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Genomic architecture of asthma differs by sex

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ABSTRACT

Asthma comprised of highly heterogeneous subphenotypes resulting from complex interplay between genetic and environmental stimuli. While much focus has been placed on extrinsic environmental stimuli, intrinsic environment such as sex can interact with genes to influence asthma risk. However, few studies have examined sex-specific genetic effects. The overall objective of this study was to evaluate if sex-based differences exist in genomic associations with asthma. We tested 411 asthmatics and 297 controls for presence of interactions and sex-stratified effects in 51 genes using both SNP and gene expression data. Logistic regression was used to test for association. Over half (55%) of the genetic variants identified in sex-specific analyses were not identified in the sex-combined analysis. Further, sex-stratified genetic analyses identified associations with significantly higher median effect sizes than sex-combined analysis for girls (p -value = $6.5E-15$) and for boys (p -value = $1.0E-7$). When gene expression data were analyzed to identify genes that were differentially expressed in asthma versus non-asthma, nearly one third (31%) of the probes identified in the sex-specific analyses were not identified in the sex-combined analysis. Both genetic and gene expression data suggest that the biologic underpinnings for asthma may differ by sex. Failure to recognize sex interactions in asthma greatly decreases the ability to detect significant genomic variation and may result in significant misrepresentation of genes and pathways important in asthma in different environments.

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1. Introduction

Asthma is a major public health problem affecting more than 9 million children in the United States [1]. Numerous studies have indicated that susceptibility to asthma differs by sex [2]. Young boys are nearly twice as likely to develop asthma as young girls [3,4]. Animal models also support sex differences in asthma risk with male mice having greater airway hyperresponsiveness than female mice [5] and female mice having higher levels of airway inflammation and pulmonary remodeling than male mice [6,7].

It is generally understood that asthma is a complex trait influenced by both genetic and the environment as well as their interaction. While variants in more than 43 genes have been associated with asthma [8,9] most, thus far, explain only a small proportion of disease risk [10].

Similarly, while studies examining the activation and repression of asthma-related genes (e.g. via changes in gene expression) have provided critical insight into the underlying pathways associated with asthma [11,12], identifying the clinical utility of these findings has been challenging.

A major problem is that most genetic association studies fail to account for the interaction between genes and the environment, in this case, sex. Indeed, as the internal environments of males and females may differ as a result of sex-specific differences in hormones or persisting differences in maturation and physiologic performance in the developing lung [13], it is possible, and even likely, that the underlying genomic pathways leading to asthma may differ between males and females. Most genetic and genomic studies of asthma using genome-wide approach assess the main effects of genetic variants in sex-combined analyses in order to increase power. In fact, such a practice require thousands of cases and controls samples and may even reduce power to detect genetic associations with asthma, particularly those that differ by sex and are in opposing directions, thus creating a net cancelling effect. Further, these studies do not take into account interaction analysis as well as prior information gained from previously published animal and human candidate genes studies. Including sex in the analysis as a covariate may not help as this approach assumes that differences in asthma risk between the sexes are not due to underlying

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genetic or genomic factors. Finally, although sex-specific associations are often attributed to variants on the sex chromosomes [14,15], numerous studies have shown that autosomal variants may result in differences by sex as well [15–20]. Taken together, it is plausible that sex may modify genetic and/or genomic associations with asthma. Common diseases such as asthma are heterogeneous and may actually be a compilation of disorders with numerous subphenotypes. Current studies are not designed to examine specific subphenotypes of disease due to the large sample sizes required for genome-wide approaches. Recent genome-wide and meta-analysis with sample size over 5,000 individuals showed nominally significance of genotype-by-sex interactions, which could imply sample and phenotype heterogeneity within and between studies, respectively [21]. By focusing on regions with a priori evidence of gene involvement, the candidate gene approach allows researchers to reduce the sample size and the number of genes to be tested with the potential to gain a higher statistical power compared to the unbiased approach of investigating the whole genome. The reduced sample requirement might permit better phenotyping and reduced heterogeneity and hence increases statistical power to detect sex-specific main as well as interaction association by lowering the multiple testing burden [22].

Therefore, the purpose of this study was to determine how sex impacts the detection of genetic and genomic associations. To this end, we compared the results of sex-combined and sex-stratified genetic and RNA expression association analyses among 507 SNPs and 136 RNA probe sets within 51 genes. Importantly, we evaluated gene-by-sex interactions to better understand how sex modifies the underlying associations identified, particularly in genes whose effects were in opposite directions in boys and girls.

2. Results

2.1. Description of study populations

The population for the genetic association analyses included 402 asthmatic cases (180 girls and 222 boys) and 297 controls (154 girls and 143 boys) after exclusions for missing call rates. Asthmatics were significantly younger than controls for both sexes (10.45 years vs. 12.30 years for girls and 9.64 years vs. 11.62 years for boys, p -value < 0.0001) (Table 1). The population for the gene expression analyses included 163 asthmatic boys and 105 asthmatic girls, and 58 control boys and 78 control girls. As these were from a public dataset, no other information was available.

2.2. Magnitude of association and effect sizes are stronger in sex-stratified analyses

Sex-combined (Fig. 1a) and sex-stratified analyses (Fig. 1b and c) identify genes that are common as well as specific to either boys or

girls. Odds ratios of asthma are shown on the y-axis. The x-axis represents the candidate SNPs genotyped across the genome. The horizontal dotted line represents the level at which we are sufficiently powered to detect effect sizes greater than 1.44 in the sex-combined analysis. The median odds ratios in both the sex-combined and sex-stratified analyses are shown in Fig. 1d. When examining the pattern of effect sizes across the 507 genetic variants, the highest effect sizes were seen in the sex-specific analyses (Fig. 1b & c). Six SNPs in the analysis of girls and one SNP in boys had effect sizes greater than any SNP identified in the sex-combined analysis.

To understand the impact of sex-stratified association on the magnitude of the genetic effect, the median effect sizes of both the sex-combined genetic and expression analyses were compared to the boy-only and girl-only analyses. Both sex-stratified genetic analyses revealed a significantly higher median effect size (odds ratio) than in the sex-combined analysis (1.163 vs. 1.098 (p -value = $6.5E-15$) for girls and 1.139 vs. 1.098 (p -value = $1.0E-7$) for boys; Fig. 1d).

To account for differences in power, we selected an effect size threshold (OR = 1.44) in which we would have 80% power to detect an effect for a common variant (MAF = 20%). In the sex-stratified analysis, 36 and 38 SNPs in the girl and boy analysis, respectively, exceeded this threshold compared to only 18 variants in the sex-combined analysis. Not surprising, SNPs that were consistently identified in both the sex-combined and sex-stratified analyses have similar effect sizes between the sexes. When considering a nominal significance level (p -value < 0.05), there were 26 variants identified in the sex-stratified, but not in the combined analysis (additional file 1: Supplementary Fig. 1). For these 26 variants, permutation testing suggested that the elevated effects sizes were unlikely due to chance (p -value ≤ 0.0478). Details about the sex*SNP interactions including patterns, genes, variants, chromosome, combined or sex-stratified odds ratio, p -value as well as SNP*Sex interactions are presented in Supplementary Table 1.

We also analyzed gene expression data to identify genes that were differentially expressed in asthma versus non-asthma. Sex-combined (Fig. 4a) and sex-stratified analyses (Fig. 4b and c) identify genes that are common as well as specific to either boys or girls. When examining the pattern of effect sizes across the 136 expression probes, the largest effect sizes were present in the sex-specific analyses (Fig. 4b and c). Similar to the results from the genetic variants analysis, the sex-stratified expression analyses also supported a significantly higher median effect size (beta coefficient) in the boy-only analysis compared to the sex-combined (Wilcoxon rank sum test; p -value = $1.2E-08$; Fig. 1d). Although the median beta in girls did not significantly differ from the sex-combined analysis (p -value = 0.19), a higher effect size was still observed.

Specifically, 3 probes in the analysis of girls and 4 probes in the analysis of boys exceeded the largest effect size in the combined analysis. In addition, while 13 probes in the sex-combined analysis exceeded our 80% power threshold for effect, 17 and 42 probes in girls and boys, respectively, exceeded the threshold. Importantly, at the nominal significance level (p -value < 0.05), there were 11 probes identified in the sex-stratified, but not in the combined analysis (additional file 1: Supplementary Fig. 2). For these 11 probes, permutation testing suggested that the elevated effects sizes were unlikely due to chance (p -value ≤ 0.045). Details about the sex*probe interactions including patterns, genes, probe ID, beta value combined or sex-stratified beta value, p -value as well as probe*Sex interactions are presented in Supplementary Table 2.

2.3. Sex interactions likely to have non-significant main effects in sex-combined analyses

Of the 507 SNPs, 33 SNPs showed nominal associations (p -value < 0.05) with asthma in the sex-combined analysis. In contrast, sex-stratified analyses revealed 47 SNPs associated with asthma (32 and 24 SNPs in girls and boys). Over half (55%, 26/47) of the variants identified in sex-specific analyses were not identified in the sex-combined analysis. Among all the 59 SNPs identified either in sex-

Table 1
Population characteristics.

Population	Variable	Girls		Boys	
		Asthma	Control	Asthma	Control
GCPCR/GCC (genetic data)	Total children (n)	185	154	226	143
	After exclusions (n) ^a	180	154	222	143
	Mean age (years)	10.45 ^b	12.3	9.64 ^b	11.62
GEO (expression data)	Total children (n) ^c	105	78	163	58

^a Indicates the number of children from the Greater Cincinnati Pediatric Clinic Repository (GCPCR) and Geneomic Control Cohort (GCC) after excluding those with missing call rates above 20%.

^b Indicates significant mean age differences ($p < 0.0001$) between asthmatic and non-allergic children within same sex.

^c Includes all children available from accession number GSE8052 in the Gene Expression Omnibus.

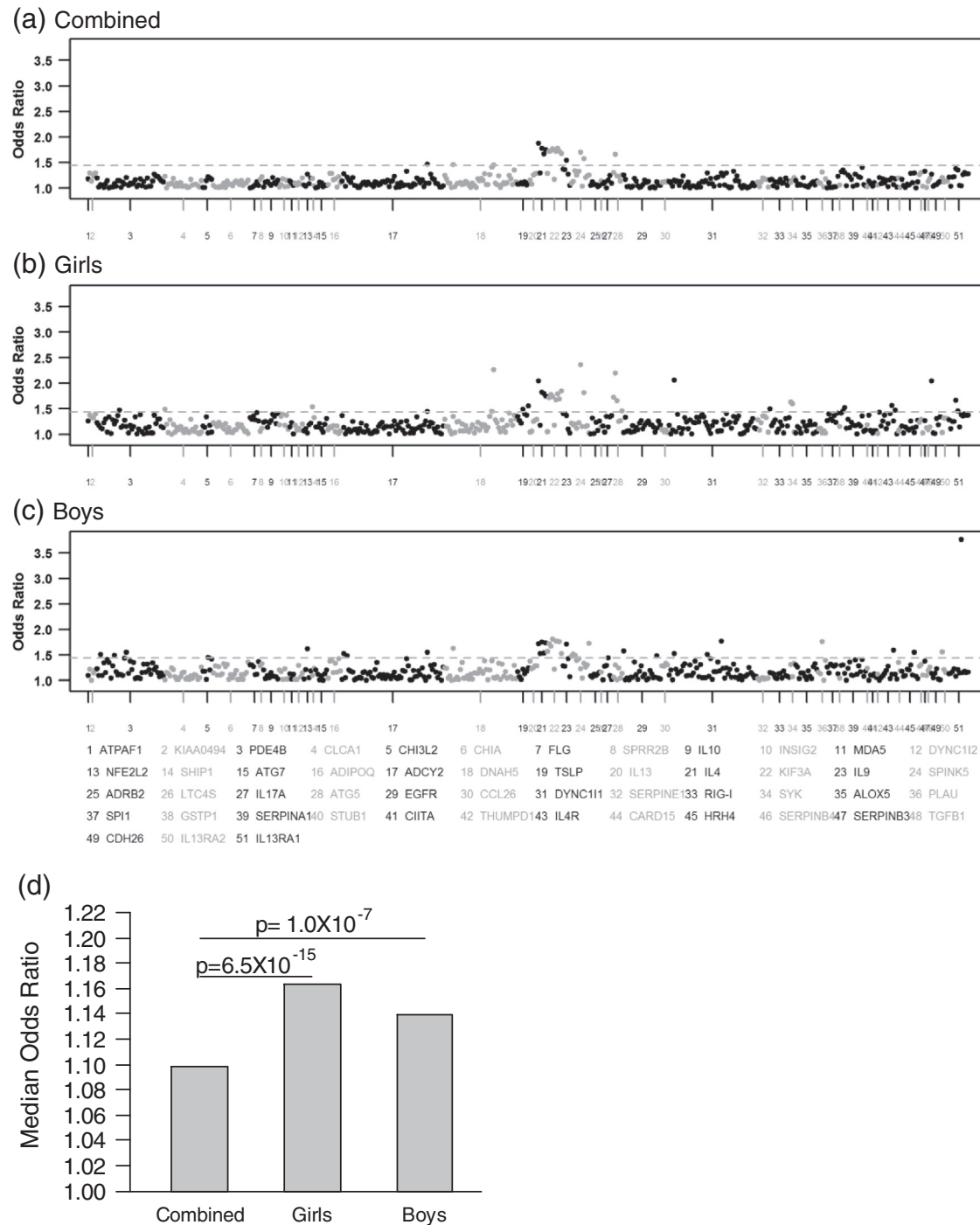


Fig. 1. Sex-combined and sex-stratified analyses identify unique asthma-associated SNPs. Logistic regression was performed adjusting for sex, age and population stratification. Odds ratios of asthma are shown on the y-axis. The x-axis represents the candidate SNPs genotyped across the candidate genes studied. (a) Sex-combined results (boys and girls together). (b) and (c) Sex-stratified results (only girls and boys, respectively). The horizontal dotted line represents the level at which we are sufficiently powered to detect effect sizes greater than 1.44 in the sex-combined analysis. (d) The median odds ratios in both the sex-combined and sex-stratified analyses.

combined analysis or sex-stratified analysis, nearly half (44%, 26/59) were only identified in sex-stratified analysis (Fig. 2). For the gene expression analysis, 24 probes showed nominal associations with asthma in a sex-combined analysis. In contrast, sex-stratified analyses revealed 29 probes associated with asthma (8 and 22 probes in girls and boys). Nearly one third (38%, 11/29) of the probes identified in the sex-specific analyses were not identified in the sex-combined analysis. Among all the 35 probes identified either in the sex-combined analysis or sex-stratified analysis, 31% (11/35) were only identified in sex-stratified

analysis (Fig. 2). Thus, performing only sex-combined analyses results in missing a substantial portion of associations, which could be detected in the sex-specific analysis. This could result in significant misrepresentation of genes and pathways important in asthma different gender environments.

To further understand the ability to detect variants with sex-specific effects, we also performed formal interaction analyses. Importantly, we identified 21 SNPs and 15 probes that exhibited nominal ($p \leq 0.05$) interaction effects. Several of these (8 SNPs and 5 probes) had not

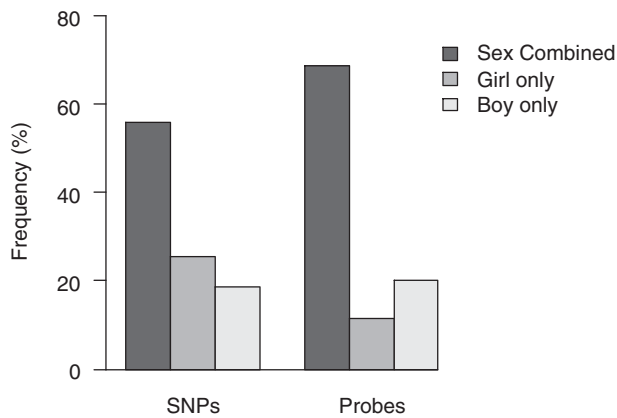


Fig. 2. Sex-specific analyses account for a substantial proportion of the detected associations. The bars represent the cumulative frequency of identified SNPs and probes with nominal association from either the sex-combined or sex-specific analyses.

been identified in the sex-combined or stratified analyses. The unique interactive effects demonstrated opposing effects in the sex-specific analyses. After accounting for overlapping findings, a total of 55 SNPs and 34 probes had sex-stratified or sex interaction effects. SNPs and probes without interaction were identified significantly more often in the sex-combined analyses than those with interactions (Fig. 3; p -value = 0.0002 and p -value = 0.002, for SNPs and probes, respectively). Our data suggest that variants/probes with interaction effects are likely to be missed in sex-combined analyses. Interestingly, those without interactions are more likely to be missed in the stratified analyses.

3. Discussion

While it has long been recognized that the underlying etiology of childhood asthma differs by sex [3,4,23], most human genomic studies of childhood asthma simply include sex as a covariate. This study shows that genomic associations with asthma differ by sex, thereby suggesting a distinct biologic underpinnings for asthma in boys versus girls. Using both genetic and gene expression data and a case-control design, we found that the overall magnitude of genomic effect is stronger in the sex-specific than in the sex-combined analyses. Importantly, variants and probes with sex interactions are likely to have non-significant main effects in sex-combined analyses, and therefore go undetected. Taken together, these results suggest that the failure to recognize sex interactions in asthma greatly decreases the ability to detect significant

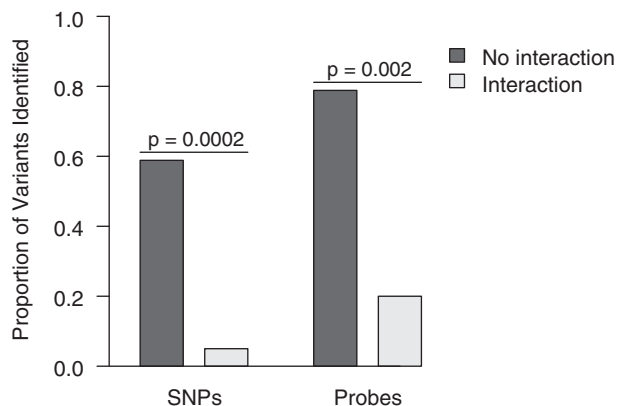


Fig. 3. SNPs and probes with sex-stratified effects and interaction are difficult to detect in combined analysis. Among all SNPs and probes identified as nominally associated in sex-stratified and interaction analyses, what proportion are also nominally associated in the sex-combined analyses (p -value < 0.05). Black bars represent SNPs and probes with no interaction effect, grey bars represent SNPs and probes with nominal interaction effects.

genomic variation. As most studies to date have utilized sex-combined analyses, our data suggest that the genes with the largest effect sizes are being missed. In fact, our data revealed several genes that have opposing effects in boys and girls, which are negated in the combined analyses.

Using genetic variants and gene expression data from 51 genes with biologic links to asthma [24], we found that effect sizes were significantly higher in sex-stratified compared to sex-combined analyses. The larger effect sizes in the sex-specific analyses are suggestive of the presence of interactive effects [25]. The presence of SNP-by-sex and probe-by-sex interaction is supported by previous studies. Genetic association studies of have implicated sex-specific effects in *INFG* [19], *TSLP* and asthma [26] and *CTLA4* with allergic response [27]. Gene expression studies also implicate sex interactions in placental global gene expression in response to asthma [28], *IL17BR* in allergic response [29] and overall gene expression in humans [30], as well as for asthma-related genes in mice [6,7,31]. Collectively, these data support a role of sex interaction in the genomics of asthma.

The discovery of new asthma-related genes is hindered because the median effect sizes are lower in sex-combined analyses compared to sex-stratified analyses (Fig. 1). While these differences are modest, they are likely driven by the fact that the majority of variants and probes have negligible effects. Indeed, examination of the 90th percentile of effect sizes demonstrates substantial differences between the sex-combined and sex-stratified analyses. The larger effect sizes in the sex-stratified analyses result in identification of more asthma-related genes and lower sample size requirements for sufficient power. Previous studies have demonstrated that failure to account for interaction may increase sample size requirements by 3 fold [32]. Indeed, the utility of smaller cohorts with more rigorous phenotyping may be a superior approach to large population studies to identify significant genes with large effect sizes. It is striking that analyses that combined both sexes failed to identify about half of the sex-specific genetic factors in our study. Interestingly, SNPs and probes without evidence of sex interaction effects were identified significantly more often in the sex-combined analyses compared to the stratified analyses. This is likely due to the fact that these effect sizes were generally smaller and, thus, would require larger sample sizes.

Another potential reason that asthma-related SNPs and probes are missed in sex-combined analyses is that SNPs and probes that interact with sex may have opposite effects on asthma, thus cancelling each other out [33]. This is problematic because the majority of genomic studies do not implicitly model sex interaction effects [10,34,35]. Further, many two-step genome-wide approaches to test interaction assume main (marginal) effects [36,37]. As our study and others have shown, requiring main effects may limit success in interaction studies [19,38]. Failure to account for interaction effects results in a considerable portion of the variation remaining unexplained. For many complex traits, researchers have recognized that the amount of variation explained by genome-wide association studies is dramatically lower than the proportion of expected variation (termed missing heritability) [39]. It has been demonstrated that much of this missing heritability can be accounted for by untested interaction effects [40].

Unexpectedly, we observed an overall trend between SNPs and probes with larger effect sizes and failure to detect the variants and probes with interactive effects in the combined analyses; however, our findings were not consistent within genes (e.g. the SNPs that showed sex-specific effects and interaction were not in the same genes that showed interaction in the probes). There are three possible reasons for this. First, the SNPs selected are tagging SNPs and unlikely to directly influence protein structure. Indeed, most variants which have been identified from genome wide association studies for complex traits are regulatory [41,42]. Second, the SNP and probe data were derived from non-overlapping populations of children (one from Cincinnati, the other from a public database). Genetic and environmental heterogeneity between populations are likely to differentially impact

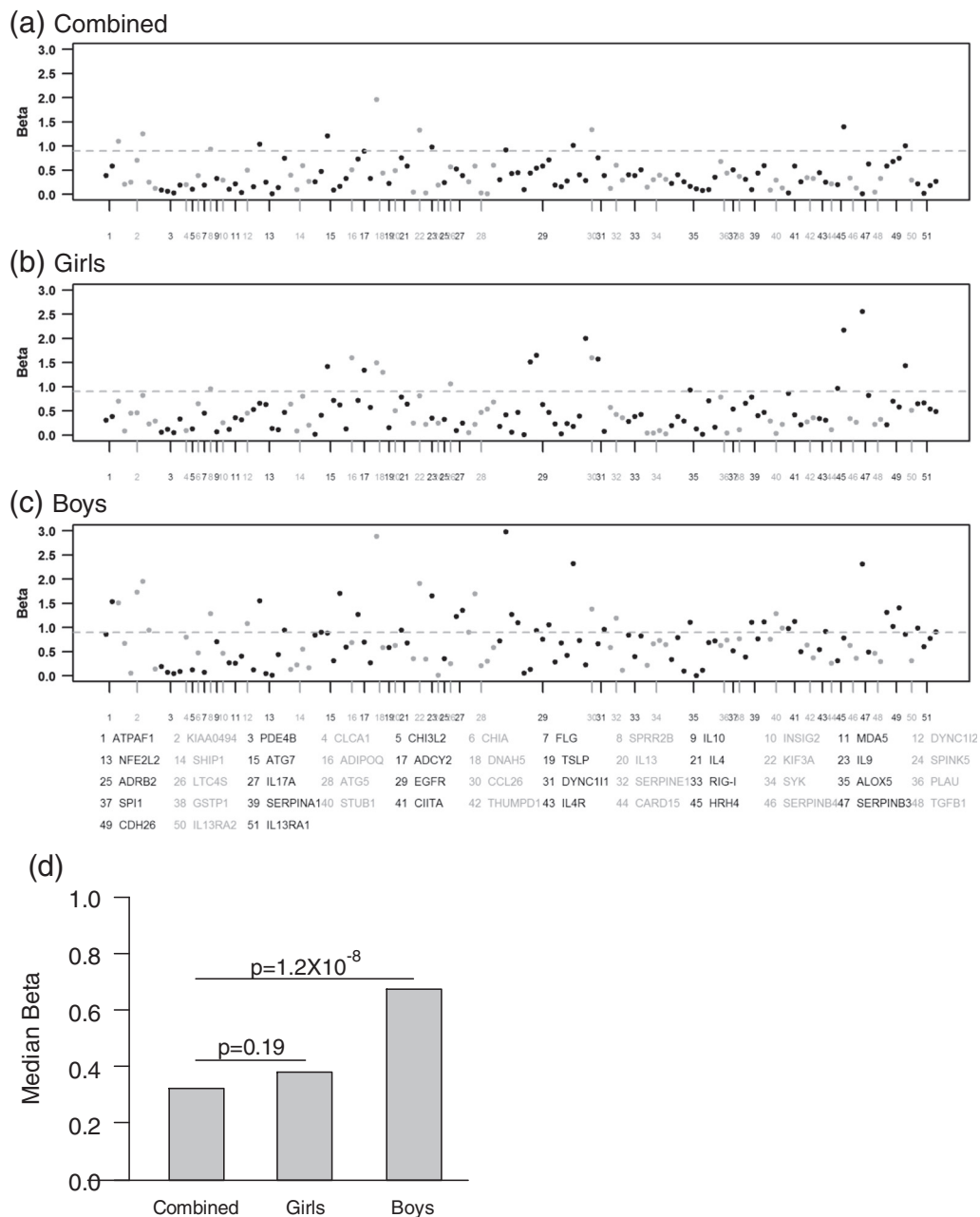


Fig. 4. Sex-combined and sex-stratified analyses identify unique asthma-associated expression profiles. Logistic regression was performed adjusting for sex, age and population stratification. Beta values are shown on the y-axis. The x-axis represents the candidate probe across the candidate genes studied. (a) Sex-combined results (boys and girls together). (b) and (c) Sex-stratified results (only girls and boys, respectively). The horizontal dotted line represents the level at which we are sufficiently powered to detect effect sizes greater than 0.90 in the sex-combined analysis. (d) The median odds ratios in both the sex-combined and sex-stratified analyses.

gene expression and the ability to identify overlap. Third, the sample sizes for our analyses were underpowered to detect small effects. Thus, it is possible that some overlap would have been identified in a larger population.

4. Conclusions

In conclusion, we have shown that the underlying genomic architecture of asthma differs in part by sex. Through the use of both SNP and gene expression data from populations of childhood asthmatics and non-asthmatics, we conclude that genomic variants with interaction are those most likely to be missed in sex-combined analyses. In addition, we demonstrated the presence of multiple sex interactions through the larger median effect sizes of the sex-specific analyses. The failure to account for sex interactions may in-part explain why

genome-wide association studies have had limited success in identifying variants associated with asthma and overlook genomic effects which may be useful in advancing personalized treatment regimens.

5. Materials and methods

5.1. Study population and study design

The study population included 411 European American asthmatic cases (185 girls and 226 boys) and 297 non-asthmatic, non-allergic control children (154 girls and 143 boys) enrolled in the Greater Cincinnati Pediatric Clinic Repository (GCPCR) or the Cincinnati Children's Hospital Medical Center (CCHMC) Cincinnati Genomic Control Cohort (GCC). All study participants were 4 to 17 years old. Asthmatic cases were GCPCR children with asthma diagnosed by a physician in CCHMC allergy or

pulmonary-based specialty clinics using the ATS criteria [43]. Children from either the GCPCR or the GCC were included as controls if they reported not having any personal or family history of asthma, and did not have physician diagnosed allergic rhinitis or atopic dermatitis (GCPCR) or report a personal history of any environmental allergies, hay fever or eczema (GCC). Written informed consent was obtained from all interested patients and their parents/guardians prior to participation. Recruitment criteria and cohort descriptions have been described elsewhere [24,44,45]. The study was approved by the CCHMC Institutional Review Board.

5.2. Candidate gene and SNP selection, and genotyping

Genomic DNA was isolated from either buccal swabs with either the Zymo Research Genomic DNA II Kit (Zymo Research Corp., Orange, CA) or the Purgene DNA Purification System (Gentra Systems Minneapolis, MN), or from Oragene saliva samples per the kit's instructions (GCPCR). For the GCC, genomic DNA was extracted from blood samples using Manual PerfectPure DNA Blood Kit (Invitrogen, Carlsbad, CA). A total of 738 SNPs and 30 ancestry-informative markers (AIMs) in 51 candidate genes were selected for inclusion on a custom Illumina GoldenGate™ assay. These candidate genes were chosen based on a high number of replications in the literature (>10 studies) and biologic relevance in the pathogenesis of asthma or allergy [46]. SNPs within a gene were selected in one of two ways. First, non-synonymous SNPs or SNPs in regulatory or coding regions of interest were selected. Second, tagging SNPs that captured the common genetic variation in a gene were selected using Haploview and Tagger (<http://www.broad.mit.edu/mpg/haploview>). Genotyping using the Illumina GoldenGate Assay (<http://www.illumina.com>) system was performed at the CCHMC Genetic Variation and Gene Discovery Core. Genotypes were assigned using Illumina's BeadStudio v3.2 Software (San Diego, CA).

5.3. GEO expression profiling data of childhood asthma

To examine whether gene expression profiles differed between boys and girls with and without asthma, we searched Gene Expression Omnibus (GEO) at <http://www.ncbi.nlm.nih.gov/geo> for studies related to childhood asthma. We analyzed data deposited under accession number GSE8052, which contained gene expression profiles from peripheral blood lymphocytes [47]. There were 136 probe sets on the Affymetrix GeneChip Human Genome U133 Plus 2.0 [HG-U133_Plus_2] microarray representing the 51 genes tested in our SNP analysis.

5.4. Statistical analysis

5.4.1. SNP analysis

Prior to analysis, SNPs which failed Hardy Weinberg Equilibrium (HWE) in the control dataset ($p < 0.0001$), had poor genotype calling (missing rate greater than 10%) or minor allele frequencies (MAF) below 10% were excluded. In addition, individuals with more than 20% missing genotypes were removed from the analysis. Principal component analyses were performed on the 30 AIMs using EIGENSTRAT to infer principal components and account for population stratification. The first four principal components (pcs) scores were included as covariates.

To test SNP associations with asthma, assuming additive genetic effects, logistic regression were performed using PLINK v1.07 [48]. Age and pcs were included as covariates. Three types of analysis were performed. First, we evaluated associations with asthma in the sex-combined cohort with sex included as a covariate to generate reference results. Then, we evaluated associations in sex-stratified analyses. Lastly, we performed sex by SNP interaction. To determine whether there was evidence of sex-specific variants, we first looked at the pattern of variants which reached nominal significance ($p \leq 0.05$), across the three analysis groupings. However, as statistical significance is a function of sample size, we also sought to understand how sex influenced

the magnitude of the effect, thus we also examined the odds ratios. Because the direction of effect for a MAF may differ, when comparing effect sizes across the groups the odds ratios for the risk variant were used. We then compared the median effect size across all 507 variants from the sex-combined and sex-stratified analysis using Wilcoxon rank sum. Additionally, to have further insight on the pattern of such effects the effect size was plotted similar to a Manhattan plot.

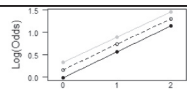
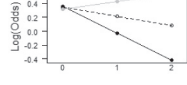
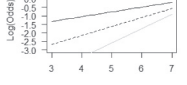
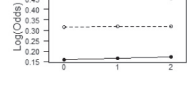
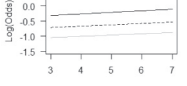
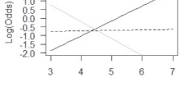
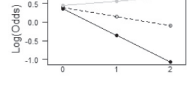
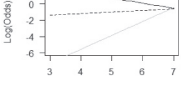
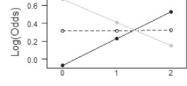
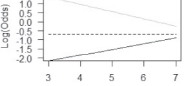
The challenge with using effect sizes rather than p-values is that there is no pre-specified threshold. Thus, we set our threshold for variants in which we have 80% power to detect at $\alpha = 0.05$ and MAF = 0.2 using the sex-combined study (OR = 1.44). Thus variants which surpassed this threshold should have been detected as significant in the sex-combined cohort if sex differences did not exist.

For those variants that were identified in stratified analysis but not in combined cohort, we adopted permutation tests to estimate the probability distribution of the statistic under the null hypothesis that sex differences did not exist. The purpose of the permutation test is to show that it is very unlikely that a permuted dataset will achieve the same significance and the observed effect is not due to chance. We permuted sex while keeping the case/control sample size fixed as in the observed data. From 10,000 permutations, we conducted sex-stratified and sex-combined analysis in each permutation. The empirical p value was defined as the probability of observing permuted statistics larger than the observed statistics.

To further explore the results from sex-stratified and sex interaction analyses, we attempted to classify SNPs/probes into six categories (Table 2) based on all possible combinations of stratified effects (neither sex, one sex or both sexes) and interaction effects (present, not present): 1) no sex by SNP interaction or sex-stratified association, 2) no sex by SNP interaction, but an association in a single sex, 3) no sex by

Table 2

Sex-stratified analyses identify more SNPs and probes associated with asthma than when the sexes are combined.

SNP (or probe) × sex interaction	SNP (or probe) effect	Common pattern of a Representative SNP ^a	Common pattern of a Representative probe ^b
No Interaction	Both sexes		N/A
	Single sex only		
	Neither sex		
Interaction	Both sexes	N/A	
	Single sex only		
	Neither sex		

^a Patterns of SNP*sex interaction and no SNP*sex interaction analysis. N/A refers no SNPs or Probes in this category. See Supplementary Table 1 for detailed listing of SNPs and p-values.

^b Patterns of probe*sex interaction and no-probe*sex interaction analysis. See Supplementary Table 2 for detailed listing of probes and p-values. The straight lines are regression lines (black line: girls; gray line: boys; dashed line: girls and boys combined).

SNP interaction, but association in both sexes, 4) a sex by SNP interaction, but no sex-stratified associations, 5) a sex by SNP interaction, with an asthma association in a single sex, and 6) a sex by SNP interaction with associations in both sexes.

5.5. Gene expression analysis

Logistic regression was performed using R. Three groupings were performed: sex-combined analysis, sex-stratified analysis and sex by probe interaction analyses. Sex was used as covariate in the combined analysis. We also compared the median effect size (beta) across all 136 probes from the sex-combined and stratified analysis using Wilcoxon rank sum. The effect sizes were plotted similar to the Manhattan plots.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ygeno.2015.03.003>.

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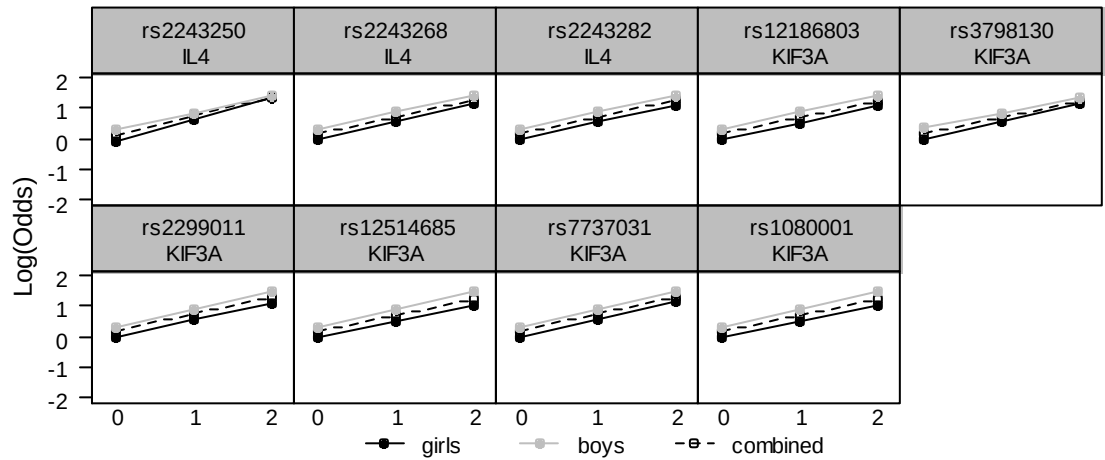
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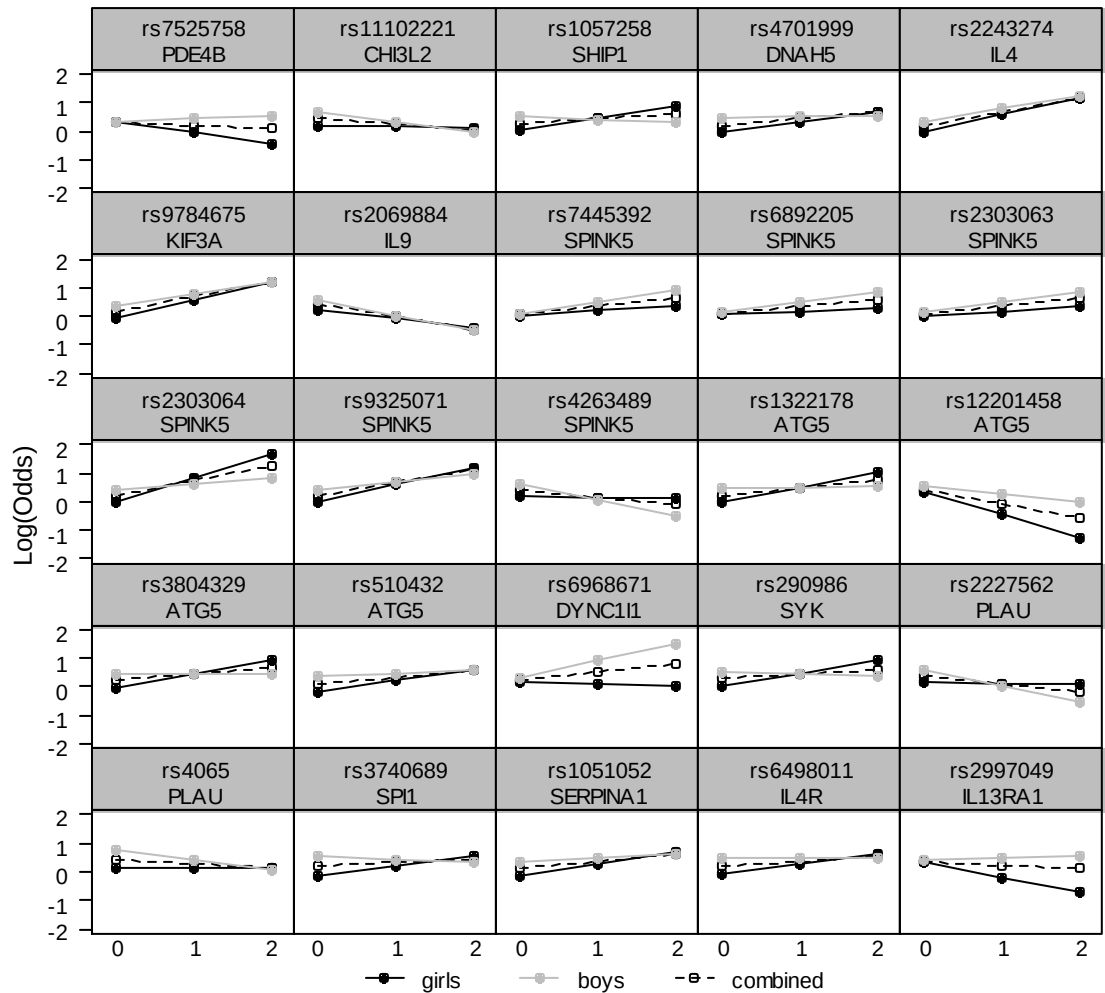
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Supplemental Figure 1: Exploring SNPs with significant effects in girls or boys or have significant gene-by-sex interaction

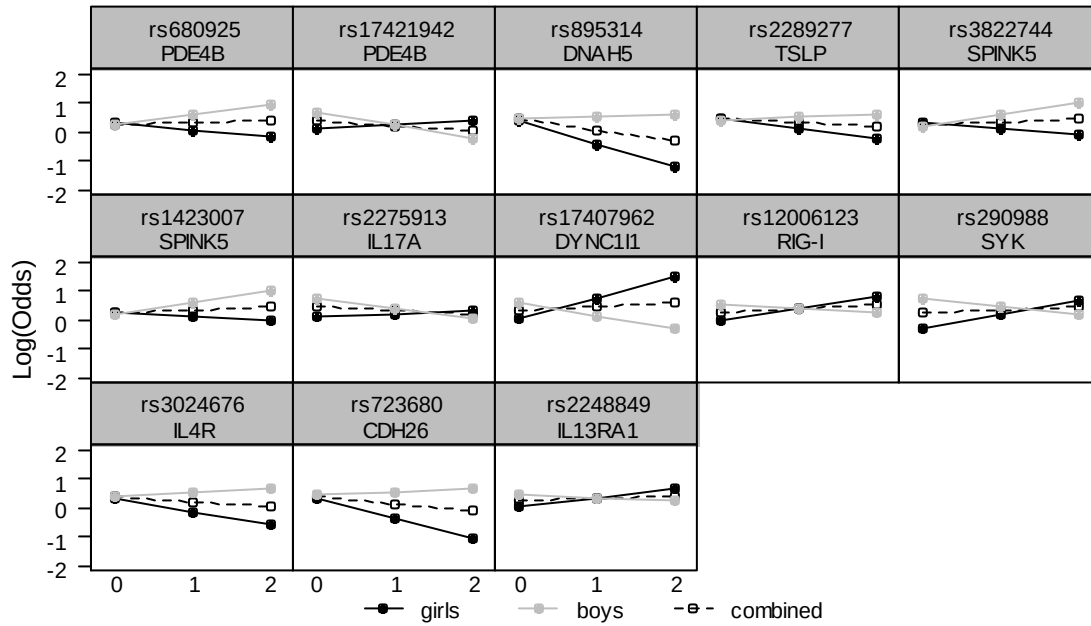
A. No SNP × Sex interaction, associations in both sexes (9 SNPs)



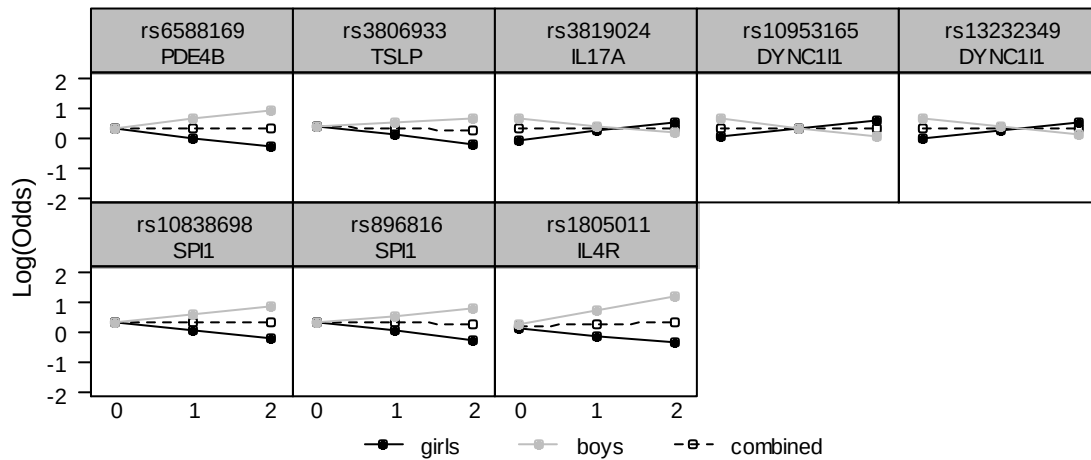
B. No SNP × Sex interaction, single sex association (25 SNPs)



C. SNP × Sex interaction, single sex association (13 SNPs):

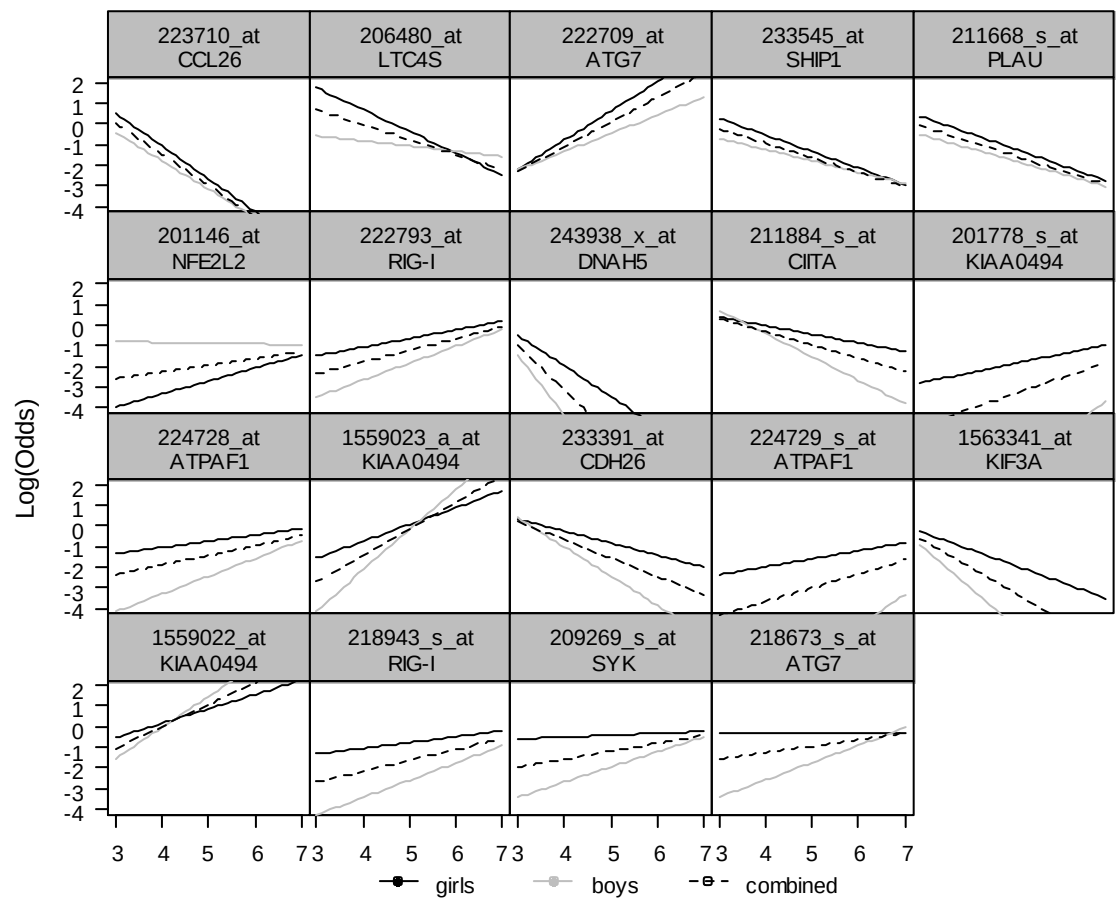


D. SNP × Sex interaction, no sex association (8 SNPs)

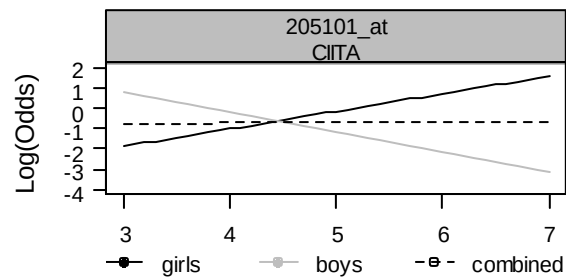


Supplemental Figure 2: Exploring Probes with significant effects in girls or boys or have significant sex-by-gene interaction

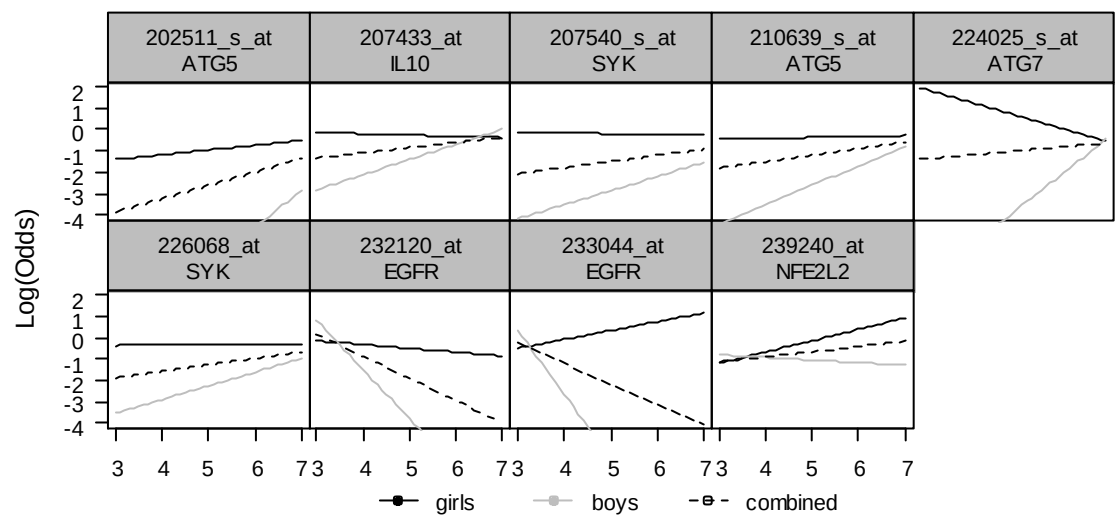
A. No Probe $\times$ Sex interaction, single sex association (19 probes)



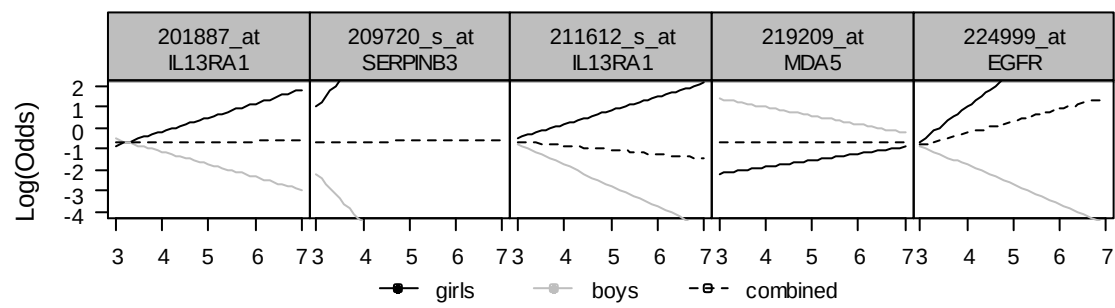
B. Probe $\times$ Sex interaction, associations in both sexes (1 probe)



C. Probe × Sex interaction, single sex association (9 probes):



D. Probe × Sex interaction, no sex association (5 probes)



Supplemental Table 1. Detailed Sex*SNP interactions

Pattern	#	Gene	SNP	CHR	Combined		Girls		Boys		SNP*Sex interaction
					OR	P-value	OR	P-value	OR	P-value	P-value
No Sex*SNP interaction, associations in both sexes	1	IL4	rs2243250	5	1.87	0.00016	2.04	0.0026	1.72	0.02	0.58
	2	IL4	rs2243268	5	1.78	0.00053	1.82	0.012	1.75	0.016	0.84
	3	IL4	rs2243282	5	1.75	0.00081	1.74	0.019	1.74	0.018	0.94
	4	KIF3A	rs12186803	5	1.72	0.0013	1.71	0.023	1.72	0.023	0.99
	5	KIF3A	rs3798130	5	1.72	0.0011	1.78	0.015	1.67	0.029	0.83
	6	KIF3A	rs2299011	5	1.77	0.00062	1.73	0.021	1.81	0.012	0.9
	7	KIF3A	rs12514685	5	1.72	0.0011	1.67	0.03	1.77	0.015	0.92
	8	KIF3A	rs7737031	5	1.77	0.00057	1.79	0.015	1.77	0.014	0.94
	9	KIF3A	rs1080001	5	1.72	0.0011	1.69	0.027	1.76	0.016	0.91
No Sex*SNP interaction, single sex association	1	KIF3A	rs9784675	5	1.68	0.0016	1.85	0.01	1.53	0.06	0.55
	2	IL4	rs2243274	5	1.66	0.0019	1.79	0.013	1.54	0.06	0.6
	3	SPINK5	rs2303064	5	1.7	0.003	2.36	0.001	1.23	0.42	0.09
	4	ATG5	rs12201458	6	0.6	0.0047	0.46	0.003	0.77	0.31	0.13
	5	SPINK5	rs9325071	5	1.58	0.009	1.81	0.013	1.33	0.26	0.41
	6	SPINK5	rs7445392	5	1.33	0.01	1.2	0.27	1.49	0.01	0.35
	7	SPINK5	rs2303063	5	1.32	0.014	1.23	0.23	1.43	0.023	0.49
	8	IL9	rs2069884	5	0.65	0.017	0.73	0.22	0.59	0.04	0.57
	9	SERPINA1	rs1051052	14	1.3	0.023	1.52	0.014	1.14	0.43	0.23
	10	ATG5	rs510432	6	1.25	0.048	1.45	0.026	1.13	0.43	0.21
	11	SPINK5	rs6892205	5	1.25	0.048	1.11	0.52	1.4	0.032	0.3
	12	ATG5	rs1322178	6	1.3	0.06	1.72	0.011	1.06	0.76	0.07
	13	DNAH5	rs4701999	5	1.24	0.08	1.42	0.045	1.06	0.73	0.27
	14	IL13RA1	rs2997049	23	0.73	0.09	0.6	0.029	1.08	0.81	0.11
	15	PLAU	rs2227562	10	0.77	0.09	0.98	0.91	0.57	0.015	0.14
	16	CHI3L2	rs11102221	1	0.82	0.12	0.98	0.91	0.69	0.035	0.28
	17	IL4R	rs6498011	16	1.2	0.14	1.43	0.035	1	0.98	0.14
	18	ATG5	rs3804329	6	1.23	0.15	1.65	0.02	0.99	0.98	0.06
	19	PLAU	rs4065	10	0.85	0.15	0.98	0.88	0.72	0.036	0.25
	20	DYNC1I1	rs6968671	7	1.29	0.18	0.94	0.83	1.77	0.037	0.11
	21	SPINK5	rs4263489	5	0.8	0.19	0.98	0.93	0.58	0.034	0.13
	22	SHIP1	rs1057258	2	1.18	0.27	1.54	0.044	0.91	0.68	0.11
	23	PDE4B	rs7525758	1	0.88	0.3	0.68	0.038	1.12	0.52	0.06
	24	SYK	rs290986	9	1.15	0.34	1.59	0.047	0.94	0.75	0.11
	25	SPI1	rs3740689	11	1.1	0.42	1.39	0.042	0.88	0.43	0.06
Sex*SNP interaction, single sex association	1	DNAH5	rs895314	5	0.69	0.023	0.44	0.0006	1.05	0.84	0.01
	2	CDH26	rs723680	20	0.79	0.15	0.49	0.004	1.13	0.59	0.017
	3	PDE4B	rs17421942	1	0.83	0.21	1.15	0.52	0.65	0.028	0.034
	4	IL4R	rs3024676	16	0.84	0.23	0.64	0.027	1.12	0.59	0.049
	5	IL17A	rs2275913	6	0.87	0.25	1.14	0.45	0.69	0.029	0.041
	6	RIG-I	rs12006123	9	1.16	0.28	1.49	0.042	0.87	0.48	0.046
	7	SPINK5	rs1423007	5	1.13	0.32	0.85	0.35	1.52	0.016	0.022
	8	TSLP	rs2289277	5	0.9	0.33	0.72	0.037	1.12	0.48	0.041
	9	SYK	rs290988	9	1.11	0.39	1.63	0.004	0.76	0.1	0.001
	10	DYNC1I1	rs17407962	7	1.15	0.45	2.06	0.008	0.66	0.11	0.002
	11	SPINK5	rs3822744	5	1.09	0.45	0.83	0.27	1.47	0.024	0.02
	12	PDE4B	rs680925	1	1.08	0.55	0.79	0.18	1.43	0.038	0.018
	13	IL13RA1	rs2248849	23	0.96	0.83	1.38	0.19	0.27	0.01	0.004
Sex*SNP interaction, no sex association	1	PDE4B	rs6588169	1	1	0.99	0.75	0.15	1.35	0.12	0.038
	2	TSLP	rs3806933	5	0.93	0.53	0.74	0.06	1.17	0.35	0.043
	3	IL17A	rs3819024	6	1	0.98	1.35	0.09	0.77	0.13	0.027
	4	DYNC1I1	rs10953165	7	0.98	0.88	1.32	0.15	0.75	0.14	0.037
	5	DYNC1I1	rs13232349	7	1	0.97	1.28	0.16	0.77	0.14	0.039
	6	SPI1	rs10838698	11	1.01	0.95	0.77	0.14	1.32	0.11	0.047
	7	SPI1	rs896816	11	0.97	0.81	0.73	0.07	1.28	0.16	0.04
	8	IL4R	rs1805011	16	1.07	0.71	0.8	0.33	1.59	0.07	0.046

*Numbers in bold are those P-values less than 0.05

Supplemental Table 2. Detailed Sex*Probe interactions

Pattern	#	Gene	Probe ID	Combined		Girls		Boys		probe*Sex interaction
				Beta	P-value	Beta	P-value	Beta	P-value	P-value
No Sex*SNP interaction, single sex association	1	CCL26	223710_at	-1.34	0.004	-1.59	0.008	-1.38	0.07	0.82
	2	LTC4S	206480_at	-0.56	0.11	-1.05	0.029	-0.25	0.64	0.27
	3	ATG7	222709_at	1.20	0.019	1.42	0.039	0.88	0.27	0.61
	4	SHIP1	233545_at	-0.59	0.047	-0.8	0.043	-0.55	0.26	0.68
	5	PLAU	211668_s_at	-0.67	0.019	-0.78	0.047	-0.62	0.15	0.79
	6	NFE2L2	201146_at	0.25	0.25	0.63	0.049	-0.04	0.9	0.13
	7	RIG-I	222793_at	0.51	0.007	0.42	0.07	0.82	0.011	0.31
	8	DNAH5	243938_x_at	-1.96	0.011	-1.49	0.17	-2.88	0.015	0.39
	9	CIITA	211884_s_at	-0.58	0.024	-0.42	0.17	-1.12	0.019	0.21
	10	KIAA0494	201778_s_at	0.70	0.034	0.46	0.19	1.73	0.028	0.14
	11	ATPAF1	224728_at	0.39	0.06	0.3	0.21	0.86	0.039	0.25
	12	KIAA0494	1559023_a_at	1.25	0.015	0.82	0.23	1.95	0.017	0.28
	13	CDH26	233391_at	-0.74	0.049	-0.58	0.23	-1.41	0.034	0.31
	14	ATPAF1	224729_s_at	0.58	0.047	0.38	0.24	1.53	0.025	0.13
	15	KIF3A	1563341_at	-1.33	0.023	-0.81	0.29	-1.91	0.037	0.35
	16	KIAA0494	1559022_at	1.09	0.027	0.7	0.3	1.5	0.042	0.42
	17	RIG-I	218943_s_at	0.40	0.05	0.28	0.33	0.84	0.015	0.21
	18	SYK	209269_s_at	0.39	0.014	0.09	0.67	0.73	0.0025	0.05
	19	ATG7	218673_s_at	0.25	0.21	0.01	0.96	0.84	0.023	0.07
Sex*SNP interaction, associations in both sexes	1	CIITA	205101_at	-0.03	0.91	0.86	0.021	-0.98	0.017	0.001
Sex*SNP interaction, single sex association	1	ATG5	202511_s_at	0.58	0.018	0.22	0.41	1.69	0.001	0.012
	2	IL10	207433_at	0.33	0.06	-0.06	0.79	0.7	0.013	0.035
	3	SYK	207540_s_at	0.30	0.07	-0.04	0.87	0.66	0.01	0.043
	4	ATG5	210639_s_at	0.26	0.13	0.04	0.83	0.9	0.01	0.033
	5	ATG7	224025_s_at	0.16	0.59	-0.62	0.11	1.7	0.003	0.001
	6	SYK	226068_at	0.31	0.028	0.02	0.92	0.64	0.003	0.032
	7	EGFR	232120_at	-1.01	0.013	-0.18	0.73	-2.32	0.0015	0.017
	8	EGFR	233044_at	-0.91	0.16	0.41	0.63	-2.98	0.0081	0.017
	9	NFE2L2	239240_at	0.15	0.32	0.53	0.015	-0.12	0.61	0.042
Sex*SNP interaction, no sex association	1	IL13RA1	201887_at	-0.02	0.93	0.66	0.09	-0.6	0.13	0.023
	2	SERPINB3	209720_s_at	-0.01	0.99	2.56	0.08	-2.31	0.15	0.025
	3	IL13RA1	211612_s_at	-0.21	0.58	0.65	0.27	-0.99	0.09	0.049
	4	MDA5	219209_at	-0.04	0.79	0.32	0.13	-0.4	0.08	0.021
	5	EGFR	224999_at	0.54	0.37	1.64	0.06	-0.94	0.34	0.049

*Numbers in bold are those P-values less than 0.05