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Linkage Analysis Incorporating Gene-Age Interactions Identifies Seven Novel Lipid Loci: The Family Blood Pressure Program

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Abstract

OBJECTIVE—To detect novel loci with age-dependent effects on fasting (8 hours) levels of total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides using 3,600 African Americans, 1,283 Asians, 3,218 European Americans, and 2,026 Mexican Americans from the Family Blood Pressure Program (FBPP).

METHODS—Within each subgroup (defined by network, race, and sex), we employed stepwise linear regression (retention $p = 0.05$) to adjust lipid levels for age, age-squared, age-cubed, body-mass-index, current smoking status, current drinking status, field center, estrogen therapy (females only), as well as antidiabetic, antihypertensive, and antilipidemic medication use. For each trait, we pooled the standardized male and female residuals within each network and race and fit a generalized variance components model that incorporated gene-age interactions. We conducted FBPP-wide and race-specific meta-analyses by combining the p -values of each linkage marker across subgroups using a modified Fisher's method.

RESULTS—We identified seven novel loci with age-dependent effects; four total cholesterol loci from the meta-analysis of Mexican Americans (on chromosomes 2q24.1, 4q21.21, 8q22.2, and 12p11.23) and three high-density lipoprotein loci from the meta-analysis of all FBPP subgroups (on chromosomes 1p12, 14q11.2, and 21q21.1). These loci lacked significant genome-wide linkage or association evidence in the literature and had logarithm of odds (LOD) score ≥ 3 in the meta-analysis with LOD ≥ 1 in at least two network and race subgroups (exclusively of non-European descent).

CONCLUSION—Incorporating gene-age interactions into the analysis of lipids using multi-ethnic cohorts can enhance gene discovery. These interaction loci can guide the selection of families for sequencing studies of lipid-associated variants.

Keywords

Lipids; aging; genetics; meta-analysis; gene-age interactions; linkage; triglyceride; cholesterol

INTRODUCTION

Lipid levels are important determinants of cardiovascular disease (CVD), an ailment that causes one of every three deaths in the United States and contributes to \$312.6 billion in health care costs and lost productivity annually [1]. Although plasma lipid levels are highly heritable (40% to 80%) [2-3], genetic association studies have identified (mostly) common variants in 157 loci that collectively explain less than 15% of the variance in lipid levels [4-6], leaving a substantial proportion of the lipid heritability missing [7]. The most recent (and largest) genetic association study of lipids to date included 188,577 participants of European descent and discovered 62 novel loci that collectively explained less than 2.6% of the lipid variance [5]. This insatiable need for increasingly bigger samples to detect common variants with diminishing effect sizes suggests that alternative approaches may facilitate the discovery of novel lipid loci with potentially larger effect sizes.

We leveraged the dynamic nature of genetic effects over a lifetime to enhance the discovery of lipid loci which may contain rare (minor allele frequency (MAF) <1%) and low-frequency (1% < MAF < 5%) variants. Plasma lipids vary by age [8] and exhibit greater correlations among first-degree relatives with similar ages [9], a promising but insufficient indicator of age-dependent genetic effects. Additional evidence has been rendered by candidate gene studies and a small-scale genome-wide association study (GWAS) which identified a handful of lipid loci with age-dependent effects [6, 8, 10-20]. Some of these were qualitative ("crossover") gene-age interactions, with opposite directions of the genetic effect in the young versus the elderly; for example, a single nucleotide polymorphism (SNP) in *SGSM2* was associated with increased low-density lipoprotein levels at ages 12-20 year old but decreased levels in 72-80 year olds [6]. Failing to account for gene-age interactions may prevent lipid loci from being detected, particularly in samples with wide age distributions. We incorporated gene-age interactions into genome-wide linkage analyses which may have a greater ability to detect low-frequency and rare variants than genome-wide association studies. Rare and low-frequency polymorphisms compose $\approx 74.6\%$ of all variants [21] and may have larger effects sizes than common variants and explain substantial proportions of lipid variance. One recent study suggested that rare variants may collectively explain up to 7.8% of the variance in high-density lipoprotein levels [21], while another found that rare variants in four genes (*LPL*, *GCKR*, *APOB*, and *APOA5*) explained 1.1% of total variation in the diagnosis of hypertriglyceridemia [22].

In this investigation, our primary aim was to detect novel quantitative trait loci (QTLs) with age-dependent effects on fasting (8 hours) serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG). We fit a highly flexible generalized variance components model [23], which allowed both the heritability and the correlations among relatives to depend on age [23], in each of nine population groups from the Family Blood Pressure Program (FBPP) [24]. These nine groups represented 3,600 African Americans (AAs), 1,283 Asians, 3,218 European Americans (EAs), and 2,026 Mexican Americans (MAs) [24]. We subsequently performed overall (across all races) and race-specific meta-analyses of these genome-wide linkage analyses to capitalize on the sharing of lipid loci across populations [3]. The newly discovered lipid loci may guide the selection of families to sequence using next-generation methods in order to increase the effect size and power to detect the causal variants underlying the linkage peak. This investigation may also provide further insight into the pervasiveness of age-dependent effects across the genome.

METHODS

Subjects

The FBPP is comprised of four separate multicenter networks: GenNet, GENOA, HyperGEN, and SAPHIRE. Although the recruitment method varied by the network, each recruited families ascertained through probands with high or low blood pressure. GenNet recruited nuclear families of young or middle-aged AAs, EAs (no lipid data available), and MAs through probands with untreated high-normal blood pressure. GENOA recruited AA and EA sibships containing at least two hypertensives, MA sibships containing at least 2

hypertensives who are also adult onset diabetics, and all full siblings of each index pair. HyperGEN recruited AA and EA sibships each containing at least two hypertensives, their parents, and one or more of a sibling's untreated offspring. SAPHIRE recruited Chinese (CHI) and Japanese (JAP) sibpairs highly concordant or discordant for hypertension. More details on the participants are published elsewhere[24]. All FBPP studies were approved by their institutional review committees and all included participants provided informed consent.

Genotyping

The Mammalian Genotyping Service in Marshfield, Wisconsin, performed all genome-wide linkage scans[25]. For all cohorts except GenNet MAs, we used 362 autosomal markers that passed quality control criteria (had missing rate <70%, mapped to one location in the genome, and had a location not duplicated by other markers). GenNet MAs were genotyped later using a different screening set that contained 372 quality controlled autosomal markers; of which, 71% matched (by name and/or genetic distance) the autosomal markers from the other cohorts. The average intermarker distances for the two screening sets were 9.5 cM and 9.4 cM, respectively. MapMaker/SIBS[26] and Pedcheck[27] were utilized to remove Mendelian errors, while GRR (graphical representation of relationships)[28] and ASPEX (affected sibpair exclusion mapping)[29] were used to verify family relationships.

Phenotype Adjustment

We analyzed four fasting (8 hours) plasma lipid concentrations: TC, HDLC, LDLC, and the natural logarithm of TG (transformed to reduce the skewness and excess kurtosis). GenNet AAs and MAs had only one available lipid level, TC, measured by a finger stick (ProAct cholesterol system) [30]; GenNet EAs did not have any lipid information available. All racial groups from GENOA, HyperGEN, and SAPHIRE had TC, HDLC, and TG measured by standard laboratory enzymatic methods. In general, LDLC was derived using the Friedewald equation [31] if TG < 400 mg/dL and directly measured if TG ≥ 400 mg/dL. When multiple visit data were available, we included only one visit per individual (that which minimized missing lipid and covariate data). We excluded the lipid phenotypes of 87 participants with body-mass-index (BMI) or any lipid value that was four or more standard deviations away from the means of their subgroup (defined by network, race, and sex). Stepwise linear regression within each subgroup adjusted lipid phenotypes for age, age-squared, age-cubed, BMI, current smoking status (yes/no), current drinking status (yes/no), estrogen therapy (yes/no for females only) and field center (if multi-center), as well as dichotomous (yes/no) indicators of antidiabetic, antihypertensive, and antilipidemic medication use. We retained terms significant at the 0.05 level and standardized the residual lipid values to a mean of zero and standard deviation of one. We excluded any covariate-adjusted lipid value that exceeded four standard deviations from the mean of the subgroup (10 values were excluded for any trait).

Statistical Analysis

We pooled the male and female lipid residuals within each network-race group before conducting linkage analysis. We used MERLIN[32] to estimate the kinship coefficients for all members of a pedigree, as well as to compute the identity-by-descent (IBD) coefficients

for all markers using the multipoint method, sex-averaged genetic distances from the Marshfield map, and allele frequencies estimated from our samples. The multipoint method allowed the calculation of the IBDs for markers missing in GenNet MAs due to discrepancies in the screening set. Within each network and race group, we first employed MERLIN to test all markers for additive genetic variance due to the QTL in the absence of age interactions. We then imported the estimated kinship coefficients and IBDs into the MATLAB-based QTLtrends package [23, 33] and fit a generalized variance components model that allowed the polygenic and QTL components to vary as Gaussian functions of age [23, 33]. This Gaussian interaction function defined a unimodal heritability (as a function of age) with parameters indicating the age at which the heritability peaked for the QTL component, the age at which the heritability peaked for the polygenic component, and the spreads of these heritability peaks (i.e., variances); however, this model could mimic a linearly increasing or decreasing heritability by selecting extreme values of model parameters (if the age at which the peak heritability was reached was estimated to be extremely large or small). For both analyses with and without interactions, FBPP-wide and race-specific meta-analyses were conducted by combining the p-values of each of the 362 common markers across subgroups using a modification of Fisher's method [34].

DEFINITION OF A QTL

For each lipid trait, we defined a quantitative trait locus (QTL) as a genomic segment with logarithm of odds (LOD) score ≥ 3 in any race-specific or FBPP-wide meta-analysis and with LOD ≥ 1 in at least two subgroups (defined by race and network).

RESULTS

Our analysis included 3,600 AAs, 1,283 Asians, 3,218 EAs, and 2,026 MAs (for a total sample size of 10,127 participants) with age and at least one covariate-adjusted lipid value. The descriptive statistics stratified by network, race, and sex are displayed in **Table 1**. Overall, 54 % of the total sample had desirable total cholesterol levels (<200 mg/dl) and 59% had normal triglyceride levels (<150 mg/dl). However, the mean levels of triglycerides differed markedly across subgroups, ranging from 100.0 to 222.7 mg/dl, as did the prevalence of antilipidemic medication use, which ranged from 1.1% to 27.3%. The GenNet subgroups had the youngest mean ages and the lowest prevalences of antilipidemic medication, antihypertensive medication, and estrogen replacement use. GENOA MAs had a high ($>51\%$) prevalence of antidiabetic medication use as expected due to their ascertainment strategy (recruited sibships containing at least 2 hypertensives with adult onset diabetes). Lifestyle behaviors varied widely across the subgroups; the prevalence of smoking ranged from 5.2% to 63.8% and the prevalence of alcohol drinking ranged from 5.9% to 81.7%.

In order to determine whether we experienced enhanced gene discovery using gene-age interactions, we first established the “baseline” of how many QTLs were discovered without age interactions. Performing overall and race-specific meta-analyses without gene-age interactions identified two QTLs (1 novel and 1 known; see **Table 2**). The first QTL was a novel Asian-specific HDL C locus on chromosome 1p12 (D1S534) which had supporting evidence (LOD ≥ 1) from the SAPHIRE Chinese and Japanese subgroups. The second QTL

was a known lipid locus on chromosome 9p21.3 (D9S1121) that was significantly linked to TC in the EA-specific meta-analysis but garnered some support from one EA subgroup (HyperGEN EAs) and one MA subgroup (GENOA MAs). Previous family studies have yielded significant linkage evidence of chromosome 9p21.3 with TG ($p=4E-05$; EAs; [35]) and HDLC (LOD=3.4; MAs; [36]), also demonstrating biracial support for this lipid locus. The 9p21.3 region has also been suggestively linked to TC (LOD=2.1; EAs; [37]) and a SNP contained in the 1-LOD drop (≈ 21.2 Mb to 42.1 Mb) interval was significantly associated with coronary heart disease ($p=7E-58$; [38]).

Sixteen additional distinct QTLs (6 novel and 10 known; see **Table 2**) were identified through the overall and race-specific meta-analyses that incorporated gene-age interactions. One additional novel HDLC locus (on chromosome 1p12) was discovered during both the Asian-specific meta-analysis without interactions and the FBPP-wide meta-analysis with interactions; three different racial groups (MAs, AAs, and Japanese) supported the QTL when interactions were included but only Asians (Chinese and Japanese) supported the QTL when interactions were omitted, demonstrating that age-dependent effects can impact consistency of findings across populations. Thus, in total, the effects of seven novel QTLs were significantly modulated by age: four TC loci from the MA-specific meta-analysis (on chromosomes 2q24.1, 4q21.21, 8q22.2, and 12p11.23) and three HDLC loci from the FBPP meta-analysis (on chromosomes 1p12, 14q11.2, and 21q21.1). All novel QTLs were supported exclusively by non-EA subgroups and were significantly linked with only one lipid trait.

Of the ten known lipid loci identified through the use of interactions, 5, 5, and 3 were associated with TC, HDLC, and LDLC, respectively; three known lipid QTLs influenced two different lipid traits. These ten known QTLs lacked even suggestive evidence (LOD 2) in our investigation when interactions were omitted and were largely supported by subgroups of non-European descent. In fact, EAs subgroup contributed to only two of the QTLs that interacted with age. For each novel and known QTL, **Supplementary Table 1** displays the age at which the maximum heritability was achieved in all supporting network-race subgroups. The age affiliated with the peak heritability differed across subgroups for most QTLs; however, the TC loci on chromosomes 8q22.2 and 18q22.3 attained peak heritability in the late twenties to mid-thirties, the LDLC locus on chromosome 6q12 reached maximum heritability in the mid-forties to early fifties, and the LDLC locus on chromosome 8q13.1 achieved maximum heritability in the late thirties to mid-forties,

The LOD score plots for all (novel and known) QTLs that significantly interacted with age are displayed in **Figures 1-2** along with their supporting network-race subgroups. As shown in **Figure 1**, the MA subgroups contributed greatly to the discovery of QTLs for the only lipid trait (TC) for which meta-analysis was performed (the other lipids were unavailable in GenNet MAs). The MA-specific meta-analysis led to the identification of four novel (on chromosomes 2q24.1, 4q21.21, 8q22.2, and 12p11.23) and three known (on chromosomes 11p13, 17q22, and 18q22.3) QTLs that interacted with age to influence TC. Six of these seven loci, including all four novel loci, lacked support (LOD 1) from any other racial subgroup at the peak marker. These loci were supported by both the GenNet MAs, which

had a low prevalence of antidiabetic medication use, and the GENOA MAs which were ascertained also on diabetic status.

The three novel HDLC loci discovered via the meta-analysis of all FBPP subgroups are displayed in **Figure 2a** (represented by marker D21S1437 on 21q21.1) and **Figure 2b** (on chromosomes 1p12 and 14q11.2). **Figure 2a** also shows the three double-trait QTLs, all previously known, on chromosomes 1p33 (D1S2134; LDLC and TC), 6q12-6q14.1 (the region encompassed by D6S1053 to D6S1031; LDLC and HDLC), and 21q22.3 (D21S1446; HDLC and TC). The association of QTLs with both TC and one of its components (HDLC, LDLC) was not surprising. However, the association of chromosome 6q12-6q14.1 with LDLC in the AA- specific meta-analysis and HDLC in the meta-analysis of the FBPP subgroups (with support from HyperGEN AA and the SAPPHiRe Chinese) suggested either pleiotropy or two distinct QTLs (one for each trait).

Published linkage findings that corroborated our lipid loci are shown in **Supplementary Table 2**, while selected genome-wide association findings in the 1-LOD drop support interval of our QTLs are presented in **Table 3**. QTLs were deemed “known” if they had been previously linked (with LOD ≥ 3 or p-value reported as genome-wide significant) to any lipid trait or contained a SNP previously associated (p-values $\leq 5E-08$) with a lipid trait in their 1-LOD drop support interval. The QTLs with both significant linkage and association evidence were 1p33 (linkage:[39]; lipid-associated genes (see **Table 3**): *CYP4A11*, *PCSK9*, and *GOT2P1-RPSAP20*) and 2q14.1 (linkage:[35]; lipid-associated genes: *INSIG2* and *LOC84931*). The QTLs on chromosomes 6q12 [40], 9p21.3 [35-36], 11p13 [41], 18q22.3 [41], and 21q22.3 [42] had significant linkage evidence only, while the QTLs on 6p22.3 (cited genes: HFE, ZNRD1-AS1, WASF5PHLA-B, HCG26-MICB, AGPAT1-EGFL8, HLA-DRA, COL11A2, and *SPDEF-RPL7P25*, *KCNK17*), 8p12 (*DUSP4-RPL17P33*), 8q13.1 (*UBXN2B-CYP7A1*), and 17q22 (*VMP1*, *APOHPRXCA*, and *ABCA8*) had significant association evidence only (see **Table 3**). Three of our novel loci (on chromosomes 2q24.1, 4q21.21, and 12p11.23) contained SNPs with some (p<1E-05) evidence of associations with lipids.

DISCUSSION

Incorporating gene-age interactions into genome-wide linkage analyses of a large (N=10,127) multi-ethnic sample enabled the identification of 6 novel loci that would have been missed through the traditional (interaction-free) approach; one additional novel locus was discovered both with and without interactions, bringing the total number of novel loci detected to seven. We also identified ten known lipid loci with interactions that lacked even suggestive evidence without interactions, showing the impact that age-dependent effects may have on the consistency of results across populations. Other investigations have reported gene-age interactions in three of our known QTLs [6, 11, 13], lending some credibility to the results from our modeling method. Known loci on chromosomes 1p33, 2q14.1, and 6p22.3 contained SNPs (in *PCSK9* [11], *INSIG2* [13], and an intergenic region [6], respectively) that yielded nominal evidence (p-value<0.05) of age interactions in prior lipid investigations. The modulation of genetic effects by age is biologically plausible, perhaps even expected. Aging encompasses the cumulative exposure to lifestyle choices, particularly diet, exercise,

stress, alcohol consumption, and smoking, which may affect lipid metabolism [8] and the expression of lipid-influencing genes [2](possibly through epigenetic mechanisms [43]). Identifying gene-age interactions in both novel and known genes may enhance our understanding of the biological basis of lipid homeostasis and potentially facilitate age-based antilipidemic interventions.

We can use our linkage results to capitalize on the overlap of rare/low-frequency and common lipid-associated variants in the same gene(s) [22]. The rationale is that once a gene is associated with a trait, it may become more likely that there are additional variants that alter the expression or function of the same gene that influences that trait [44]. For example, hypertriglyceridemia-associated genes discovered through GWAS contained a significant excess of rare variants when resequenced [22]. Thus sequencing the genes that are contained in our QTLs and have suggestive or significant GWAS evidence (as listed in **Table 3**) may harvest the “low hanging fruit” to discover rare/low-frequency variants interacting with age to affect lipid levels. This approach may identify new variants with large effects sizes even in the “known” lipid loci, as the strongest association often differs in effect size and allele frequency from the variant identified through GWAS [5]; fine mapping of the known *PCSK9* locus on 1p33 revealed that a low-frequency variant (MAF=0.03; $p=2E-136$) was more strongly associated with lipids than the original common variant (MAF=0.24; $p=9E-24$) identified through GWAS [5]. This approach is not a comprehensive investigation of the overlap between rare and common variants because the GWAS-associated variant may be a long distance away from (but in linkage disequilibrium with) the causal variant rather than the causal variant itself. In addition, QTLs may contain multiple lipid-influencing loci and may be driven by important regulatory variants not fully interrogated through GWAS.

To identify novel loci not implicated by previous GWAS, we can perform next-generation sequencing to examine all the protein-coding and regulatory regions in the 1-LOD drop support intervals of our QTLs [45]. We can further enhance causal variant discovery by using the linkage information to select the families to sequence with these next-generation methods [45]. Bowden et al. [46] followed up a linkage peak for adiponectin by exome sequencing three members of two families that were chosen by family-specific LOD scores; this narrowed the suspected region to the *ADIPOQ* gene [46]. Through conventional DNA sequencing of the gene in the whole family and genotyping of the identified causal variant in the whole sample, they identified a low frequency mutation that explained 17% of the variance in plasma adiponectin levels in the total sample of Hispanic Americans and 63% of the variance in families carrying the mutation. Linkage analyses incorporating gene-age interactions can offer even greater refinement by identifying the ideal ages of family members to sequence, if so desired, in order to enhance the effect size and power to detect the causal variants in a specific QTL.

Our findings underscore the importance of including underrepresented populations as a rich source of untapped trait-associated variants. We found four novel and three known TC loci by meta-analyzing the MA subgroups; all four of the novel loci and two of the known loci lacked supporting evidence at the peak marker in any other race. Similarly, Bowden et al could not find the aforementioned Hispanic adiponectin variant in 4,000 AA chromosomes or 3,000 EA chromosomes [46]. In our investigation, the EAs did not contribute to the

discovery of any novel loci but did provide support for three known loci (two that interacted with age and one that did not). The overlap of our linkage results (which were mainly supported by AA, Asian, and MA subgroups) with common variants from GWAS (which were mainly conducted on individuals of European descent) was supported in the literature; other investigations have demonstrated substantial overlap in the direction of effect and magnitude of lipid loci across ethnicities [3] but with disparate frequencies and allelic heterogeneity of the loci due to population-specific rare variants [3]. Linkage analysis may be better suited than GWAS to identify these population-specific variants (which tend to be rare [3]) which may be enriched in families. From a public health perspective, minorities suffer disproportionately higher rates of CVD [3, 47]; thus improved understanding of the biological basis of risk factors (such as lipid levels) specific to those populations may help inform interventions to reduce health disparities.

There are a few limitations to this study. The FBPP participants were ascertained based on hypertension or high blood pressure levels, thus the identified QTLs may relate more to dyslipidemia and hypertension [48] and may not be generalizable to healthy populations. On the other hand, families enriched for hypertension may also be enriched for lipid variants, increasing the power to detect lipid loci. A recent study showed that lipid-associated loci are strongly associated with other components of the metabolic syndrome, including SBP and DBP with excess associations of 2.5-fold and 3.7-fold [5], respectively. We ignored the possible modulation of gene-age interactions by gender [16-17] and adjusted for the use of lipid-altering medications through a dichotomous (yes/no) covariate; this crude adjustment ignored both the dosage (which is not available in this study) and exact medication taken.

The approach we used to examine gene-age interactions was quite flexible. The Gaussian function embedded in the variance components models could accommodate a wide range of interaction patterns, including those linearly increasing or decreasing (by having an extremely high or low age at which the peak heritability was obtained). By using a modification of Fisher's method to combine the p-values from the linkage analyses, we allowed the age at which the QTL reached maximum heritability, as well as the shape of the heritability, to vary across network and race subgroups. As shown in **Supplementary Table 1**, this model flexibility may be vitally important as the peak age differed substantially across the network and race subgroups supporting a QTL. However, our model was not without drawbacks; it was not suited to capture genes with bimodal or U-shaped effects, such as those that might occur in two disparate stages of life (i.e. influencing the young and old but not those in middle age). Also, the increase in the degrees of freedom due to the extra parameters used to model interactions can reduce the power to identify QTLs if no true interaction exists; the novel QTL on chromosome 1p12 was significant in Asians when interactions were omitted but was not even suggestive in Asians when interactions were included.

In summary, we capitalized on rare and low-frequency variants, interactions between genes and age, and the sharing of loci across minority populations to identify 18 QTLs (7 novel and 11 known) for TC, HDLC, and LDLC. With evidence that rare and low frequency polymorphisms compose $\approx 74.6\%$ of all variants [21], a strategy that employs genome-wide linkage analysis with gene-age interactions as a tool to select

families for whole-genome sequencing may facilitate the identification of novel rare and low-frequency variants with large effects involved in lipid homeostasis. These non-common variants may explain a non-negligible proportion of the trait variance and suggest novel pathways underlying the regulation of lipid levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Seven novel and ten known loci were linked to age-dependent effects on lipid levels
- Sixteen of the loci lacked even suggestive evidence without gene-age interactions
- The novel loci were supported exclusively by populations of non-European descent
- The novel linkage loci lacked published genome-wide significant associations
- Gene-age interactions, diverse samples, and non-common variants aid gene discovery

Figure 1.

LOD plots for the four novel (on chromosomes 2q24.1, 4q21.21, 8q22.2, and 12p11.23) and three known (on chromosomes 11p13, 17q22, and 18q22.3) QTLs that interacted with age to influence TC in the meta-analysis of MAs.

Figure 2.

The remaining QTLs that interacted with age to influence lipids: a) LOD plots for genomic regions containing QTLs for more than one trait. The HDLC locus represented by marker D21S1437 on 21q21.1 was novel. b) LOD plots for two novel (on chromosomes 1p12 and 14q11.2) and one known HDLC locus (on chromosome 2q14.1) discovered through the meta-analysis of all FBPP subgroups.

Table 1

Descriptive Statistics for Each FBPP Network, Race, and Sex Subgroup

Network	Race	Sex	N	Age (in years)		BMI (kg/m ²)		Total Cholesterol (mg/dl)		HDL C (mg/dl)		LDL C (mg/dl)		Triglycerides (mg/dl)		% Lipid Meds	% Diabetes Meds	% Blood Pressure Meds	% Drinkers	% Smokers	% Estrogen
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD						
GenNet	AA	F	181	42	12	33.4	9.0	204.6	47.2							1.1	5.5	23.8	22.7	35.9	5.0
		M	94	39	10	27.5	6.7	193.0	43.8							1.1	3.4	12.5	47.9	63.8	0.0
	MA	F	255	36	15	29.0	6.6	188.7	32.6							3.1	6.3	8.6	29.8	7.1	1.6
		M	162	34	16	28.2	5.6	188.1	32.1							1.2	7.4	6.2	52.5	18.5	0.0
GENOA	AA	F	1,104	58	11	32.1	6.9	207.2	43.1	58.5	17.2	122.6	39.4	131.4	53.0	7.9	17.8	65.0	30.4	13.3	24.6
		M	477	60	10	28.4	4.7	196.8	43.8	49.1	14.1	122.8	41.2	129.6	56.3	8.6	17.8	58.1	46.6	24.3	0.0
	EA	F	757	56	11	30.3	7.0	210.5	37.1	57.4	16.7	120.5	34.3	177.8	82.2	16.6	7.5	67.9	69.5	10.8	37.4
		M	618	56	11	30.4	4.9	200.9	35.9	44.3	11.1	123.7	32.3	180.5	88.9	27.3	8.9	66.3	76.1	11.7	0.0
	MA	F	947	56	12	31.8	6.2	202.6	43.8	47.9	11.9	119.3	39.6	194.5	100.2	17.4	55.0	41.3	5.9	9.2	11.1
		M	662	56	12	29.7	5.3	197.3	41.1	40.7	10.1	120.6	36.8	222.7	159.1	17.5	51.4	33.7	49.3	29.0	0.0
HyperGEN	AA	F	1,135	47	13	33.5	8.1	194.4	39.1	55.9	14.3	118.3	35.2	100.0	53.9	4.8	13.4	64.1	20.3	22.6	18.9
		M	609	45	13	29.7	6.2	190.2	39.9	48.9	13.3	119.0	36.7	113.2	82.3	4.4	9.9	53.5	47.5	37.1	0.0
	EA	F	952	53	13	29.1	6.6	200.4	38.0	53.8	14.3	117.2	32.9	149.9	92.4	11.7	7.5	54.4	28.0	10.0	30.8
		M	891	52	14	29.1	4.6	191.8	34.1	42.2	10.1	117.5	30.2	166.7	105.4	17.2	7.1	54.0	39.5	9.3	0.0
SAPPHIRE	CH	F	464	50	9	24.8	3.7	193.3	38.6	47.1	12.0	123.7	36.5	115.9	73.5	5.2	0.9	53.8	14.7	5.2	6.1
		M	394	49	9	25.8	3.1	187.9	36.4	38.4	9.1	121.8	35.0	141.7	83.2	3.0	0.5	56.3	48.0	33.2	0.0
	JP	F	250	55	8	26.4	4.5	208.3	36.7	52.4	13.7	124.9	32.3	165.2	94.0	17.6	1.6	73.2	69.6	10.0	40.0
		M	175	56	8	27.3	3.9	196.1	34.4	43.5	11.7	117.1	34.1	186.0	107.4	26.9	3.4	74.9	81.7	9.7	0.0

Table 2

QTLs Identified in the Linkage Analyses Including and Omitting Gene-Age Interactions

Chr	Distance in cM	Marker Name	Marker Position in Basepairs (NCBI Build 37)	Novel?	Trait	Meta Group	LOD for the Model:		Network-Race Subgroups with LOD 1 at the Specified Marker (LOD score in that subgroup)
							With Interactions	Without Interactions	
1p33	75.66	DIS2134	48,281,388	No	TC	FBPP	3.36	1.12	GENOA EA (1.68), GENOA MA (2.28), SAPHIRE CHI (1.22)
					LDLC	FBPP	5.08	0.77	GENOA AA (2.01), SAPHIRE CHI (2.17), SAPHIRE JAP (1.14)
1p12	151.88	DIS534	119,678,412	Yes	HDLC	FBPP	4.20	2.58	GENOA MA (2.34), HyperGEN AA (1.13), SAPHIRE JAP (1.57)
					HDLC	Asian	1.98	3.27	SAPHIRE CHI (1.76), SAPHIRE JAP (1.85)
2q14.1	125.18	D2S410	116,241,064	No	HDLC	FBPP	3.52	0.13	GENOA MA (2.59), HyperGEN AA (1.51)
2q24.1	164.51	D2S1353	159,559,007	Yes	TC	MA	3.85	0.35	GenNet MA (1.95), GENOA MA (2.27)
4q21.21	88.35	D4S3243	80,932,385	Yes	TC	MA	3.86	0.15	GenNet MA (2.39), GENOA MA (1.84)
6p22.3	34.23	D6S1959	19,912,251	No	HDLC	FBPP	3.28	0.33	GENOA MA (3.46), HyperGEN AA (1.86)
6q12	80.45	D6S1053	64,590,399	No	LDLC	AA	3.68	1.86	GENOA AA (2.91), HyperGEN AA (1.16)
6q14.1	88.63	D6S1031	77,461,468		HDLC	FBPP	3.49	1.67	HyperGEN AA (2.89), SAPHIRE CHI (1.95)
8p12	60.34	D8S1477	32,067,231	No	HDLC	FBPP	3.64	0.40	GENOA MA (1.08), HyperGEN AA (2.44), SAPHIRE CHI (1.45)
8q13.1	82.26	D8S1136	66,068,296	No [‡]	LDLC	FBPP	3.09	0.30	GENOA AA (1.49), SAPHIRE CHI (3.20)
8q22.2	110.20	GAAT1A4	99,189,924	Yes	TC	MA	4.39	0.32	GenNet MA (2.77), GENOA MA (2.01)
9p21.3	44.28	D9S1121	25,403,184	No	TC	EA*	2.99	3.42	GENOA MA (1.72), HyperGEN EA (4.37)
11p13	43.16	D11S1392	34,640,151	No	TC	MA	3.60	0.90	GenNet MA (2.85), GENOA MA (1.14)
12p11.23	48.70	D12S1042	27,647,464	Yes	TC	MA	3.56	0.23	GenNet MA (2.59), GENOA MA (1.34)
14q11.2	12.46	D14S742	22,201,187	Yes	HDLC	FBPP	3.04	0.06	GENOA AA (1.11), HyperGEN AA (1.74)
17q22	82.00	D17S1290	56,331,564	No	TC	FBPP	3.09	1.72	GenNet MA (2.54), GENOA AA (1.66), GENOA MA (1.73)
					TC	MA	3.90	0.70	

Chr	Distance in cM	Marker Name	Marker Position in Basepairs (NCBI Build 37)	Novel?	Trait	Meta Group	LOD for the Model:		Network-Race Subgroups with LOD 1 at the Specified Marker score in that subgroup
							With Interactions	Without Interactions	
18q22.3	106.81	ATA82B02	68,913,612	No	TC	MA	4.28	0.05	GenNet MA (2.63), GENOA MA (2.03)
21q21.1	13.05	D21S1437	21,646,843	Yes	HDLC	FBPP	3.65	0.16	GENOA MA (1.80), HyperGEN AA (1.68)
21q22.3	57.77	D21S1446	48,037,723	No	TC	FBPP	4.27	1.76	GenNet MA (2.77), HyperGEN EA (1.14), HyperGEN AA (1.91)
					HDLC	FBPP	3.45	0.04	GENOA MA (3.88), HyperGEN AA (1.16)

* This QTL was found during the EA-specific meta-analysis but was supported by one EA subgroup and one MA subgroup. The basepair positions refer to the center of the microsatellite marker. The “Novel” column indicates whether the QTL has been significantly linked (LOD 3or p-value reported as genome-wide significant) to any lipid trait or harbors a SNP previously associated (p-values 5E-08) with a lipid trait in the 1-LOD drop support interval.

‡ This locus was also associated with HDLC in an admixture analysis that included some AA FBPP participants (Z-score of 4.13 at marker D8S1136[49]).

Selected GWAS Findings for Lipids and Coronary Heart Disease in the 1-LOD Support Interval For Each Linkage Peak

Table 3

Chr	Marker Name	Trait	1 LOD-drop Support Interval		Trait	Selected GWAS Associations in the NIH GWAS catalog (Accessed November 20,2013; see http://www.genome.gov/26525384#download), Supplemented with Novel Loci From the Largest Genetic Association Study of Lipids to Date[5]				
			Lower Bound	Upper Bound		SNP ID	Position (Build 37)	GENE	P-value	PubMed ID
1p33	D1S2134	TC	46,332,010	59,791,499	10-nonadecenoate/10-undecenoate	rs9332998	47404186	CYP4A11	5.E-32	21886157
		LDLC	46,869,997	52,491,021	LDL cholesterol	rs685001	48025063	ATPGV0E1P4 - TRABD2B	9.E-06	23251661
					Response to statin therapy	rs6588480	53978119	GLIS1	8.E-06	20339536
					Nonalcoholic fatty liver disease	rs11206226	54320337	YIPF1	5.E-06	22719876
					Coronary heart disease	rs11206510	55496039	BSND - PCSK9	9.E-08	21378990
					LDL cholesterol	rs11206510	55496039	BSND - PCSK9	4.E-11	18193043
					Cholesterol, total	rs2479409	55504650	PCSK9	4.E-24	20686565
					LDL cholesterol	rs2479409	55504650	PCSK9	2.E-28	20686565
					Response to statin therapy (LDL-C)	rs11591147	55505647	PCSK9	5.E-09	22331829
					LDL cholesterol	rs11591147	55505647	PCSK9	2.E-44	18193044
					Coronary heart disease	rs12239436	55742837	MIR4422 - GOT2P1	5.E-06	21347282
					IDL-FC	rs1998013	55958030	GOT2P1 - RPSAP20	5.E-13	22916037
					Lipid metabolism phenotypes	rs72669744	56116505	GOT2P1 - RPSAP20	1.E-19	22286219
					Coronary heart disease	rs17114036	56962821	PPAP2B	4.E-19	21378990
1p12	D1S534	HDLc (FBPP)	116,035,560	138,848,755	Fat mass change	rs10776733	112107669	ADORA3	7.E-06	23251661
		HDLc (Asian)	112,102,917	137,170,945	Non-alcoholic fatty liver disease	rs7552722	115921355	NGF-TCEB1P20	7.E-06	23535911
2q14.1	D2S410	HDLc	107,947,342	126,892,597	LDL cholesterol	rs10490626	118835841	INSIG2	2E-12	24097068
					Cholesterol, total	rs10490626	118835841	INSIG2	6E-09	24097068
					Diet fat	rs1374313	120145655	DBI - TMEM37	9.E-07	23251661
					LDL cholesterol	rs2030746	121309488	LOC84931	9E-09	24097068
					Cholesterol, total	rs2030746	121309488	LOC84931	4E-08	24097068
					Response to statin therapy	rs2901331	125327950	CNTNAP5	6.E-06	20339536
2q24.1	D2S1353	TC	146,714,592	162,175,538	Response to statin therapy	rs7584099	148478336	PABPC1P2 - RPL26P14	5.E-07	20339536
					Response to statin therapy	rs16839962	156925286	MTNDS5P30 - NR4A2	9.E-06	20339536
4q21.21	D4S3243	TC	77,655,414	83,053,702	Triglycerides	rs10518224	80564508	OR7E94P - GDEP	7.E-06	19197348

Chr	Marker Name	Trait	1 LOD-drop Support Interval		Trait	SNP ID	Position (Build 37)	GENE	P-value	PubMed ID
			Lower Bound	Upper Bound						
6p22.3	D6S1959	HDL C	16,244,851	40,067,808	LDL cholesterol	rs2274136	17633061	NUP153	3.E-06	23251661
					Total cholesterol	rs2274136	17633061	NUP153	5.E-06	23251661
					Response to statin therapy	rs16883019	19969638	ID4 - RPL29P17	7.E-07	20339536
					HDL-C	rs10498712	22743403	HDGFL1 - RPL6P18	9.E-06	19197348
					Cholesterol, total	rs1800562	26093141	HFE	2.E-08	20686565
					LDL cholesterol	rs1800562	26093141	HFE	6.E-10	20686565
					Lipid metabolism phenotypes	rs6917603	30017071	ZNRD1-AS1	3.E-29	22286219
					Coronary heart disease	rs3869109	31184196	HCG27 - USP8P1	1.E-09	22319020
					Triglycerides	rs2247056	31265490	WASFSP - HLA-B	2.E-15	20686565
					HDL cholesterol	rs3099844	31448976	HCG26 - MICB	2.E-08	22399527
					Phospholipid levels (plasma)	rs3117181	32071017	TNXB	5.E-07	21829377
					Oleic acid (18:1n-9) plasma levels	rs3134950	32127477	PPT2;PPT2-EGFL8	1.E-06	23362303
					Phospholipid levels (plasma)	rs1061808	32136547	AGPAT1;EGFL8;PPT2-EGFL8	8.E-10	22359512
					Coronary heart disease	rs9268402	32341353	C6orf10	3.E-15	22751097
					Cholesterol, total	rs3177928	32412435	HLA-DRA	4.E-19	20686565
					LDL cholesterol	rs3177928	32412435	HLA-DRA	2.E-15	20686565
					Coronary heart disease	rs11752643	32669373	HLA-DQB1 - MTCO3P1	5.E-07	21971053
					LDL cholesterol	rs2254287	33143948	COL11A2	5.E-08	18193043
					Cholesterol, total	rs2814982	34546560	SPDEF - RPL7P25	5.E-11	20686565
					HDL cholesterol	rs2814944	34552797	SPDEF - RPL7P25	4.E-09	20686565
					Coronary heart disease	rs17609940	35034800	ANKS1A	1.E-08	21378990
					Cholesterol, total	rs2758886	39250837	KCNK17	3.E-08	24097068
					Ratio of Triglyceride/HDL	rs9296295	39361854	KIF6	6.E-06	23251661
6q12	D6S1053	LDLC	46,783,929	73,422,533	Phospholipid levels (plasma)	rs2585617	69337787	NUFIP1P - BA13	6.E-07	21829377
6q14.1	D6S1031	HDL C	47,592,257	84,904,609	Response to statin therapy	rs3757057	70074232	BA13	4.E-06	20339536
					Triglyceride response to thiazides	rs10943724	81302805	RPL17P25 - FAM46A	7.E-06	23400010
					Coronary heart disease	rs16893526	82515315	FAM46A - IBTK	5.E-06	21606135

Chr	Marker Name	Trait	1 LOD-drop Support Interval		Trait	Selected GWAS Associations in the NIH GWAS catalog (Accessed November 20,2013; see http://www.genome.gov/26525384#download), Supplemented with Novel Loci From the Largest Genetic Association Study of Lipids to Date[5]					P-value	PubMed ID
			Lower Bound	Upper Bound		SNP ID	Position (Build 37)	GENE				
8p12	D8S1477	HDL C	28,999,923	47,686,319	Response to statin therapy	rs10091038	29360305	DUSP4 - RPL17P33	3.E-08	20339536		
8q13.1	D8S1136	LDL C	58,678,139	69,733,647	Cholesterol, total	rs2081687	59388565	UBXN2B-CYP7A1	9.E-13	20686565		
					LDL cholesterol	rs2081687	59388565	UBXN2B-CYP7A1	4.E-09	20686565		
					Triglyceride response to thiazides	rs2980003	65999963	CYP7B1 - RPL31P41	5.E-06	23400010		
					Response to statin therapy	rs13279522	66974252	DNAJC5B	5.E-07	22666496		
9p21.3	D9S1121	TC	21,192,238	42,123,268	Coronary heart disease	rs1333049	22125503	CDKN2B-AS1 - DMRTA1	7.E-58	21606135		
					Heart failure	rs10812610	27533984	IFNK - C9orf72	5.E-06	20445134		
					HDL	rs10969375	29783989	MIR873 - RBMXP2	6.E-06	23251661		
11p13	D11S1392	TC	31,507,065	37,630,142	Triglyceride response to thiazides	rs1559759	35123051	PDHX-CD44	9.E-06	23400010		
12p11.23	D12S1042	TC	23,650,924	33,687,130	Response to statin therapy	rs7979575	24278821	SOX5	2.E-06	20339536		
					Heart failure	rs2046383	30104142	RPL21P99 - IPO8	3.E-06	20445134		
					Fat mass change	rs1454933	33017043	PKP2	9.E-06	23251661		
17q22	D17S1290	TC (FBPP)	50,869,616	69,149,069	Lipoprotein-associated phospholipase A2 activity and mass	rs11650106	57850251	VMP1	3.E-09	23118302		
		TC (MA)	52,897,724	67,307,204	Coronary heart disease	rs6504218	62408299	PECAM1	1.E-06	21378988		
					LDL cholesterol	rs1801689	64210580	APOH-PRXCA	1E-11	24097068		
					HDL cholesterol	rs4148008	66875294	ABCA8	2.E-10	20686565		
					Palmitoleic acid (16:1n-7) plasma levels	rs2366017	67850901	MAP2K6 - KCNJ16	3.E-07	23362303		
					LDL cholesterol	rs16975985	68634065	CALM2P1 - SOX9	5.E-06	23251661		
21q21.1	D21S1437	HDL C	19,043,966	23,613,731	Fat-free mass change	rs1735884	21820408	KRT18P2 - LINC00320	7.E-06	23251661		
21q22.3	D21S1446	TC	45,844,270	end of chr	Phospholipid levels (plasma); docosapentaenoic acid	rs7435	45404338	AGPAT3	2.E-07	21829377		
		HDL C	45,320,004	end of chr								

NOTE: For three QTLs, no pertinent GWAS associations were noted in the 1-LOD drop support interval. The three QTLs included the TC locus on chromosome 8q22.2 (1-LOD drop from 93,359,802 to 103,411,847 basepairs), the HDL C locus on chromosome 14q11.2 (1-LOD drop from 0 to 23,704,701 basepairs), and the TC locus on chromosome 18q22.3 (1-LOD drop from 66,646,399 to 70,895,238 basepairs). For these three QTLs, there are several candidate genes associated with other components of the metabolic syndrome such as type 2 diabetes (*TP53INP1* in 8q22.2), obesity and obesity-related traits (*LOC100616530*, *PTDSS1* - *SDC2*, and *C8orf47* - *HRSPI2* for 8q22.2; *RVASE6* - *RVASE1*, *TRA* for 14q11.2), and pulmonary arterial hypertension (*RPS2P6* - *CBLN2* for 18q22.3).