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Evaluation of the gene-age interactions in HDL cholesterol, LDL cholesterol, and triglyceride levels: The impact of the *SORT1* polymorphism on LDL cholesterol levels is age dependent

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Abstract

Several genes that influence HDL-C, LDL-C, and triglyceride levels have been identified. The effects of genetic polymorphisms on lipid levels may be age dependent. We replicated 17 of these previously identified associations and then used cross-sectional and longitudinal analysis to investigate age-SNP interaction effects. The rs646776 SNP at the *SORT1* locus showed an age interaction that was significant in cross-sectional analyses of 1350 individuals from Utah (p=0.0003) and in 2977 individuals from the NHLBI Family Heart Study (p=0.007) as well as in longitudinal analysis of a subsample of 1099 individuals from the Utah cohort that had been followed for over 20 years (p=0.0001). The rs646776 genotype-specific difference in LDL-C levels was significantly greater for younger individuals than for older individuals. These findings may help elucidate the mode of action of the *SORT1* gene and impact potential therapeutic interventions targeting this pathway.

Keywords

SORTILIN 1; AGE-GENE INTERACTION; CARDIOVASCULAR DISEASE; CARDIOVASCULAR GENETICS; HERITABILITY

Introduction

Over 90 genes have been identified that influence lipid levels [1–2]. The molecular mechanism and clinical relevance of causative polymorphisms at several associated loci are being elucidated [1, 3–4]. These genes only explain a small portion of lipid heritability suggesting other mechanisms such as gene-gene and gene-environment interaction may be important [5]. The effects of genetic polymorphisms on lipid levels may be age dependent. Although age is not inherited or modifiable, a portion of heritability may be attributable to gene-age interactions, as described by Guo and Shi, et al [6–7].

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In a family-based sample, we genotyped 14 of the most significant SNPs previously associated with high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), or triglyceride levels in genome-wide studies [1–2]. We then investigated SNP-age interaction effects at nominally replicated loci in this sample, a replicate sample, and in a longitudinal subsample of the initial sample. This allowed us to evaluate genotype-specific age effects comparing subjects of different ages in cross sectional samples as well as comparing inter-individual change over time in the longitudinal sample. Family based samples also allowed estimation of the proportion of heritability attributable to the variants and interactions.

Methods

Study samples

Cardiovascular Disease in Utah Pedigrees Sample (Utah Pedigree Study)—In 1980 and 1982, approximately 2500 subjects in 98 Utah pedigrees were recruited by identifying 2 or more probands with early stroke, coronary heart disease death, or hypertension in each pedigree[8]. Subjects were reexamined in 1991 as described previously [9]. An additional follow-up visit was performed between 2004 and 2008 where DNA was obtained for genetic studies and a dietary survey was administered. Lipid measures and standard cardiovascular risk measures were obtained for early visits using standard methods as described previously [9]. For follow-up visits between 2004 and 2008 lipid measures were performed using the vertical auto profile (VAP) method (Atherotech, Birmingham, Alabama), with a subset of samples also receiving standard lipid measures to enable adjustment for bias between different methods of lipid measurement. For this study, initial analysis was performed on individuals for whom measures on lipids and genetic polymorphisms both were available and who were not taking cholesterol modifying medications (n=1350 individuals). We used a subset of this sample for longitudinal confirmation, selecting individuals for whom at least 2 LDL-C measures taken at least 15 years apart were available. Two, 3, and 4 LDL-C measures were available for 159, 308, and 632 individuals, respectively, for a total of 1099 individuals with at least 2 measures.

Family Heart Study sample (FamHS sample)—A replication cohort consisted of 2977 white individuals from the Family Heart Study who were not taking cholesterol modifying medications and for whom lipid measures and genotyping was available as described previously [10]. These population-based families were gathered at 4 clinical centers: the Framingham Heart Study, the Utah Family Tree Study, and ARIC centers in Minneapolis and Forsyth County, NC [11].

SNP selection and genotyping

We genotyped 14 SNPs in 2302 individuals from the Utah Pedigree Study by real-time PCR melting curve analysis on a LightScanner instrument (Idaho Technology, Salt Lake City, Utah) using SimpleProbes and genotyping reagents (Idaho Technology, Salt Lake City, Utah). The 14 SNPs selected were among the most significant replicated loci that had been implicated in previous genome-wide association studies or were in linkage disequilibrium with implicated SNPs, and were at genomic locations where successful assay probes could be developed 1–2. These were: rs102275, rs10468017, rs1260326, rs17321515, rs2228671, rs3135506, rs3846662, rs402275, rs4420638, rs4939883, rs646776, rs6756629, rs693, and rs714052.

Genotyping data from genome-wide studies using FamHS sample has been described previously [12].

Statistical Analysis

For all statistical analyses additive genetic models were used and natural log transformations of HDL-C, LDL-C, and triglyceride levels were used as transformed distributions more closely approximated Gaussian distributions. We evaluated associations with HDL-C, LDL-C, and triglyceride levels using jPAP software [13], a tool for maximum likelihood analysis of large pedigrees, to evaluate the interaction while correcting for age, sex, and BMI and family structure. We considered $p<0.05$ adequate to justify further analysis, with $p<0.001$ consistent with definitive replication after correction for multiple comparisons.

We then investigated potential age interactions for 17 associations (see Table 1) using R statistical software (R) for multiple linear regression to evaluate the effect of age-genotype interaction while correcting for BMI and sex. We only investigated interactions where associations were at least nominally replicated to maximize the likelihood of finding gene-age interactions. Subsequent confirmatory analysis was performed only for the gene-age interactions that remained significant after Bonferroni correction for multiple comparisons ($p<0.003$). Replication of the initial results was performed using general linear models in SAS (v9.2) using the FamHS data and correcting for BMI, gender, study center, and relatedness ('PROC GENMOD, corr = exchange' to correct for relatedness).

We combined FamHS and Utah Pedigree Study families and performed maximum likelihood analysis using jPAP software to estimate heritability attributable to genetic and interaction factors measured as change in polygenic variance. LDL-C levels were used as the dependent variable with sex, BMI, and study center as baseline covariates.

For the subsample of the Utah Pedigree Study with longitudinal LDL-C measures (Table 2), we used simple linear regression to obtain estimates of linear trend in LDL-C over time (beta from linear regression) for each individual separately. Beta in this regression is the best estimate of individual's change in LDL-C per year regardless of number of LDL-C measures available. We then used SAS general linear models to determine if an individual's trend in LDL-C per year (beta) was associated with genotype while adjusting for sex, BMI, and relatedness.

Results

Of the 14 SNPs we investigated, we replicated 17 associations for 12 genetic loci identified by previous GWAS at a nominal significance of $p<0.05$ with four SNPs associated with multiple lipid measures. Six associations survived strict correction for multiple comparisons (Table 1). Of the 17 associations evaluated for age interaction, only the effect of rs646776 at the *SORT1* locus on LDL-C was significant ($p=0.0003$, Table 1). Linear models showed the interaction effect increased the significance of both age and genotype ($p=0.57$ and 0.011, respectively, without interaction; $p=0.0004$ and 0.00003, respectively, with interaction). LDL-C variance attributed to *SORT1* was 1.8% and to the age-*SORT1* interaction was 0.2%.

In the complete model using the confirmation FamHS sample, age, rs646776, and the age-rs646776 interaction each had a significant effect on LDL-C levels ($p=0.03$, $p=1\times10^{-6}$, and $p=0.007$ respectively).

Maximum likelihood analysis of the combined Utah Pedigree Study and FamHS sample using jPAP software estimated LDL-C heritability to be 43%, with the heritability portion due to rs646776 and the age-rs646776 interaction 4.3% and 0.2%, respectively.

Within-individual longitudinal change in LDL-C was analyzed in 1099 individuals (681 AA, 364 AG, and 54 GG for rs646776) who had multiple visits in the Utah Pedigree Study. An

average of 22.5 years (min 17, max 27) separated initial and final cholesterol measures on individuals who were 3 to 67 years at initial visit and 27 to 90 years at final visit (see Table 2). Primarily for the younger ages, each copy of the G allele was associated with lower LDL-C levels, whereas this effect diminishes with age ($p=8\times10^{-6}$ for LDLC difference at initial visit, p=0.06 for LDL-C difference at final visit, Table 2). The gene-age interaction effect was significant after correcting for sex, change in BMI, relatedness, and age at initial visit (p=0.0001).

Discussion and Conclusions

We replicated several previously identified genetic associations with lipid markers of cardiovascular disease. Of 17 nominally replicated associations with HDL-C, LDL-C, and triglyceride levels, we identified one significant gene-age interaction. This interaction replicated in a larger cohort and in a subset of the Utah cohort followed longitudinally for two decades. The effect of rs646776, at the *SORT1* locus, on LDL cholesterol is age dependent with *SORT1* genotype-associated differences in LDL-C levels decreasing with increasing age.

As expected, the rs646776 variance was heritable, and a portion of the interaction variance was heritable, indicating that at least a small portion of "missing" heritability is due to gene-age interactions. However, heritability estimates vary from study to study and are associated with large standard error. Suggestive trends for gene-age interaction were seen at several other SNPs; failure to find significant age effects at these may have been due to lack of power (see Table 1).

The noncoding polymorphism, rs12740374, which alters a C/EBP transcription factor binding site, was recently identified as the causative polymorphism at the *SORT1* locus [3]. The minor allele increases transcription factor binding, which is associated with higher SORT1 protein and lower LDL-C levels. SNPs rs646776 and rs12740374 are less than 1kb apart and are almost perfectly correlated in Caucasian populations [3]. Levels of different CCAAT/Enhancer Binding Protein Alpha (CEBPA) isoforms have been found to vary with age and be involved in age-associated chromatin remodeling in mice and rats, suggesting a molecular mechanism for this interaction [14–15]. As the effect of the *SORT1* locus may be greater than previously reported in young individuals and less in older individuals, future confirmatory studies and risk prediction algorithms should take this effect into account. These findings may also contribute to the development of potential therapeutic interventions targeting this pathway. For example, treatments that prevent levels of CEBPA from decreasing with age may prevent age-related increase LDL-C levels in individuals carrying the CEBPA responsive variant.

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Table 1

Replicated associations with HDL-C, LDL-C, and triglyceride levels and age-SNP interaction p-values in the Utah Pedigree Study.

Analyte	SNP (gene)	Main association p-value	Age-SNP interaction p-value
HDL-C	rs328(*LPL*)	0.014	0.35
	rs4420638 (*APOE*)	0.014	0.42
	rs4939883 (*LIPG*)	0.0013	0.15
	rs693 (*APOB*)	0.021	0.08
LDL-C	rs646776 (*SORT1*)	0.000004*	0.0003*
	rs693 (*APOB*)	0.0066	0.25
	rs3846662 (*HMGCR*)	0.018	0.010
	rs17321515 (*TRIB1*)	0.0098	0.22
	rs102275 (*FADS*)	0.018	0.4
	rs2228671 (*LDLR*)	0.026	0.045
	rs4420638 (*APOE*)	0.0007*	0.22
Triglycerides	rs1260326 (*GCKR*)	0.0010*	0.89
	rs714052 (*MLXIPL*)	0.00003*	0.24
	rs17321515 (*TRIB*1)	0.014	0.73
	rs3135506 (*APOA1*)	0.00008*	0.38
	rs4420638 (*APOE*)	0.0002*	0.45
	rs646776 (*SORT1*)	0.0047	0.67

*Significant after correction for multiple comparisons

Table 2

Changes in lipid measures overtime in the Utah Pedigree Study by *SORT1* genotype

	rs646776AA (n = 681)		rs646776AG (n = 364)		rs646776GG (n = 54)	
	Initial Visit Mean (SD)	**Change Mean [95% CI]**	**Initial Visit Mean (SD)**	**Change Mean [95% CI]**	**Initial Visit Mean (SD)**	**Change Mean [95% CI]**
Sex (M)	48.8%	-	51.1%	-	61.1%	-
Age Years	26.9 (14.7)	22.5 [22.4_22.6]	26.3 (14.7)	22.5 [22.4_22.6]	28.0(14.1)	22.3 [22.0_22.6]
BMI	22.4(5.3)	6.3 [5.9_6.6]	22.1(5.1)	6.3 [5.8_6.8]	23.8(6.0)	5.5 [4.5_6.6]
LDL (mg/dL)	120.5(38.0)*	15.1 [11.8_18.3]*	112.7(34.7)*	19.4[15.7_23.2]*	105.3(35.7)*	26.3 [17.3_35.2]*
HDL (mg/dL)	48.1(11.4)	−2.3 [−3.1_−1.3]	48.1 (11.1)	−1.3 [−2.3_−0.2]	46.2 (11.6)	−0.5 [−3.5_2.4]
Trig (mg/dL)	95.2(53.8)	42.2 [36.0_48.4]	90.4(50.7)	54.9 [40.5_69.2]	85.4(49.5)	55.5 [29.8_81.2]

*Significant difference between genotypes for difference at initial visit and difference in change over time using PROC GENMOD, $p \leq 0.001$