of the five lipids or lipid proteins) in the three treatment groups. The Q–Q plots for *p*-values of the association studies are shown in Supplemental Fig. 1. Power calculation shows that SKAT method has enough power to detect significant associations with ≥ five rare variants. Three significant associations were found using SKAT method. These significant results were then further confirmed by 100,000 permutations.

The synonymous rare variants in the LPL gene region were found to be significantly associated with the absolute HDL-C change ($P = 9.00 \times 10^{-4}$, $P_c = 0.023$ after Bonferroni correction, *P*perm = 0.00196 using permutation test) and TG percent change ($P = 6.76\ 10^{-4}$, $P_c = 0.017$ after Bonferroni correction, *P*perm = 0.00189 using permutation test) (Tables 1 and 2) in the FA treatment group. No association was detected with APOAI response in any of the treatment groups.

Participants with synonymous rare variants in the *LPL* gene region receiving FA only therapy had an attenuated increase of 2 mg/ dL in HDL-C as compared to 6.2 mg/dL increase in those without rare *LPL* synonymous variants (Table 3). The opposite pattern was observed for TG response in those receiving FA only therapy. Participants with synonymous rare variants in the *LPL* gene region had TG reduction of 39 mg/dL as compared to 100 mg/dL reduction in those without rare *LPL* synonymous variants (Table 4).

Combination of all of the rare variants in the *APOC-III* gene in the FA and statin combined therapy group were found to be significantly associated with the absolute change in APOB ($P = 8.72 \times 10^{-4}$, $P_c = 0.022$ after Bonferroni correction, *P*perm = 0.00469 using permutation test) (Table 5).

Participants with rare variants in the APOC-III gene in the FA-statin combination group had a 53 mg/dl reduction in APOB levels compared with 56 mg/dl in those without rare variants in APOC-III. However, there was no difference in LDL-C reduction between the groups with and without the APOC-III rare variants in all therapy groups (Table 6). The information of rare variants involved in the significant associations is listed in Supplementary Tables 3 and 4.

We used the beta coefficients from the simple collapsing method (Supplementary Table 5) to interpret the significant results found by the SKAT method. For the FA group, the individuals with rare variants in LPL synonymous category tended to have lower absolute change in HDL-C and higher percentage change in TG compared with those without any rare variant (Supplemental Fig. 2). For the combination therapy group, the individuals with rare variants in APOC-III gene tended to have higher absolute change in APOB compared with those without any rare variant (Supplemental Fig. 2). The corresponding information for simple collapsing method, including the Q–Q plot for the simple collapsing method is shown in Supplementary Table 2 and Supplemental Fig. 3.

4. Discussion

In the current study we show that rare synonymous LPL gene variants can attenuate the effects of FA therapy on TG reduction and HDL-C increase in individuals with mixed dyslipidemia. In addition, rare *APOCIII* gene variants were associated with a modest attenuation in APOB reduction following combination therapy with sta-tins and FA in the study population.

Rare genetic variants are fairly common but are usually unique for each individual. Overall the frequency of a specific rare variant is very low, but the probability of having some type of a unique rare variant is high. In fact, the overall population frequency of rare variants in a specific gene (i.e. total number of rare variants which are different) is higher than the frequency of many of the common SNP's in that gene. It has been previously shown that common SNPs in the LPL gene affect baseline triglyceride and HDL-C levels as well as response to fibrates. The additional information in this study about the effect of rare *LPL* gene variants and response to FA adds to the understanding of how LPL gene variants affect response to fibrate therapy.

TG reduction is thought to be a risk factor for coronary disease, as previously shown in a large Mendelian randomization study using common SNPs in the *LPL* gene region [19]. Although therapy with fibrates did not reduce coronary disease risk in the general study population in large clinical trials such as in the ACCORD LIPD trial, it did reduce coronary events in those with mixed dyslipidemia for those with absolute levels of TG > 204 mg/dL and HDL-C <36 mg/dL [20]. Thus, identifying genetic factors that influence response to fibrates in the mixed dyslipidemia population has the potential to predict which patients will have higher TG reduction and larger HDL-C increase with FA therapy. This has the potential to identify which patients are most likely to derive coronary risk reduction from fibrate therapy. We suggest that rare variants in the *LPL* gene region may contribute to the coronary risk reduction effect of fibrates previously observed in the population of individuals with mixed dyslipidemia.

The LPL enzyme plays a pivotal role in TG metabolism. TG hydrolysis is facilitated by a complex interaction of LPL with various proteins such as APOCII, APOCIII, APOA5, and others. There are well known recessive, single gene disorders that involve *APOCII* and *LPL* genes and result in significant hypertriglyceridemia [21]. In these conditions there is little or no response to fibrates, as there is almost no residual LPL function. Common and rare SNPs may potentially have a similar but milder effect.

A common intronic SNP, rs320, was examined in a Chinese population receiving fenofibrates and was associated with a lesser TG reduction in homozygotes as compared with wild type geno-types. Although the rs328 was not expected to change the gene product, it was thought to reside in a protein binding region that indirectly effects the protein production [7]. In the current study, we show that rare synonymous *LPL* gene variants that are not expected to change protein structure can attenuate the TG reduction and HDL-C increase effects of fenofibrate.

FA is a peroxisome proliferator-activated receptor agonist that has multiple effects. It reduces TG by increasing LPL gene transcription and increases APOA-I and HDL-C levels [22]. The rare *LPL* gene variants identified in our study may potentially affect FA response by interfering with PPAR-alpha activation of *LPL* transcription resulting in a smaller net TG reduction and HDL-C increase.

An additional finding was the association of the total rare variants in the *APOCIII* gene with APOB response in participants receiving the combination of statins and FA. This was a modest effect, and there is no known direct biological relationship between APOCIII and APOB. APOCIII is an important cofactor for TG hydro-lysis by the LPL enzyme, and a possible explanation for the effect of APOCIII rare variants on APOB response could be related to very low-density lipoprotein particle metabolism by the LPL enzyme.

Our study has limitations. The study was a randomized active-controlled trial with the most significant associations identified in the FA only group which had relatively a small sample size. This limitation was approached by using the SKAT statistical approach for which this sample size was sufficient and permutation testing that did further confirm the results.

However, replication of the significant association in an independent randomized prospective clinical trial would be important to further confirm the study's results.

In conclusion, we identified rare genetic variants that affect the response to FA and its combination with statins. Our analysis suggests that synonymous variants within the *LPL* gene region may be associated with reduced response to FA in individuals with mixed dyslipidemia, which may attenuate its potential effect to reduce coronary disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Significant association between LPL coding synonymous gene category and absolute change in HDL-C in FA group adjusted for sex, age, BMI, smoking, diabetes and baseline HDL-C level.

Group	Number of individuals	Number of rare variants	P value by SKAT
FA	318	5	9.00×10^{-4}
FA + Statin	834	11	0.50
Statin	1019	15	0.88

Table 2

Significant association between LPL coding synonymous gene category and percentage change inTG in FA group adjusted for sex, age, BMI, smoking, diabetes and baseline TG level.

Group	Number of individuals	Number of rare variants	P value by SKAT
FA	343	5	$6.76 \chi 10^{-4}$
FA + Statin	899	11	0.48
Statin	1071	15	1.00

Table 3

Mean HDL-C before treatment, after treatment and change in HDL-C for individuals in FA group with and without rare variants in LPL coding synonymous gene category.

Group	Mean ± SE HDL-C before treatment (mg/dL)	Mean ± SE HDL-C after treatment (mg/dL)	Mean ± SE HDL-C change (mg/dL)
Without rare variants (n = 313)	38.56 ± 0.38	44.78 ± 0.55	6.22 ± 0.35
With rare variants (n = 5)	39.80 ± 1.65	41.80 ± 6.08	2.00 ± 6.50

Abreviations: SD, standard deviation; FA, fenofibric acid; HDL-C, high-density lipoprotein cholesterol

Table 4

Mean TG before treatment, after treatment and change in TG for individuals in FA group with and without rare variants in LPL coding synonymous gene category.

Group	Mean ± SE TG before treatment (mg/dL)	Mean ± SE TG after treatment (mg/dL)	Mean ± SE TG change (mg/dL)
Without rare variants (n = 338)	272.46 ± 7.82	172.27 ± 5.07	–100.19 ± 6.48
With rare variants (n = 5)	279.60 ± 65.31	240.20 ± 54.53	–39.40 ± 40.19

Abbreviations: TG, triglycerides; FA, fenofibric acid

Table 5

Significant association between gene APOC-III and absolute change in APOB in FA and statin combined group adjusted for sex, age, BMI, smoking, diabetes and baseline APOB level.

Group	Number of individuals	Number of rare variants	P value by SKAT
FA + Statin	887	7	8.72×10^{-4}
FA	342	0	–
Statin	1055	5	0.62

Table 6

Mean APOB before treatment, after treatment and change in APOB for individuals in FA and statin combined group with and without rare variants in APOC-III gene.

Group	Mean ± SE APOB before treatment (mg/dL)	Mean ± SE APOB after treatment (mg/dL)	Mean ± SE APOB change (mg/dL)
Without rare variants (n = 881)	142.63 ± 0.88	86.14 ± 0.87	–56.49 ± 0.95
With rare variants (n = 6)	138.00 ± 13.57	85.33 ± 7.34	–52.67 ± 8.93

Abbreviations: APO, apolipoprotein; FA, fenofibric acid