Integrating epigenetic, genetic, and phenotypic data to uncover gene-region associations with triglycerides in the GOLDN study

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Abstract

Background: There has been significant interest in investigating genome-wide and epigenome-wide associations with lipids. Testing at the gene or region level may improve power in such studies.

Methods: We analyze chromosome 11 cytosine-phosphate-guanine (CpG) methylation levels and single-nucleotide polymorphism (SNP) genotypes from the original Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study, aiming to explore the association between triglyceride levels and genetic/epigenetic factors. We apply region-based tests of association to methylation and genotype data, in turn, which seek to increase power by reducing the dimension of the gene-region variables. We also investigate whether integrating 2 omics data sets (methylation and genotype) into the triglyceride association analysis helps or hinders detection of candidate gene regions.

Results: Gene-region testing identified 1 CpG region that had been previously reported in the GOLDN study data and another 2 gene regions that are also associated with triglyceride levels. Testing on the combined genetic and epigenetic data detected the same genes as using epigenetic or genetic data alone.

Conclusions: Region-based testing can uncover additional association signals beyond those detected using single-variant testing.

Background

Many authors have called for greater use of gene-based approaches to detect candidate regions at the genome-wide discovery stage, raising concerns that exclusive marginal single-variable testing may miss more complex associations. For example, Yoo et al. [1] report that region tests can be more sensitive to genetic architectures with multiple causal components, and find that reduced-dimension test statistics, such as that proposed by Gauderman et al. [2], can improve power compared to tests in full multivariable regressions. To some extent, this argument also applies to genome-wide epigenetic studies, but conclusive evidence is lacking for it. Specification of the constituent variables for a gene region, however, is a major challenge in implementation for both genetic and epigenetic gene-region modeling, and is critical for integration of the 2 data sources when the molecular technology platforms differ.

In their investigation of epigenome-wide association of fasting blood lipids in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study, Irvin et al. [3] model the percentage methylation separately at each individual cytosine-phosphate-guanine (CpG) site as a function of triglyceride levels. They report genome-wide significant associations of 4 CpG sites in intron 1 of the CPT1A gene located on chromosome 11. In this article, we apply gene-region association methods to the original chromosome 11 epigenetic data from the GOLDN study [3], supplemented with chromosome 11 genome-wide association...
study single-nucleotide polymorphism (SNP) data available in a common subset of individuals. Our aims are to explore the association between baseline triglyceride (TG) levels and genetic/epigenetic factors using gene-region analysis methods, and to investigate 1 approach to integration of 2 omics data types (SNP genotype and CpG methylation) by comparing the integrated approach with separate analyses.

**Methods**

**Data**

We take the phenotype to be log-transformed triglyceride (lnTG) using averaged TG measurements before treatment with fenofibrate. To investigate genetic association with the phenotype, we convert the Affymetrix platform SNP genotypes to allele counts coded as 0, 1, or 2. In total, we consider 36,796 SNPs on chromosome 11. The methylation data for the same chromosome consists of 28,285 CpG sites in total. The number of participants from the original study with sufficiently complete epigenetic data are 995. Of the 995 participants, 717 had genotype information as well.

**Specification of gene regions**

Sets of CpG sites and SNPs corresponding to each gene region were obtained using the GENCODE [4] annotation file bundled with LocusZoom standalone software [5], and expanding each genetic region by 20 kb before the start, and after the end, of the annotated base-pair positions. This was done to include any possibly related functional SNPs or CpG sites from each gene neighborhood. In all, we defined 2621 gene regions on chromosome 11. The number of component variables per gene region ranged from 2 to 544 for SNPs and from 2 to 372 for CpG sites. For computational reasons, we excluded 6 genetic regions (Metazoa_SRP, SNORA1, SNORA7, U3, Y RNA, snoU13) that had more than 2000 CpG or SNPs from the subsequent gene-region regression analysis.

**Single CpG epigenetic association**

To investigate association between TGs and methylation on chromosome 11, we regress percentage methylation on lnTG measurements as in Irvin et al. [3], and include age, study site, sex, and cell purity as fixed effects, and family as a random effect. TG values are first averaged over the measurements pretreatment (at most 2 per participant), as this yields the most complete data set (995 cases). Cell purity variables estimated as the top 4 principal components of the methylation data, are included as fixed effects. The model reads (in R notation):

$$\text{CpG} \sim \text{ln(TG)} + \text{age} + \text{center} + \text{sex} + \text{PC}\text{G}^1 + \text{PC}\text{G}^2 + \text{PC}\text{G}^3 + \text{PC}\text{G}^4$$

with a random effect for GPEDID, the family ID from the pedigree file, used to account for familial correlation. Here, the superscript \(G\) indicates that the principal component (PC) for cell purity is computed globally for chromosome 11. We note 2 differences from the original GOLDN study. First, the chromosome 11 methylation data we use to calculate cell purity PCs has 28,285 CpG sites, whereas in Irvin et al. [3] the same procedure was based on 461,281 CpG sites from the whole genome (after quality control). Second, model (1) assumes a common correlation among members of the same family, whereas the original analysis used the kinship coefficient to define the correlation of random effects. Our approach is much faster computationally as it uses the inner function rather than lmekin (as in Irvin et al. [3]). We also confirmed the fit using the kinship coefficient, but note that the \(p\) values obtained using model (1) already match those in the original paper fairly closely.

**Gene-region testing of SNP genetic and CpG epigenetic association**

To assess the value of integrating the 2 types of data in detecting gene regions associated with the TG phenotype, we regress lnTG on SNP-derived and CpG-derived predictors. We employ the method of Gauderman et al. [2], which computes the PCs of the regressors, and tests for association between the response (\(\text{Y} = \text{lnTG}\)) and the PCs of the “\(X\)” variables that explain at least 80% of their total variation. This method takes advantage of the correlation structure within a gene region, and may increase power by reducing the dimensionality of the regressor set, such that more genes achieve significance even if their component CpG sites/SNPs are not detected in marginal regression.

For a given gene, let \(\{\text{PC}_{1}^G, \text{PC}_{2}^G, \ldots, \text{PC}_{k}^G\}\) be the first \(k\) PCs of the SNP variables associated with that gene, which explain 80% of their variation. Similarly, define \(\{\text{PC}_{m1}^n, \text{PC}_{m2}^n, \ldots, \text{PC}_{mn}^n\}\) as the first \(l\) PCs of the methylation data. With this reduced data set, we fit the following regression models, including random effects for family:

\[
\text{ln(TG)} \sim \text{PC}_{1}^G + \text{PC}_{2}^G + \ldots + \text{PC}_{k}^G + \text{age} + \text{center} + \text{sex} \quad (2)
\]

\[
\text{ln(TG)} \sim \text{PC}_{m1}^n + \text{PC}_{m2}^n + \ldots + \text{PC}_{mn}^n + \text{age} + \text{center} + \text{sex} + \text{PC}_{1}^G \quad (3)
\]

\[
\text{ln(TG)} \sim \text{PC}_{1}^G + \ldots + \text{PC}_{k}^G + \text{PC}_{1}^n + \ldots + \text{PC}_{mn}^n + \text{age} + \text{center} + \text{sex} + \text{PC}_{1}^G \quad (4)
\]

We opted to use the first chromosome 11 global \(\text{PC}_{1}^G\) of the methylation data as a measure of cell purity, as we found this produces a CpG test \(p\) value
distribution close to that expected under the null hypothesis. In each of the 3 models [models (2), (3), and (4); fitted via R function `lmer`], a global Wald test is performed on the coefficient estimates \( \hat{\beta} \) of the SNP and/or CpG PC terms. Model (4) is designed to assess the combined contribution of the CpG and SNP PCs and determine whether the 2 sets of PCs make independent contributions. Although we limited testing to chromosome 11, to control the overall Type 1 error level, we specify a genome-wide significance threshold for testing. Counting approximately 20,000 to 30,000 genes (and thus tests) yields a threshold of 2 × 10^-6.

### Integration of predictors at the gene-region

To further differentiate the relative contribution of SNPs and CpG sites in model (4), we compute variance inflation factors (VIFs) for each PC as a means to identify multicollinearity among the variables in the joint regression model. High correlation between SNP and CpG components may be undesirable because it can inflate standard error estimates. The VIF in a linear regression is computed as \( VIF_i = (1-R_i^2)^{-1} \), where \( R_i^2 \) is obtained by regressing the \( i^{th} \) predictor on all the other predictors. In our case, as PCs in each data set are orthogonal, the VIF for a SNP PC will be based on its correlation with all the CpG PCs, and conversely.

| Mark name   | Genes | Position | p Value (lmer) | p Value (lmekin) |
|-------------|-------|----------|----------------|-----------------|
| cg00574958  | CPT1A | 68,607,622 | 6.52e – 31     | 1.23e – 35      |
| cg17058475  | CPT1A | 68,607,737 | 1.61e – 20     | 1.31e – 21      |
| cg01082498  | CPT1A | 68,608,225 | 2.21e – 11     | 2.85e – 12      |
| cg09737197  | CPT1A | 68,607,675 | 7.30e – 10     | 9.34e – 10      |
| cg11376147  | SLCA43A1 | 57,261,198 | 2.51e – 09     | 7.53e – 09      |
| cg26989316  | CPT1A | 68,607,257 | 1.90e – 08     | 7.56e – 09      |
| cg12556569  | APOA5 | 116,664,039 | 2.25e – 10     | 4.63e – 10      |
| cg00264754  | LRR4C | 40,136,810 | 9.30e – 08     | 3.39e – 07      |

### Results

#### Single CpG testing

We reproduced the original study associations [3] for chromosome 11 by fitting model (1) to %methylation for each CpG. Eight CpG sites achieved significance (p value < 10^-7) with the top 4 sites the same as those found in the GOLDN study in CPT1A (Table 1).

#### Gene-region testing of CpG’s and SNPs

We fit models (2) to (4) to each gene region in turn, and test the corresponding global association hypotheses for CpG’s and SNPs using generalized Wald tests. We detect gene CPT1A using gene-region testing, but in addition we find 2 other genome-wide significant regions: AP006216.5 using methylation data, and BUD13 using genetic data (Table 2). These gene-regions are in the same neighborhood that also contains APOA5, detected in the single CpG analysis reported in Table 1 (Fig. 1).

The integration of the 2 data types does not seem to improve the overall association signal: testing found the roughly the same gene set to be significant as in the separate epigenetic and genetic analyses, with 3 of the top 4 genes having larger p values (see Tables 2 and 3). The examination of pairwise correlations between CpG and SNP PCs within gene regions suggests that this can be explained by relationships of higher order CpG PCs with SNP PCs, particularly for CPT1A and APOA5. APOA5 was affected in both epigenetic and genetic components. The gene BUD13 detected in the genetic SNP analysis dropped below the detection threshold after adding CpG data, most likely a consequence of the increase in model degrees of freedom. Remarkably, we observe little Spearman rank correlation between the epigenetic and genetic gene-region p values across the 2615 gene regions.

To address multicollinearity in predictor integration, we fit a reduced model (4) to the 2 genes in Table 3 with high VIFs (Fig. 2) by sequentially dropping high VIF PCs, until no term remains with a VIF larger than 2 (this corresponds to excluding those PCs with ≥50% variation explained by the other predictors). This removes SNP PCI and PC2 from the CPT1A model.

### Table 2 Gene-region testing applied to separate epigenetic and genetic regressions (Models 2 and 3; n = 717)

| Gene | Gene region (BP) | Epigenetic | Genetic |
|------|------------------|------------|---------|
|      | Start | End | Degrees of freedom | p Value | Degrees of freedom | p Value |
| CPT1A | 68,522,088 | 68,611,878 | 33 | 3.44e – 14 | 5 | 0.436 |
| AP006216.5 | 116,683,920 | 116,684,719 | 7 | 3.51e – 06 | 4 | 0.045 |
| BUD13 | 116,618,886 | 116,643,704 | 17 | 0.538 | 5 | 1.48e – 07 |
| APOA5 | 116,660,083 | 116,663,136 | 17 | 1.95e – 04 | 6 | 1.47e – 05 |
and CpG PC9 from the APOA5 model, (with VIFs for all remaining terms below 1.4), but does not improve the overall association signal ($p$ values of 1.57e-13 and 9.25e-04 for CPT1A and APOA5, respectively). For APOA5, the most highly collinear CpG PC is also one of the strongest predictors of TG, suggesting that VIF pruning is not advisable for improving power, but can help clarify variable importance.

**Discussion**

In this contribution to GAW20, we investigate associations between a lipid phenotype (TG level) and epigenetic (methylation CpG sites) and/or genetic (SNP) markers. As an alternative to single-marker analysis, we apply a gene-region testing method based on multiple regressions of PCs summarizing CpG sites and SNPs in each gene region. The dimension reduction fraction achieved (number of PCs that explain at least 80% of data variability over the number of original variables) was often greater than 50%, with greater data compression for larger genes, and SNP sets showing slightly more dimension-reduction capacity than CpG sites, despite having similar number of original variables (Fig. 3).

In separate and combined epigenetic and genetic regression analyses, we detected genome-wide significant gene-region CpG signals for the CPT1A gene reported in the original GOLDN study [3], as well as for 2 other genes. The 2 other gene regions lie within 50 kb of a single significant CpG detected in our single-variable CpG analysis, which suggests that this entire region harbors signals of association with TGs. For the CPT1A gene, the epigenetic component clearly leads the results, with no detectable genetic signal. Associations detected with AP006216.5, BUD13, and APOA5, all located in a different region of chromosome 11, also included epigenetic and/or genetic components. For AP006216.5, the epigenetic component leads the overall association, with an independent nominal genetic component. In contrast, for BUD13, the genetic component is the sole contributor. For the APOA5 gene, which is located midway between AP006216.5 and BUD13, there is suggestive genome-wide association resulting from both epigenetic and genetic components, which are not independent, and we find evidence for relationships between certain CpG PCs and SNP PCs. Notably, APOA5 is a known genetic determinant of TG variation, and recent data points to joint genetic and epigenetic regulation of TG [6].

We attempt to increase power in the combined epigenetic and genetic regression using VIFs to eliminate multicollinearity among predictors. This produces 2 sets of regressors that are approximately orthogonal.

**Table 3** Gene-region testing applied to integrated epigenetic-genetic regressions (Model 4; $n = 717$)

| Gene     | BP Start | Epigenetic | Genetic | Epigenetic + Genetic |
|----------|----------|------------|---------|----------------------|
|          | Start    | Degrees of freedom $p$ value | Degrees of freedom $p$ value | Degrees of freedom $p$ value |
| CPT1A    | 68,522,088 | 33 | 2.47e–14 | 5 | 0.276 | 38 | 9.44e–14 |
| AP006216.5 | 116,683,920 | 7 | 2.62e–06 | 4 | 0.034 | 11 | 1.16e–06 |
| BUD13 | 116,618,886 | 17 | 0.711 | 5 | 7.13e–07 | 22 | 2.23e–04 |
| APOA5 | 116,660,083 | 17 | 0.088 | 6 | 0.064 | 23 | 7.19e–05 |
facilitating evaluation of independent contributions of SNP- and CpG-based PCs, but this approach does not strengthen association signals in the combined regression. We speculate that this may be partly because the PCs which are highly correlated between data types are likely to share causal etiology, so excluding them reduces power; and partly because most PCs are largely uncorrelated, and the VIF approach does not eliminate these PCs. Our recommendation for future studies is that Gauderman’s method works well at the gene level for separate analysis of both genetic and epigenetic data types, and integration of the 2 data sources, with assessment of their intercorrelation, can give further insight.

**Conclusions**

Using a gene-region testing approach that effectively reduced predictor dimensionality, we recovered the gene *CPT1A* as having significant association between methylation and TG levels. In addition we identified 2 other genes that were not detected in the single CpG analysis: gene *BUD13*, genetically significant, and region AP006216.5, epigenetically significant. In integration of the genetic and methylation data types when testing for association with TG levels at the gene level, although we found no evidence of improvement in association signal strength over separate analyses, use of a combined model helps clarify the relative contribution of epigenetic and genetic components.
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Availability of data and materials
The data that support the findings of this study are available from the Genetic Analysis Workshop (GAW), but restrictions apply to the availability of these data, which were used under license for the current study. Qualified researchers may request these data directly from GAW.

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Authors' contributions
RGR, OEG, and SBB designed the overall study. RR and JM conducted statistical analyses. OEG supplied gene definitions. RR participated in the workshop and drafted the manuscript. SBB revised the manuscript for critical content. All authors read and approved the final manuscript.

Ethics approval and consent to participate
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