

Interaction between SNPs in the *RXRA* and near *ANGPTL3* gene region inhibits apoB reduction after statin-fenofibric acid therapy in individuals with mixed dyslipidemia[§]

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Abstract The mixed dyslipidemia phenotype is characterized by elevated triglycerides (TG), low HDL cholesterol (HDL-C), increased ApoB levels, and premature coronary atherosclerosis. Fibrate-statin combination therapy reduces ApoB levels and coronary events in the mixed dyslipidemia population. We sought to identify gene-gene interactions that affect ApoB response to statin-fenofibric acid therapy in the mixed dyslipidemia population. Using a predefined subset of single-nucleotide polymorphisms (SNPs) that were previously associated with TG, VLDL, or HDL-C, we applied gene-gene interaction testing in a randomized, double-blind, clinical trial examining the response to fenofibric acid (FNA) and its combination with statin in 1,865 individuals with mixed dyslipidemia. Of 11,783 possible SNP pairs examined, we detected a single significant interaction between rs12130333, located within the *ANGPTL3* gene region, and rs4240705, within the *RXRA* gene, on ApoB reduction after statin-FNA therapy ($P = 4.0 \times 10^{-6}$). ApoB response to therapy gradually reduced with the increasing number of T alleles in the rs12130333 but only in the presence of the GG genotype of rs4240705. Individuals doubly homozygous for the minor alleles at rs12130333 and rs4240705 showed a paradoxical increase of 1.8% in ApoB levels after FNA-statin combination therapy. No gene-gene interaction was identified other than an interaction between SNPs in the *ANGPTL3* and *RXRA* regions, which results in the inhibition of ApoB reduction in response to statin-FNA therapy. Further study is required to examine the clinical applicability of this genetic interaction and its effect on coronary events.—Ma, L., C. M. Ballantyne, J. W. Belmont, A. Keinan, and A. Brautbar. Interaction between SNPs in the *RXRA* and near *ANGPTL3* gene region inhibits apoB reduction after statin-fenofibric acid therapy in individuals with mixed dyslipidemia. *J. Lipid Res.* 2012. 53: 2425–2428.

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The mixed dyslipidemia phenotype comprised of elevated triglycerides (TG), reduced HDL cholesterol (HDL-C), and increased ApoB is associated with diabetes and increased risk of coronary artery disease. One of the commonly used agents to treat individuals with mixed dyslipidemia are fibrates, including fenofibric acid (FNA), which are peroxisome proliferator-activated receptor- α (PPAR- α) agonists. FNA decreases TG, ApoB containing VLDL particles, and APOC-III while increasing HDL-C (1). Among the factors that determine the individual response to FNA and its combination with statins are single nucleotide polymorphisms (SNPs), which were previously shown to affect HDL-C, TG, and APOC-III levels in response to fibrates and statins (2–4). Going beyond the individual, additive effect of single loci, gene-gene interaction, or epistasis, is an important mechanism in determining an individual response to FNA therapy (5, 6).

In this study, we sought to identify gene-gene interactions affecting the response of ApoB to therapy with FNA. To improve statistical power, we focused on a small set of SNPs that we identified as known to be highly relevant to the mixed dyslipidemia phenotype.

MATERIALS AND METHODS

Study population

Our study population included DNA from 1,865 participants from three concurrent prospective, randomized, clinical trials examining the response to FNA, all of which had the same

Abbreviations: FNA, fenofibric acid; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; LXR, liver X receptor; MAF, minor allele frequency; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR- α response element; RXR- α , retinoid X receptor alpha; SNP, single-nucleotide polymorphism; TG, triglycerides.

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design. The study received Institutional Review Board approval from Baylor College of Medicine, and informed consent was obtained by Abbott Laboratories. Only individuals with mixed dyslipidemia were included, defined as TG \geq 150 mg/dl, HDL-C $<$ 40 mg/dl for men or $<$ 50 mg/dl for women, and LDL cholesterol (LDL-C) \geq 130 mg/dl. Exclusion criteria included active or recent ulcer, type 1 diabetes (type 2 diabetes was included unless uncontrolled), unstable cardiovascular disease, severe heart failure, renal failure, and elevated liver or muscle enzymes at baseline.

Participants were randomized into groups receiving FNA monotherapy, statin monotherapy, or statin-FNA combination. After a washout period of 6 weeks, participants received the assigned treatment for 12 weeks. ApoB levels were measured before and after the 12 weeks of therapy (7, 8). The statin that was used in each clinical trial was different and was either simvastatin, rosuvastatin, or atorvastatin. Baseline characteristics for the three clinical studies separately and combined are presented in supplementary Tables I–IV.

Candidate SNP selection

Genes that are involved in TG, VLDL, and HDL-C pathways were selected (1, 9). Common ($>$ 5%) and uncommon (1–5%) tag SNPs within up to 10 Kb upstream and downstream of these gene boundaries were selected using the Genome Variation Server (<http://gvs.gs.washington.edu/GVS/>). Criteria for tag SNP selection included minor allele frequency (MAF) \geq 1% in the HapMap-CEU dataset and tagging with linkage disequilibrium of $r^2 \geq 0.8$ for of any SNP in the target genes. Additional SNPs that were previously identified in a genome-wide association study as associated with TG and HDL-C levels were added (10–13), for a total of 338 SNPs used to examine possible epistatic effects in therapy response. Thirty-four additional known ancestry informative SNPs were used to correct for possible population stratification within the European-American population, with no significant substructure observed as previously shown (2). SNPs, their relative location in relation to genes, and their frequency in our study population are detailed in supplementary Table V.

Genotype data

SNPs were genotyped with the Golden Gate chemistry platform on an Illumina Bead Express system (Igenix, Seattle, WA). Individuals with $>$ 10% missing data were excluded, as well as subjects with SNPs with call rates $<$ 90%, MAF \leq 1%, or subjects who departed from Hardy-Weinberg Equilibrium genotype proportions ($P < 10^{-6}$). For the interaction test of each pair of SNPs, we also required a sample size of each of the nine possible genotype-combinations \geq 3 and linkage disequilibrium of $r^2 < 0.1$ between the two SNPs. The first requirement is a generalization of the MAF requirement in a single-marker analysis.

Gene-gene interaction testing

We tested for the existence of gene-gene interaction between a pair of SNPs effects using an F -test with four degrees of freedom that compares two models with and without interaction (5, 14). This F -test does not assume a certain type of deviation from additive effects and allows for epistatic interaction of any type; hence, when little is known about the underlying epistatic effect, it tends to be more robust and powerful than tests that make such assumptions (14). In total, we tested 11,783 pairwise interactions between 304 SNPs in their effect on percent change in levels of ApoB in each of the three treatment groups: FNA monotherapy, statin monotherapy, and statin-FNA combination. Gender, age, body mass index, and diabetes were included as covariates in the model.

Multiple hypothesis testing correction

We estimated the effective number of tests out of the 11,783 interaction tests and used it to apply a Bonferroni correction on significance levels. First, by using the pairwise correlation of SNP genotypes, we estimated the effective number of SNPs from the original 304 markers to be 227 (15). Assuming the number of independent interaction tests is equivalent to all possible pairs out of the 227 markers ($227 \times 226/2$), it is equivalent to 56% of tests, which is slightly larger than the estimated 40% from Illumina HumanHap 550 data (16), presumably due to differences in the procedure of SNP selection. Thus, we estimated the effective number of interaction tests as $11,783 \times 0.56 = 6,599$, which is the number of hypotheses we corrected for in our multiple testing correction.

RESULTS

In total, we tested 11,783 epistatic interactions on ApoB response to statin-FNA combination therapy. Of the interactions examined, two interactions were found significant after multiple testing correction. The two interactions are between rs12130333 and rs4240705 and between rs12130333 and rs3118571. rs4240705 and rs3118571 are in high linkage disequilibrium. The most significant interaction was observed between rs12130333 in the *ANGPTL3* gene region and rs4240705 in the *RXRA* gene ($P = 4.0 \times 10^{-6}$; $P_c = 0.026$ after multiple-testing correction). Examining the ApoB response of each of the nine genotype combinations of the two SNPs suggests that the double homozygotes for the minor allele for each of the two SNPs is expected to exhibit an average increase in APOB, paradoxical to the expected drug response (Fig. 1). An additive effect was observed as a function of the number of T alleles in the rs12130333 but only in the presence of GG genotype for rs4240705. Conditioning on the latter, ApoB response to therapy was gradually reduced with the increasing number of T alleles in the rs12130333: -39.5% with CC, -29.99% with CT, and $+1.8\%$

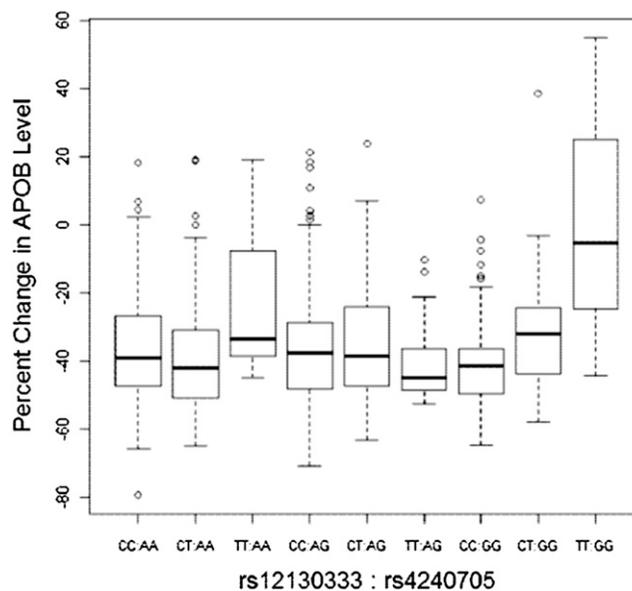


Fig. 1. Marginal and interaction effect sizes on percent change of uncorrected ApoB levels in the treatment group of statin-FNA combination.

with TT. All other genotype combinations predicted a considerable reduction in ApoB levels, although there was some attenuated response for the combination of the rs12130333 TT and the rs4240705 AA genotype (Table 1). Thus, the most prominent interaction was observed in individuals doubly homozygous for the minor alleles at rs12130333 and rs4240705 with a paradoxical increase of 1.8% in ApoB levels on average. A similar interaction was not observed for the FNA or statin monotherapy groups.

DISCUSSION

In this study, we examined multiple gene-gene interactions between multiple candidate genes associated with ApoB, HDL-C, and TG with identifying a single novel interaction between the alleles in the *RXRA* and *ANGPTL3* gene regions. This interaction resulted in attenuation of the ApoB reduction in response to combination of FNA-statin therapy.

PPAR- α is activated by FNA and to a minor degree by statins (17). Multiple downstream target genes with a PPAR- α response element (PPRE) are activated when PPAR- α forms a heterodimer with retinoid X receptor α (RXR- α), which is coded by the *RXRA* gene. Although PPAR- α alone can activate its PPRE targets, the PPAR- α /RXR- α heterodimer has a better ability to enhance gene transcription. The SNP rs4240705 is located in an *RXRA* intron and is not expected to affect RXR- α protein structure. However, this is a tag SNP that is possibly a marker of a coding variant. Another possibility is that the rs4240705 has an impact as a regulatory element on RXR- α expression.

The rs12130333 is an intergenic SNP upstream of *ANGPTL3* that was previously associated with reduced TG in a number of genome-wide association and other studies (18–20) and with reduced LDL-C (21). Mutations in the *ANGPTL3* gene have been previously shown in families and sporadic cases of low ApoB and in hypobetalipoproteinemia (22, 23). *ANGPTL3* is an LPL enzyme inhibitor, and mutations in *ANGPTL3* in humans have been associated with low TG levels (24).

There is an interesting connection between *ANGPTL3* and the PPAR- α pathway. PPAR- α agonists, such as FNA, induce the nuclear liver X receptor (LXR) via a PPRE in its promoter (25). The *ANGPTL3* promoter has a known LXR binding site, and it has been shown that *ANGPTL3* expression is induced by LXR (26–28). Thus, FNA can possibly induce *ANGPTL3* expression via LXR, which is expected to increase ApoB levels. However, as observed in this study, the net effect of FNA and statin was reduction in ApoB levels, probably via reduction in TG and VLDL-C after the direct activation of the LPL enzyme with FNA.

It is possible that in the presence of both FNA and statins, the minor allele of the rs4240705 in *RXRA* helps in the induction of LXR, which in turn has a stronger effect on *ANGPTL3* induction due to the presence of the rs12130333. Thus, there is a larger *ANGPTL3*-related ApoB increase that possibly attenuates the ApoB reduction effect of the statin-FNA combination.

The attenuated ApoB in the mixed dyslipidemia phenotype in response to the FNA-statin combination with certain genotypes of the rs12130333 and rs4240705 may possibly affect cardiovascular outcomes. In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, a combination of statin and fenofibrate did not improve coronary outcomes in the overall study population (29). However, improved outcomes were observed in participants with mixed dyslipidemia (TG \geq 204 mg/dl and HDL-C \leq 34 mg/dl). Thus, statin-fibrate therapy appears to provide more benefit in individuals with mixed dyslipidemia. If ApoB reduction contributed to the improved outcomes from statin-fibrate combination therapy in the ACCORD trial, then individuals with mixed dyslipidemia who are carriers of the minor alleles of rs4240705 and rs12130333 may have reduction in that outcome benefit because their ApoB will not be reduced as effectively compared with individuals who are not carriers of these minor alleles. However, we were not able to examine coronary outcomes in this study because the clinical trial was conducted for a relatively short period of time with no long-term cardiovascular outcome information.

A possible limitation of our study is the fact that three doubly homozygous individuals for the minor alleles (TT for *ANGPTL3* rs12130333 and GG for *RXRA* rs4240705) had a substantial effect on the overall interaction statistics. However, the rs12130333 T allele in combination with the rs4240705 C allele contributed to the overall effect on the ApoB response.

In summary, after testing of multiple gene-gene interactions, we have identified only a single interaction that has a modest effect on ApoB response to therapy. We have shown that individuals with mixed dyslipidemia who are receiving a combination of FNA and statins may have an attenuated ApoB reduction if they are homozygous for the minor allele of SNPs in both the *ANGPTL3* and *RXRA* gene regions. This may attenuate the cardiovascular risk reduction observed with the combination of FNA and statins compared with statins alone. Further study is required to test the significance of this result in a larger sample size and to examine the clinical utility of these findings, and functional studies are needed to examine the biological effect of the interactions between *ANGPTL3* and *RXRA* to influence ApoB response to fibrate-statin therapy.

TABLE 1. Two-SNP (rs12130333 and rs4240705) joint-genotype frequencies, mean percent change of ApoB levels, and standard error in the combination therapy group

Genotype	CC:AA	CT:AA	TT:AA	CC:AG	CT:AG	TT:AG	CC:GG	CT:GG	TT:GG
Frequency	191	88	8	208	68	17	62	23	3
Mean percent change	-36.54	-38.07	-23.12	-36.33	-34.50	-40.00	-39.53	-29.99	1.84
Standard error	1.18	1.82	8.71	1.14	2.20	3.27	1.84	4.41	28.95

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