SNPs located at CpG sites modulate genome-epigenome interaction

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Recommended Citation
Zhi, Degui; Aslibekyan, Stella; Irvin, Marguerite R.; Claas, Steven A.; Borecki, Ingrid B.; Ordovas, Jose M.; Absher, Devin M.; and Arnett, Donna K., "SNPs located at CpG sites modulate genome-epigenome interaction." Epigenetics. 8, 8. 802-806. (2013).
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SNPs located at CpG sites modulate genome-epigenome interaction

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Keywords: DNA methylation, meSNP, meQTL, epigenome-wide study, Infinium Human Methylation 450K BeadChip

DNA methylation is an important molecular-level phenotype that links genotypes and complex disease traits. Previous studies have found local correlation between genetic variants and DNA methylation levels (cis-meQTLs). However, general mechanisms underlying cis-meQTLs are unclear. We conducted a cis-meQTL analysis of the Genetics of Lipid Lowering Drugs and Diet Network data (n = 593). We found that over 80% of genetic variants at CpG sites (meSNPs) are meQTL loci (P value < 10−9), and meSNPs account for over two thirds of the strongest meQTL signals (P value < 10−200).

Beyond direct effects on the methylation of the meSNP site, the CpG-disrupting allele of meSNPs were associated with lowered methylation of CpG sites located within 45 bp. The effect of meSNPs extends to as far as 10 kb and can contribute to the observed meQTL signals in the surrounding region, likely through correlated methylation patterns and linkage disequilibrium. Therefore, meSNPs are behind a large portion of observed meQTL signals and play a crucial role in the biological process linking genetic variation to epigenetic changes.

DNA methylation plays an important role in gene regulation. As such, it is an important intermediate molecular phenotype that links genotypes and other macro-level phenotypes and may contribute to the missing heritability.1 Despite their prominent physiologic role, the genetic determinants of DNA methylation patterns are poorly understood. There is evidence of correlation between genetic variation at specific loci and the quantitative trait of DNA methylation.2-3 Additionally, prior studies4,5 revealed that genetic variants at CpG sites (meSNPs) are likely to disrupt the substrate of methylation reactions and thus drastically change the methylation status at a single CpG site. However, it is unclear if meSNPs represent a major class of methylation-associated loci (meQTLs). Moreover, it is unclear if meSNPs modulate the methylation status of nearby CpG sites. Most existing meQTL studies2,5 are based on relatively small-sized samples and low-resolution methylation microarrays, wherein meSNPs are sparsely represented. Additionally, current practices of meQTL analysis typically exclude probes that contain sequence variants to limit misclassification due to alterations in probe hybridization.2,3 Recently, we generated high-resolution genotyping and epigenetic data from the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN),4 yielding a considerable amount of meSNPs with information on both genotype and methylation status. These data enabled us to investigate the impact of meSNPs on the methylation status of the CpG site harboring the SNP, as well as on surrounding CpG sites.

We used CD4+ T-cells harvested from frozen buffy coat samples isolated from peripheral blood for methylation measurement. We used CD4+ T-cells simply due to their abundance and physiologic role in the immune response. While that ensures higher validity of findings, our results may not be generalizable to other cell populations. After genotype imputation and quality control (see Supplemental Methods), we had data for 461 281 CpGs of n = 593 GOLDN participants. All participants had previously been genotyped at 906600 loci using the Affymetrix Genome-Wide Human SNP Array 6.0, as described in prior publications.9,10 MACH software (Version 1.0.16) was used to impute non-genotyped SNPs using HapMap Phase II (release 22, Human Genome build 36, hg18) as a reference, resulting genotypes of 2529001 SNPs.

We first performed a genome-wide cis-meQTL analysis of GOLDN data. We restricted the analysis to SNP/CpG site pairs within 20 kb of each other, and removed the pairs in which the SNP was located on the methylation probe as described below, yielding 12347772 pairs for the final analysis. We used linear mixed models, fit using the lmekin function of the Kinship package in R,10 to regress the methylation level (β value) of a CpG site on the genetic variant at a SNP site, adjusting for covariates (age, sex, and recruitment center) as fixed effects and family structure as a random effect. Adjusting top principal components of phenotype matrix is an effective approach to reduce experimental artifacts and enhance power for gene expression quantitative

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Submitted: 02/28/13; Revised: 06/19/13; Accepted: 06/21/13
http://dx.doi.org/10.4161/epi.25501

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We subsequently investigated the potential impact of meSNPs on the methylation status of nearby CpG sites, located within 100 bp. It is notable that the M450K chip only directly measures the methylation status at CpG sites of the reference genome, and thus only SNPs that disrupting CpG sites are directly observable. To study the potential effect of all CpG-gain SNPs to nearby CpG sites, we conducted a bioinformatics annotation of all SNPs in the GOLDN and the 1000 Genomes Project phase 1 release. All SNPs were classified into four categories in terms of changing CpG sites on the reference genome: CpG-loss, which eliminates a CpG site; CpG-gain, which creates a CpG site; CpG-shift, which represents a variant between CCG and CGG that shifts the position of a CpG site by 1 bp; and CpG-irrelevant. CpG-loss and CpG-gain SNPs are only defined relative to the reference genome; both CpG-loss and CpG-gain have an allele that disrupts the substrate of methylation and thus are likely to be meQTLs. All 812 meSNPs represented on the M450K chip and were successfully genotyped in GOLDN are either CpG-loss (803, 99%) or CpG-shift (9, 1%). We scanned all 39 million SNPs from the 1000 Genomes Project phase 1 release and all 2.5 million SNPs in the GOLDN imputed variant set, resulting in the classification of SNPs as shown in Figure S3. Overall, we found that 14% of GOLDN SNPs are CpG-loss and 14% are CpG-gain. Similar proportions were observed in the 1000 Genomes Project Phase 1 SNPs (Fig. S3).

Although the methylation measurements at CpG-gain SNPs and many of the CpG-loss SNPs are not directly observed, we reasoned that they likely follow the same pattern as the 812 CpG-loss SNPs discussed above. To investigate the effect of meSNPs to the methylation at immediate nearby sites, we looked for meQTLs ($P < 10^{-9}$) between 701 CpG-gain or 861 CpG-loss SNPs with allele frequency between 5% and 95% represented in the GOLDN genotype set and CpG sites within 100 bp (but not at the SNP directly) with M450K measurements in Infinium II chemistry, and found 1562 such pairs. Through a linear regression of the effect sizes of these meQTL and the distance between the SNP-CpG pairs, we found that, for each CpG-disrupting allele, the methylation of CpG sites at very close distance is also lowered for about 2% ($P$ value = $3 \times 10^{-11}$ for the interect), but the effect is diminishes to flat and even a slight elevation beyond 60 bp ($P$ value = $4.0 \times 10^{-10}$ for the slope) (Fig. 2). This result suggests that a CpG-changing SNP can affect not only the methylation status of its own CpG site, but also the methylation patterns surrounding it at very close distance. However, while this may point to an interesting methylation regulation mechanism, additional investigations are warranted to rule out potential technical artifacts such as local hybridization competition.

Finally, we investigated whether meSNPs may explain meQTL signals at surrounding sites located within 10 kb. Not all meQTL signals are due to independent association signals. In particular, we observe that meQTL signals show a prominent correlation pattern (Fig. 2B). We therefore hypothesize that, in the neighborhood of a meSNP with genotype X and CpG methylation Y, the meQTL signal between genotype X’ and CpG methylation Y is due to their respective correlations to the
We found a considerable attenuation of meQTL signals surrounding meSNPs (Fig. 2C vs. B). In the 10 kb neighborhood of 812 observed meSNPs, the total numbers of meQTLs decreased by 32.8%, from 8017 to 5384 after the adjustment. Given the genome-wide distribution of meSNPs, most of which are not directly observed on the M450K chip, we estimate that meQTLs at meSNPs may explain a large portion of observed cis-meQTL signals.

Overall, our analysis provides the first genome-wide profiling of methylation changes at and surrounding SNPs. These meSNP pair X and Y: X’ to X by linkage disequilibrium, Y’ to Y by methylation correlation. Unlike standard mediation analyses, we are not claiming a chain of causative links, but rather to test if the associations X’ to Y’ and X to Y are independent. Instead of running a full mediation analysis, we conducted a partial mediation analysis, i.e., the conditional regression of Y’ to X’ given Y, in addition to the pairwise meQTL analysis. If they are truly independent associations, regressing Y’ to X’ conditional on Y should not eliminate the significant meQTL association between Y’ and X’.

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Overall, our analysis provides the first genome-wide profiling of methylation changes at and surrounding SNPs. These

Table 1. Summary of 812 cis-meQTLs at meSNPs probed by Infinium II chemistry

<table>
<thead>
<tr>
<th>Strand</th>
<th>Position within CpG</th>
<th>Ref</th>
<th>Alt</th>
<th>Count</th>
<th>Mean $-\log_{10} P$ val</th>
<th>Mean effect size $\beta$</th>
<th>%meQTL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>0</td>
<td>C</td>
<td>A</td>
<td>6</td>
<td>102.0</td>
<td>-0.207</td>
<td>83.33%</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>C</td>
<td>G</td>
<td>11</td>
<td>95.2</td>
<td>0.151</td>
<td>90.91%</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>C</td>
<td>T</td>
<td>79</td>
<td>108.3</td>
<td>-0.229</td>
<td>84.81%</td>
</tr>
<tr>
<td>R</td>
<td>1</td>
<td>G</td>
<td>A</td>
<td>606</td>
<td>115.4</td>
<td>-0.216</td>
<td>92.41%</td>
</tr>
<tr>
<td>R</td>
<td>1</td>
<td>G</td>
<td>C</td>
<td>48</td>
<td>74.5</td>
<td>0.111</td>
<td>87.50%</td>
</tr>
<tr>
<td>R</td>
<td>1</td>
<td>G</td>
<td>T</td>
<td>62</td>
<td>103.1</td>
<td>-0.217</td>
<td>88.71%</td>
</tr>
</tbody>
</table>

*% of pairs with $P < 10^{-9}$. See Supplemental Methods for an interpretation of the effect sizes.
meSNPs contribute to the strongest methylation QTL signals as they disrupt the methylation status at CpG sites. Moreover, meSNPs also modulate methylation status at nearby CpG sites and a large proportion of variability in DNA methylation can be explained by genetic variation at the CpG site itself. Our data suggest a potential biological mechanism underlying associations between common genetic variants, epigenetic processes, and disease phenotypes. We conclude that meSNPs may be a crucial class of variants to be considered in interpretation of genomic and epigenomic association results.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Figure 2. Effect of meSNPs on methylation at nearby CpG sites. (A) Near-range effect of meSNPs. For 1,562 CpG-changing (including CpG-loss and CpG-gain) SNPs that have significant meQTL (P value < 10^{-9}) to a CpG site within 100 bp, we plot their SNP-CpG distance (X-coordinate) vs. their meQTL effect sizes (Y-coordinate). Both CpG-loss and CpG-gain SNP genotypes are oriented such that the CpG-carrying allele is coded as 0 and the non-CpG-carrying allele is coded as 1. (B and C) Effect of meSNP beyond immediate neighborhood. We plotted the distribution of cis-meQTL signals surrounding 812 meSNPs in the GOLDN data, before (B) and after (C) adjusting for meSNPs. The center is the location of the meSNPs. The X-axis represents the relative position of the SNP to the central meSNPs, organized into 101 equal-sized bins, each covering 198 bp. The Y-axis represents similar bins for the relative position of the CpG to the meSNPs. Color of the heatmap represents the number of meQTLs found at the distance bin.
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