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Variants in the *APOA5* gene region and the response to combination therapy with statins and fenofibric acid in a randomized clinical trial of individuals with mixed dyslipidemia

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ABSTRACT

Objective: Atherogenic dyslipidemia is highly associated with coronary heart disease and is characterized by elevated triglycerides (TG), low high-density lipoprotein cholesterol (HDL-C), and elevated low-density lipoprotein cholesterol (LDL-C). The combination of statins and fibrates is a common modality to treat individuals with atherogenic dyslipidemia.

We sought to identify single nucleotide polymorphisms (SNPs) associated with HDL-C, TG, and apolipoprotein A1 (ApoA-I) response to combination therapy with statins and fenofibric acid (FA) in individuals with atherogenic dyslipidemia.

Methods: 2228 individuals with mixed dyslipidemia who were participating in a multicenter, randomized, double-blind, active-controlled study comparing FA alone, in combination with a statin, or statin alone for a 12-week period, were genotyped for 304 candidate SNPs. A multivariate linear regression analysis for percent change in HDL-C, ApoA-I and TG levels was performed.

Results: SNPs in the apolipoprotein (*APO*) *A5-ZNF259* region rs3741298 ($P = 1.8 \times 10^{-7}$), rs964184 ($P = 3.6 \times 10^{-6}$), rs651821 ($P = 4.5 \times 10^{-5}$), and rs10750097 ($P = 1 \times 10^{-4}$), were significantly associated with HDL-C response to combination therapy with statins and FA, with a similar association identified for ApoA-I. A haplotype composed of the minor alleles of SNPs rs3741298, rs964184, and rs10750097, was associated with a positive response to statins and FA ($P = 8.7 \times 10^{-7}$) and had a frequency of 18% in the study population.

Conclusion: In a population with atherogenic dyslipidemia, common SNPs and haplotypes within the *APOA5-ZNF259* region are highly associated with HDL-C and ApoA-I response to combination therapy with statins and FA.

1. Introduction

Elevated triglycerides (TG) and low levels of high-density lipoprotein cholesterol (HDL-C), with or without high levels of low-density lipoprotein cholesterol (LDL-C), characterize a dyslipidemia that places people at high risk for coronary heart disease (CHD). This phenotype, referred to as atherogenic dyslipidemia or mixed dyslipidemia, occurs frequently among individuals with insulin resistance such as the metabolic syndrome and type 2 diabetes. Monotherapy with statins is seldom sufficient to improve all lipid levels, including achieving non-HDL-C targets, in those with atherogenic dyslipidemia, and the addition of other agents such as niacin or a fibrate such as fenofibric acid (FA) is often required. Although recent clinical trials have questioned the utility of fenofibrate alone or in combination with statins to lower CHD events, individuals with mixed dyslipidemia in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial had incremental benefit in CHD event reduction from combination of fenofibrate and statin therapy compared to statin monotherapy [1–3]. A similar benefit was observed in individuals with mixed dyslipidemia participating in the Fenofibrate Intervention and Event Lowering in Diabetes

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(FIELD) study with a reduction in CHD events following fenofibrate treatment [2,3].

FA is a PPARA receptor agonist which modulates multiple downstream targets including ABCA1 and apolipoproteins AI, AII, and CIII. The lipid response to fenofibrate treatment varies both by individual and by lipid phenotypes (e.g., normo-lipidemic vs. mixed dyslipidemia), and its specific mechanism of action supports the notion that genetic variation may have an important effect on determining individual response to therapy. In previous studies, a few polymorphisms have been identified that are associated with TG and HDL-C response to fenofibrate therapy. However, most of these studies did not examine individuals with mixed dyslipidemia who may be, the individuals most likely to benefit from the addition of fibrates to statins, and none of the prior studies have examined combination therapy of fibrates and statins [4–6].

In this study, we examined the association of single nucleotide polymorphisms (SNPs) and the response to the combination of FA and statins in subjects with mixed dyslipidemia who enrolled in a randomized, double-blind, active-controlled clinical trial program. We hypothesized that common genetic variants will have a significant effect on the lipid response to combination therapy with FA and statins in individuals with mixed dyslipidemia.

2. Methods

2.1. Study population

The study population included men and women who participated in a program which included 3 concurrent prospective, randomized, double-blind, phase-3 studies designed to examine the efficacy for a new FA formulation (fenofibric acid, TriLipix, Abbott, Abbott Park, IL, USA). A detailed description of the study design has been published elsewhere [7,8]. In brief, inclusion criteria included TG $\geq$ 150 mg/dL, HDL-C < 40 mg/dL for men or <50 mg/dL for women, and LDL-C $\geq$ 130 mg/dL. In the three studies, participants were randomized into groups that received either FA alone, statin alone, or combination of FA and statins. Participants had a washout period of 6 weeks in which no lipid-modifying therapy was given. A 12-week treatment phase followed the washout period, and lipid measurements were obtained at the beginning and end of the treatment period. Additional details regarding the clinical study design are included in the Supplement. Multidimensional scaling revealed no population substructure in the combined study population.

2.2. Selection of genes and SNPs

Genes were selected based on their involvement in HDL-C and TG metabolism [9,10]. In order to evaluate most of the common variations in each gene, tag SNPs were selected using the Genome Variation Server (http://gvs.gs.washington.edu/GVS/) based on the following criteria: HapMap-CEU dataset, $r^2 \geq 0.8$ for each bin, minor allele frequency $\geq$1%, and a 4–10 kB margin from gene boundary. Additional SNPs were added based on published genome-wide association studies for associations with HDL-C and TG [11–14]. A total of 350 SNPs to be tested for therapy response were selected, and 34 SNPs with inter-ethnic difference in allele frequencies were added to aid in correcting for potential population stratification within the European-American population [15]. The list of SNPs and the reason for their inclusion is detailed in Supplemental Table 1. SNPs were genotyped using Golden Gate chemistry on an Illumina Bead Express system (Igenix, Seattle, WA). Samples with call rate <90% were excluded. SNPs were excluded if they had a call rate <95%, showed evidence of deviation from Hardy–Weinberg equilibrium (HWE) at $P < 0.001$ using the exact test, or were monomorphic.

2.3. Statistical analysis

Statistical analysis was performed using PLINK (version v1.07, http://pngu.mgh.harvard.edu/purcell/plink/) [16]. In order to maximize statistical power, treatment groups of the original study design were collapsed into 3 major therapy groups: FA alone ($n = 341$); statin alone ($n = 864$); and, combination therapy of statins and FA ("CG" group, $n = 674$). Analysis was restricted to the European-American population in order to avoid increased type I and type II errors due to population stratification. Samples designated "European-American" were selected and subjected to multidimensional scaling analysis. Uncorrelated SNPs were identified by pruning out those with pair-wise linkage disequilibrium $r^2 > 0.5$. The remaining SNPs (including all 34 ancestry-informative SNPs) were then used to cluster the individuals based on identity by state (IBS) analysis. The resulting IBS data were used in multidimensional scaling, and 4 components were saved. All procedures were carried out in PLINK. No significant structure in the sample was detected (Supplemental Fig. 1).

The initial discovery phase analysis was performed using linear regression including age and sex as covariates in order to identify the SNPs with the strongest association with percent change of HDL-C, TG, or ApoA-I in each of the groups after treatment. The second phase of the analysis included linear regression for three most significant SNPs and with the highest population frequency identified in the "CG" group. The outcome was percent change, and the covariates were age, sex, body mass index (BMI), smoking, and diabetes. Percent change was defined by the difference in trait level before and after treatment, standardized by the before-treatment level. Association testing for each examined trait with the candidate SNPs was considered a separate hypothesis. A probability value $<1.7 \times 10^{-4}$ was considered significant after Bonferroni correction for the number of SNPs tested (304 after exclusions). Permutation analysis as implemented in PLINK was performed to verify the significance of the associations. Haplotypes were inferred using the expectation-maximization algorithm method. Only haplotypes with inferred frequency >0.05 were analyzed. Risk haplotypes were identified based on discovered SNPs in the "CG" treatment group. Multivariate regression analysis of haplotype and covariates was performed, and the partial regression coefficients were estimated for each parameter. The Wald test P-value was reported for each haplotype. Covariates age, sex, BMI, smoking, and diabetes were included in the haplotype analysis.

3. Results

Of the 384 genotyped SNPs, the following were excluded from the analysis: monomorphic SNPs ($n = 2$), SNPs with minor allele frequency (MAF) <5% and missing >1% or MAF >5% and missing >5% ($n = 14$), and those with HWE <0.001 ($n = 30$). After excluding the 34 SNPs selected for stratification analysis, 304 SNPs remained for hypothesis testing.

From a total sample size of 2684 individuals, 456 samples were excluded. Samples were excluded due to a total call rate <95% ($n = 66$), duplicated DNA samples ($n = 64$), and ethnicity other than European ($n = 326$). Mean increase in HDL-C in the combination therapy group was 18.3%, and the mean TG decrease was 44.7% which is similar to other studies examining the combination of statins and fenofibrates in individuals with phenotypes similar to mixed dyslipidemia which showed an increase of 16–23% in HDL-C and a decrease of 29–50% in TG [17–20].

There was no significant difference in baseline characteristics between the three treatment groups (Supplemental Table 2).

There were a number of SNPs which had significant associations with HDL-C and ApoA-I percent change following FA treatment. The