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Genetic-Epigenetic Interactions in Asthma Revealed by a Genome-Wide Gene-Centric Search

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Abstract

Objectives—There is evidence to suggest that asthma pathogenesis is affected by both genetic and epigenetic variation independently, and there is some evidence to suggest genetic-epigenetic interactions affect risk of asthma. However, little research has been done to identify such interactions on a genome-wide scale. The aim of this studies was to identify genes with genetic-epigenetic interactions associated with asthma.

Methods—Using asthma case-control data, we applied a novel nonparametric gene-centric approach to test for interactions between multiple SNPs and CpG sites simultaneously in the vicinities of 18,178 genes across the genome.

Results—Twelve genes, *PF4*, *ATF3*, *TPRA1*, *HOPX*, *SCARNA18*, *STC1*, *OR10K1*, *UPK1B*, *LOC101928523*, *LHX6*, *CHMP4B*, and *LANCL1*, exhibited statistically significant SNP-CpG interactions (FDR = 0.05). Of these, three have previously been implicated in asthma risk, *PF4*, *ATF3*, and *TPRA1.* Follow-up analysis revealed statistically significant pairwise SNP-CpG interactions for several of these genes, including *SCARNA18*, *LHX6,* and *LOC101928523*, (P-Values = (1.33E-04, 8.21E-04, 1.11E-03), respectively).

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Conclusions—Joint effects of genetic and epigenetic variation may play an important role in asthma pathogenesis. Statistical methods that simultaneously account for multiple variations across chromosomal regions may be needed to detect these types of effects on a genome-wide scale.

Keywords

DNA methylation; statistical interactions; asthma susceptibility; SNPs; integrative genomics

Introduction

Asthma is a chronic inflammatory disorder of the airways, characterized by airway hyper-responsiveness and airflow limitation. Asthma prevalence has increased in recent years [1], afflicting 300 million worldwide, and is highly variable across population centers, ranging from 1% to 18% [2]. In the United States more than 23 million people were diagnosed with asthma as of August 2015, including over 6 million children [3].

Numerous genome-wide association studies (GWAS) have shown a substantial genomic contribution to the etiology of asthma, with heritability estimates varying between 35% and 95% [4–6]. However, only a fraction of this variation has been explained by specific causal variants, such as those near Chr17q12–21 [7,8]. Epigenetic mechanisms, another source of disease variation, may explain a portion of this missing heritability [9–11]. Epigenomic alterations, while heritable, may also occur as a response to the endogenous and exogenous environment, and contribute to asthma pathogenesis [9,12].

There is evidence to suggest that genetic and epigenetic variation interact synergistically to affect gene expression [13]. In fact, interactions between SNPs and DNA methylation in the genomic regions of T-helper 2 pathway genes *IL4R* and others were found to affect asthma risk [14,15], possibly through expression of these genes.

Methods for detecting statistical interactions in population studies often involve linear or logistic models that include a product term to represent the interaction effect. These pairwise approaches suffer from a multiple testing challenge [16,17], which is particular severe if applied on a genome-wide scale. For example, in a study with one million SNPs and 450,000 CpG sites, 450 billion tests would be required. In addition, pairwise approaches may not optimally leverage information from dependencies between SNPs and CpG sites across contiguous genomic regions. Using case-control data, we applied a novel gene-centric approach to test for interactions between multiple SNPs and CpG sites simultaneously in the neighborhood of each gene across the whole genome.

Methods

Asthma BRIDGE Data

These data are from the Asthma BioRepository for Integrative Genomic Exploration (Asthma BRIDGE) [18–21], which is publicly accessible and includes 1542 individuals with asthma and controls with comprehensive phenotype and genomic data. Asthma was defined via questionnaire by the presence of asthma symptoms, use of an inhaled bronchodilator at

least twice per week, or use of a daily asthma medication for the 6 months before the screening interview. Genome-wide SNP and DNA methylation was obtained from whole blood samples from 576 participants. Samples were randomized to avoid confounding by experimental batch. The final data set included 356 asthmatic and 220 non-asthmatic adults, for whom we had complete data.

Genotyping and DNA Methylation

Genotyping methods are described in detail in Torgerson et al. [19]. Briefly, SNPs included all Phase 2, Release 21 consensus HapMap variants and were obtained from studies participating in the EVE consortium [19,22]. The genotyping platforms, Illumina (1Mv1, 550k, 610k, 650k) and Affymetrix (500k, 6.0), varied by sample source. Quality control (QC) procedures included filtration for call rates (> 95%) and tests for agreement with Hardy-Weinberg expectations applied to SNPs oriented to the plus strand. High-density imputation using 1000 Genomes reference panel was performed using the MACH software [23]. Data were checked for consistency in reference alleles and strand orientation, and SNPs were excluded based on low quality scores and examination of QQ plots comparing the distribution of association p-values for genotyped and imputed markers. SNPs with minor allele frequencies less than 0.05 were excluded.

A total of 11 plates were run on the Illumina human 450K methylation platform. Data preprocessing steps included application of Norm-Exponential (NE) background correction on the raw methylation data, plate by plate for all 11 plates. HG19 annotations, including gene symbols, chromosome number, and sequence position, were added via the Bioconductor package, FDb.InfiniumMethylation.hg19. One sample from a non-asthmatic participant was excluded due to having an extreme value of 1.0 for all probes. Methylation of CpG sites were assessed for outliers, and observations exceeding 3 interquartile ranges (IQRs) from the 25^{th} and 75^{th} percentiles, per Tukey's outer fences [24], were removed unless they comprised greater than or equal to 5% of the data for that variable. The 11 NE corrected plates were combined and four additional samples were excluded as outliers. Dye bias (DB) correction was done using one sample as reference for all others, and quantile normalization (QN) was applied to the resulting data set.

Canonical Analysis of Set Interactions (CASI)

Here we describe and apply a new method, CASI, designed to identify statistical interactions between high-order sets of features in a simultaneous approach. In contrast to direct interactions in which DNA sequence variation affects DNA methylation, we are seeking to identify genomic regions underlying genes where there is synergism between the effects of SNPs and methylation on asthma susceptibility.

CASI is described in detail in Supplemental Methods and is freely available to the public in a downloadable R package (https://github.com/USCbiostats/CASI). Briefly, it is a hypothesis testing approach to detect differences in correlations between linear combinations of SNPs and CpG sites in cases vs. controls. In this study, SNPs and CpG sites were mapped to the nearest gene, thus defining the feature sets, with positions identified using the UCSC genome browser for all RefSeq genes. Overlapping isoforms of the same gene were

combined to form a single full length version. SNPs and CpG sites residing in flanking regions extending 2kb on either side were included. A total of 18,178 gene-centric regions were identified across the genome. Using canonical correlation analysis (CCA), coefficients that maximize the correlation, the *canonical correlation*, between linear combinations of the SNPs and CpG sites are estimated in cases. The same coefficients are then used to compute linear combinations in controls. The CASI statistic is based on the scaled difference between Fisher transformed canonical correlation coefficients in cases vs controls. Estimation is performed in cases because it leverages the superior statistical power of the case-only interaction analysis approach [25]; dependencies among predictive variables in cases may indicate multiplicative interactions in their effects on disease [26,27]. Due to the limitations of CCA in the number of variables relative to the sample size, sparse canonical correlation analysis as described by Tibshirani and Hastie [28] is applied for sets with dimensions that are too large to be accommodated by conventional CCA. The parametric distribution of this test statistic, the scaled difference between cases and controls, is unknown. Therefore we use a permutation-based procedure to estimate the null distribution by randomly permuting case status and computing the test statistic. The permutation analysis is conducted repeatedly until a sufficient number of values have been generated under the null. Rather than compute p-values, a computationally efficient FDR approach is used that requires few permutations and yields confidence intervals that account for dependencies among tests as well as the number of permutations conducted [29]. For this study, 100 permutation analyses were conducted and statistical significance was defined by an FDR threshold of 0.05.

Follow-up Analyses in Significant Regions

SNPs and CpG sites can be ranked in their contribution to the test statistic by computing correlations, *loadings*, in cases with their corresponding linear combinations, the *canonical variates*. SNP-CpG site pairs that were the greatest contributors to statistically significant tests were identified by selecting all SNP-CpG pairs among those with loadings greater than 0.5 within a gene region. The loading threshold of 0.5 has been proposed as the “operational definition” of a large effect size [30]. Using logistic regression, conventional likelihood ratio tests of multiplicative interactions were conducted, including one degree of freedom likelihood ratio tests (1-df LRT) for interaction and three degree of freedom tests (3-df LRT) for the combined main and interaction effects. Statistical significance was assessed according to FDR level of 0.05, using a parametric version of Millstein and Volfson [31]. Variables found to be significantly correlated with asthma status (Table 1) were included as adjustment covariates in the logistic models. While education level was not significant (p-value = 0.14), it was nevertheless included due to prior evidence of association with asthma. Final models were adjusted for gender, ancestry, family history of asthma, age at sample collection, and education level. The ancestry variables were composed of the top two principal components computed by the software Eigensoft [32] using 128 ancestry informative markers (AIMs) [33]. The primary results do not include adjustment for site, because two ABRIDGE sites contributed only cases (Table 1). However, sensitivity analyses were conducted by restricting to sites that included both cases and controls and including site as an adjustment covariate, and the results did not change substantially (Supplemental Table S1).

Results

Performance of the CASI Approach in Simulation Studies

The simulation results demonstrate that the CASI hypothesis test is sensitive to correlation differences between cases and controls and that sensitivity is associated with magnitude of the differences (Figures S1). We also found that CASI is a consistent test with asymptotics depending on effect size and that type I error is very low as the effect size approaches zero (Figure S2). CASI performed quite well in the simulation results as compared to other related approaches, especially in the vicinity of conventional levels of FDR that imply statistical significance (Figure S3). Applying a conventional pair-wise logistic regression approach combined with FDR evaluated significance to the same simulated data resulted in a complete failure to detect interactions, highlighting the utility of the proposed approach (Figure S3).

Demographics of the Asthma BRIDGE Population

Of the 576 participants, 62 percent had asthma (Table 1). While males and females were evenly distributed among cases, there were more females than males among the controls. Asthma cases had a greater percentage of Hispanics whereas controls had a greater proportion of African Americans. Frequency of asthma prevalence varied by clinic site by design, with some sites recruiting only cases.

CASI Analysis of Asthma BRIDGE Data

Note that p-values were not generated in the main analysis even as intermediate statistics because FDR was estimated directly from the observed and permuted CASI statistics. The estimated FDR and corresponding confidence intervals (CIs) for a series of increasingly stringent significance thresholds for the CASI test statistic demonstrate a downward trend with narrow confidence intervals (Figure 1), indicating that the CASI statistic is informative with respect to distinguishing observed from permuted data. However, this dynamic does not continue beyond about 4.35 where a minimum FDR occurs, implying that more extreme values of the CASI statistic do not correspond to lower rates of false discoveries. The threshold of 4.35 was therefore used to identify the most statistically significant results, yielding 12 genes, *HOPX*, *SCARNA18*, *PF4*, *STC1*, *ATF3*, *OR10K1*, *UPK1B*, *LOC101928523*, *LHX6*, *CHMP4B*, *TPRA1*, and *LANCL1* (FDR = 0.050, CI = (0.024, 0.104)). Though we highlight these 12 genes due to low FDR, it is clear from the tight CIs bracketing FDR estimates at more permissive significance thresholds that substantially more such interactions are implied by these results.

Pairwise SNP-CpG Interaction Analysis

Pairwise analyses of SNPs and CpG sites from the 12 significant genomic regions with loadings of 0.5 or greater using logistic regression revealed evidence of pairwise interactions for 19 top-loading pairs (Table 2), corresponding to 4 of the 12 genomic regions, *LOC101928523*, *SCARNA18*, *LHX6*, and *STC1*.

Although the 3-df tests were significant for most of these pairs, the 1-df tests tended to be more significant, implying that main effects were not appreciably contributing. Nine

individual SNPs and ten CpG sites in *LHX6*, three SNPs and two CpG sites in *LOC101928523*, and three SNPs and two CpG sites in *SCARNA18* met the criteria of having loadings greater than 0.5 (Figure 2). The SNP-CpG pair with the greatest loadings did not always elicit a significant result for multiplicative interaction (Table S2), however, this is not surprising considering that the CASI statistic is formed from linear combinations of SNPs and CpGs. Multiple genomic regions, *HOPX*, *PF4*, *ATF3*, *UPK1B*, *CHMP4B*, *TPRA1*, and *LANCL1* did not show evidence of interactions for their top loading SNP-CpG pair (Table S2). The lack of statistical significance for individual pairs may indicate that it is necessary to evaluate joint interactions between multiple SNPs and CpGs in order to have adequate power to detect the effects.

Additional pairwise interaction analyses were conducted for SNPs and CpGs with loadings greater than 0.5 underlying *LHX6*, *LOC101928523*, and *SCARNA18* (Tables 3-5). Significant interactions were found for *LHX6*, where involved SNPs had negative loadings located at the center haplotype block interacting with CpG sites clustered to the right in a CpG island (Figure 2 and Table 3). Odds ratios for the main effects of both SNPs and CpGs in *LHX6* were not statistically significant (Table 3). However, interactions for seven SNP-CpG pairs were significant, with odds ratios (ORs) ranging from 0.53 (0.36, 0.77) to 0.84 (0.74, 0.96) (Table 3). High LD between SNPs as well as dependencies among CpGs may explain the similarity in interaction effects that is apparent across SNP-CpG pairs. For *LHX6*, minor allele dose was associated with increasingly protective effects of methylation (Table 3). This trend is particularly apparent in the conditional ORs ($OR_{meth|SNP}$). For the most significant interaction in *LHX6*, individuals with the common GG genotype in rs10818651 had a 9% greater odds of asthma for a 5% difference in cg21469772 methylation. Addition of one minor allele A reverses the association to a 21% decrease in the odds of asthma for 5% greater methylation, whereas individuals with AA had a 43% decrease. Similarly, for the next most significant interaction (rs10985567 and cg21213617), the odds of asthma for an individual with 5% higher methylation and GG (common homozygote) increases by 24% whereas the odds for an individual with 5% higher methylation and AA decreases by 66%.

In *LHX6* the most statistically significant interaction involved the SNP with the greatest loading (rs10818651, loading = −0.86) (Table 2) that resides within the middle LD block (between base pair locations 124972042 and 124982500, indicated by vertical bars in Figure 2), paired with CpG site cg01363324 (loading=0.53). The opposite signs of the loadings for this pair (Figure 2) convey that these variables are negatively correlated among cases (Figure 3), which is reflected in the interaction pattern. Increasing minor allele dose of the SNP reflects an association between methylation level and asthma that is increasingly negative.

Boxplots of methylation values with jittered points can provide a visual demonstration of relationships underlying the significant interaction effects (Figure 3). For example, *LHX6* median methylation (cg04282082) is greater in individuals with asthma than controls with the GG genotype (rs10818651), but less within AG individuals. In general, for *LHX6*, increased methylation was protective in the presence of a minor allele (Table 3). In another example, median methylation of cg16688533 in *STC1* is greater in individuals with asthma vs those without in AA individuals (rs9969426), but those with GG tend to be unmethylated

and have asthma. Another clear example can be observed for *LOC101928523*, where median methylation (cg07956857) is greater in CC individuals with asthma (rs13301641), approximately equal in TC individuals but less in TT.

SNPs within the *LOC101928523* genomic region were confined to a single haplotype block (Figure 2). The highest loading SNPs, rs13301641 (loading = 0.87), rs11792474 (loading = 0.87), and rs75088949 (loading = −0.68) as well as the highest loading CpG site, cg07956857 (loading = −0.89), were the most statistically significant interactions for this genomic region (Table S3). As with *LHX6*, there was little evidence of main effects, however, unlike *LHX6*, minor allele dose was accompanied by both decreased and increased ORs. The most significant interaction for *LOC101928523*, between rs13301641 and cg07956857, involved loadings with opposite signs (Figure 2), which reflected negative correlation between methylation level and number of minor alleles among individuals with asthma.

For *SCARNA18* there were three SNPs, rs67216017, rs113665237, and rs10061690, all in high LD, with loadings greater than 0.5. Thus interaction analysis results are indistinguishable among the three (Table S4). The loadings for SNPs and CpGs are concordant (Figure 2), which is consistent with the idea that minor allele dose is associated with increasingly deleterious effects of methylation on asthma susceptibility. The range of methylation for cg14999833 is narrow (Figure 3), hence it is appropriate to estimate the effect over a 1% change. Deletion of allele ‘A’ near rs67216017 or ‘T’ near rs113665237 appears to have an equivalent effect to presence of minor allele ‘A’ at rs10061690 on the relationship between methylation at the CpG site cg14999833 and asthma. With respect to cg14999833 a biologically meaningful effect is observed for a 1% difference in methylation in contrast to cg20697188 where a 5% difference is within the range of the observed data.

Discussion

The proposed statistical approach, CASI, was able to overcome the substantial challenges imposed by high dimensional data to identify statistical interactions between methylation variation at CpG sites and DNA variation in SNPs. Part of the explanation for the adequate power that led to this success as well as the superior performance of CASI in simulated data in comparison to other similar methods is the fact that CASI leverages the concept underlying the case-only interaction method, known to be a powerful approach [25,34]. This characteristic is unique to CASI among related set based methods designed to detect statistical interactions [35–37]. Another unique characteristic of the CASI approach is the reliance on a computationally efficient permutation based FDR estimator to define statistical significance. This is a nonparametric approach that is robust to feature distributions and yields confidence intervals that bracket the FDR estimates, allowing uncertainty in those estimates to be quantified. These confidence intervals play an especially important role in studies of weak effects where an FDR level of 0.05 may not be achievable.

A limitation of the CASI procedure is the inablility to explicitly adjust for potential confounding covariates. However, this problem was mitigated in follow up analyses by applying conventional logistic regression with covariates to pairs of features implied by

CASI results. Like many conventional approaches, CASI is designed to detect linear associations, which limits the power for non-linear relationships. However, considering that we were able to identify 12 genes with evidence of interactions using a sample size in the hundreds suggests that more interactions may be detectable if the sample size is increased substantially. Though the procedure requires considerable computational resources due to its reliance on permutations, the required number of permutations is small enough to allow genome-wide applications.

Though several previous studies have investigated statistical interactions between genetic and epigenetic variation [13–15,38], we know of no others that have attempted a genome-wide assessment. CASI identified 12 genomic regions defined by the positions of 12 genes with evidence of statistical interactions between sets of SNPs and sets of methylated CpGs with respect to asthma risk. Of these 12 genes, 3 have previously been implicated in asthma risk or underlying biological pathways related to pathology of the disease, *PF4* [39], *ATF3* [40,41], and *TPRA1* [42]. Three genomic regions not previously implicated in asthma, *LOC101928523*, *SCARNA18*, and *LHX6*, contained the most statistically significant pairwise interactions between the considered individual SNPs and CpGs.

LHX6 (LIM homeobox domain 6) has not been implicated in asthma, however it is a recognized transcriptional regulator that controls the differentiation and development of lymphoid cells [43]. *LHX6* is known to be regulated epigenetically in lung cancer, where in vitro and in vivo studies found that in normal lung tissue it is readily expressed but down-regulated or silenced in lung cancer cells in which the gene is hyper-methylated [43]. Other evidence of epigenetic regulation in the vicinity of *LHX6* has been found in head and neck squamous cell carcinomas (HNSCC) [44]. Similar to the lung cancer study, hyper-methylation of the CpG island in *LHX6* was associated with transcriptional silencing of *LHX6*. These findings suggest that differential methylation near *LHX6* plays a role in lung biology and lends credence to a potential role in asthma.

The three genes previously implicated in asthma pathogenesis are *PF4*, *ATF3*, and *TPRA1*. *PF4* (Platelet Factor 4) is a protein coding gene which functions as an inhibitor of T-cell function [45]. *PF4* activation in the lung is a feature of the late inflammatory response to antigen challenge and may play an important role in allergic inflammation and asthma [46]. *Atf3* (Activating transcription factor 3) is a negative regulator of allergic inflammation in mice challenged with ovalbumin [47] and deficiency in mice leads to the development of significantly increased airway hyper-responsiveness and pulmonary eosinophilia [40]. Significant increases in *ATF3* mRNA have also been observed in patients with mild asthma as compared with non-asthmatic patients [48]. *TPRA1* (transmembrane protein adipocyte associated 1) is an irritant-sensing cation channel expressed in TRPV1-positive, capsaicin-sensitive chemosensory neurons that innervate various organs, including the airways [49]. Various exogenous chemicals have been described to activate *TRPA1*, including agents recognized to trigger and/or worsen asthma such as diisocyanates, cigarette smoke, acrolein, and chlorine [42]. A potential role of *TRPA1* in mediating allergen-induced asthmatic responses has been described in ovalbumin-sensitized mice, in which genetic deletion of *Trpa1* or pretreatment with a selective *Trpa1* antagonist reduced leukocyte infiltration, decreased cytokine and mucus production, and almost completely abolished airway

hyperactivity [49]. Bessac et al. (2008) suggested that *TRPA1* may function as an integrator of immunological stimuli modulating inflammation in the airways [50]. Furthermore, chemical irritant-induced activation of *TRPA1* may trigger the release of neuropeptides and chemokines in the airways, thereby exacerbating the cellular and tissue inflammatory response observed in allergic individuals [51].

HOPX, *OR10K1*, *UPK1B*, *CHMP4B*, and *LANCL1* were identified as significantly associated with asthma in the tests for joint interactions but require further investigation into their putative biological functions. There is scant prior evidence of a role for these genes in pulmonary or immune function, lung disease, or asthma, although *HOPX* is involved in the function of regulatory T cells [52].

Conclusions

We demonstrated that simultaneous consideration of genomic and epigenomic variation has the potential to identify genetic risk factors for asthma beyond individual GWAS studies or epigenetic screens. These results add to existing evidence suggesting a synergy between genomic and epigenomic variation affecting risk of asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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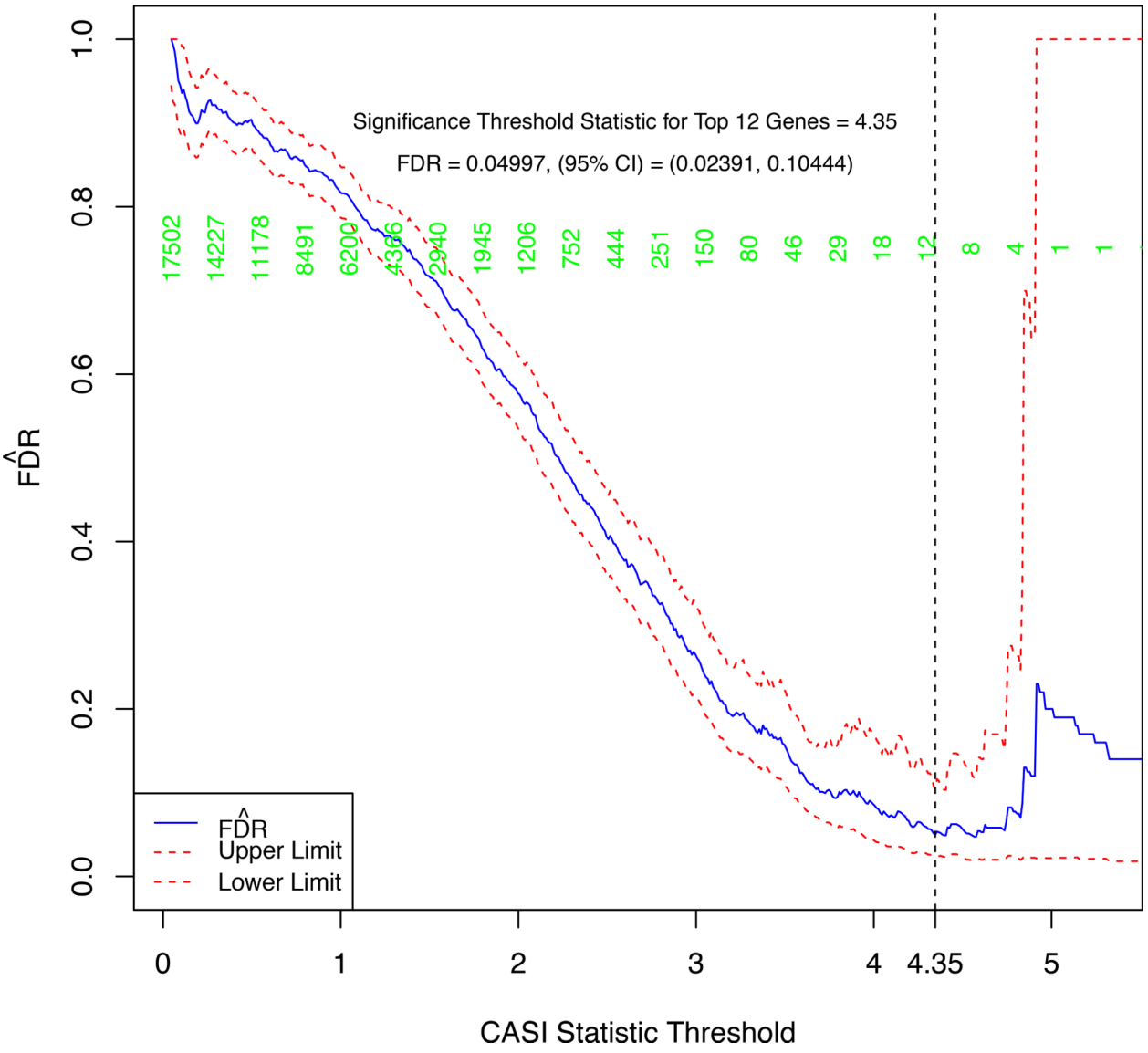

Figure 1:

FDR and confidence intervals for 18,178 genomic regions (genes) defined by 2kb up and downstream of each gene. The value of the CASI statistic (x-axis) has no direct interpretation other than that larger values are more extreme. 12 significant genomic regions correspond to a threshold of 4.35 (vertical dashed line) and FDR of 0.05, CI = (0.024, 0.104). Integer values shown in green specify the number of genomic regions with CASI statistics at least as extreme as the thresholds specified by the horizontal axis.

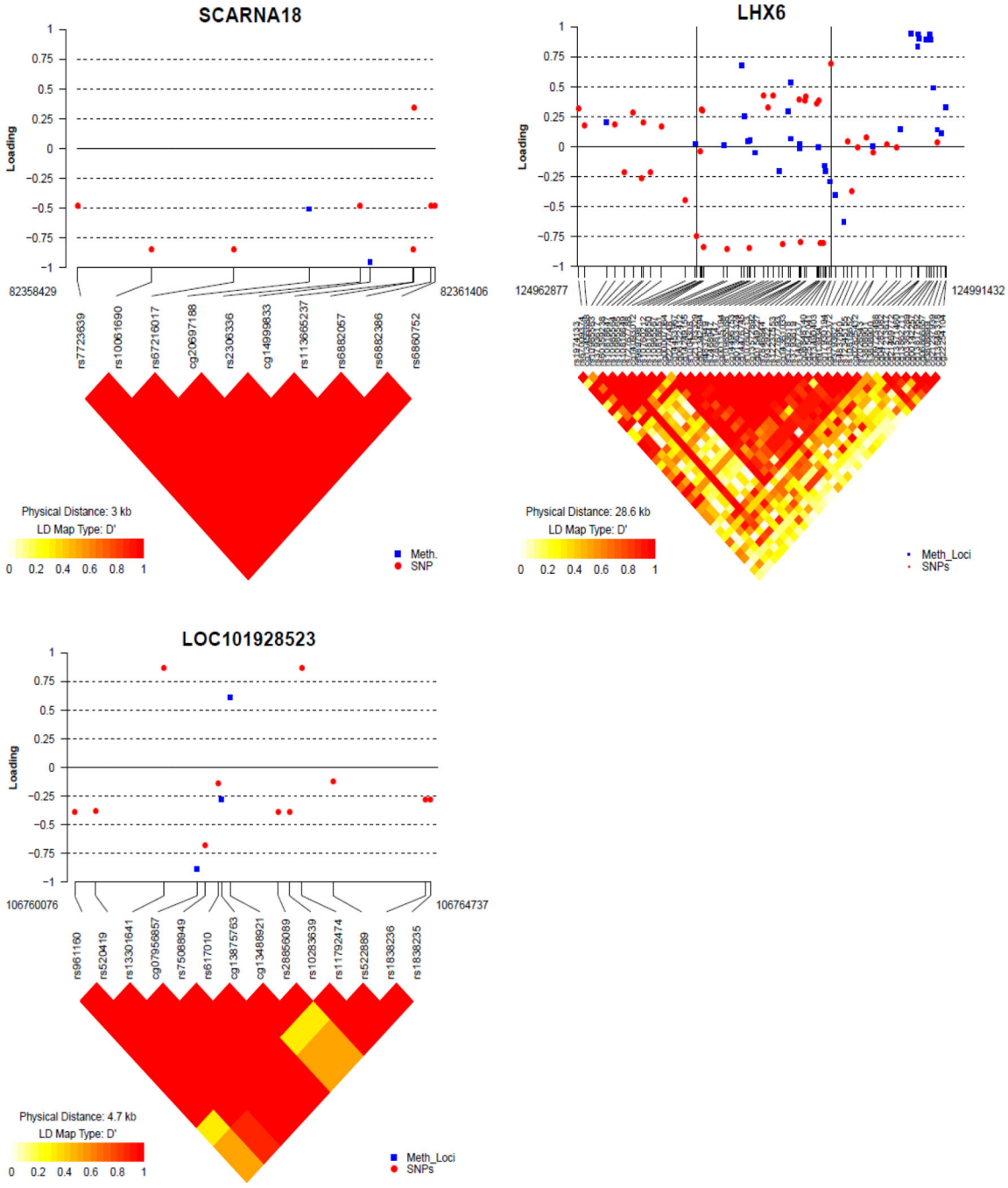

Figure 2:

SNP and methylation loadings for genomic regions *LOC101928523*, *LHX6*, and *SCARNA18* with LD heat maps. Red circles indicate SNPs and blue squares CpG sites. The horizontal axis indicates base pair (BP) positions in each of the three genomic regions. Heat maps represent dependencies between SNPs and CpGs (alignment is approximate).

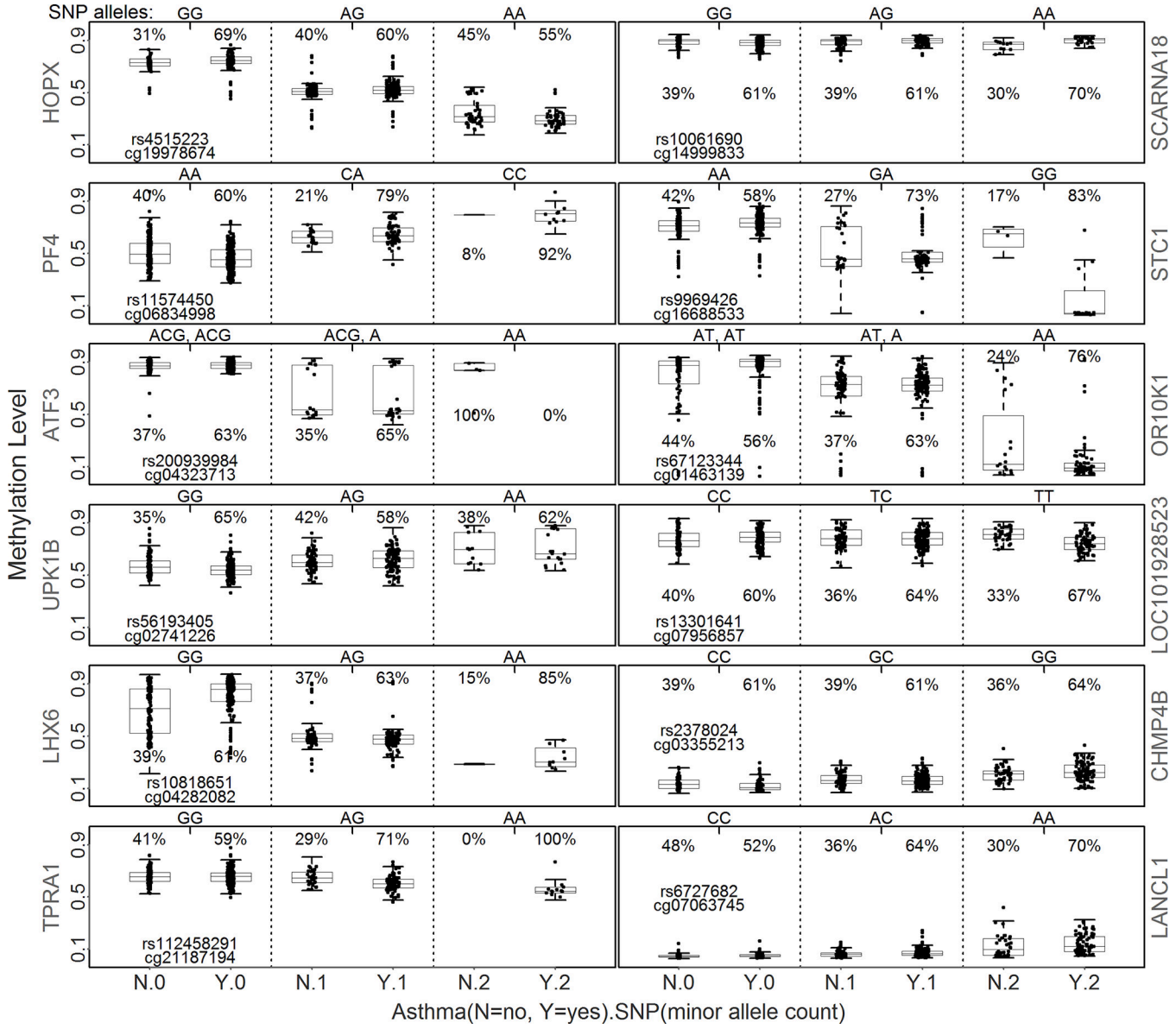

Figure 3:

Interaction box plots for SNP-methylation pairs with highest loadings in the top 12 genomic regions. (See supplemental Table S1 for adjusted odds ratios.)

TABLE 1.

Characteristics of Participants in the ABRIDGE Study.

	Asthma Case N (%) 356 (61.81)	Asthma Control N (%) 220 (38.19)
Characteristic	***n* (%)**	***n* (%)**
Gender		
Male	169 (47.5)	78 (35.5)
Female	187 (52.5)	142 (64.5)
Race/Ethnicity		
European	73 (20.5)	41 (18.6)
Hispanic/Latino	174 (48.9)	65 (29.5)
Black or African American	67 (18.8)	92 (41.8)
Other/Multiple/Uncertain	42 (11.8)	22 (10.0)
Education		
Did Not Complete High School	41 (11.5)	20 (9.1)
High School or GED Degree	96 (27.0)	51 (23.2)
Some College	97 (27.2)	74 (33.6)
College Degree	71 (19.9)	44 (20.0)
Post-College Coursework	18 (5.1)	15 (6.8)
Graduate or Professional Degree	18 (5.1)	14 (6.4)
Other	15 (4.2)	2 (0.9)
Site		
GRAAD, JH	39 (11.0)	87 (39.5)
CAG/UAC	32 (9.0)	18 (8.2)
CHS/USC	119 (33.4)	115 (52.3)
CARE; Denver NJH, Tucson/UARC, St. Louis/WUC, Madison/UWM	42 (11.8)	0 (0)
MCCAS	124 (34.8)	0 (0)
Age at Methylation Measure		
0–10	4 (1.1)	0 (0)
10–20	134 (37.6)	4 (1.8)
20–30	162 (45.5)	143 (65.0)
30–40	23 (6.5)	21 (9.5)
40–50	16 (4.5)	27 (12.3)
50–60	13 (3.7)	21 (9.5)
>60	4 (1.1)	4 (1.8)
Family History of Asthma		
Yes	173 (48.6)	51 (23.2)
No	183 (51.4)	169 (76.8)

Characteristic	Asthma Case N (%) 356 (61.81) *n* (%)	Asthma Control N (%) 220 (38.19) *n* (%)
Age at Diagnosis		
0–2	90 (25.3)	
2–5	100 (28.1)	
5–8	64 (18.0)	
8–11	57 (16.0)	
11–14	15 (4.2)	
14–17	9 (2.5)	
>17	21 (5.9)	

Table 2:

Significant interactions for SNPs and CpGs with loadings > 0.5.

SNP	Methylation	Coefficient SNP×Meth Interaction	P-Value 1-df LRT	P-Value 3-df LRT	Gene
rs67216017(G/GA)	cg14999833	19.79	2.84E-05	1.33E-04	*SCARNA18*
rs113665237(A/AT)	cg14999833	19.79	2.84E-05	1.33E-04	*SCARNA18*
rs10061690	cg14999833	19.79	2.84E-05	1.33E-04	*SCARNA18*
rs10818651	cg01363324	−24.84	1.17E-04	8.21E-04	*LHX6*
rs13301641	cg07956857	−8.11	1.28E-04	1.11E-03	*LOC101928523*
rs11792474	cg07956857	−7.86	1.79E-04	1.49E-03	*LOC101928523*
rs10818651	cg21469772	−6.47	2.49E-04	3.74E-03	*LHX6*
rs10985567	cg01363324	−23.97	2.99E-04	4.35E-04	*LHX6*
rs10818651	cg04282082	−4.99	3.59E-04	4.88E-03	*LHX6*
rs10818651	cg13832372	12.80	3.68E-04	1.44E-03	*LHX6*
rs10985567	cg21213617	−12.88	6.19E-04	4.51E-03	*LHX6*
rs10985567	cg13832372	12.61	6.42E-04	4.55E-04	*LHX6*
rs10985567	cg21469772	−6.19	8.17E-04	4.18E-03	*LHX6*
rs989798	cg04282082	−4.70	1.17E-03	7.01E-03	*LHX6*
chr8/BP:23713016 (C/CT)	cg16688533	−3.99	1.20E-03	3.87E-03	*STC1*
rs989798	cg13832372	12.33	1.42E-03	1.85E-03	*LHX6*
rs10818651	cg21213617	−11.34	1.54E-03	1.53E-02	*LHX6*
rs989798	cg21469772	−5.69	1.60E-03	1.24E-02	*LHX6*
rs10985567	cg04282082	−4.61	1.73E-03	4.51E-03	*LHX6*

Statistically significant (FDR < 0.05) pairwise interactions (1-df LRT) for top loading SNPs and CpGs from the 12 genomic regions identified as significant by CASI. Logistic regression models were adjusted for gender, ancestry/ethnicity, family history of asthma, age at methylation measure, and education level. Very similar p-values may be indicative of SNPs in high LD.

Table 3:

Tests of multiplicative interactions (P < 0.05) within LHX6 for SNP-CpG pairs.

			5% difference in methylation			
SNP (Minor/Major alleles) Methylation	**Minor Allele Freq.**	**P-Value Interaction**	**$OR_{Meth \mid SNP}$ (95% CI)**	**$OR_{Interaction}$ (95% CI)**	**OR_{SNP} (95% CI)**	**$OR_{Meth.}$ (95% CI)**
rs10818651 (A/G) cg21469772	0.25	2.49E-04	GG: 1.09 (0.97, 1.23) AG/GA: 0.79 (0.58, 1.07) AA: 0.57 (0.35, 0.94)	0.72 (0.60, 0.87)	1.00 (0.66, 1.53)	0.99 (0.93, 1.07)
rs10818651 (A/G) cg04282082	0.25	3.59E-04	GG: 1.08 (0.99, 1.18) AG/GA: 0.84 (0.67, 1.07) AA: 0.66 (0.45, 0.96)	0.78 (0.67, 0.90)	1.00 (0.66, 1.53)	1.01 (0.95, 1.06)
rs10985567 (A/G) cg21213617	0.23	6.19E-04	GG: 1.24 (0.99, 1.55) AG/GA: 0.65 (0.35, 1.20) AA: 0.34 (0.13, 0.93)	0.53 (0.36, 0.77)	1.27 (0.84, 1.96)	0.96 (0.83, 1.12)
rs10985567 (A/G) cg21469772	0.23	8.17E-04	GG: 1.13 (1.01, 1.27) AG/GA: 0.83 (0.61, 1.13) AA: 0.61 (0.37, 1.00)	0.73 (0.60, 0.89)	1.27 (0.84, 1.96)	0.99 (0.93, 1.07)
rs989798 (T/C) cg04282082	0.20	1.17E-03	CC: 1.08 (1.01, 1.17) TC/CT: 0.86 (0.69, 1.07) TT: 0.68 (0.47, 0.98)	0.79 (0.68, 0.92)	1.21 (0.78, 1.88)	1.01 (0.95, 1.06)
rs10985567 (A/G) cg04282082	0.23	1.73E-03	GG: 1.11 (1.02, 1.20) AG/GA: 0.88 (0.70, 1.11) AA: 0.70 (0.48, 1.03)	0.79 (0.68, 0.92)	1.27 (0.84, 1.96)	1.01 (0.95, 1.06)
rs10985567 (A/G) cg03363289	0.23	4.30E-03	GG: 1.17 (1.06, 1.28) AG/GA: 0.98 (0.78, 1.23) GG: 0.83 (0.58, 1.17)	0.84 (0.74, 0.96)	1.27 (0.84, 1.96)	1.03 (0.97, 1.09)

SNPs (in region chr9:124962877–124992824, GRCh37/hg19) were selected for presentation that reside in the central haplotype block and have negative loadings greater than 0.5 in absolute value. Models were adjusted for gender, ancestry/ethnicity, family history of asthma, age at methylation measure, and education level. CpG sites clustered near the CpG island toward the right of the gene as shown in Figure 2. ORs reflect a 5% change in methylation.