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Zinc Finger Protein 33B Demonstrates Sex-interaction with Atopy-related Markers in Childhood Asthma

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Abstract

Background: Sex differences related to immune responses can influence atopic manifestations in childhood asthma. While genome-wide association studies have investigated a sex-specific genetic architecture of the immune response, gene-by-sex interactions have not extensively been analyzed for atopy-related markers including allergy skin tests, immunoglobulin E, and eosinophils in child asthmatics.

Methods: We performed a genome-wide gene-by-sex interaction analysis for atopy-related markers using whole genome sequencing data based on 889 trios from the Genetic Epidemiology of Asthma in Costa Rica Study (GACRS) and 284 trios from the Childhood Asthma Management Program (CAMP). We also tested the findings in UK Biobank participants with self-reported

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Conflicts of interest statement:

Dr. Weiss reports personal fees from UpToDate, outside the submitted work; Dr. Cho reports grants from NIH, during the conduct of the study; grants from Bayer, grants from GSK, personal fees from AstraZeneca, personal fees from Illumina, personal fees from Genentech, outside the submitted work; Dr. Celedón has received research materials from GSK and Merck (inhaled steroids) and Pharmavite (vitamin D and placebo capsules) in order to provide medications free of cost to participants in NIH-funded studies, unrelated to this manuscript. Dr. DeMeo has received grants from NIH, honoraria from Novartis and grant support from Bayer, outside the submitted work.

All other authors declare that they have no potential conflicts of interest related to this work.

childhood asthma. Furthermore, downstream analyses in GACRS integrated gene expression to disentangle observed associations.

Results: SNP rs1255383 at 10q11.21 demonstrated a genome-wide significant gene-by-sex interaction ($P_{interaction} = 9.08\times10^{-10}$) for atopy (positive skin test) with opposite direction of effects between females and males. In the UK Biobank participants with a history of childhood asthma, the signal was consistently observed with the same sex-specific effect directions for high eosinophil count ($P_{interaction} = 0.0058$). Gene expression of *ZNF33B* (zinc finger protein 33B), located at 10q11.21, was moderately associated with atopy in girls, but not in boys.

Conclusions: We report SNPs in/near a zinc finger gene as novel sex-differential loci for atopy-related markers with opposite effect directions in females and males. A potential role for *ZNF33B* should be studied further as an important driver of sex-divergent features of atopy in childhood asthma.

Tweetable Abstract

We discovered a locus demonstrating a sex-differential association with atopy in childhood asthma, supporting further investigation of *ZNF33B* as a potential gene for the sex-divergent features of atopy.

Keywords

Atopic asthma; Childhood asthma; Gene-sex interaction; Family-based genome wide association study

Introduction

Atopic asthma is the most common subtype of childhood asthma and demonstrates a higher prevalence in pre-pubertal boys compared to pre-pubertal girls [1, 2]. Atopic asthma is characterized by eosinophilic airway inflammation, sensitization to environmental allergens, and an increased total immunoglobulin E (IgE) [1]. Sex differences are also known to influence innate and adaptive immune responses; for example, antibody responses to vaccination and basal levels of immunoglobulins are higher in females than males [3, 4]. Boys have a higher risk of asthma during early childhood, but also demonstrate increased rates of atopy, higher total IgE, and higher peripheral blood eosinophil counts [5–7]. After puberty, the development and control of both atopic and asthma syndromes switch in males and female.

Sex divergent features of asthma have long been recognized, highlighting the importance of investigating sex-associated genetic architecture for asthma susceptibility and severity [8, 9]. However, gene-by-environment interaction studies have mostly focused on exogenous environmental exposures such as tobacco smoke, allergens, and air pollutants rather than endogenous environments such as those related to sex and sex differences [10]. Therefore, more sex-stratified or interaction analyses are necessary to gain biological insights into sex differences in human traits. Recently, sex and immune system pathways have been shown to modulate asthma risk regardless of age of onset [11]. Genome-wide association studies (GWASs) have demonstrated that loci such as *IRF1*, *IFNγ*, and *TSLP* are associated with

sex-specific asthma risk or atopic sensitization supporting sex as a major contributor to the immune response [9, 11–15]. However, gene-by-sex interactions have not extensively been analyzed for atopy-related markers including allergy skin tests, IgE, and eosinophils in childhood asthma.

Here, we conducted a family-based GWAS of gene-by-sex interactions for atopy (allergy skin tests) in two cohorts of child asthmatics which will reveal new pathways for functional evaluation.

Methods

Study populations

The Genetic Epidemiology of Asthma in Costa Rica Study (GACRS) and the Childhood Asthma Management Program (CAMP)—The probands were from two family-based studies of childhood asthma, GACRS and CAMP. Subject recruitment and protocols for these studies have been previously described in detail [12, 16]. In brief, children in GACRS were aged 6 to 14 years and children in CAMP were aged 5 to 12 years old at enrollment who had asthma diagnosed by a physician. Both GACRS and CAMP used a family-based design focused on trios and similar protocols for phenotyping subjects [17]. Study protocols were also approved by local Institutional Review Boards (IRBs) at each recruitment site for each study, and by the IRB of Brigham and Women's Hospital.

UK Biobank—To assess the potential generalizability of our findings, significant gene-by-sex interactions in the GACRS/CAMP analysis were also tested in a subset of adults from the UK Biobank reporting childhood-onset asthma (<16 years). Subject recruitment and the study protocol for the UK Biobank have been previously described in detail [18]. Among the unrelated individuals with White British ancestry (N= 337,536) with available PC data and eosinophil counts (field 30150), we extracted data for 9,701 subjects with reported childhood asthma based on field 3786 (age at asthma onset first diagnosed on a touchscreen questionnaire) and excluded subjects with self-reported onset age difference over 10 years based on the comparison with field 22147 (the age at which asthma was first diagnosed by a doctor). For comparison, a control group without any asthma diagnosis was selected based on the fields including 3786, 22147, 6152 ("Has a doctor ever told you that you have had any of the following conditions?" asked on a touchscreen questionnaire), and 20002 (verbal interview) (N= 295,918).

Atopy-related markers

Allergy skin testing for atopy diagnosis—Allergy skin testing in GACRS and CAMP was performed according to the protocol of the International Study of Asthma and Allergies in Childhood [19]. Along with histamine and saline controls, the following antigens from ALK-Abelló (Round Rock, TX) were applied with lancets to the volar surface of the forearm: *Dermatophagoides pteronyssinus*, *D. farinae*, *Periplaneta americana*, *Blatella germanica*, mixed grass pollen, mixed tree pollen, cat hair, dog hair, and *Alternaria tenuis*. A skin test was considered positive if the maximum wheal diameter exceeded the diluent wheal

diameter by ≥3mm. Atopy was then defined as positive skin test reactivity (STR) to at least one allergen.

Serum total IgE and peripheral blood eosinophil count as atopy markers— Serum total IgE levels and peripheral blood eosinophil count as intermediate phenotypes of atopy were measured by the UniCAP 250 system (Pharmacia & Upjohn, Kalamazoo, Mich) and Coulter counter (Beckman Coulter, Fullerton, Calif) techniques in both cohorts respectively.

Elevated IgE and/or peripheral blood eosinophilia are useful biomarkers of allergic asthma in the setting of atopy [20]. Therefore, we used them as dichotomous variables with a cutoff of 100 IU/mL for IgE and 500 cells/mm^3 for eosinophil counts in the gene-by-sex interaction test given improved power for dichotomous traits in highly ascertained populations [21]. For the UK Biobank subjects, eosinophil counts were also dichotomized for analysis.

Whole genome sequencing (WGS) data and Transcriptomic Profiling in Blood

WGS data for GACRS and CAMP were generated as part of the National Heart, Lung and Blood Institute (NHLBI) Trans-Omics for Precision Medicine (TOPMed) Program (TOPMed Acc. number: phs000988 & phs001726). Details were described in the supplementary material or the previous report [22]. Whole-blood gene expression profiles in GACRS were generated using the HumanHT-12 v4 Expression BeadChip (Illumina, Inc., San Diego, CA, USA). All probes underwent quality control (QC) metrics as previously described in detail [23].

Statistical analysis

Demographic characteristics and clinical features between groups were compared using t-tests or Chi-square tests, as appropriate, using the R statistical software (version 4.03). We performed a genome-wide association analysis of STR in the merged GACRS and CAMP dataset using the family-based association tests (FBAT) software (version 2.04). We then performed a genome-wide sex-interaction analysis of STR using a robust family-based gene-by-environment interaction test (fbatge in the fbati R package) [24]. This test is performed in the set of affected offspring, here corresponding to a positive STR. The genome-wide significance threshold was set to $p = 5\times10^{-8}$. To check that candidate regions do not show a sequence homology with the sex chromosomes, we performed a local alignment (BLAST) for the candidate loci using the BLAT tool from Ensembl Genome Browser [25]. We used a flanking sequence centered around the variant of interest. The total query length was 201 base pairs, 100 base pairs from each side of the selected variant.

Since 84.65% of the children with asthma in the GACRS and CAMP were atopic (positive STR), it is an important question if we observe a sex-differential association with atopy or asthma. Therefore, to distinguish a gene-by-sex interaction for asthma or atopy, we investigated the transmission-pattern in specific subgroups (atopic vs. non-atopic and female vs. male). If the effect directions in males and females both flip between the positive STR and negative STR subset, the data suggests a sex-differential association with atopy and not

asthma. For SNPs with genome-wide significant gene-by-sex interactions for atopy, we also performed the fbatge test in offspring with other atopy markers: elevated IgE and elevated eosinophil count, respectively.

Significant gene-by-sex interactions were also tested in the UK Biobank. As described above, this analysis was performed in 1) a set of adults with reported childhood asthma history and 2) a control set with no reported asthma of any kind. The analysis incorporated elevated eosinophil count as a binary phenotype and was based on logistic regression, adjusting for SNP, sex, sex-SNP interaction, age, genotyping array, and 10 principal components (PCs).

We explored expression quantitative trait loci (eQTL) for the identified SNPs in whole-blood gene expression data based on GTEx release version 8 [26]. In the GACRS cohort, whole-blood RNA gene expression profiling was available for 242 children with asthma (male:female ratio of 1:0.692) The gene expression for samples from males or females was compared after adjusting for age and the first two PCs to evaluate sex-differential expression for atopy-related markers.

Results

Descriptive characteristics of children with asthma in GACRS and CAMP

For both datasets (N=1,173 probands), the characteristics and the prevalence of risk factors by sex are presented in Table 1. There were no differences between males and females with respect to age, age of asthma onset, self-reported ethnicity, body mass index, and disease severity related variables such as hospitalization and emergency room visit for asthma (all $P>0.05$).

The prevalence of atopy based on the STR did not differ by sex (male vs. female: 85.17% vs. 83.84%, $P=0.552$), though sensitivity to *A. tenuis* and mixed grass pollen were higher in males compared to females. Total serum IgE and the proportion of subjects with an elevated IgE were higher in males but eosinophil count was not different between the sexes (Table 1). There was a moderate correlation between total serum IgE and eosinophil count in the combined cohorts (r=0.298, $P<0.001$). Atopy was also correlated with high IgE and high eosinophil count ($\varphi=0.469$ and $\varphi=0.291$, respectively) where 831 subjects had high IgE and 519 subjects had high eosinophil count among the atopic children (N=993).

Genome-wide FBAT and gene-by-sex interaction analyses for atopy

Genome-wide FBAT results in the merged GACRS and CAMP dataset revealed no genome-wide significant loci for atopy (Supplementary Figure 1). However, gene-by-sex interaction analysis showed genome-wide significant findings which are displayed in quantile–quantile and Manhattan plots (Figure 1A, 1B). The top associations included a zinc finger protein region on chromosome 10q11.21 including SNP rs1255383 (nearest gene, *ZNF37BP*; zinc finger protein 37B, pseudogene, $P_{interaction}=9.08\times10^{-10}$), SNP rs2483695 and SNP rs2484297 (*ZNF33B*; zinc finger protein 33B, $P_{interaction}=2.07\times10^{-9}$, both), and SNP rs2484304 (*LINC02632*, $P_{interaction}=3.85\times10^{-9}$) as visualized in Figure 1C. The SNPs are in strong linkage disequilibrium (LD, $R^2\geq0.929$, Supplementary Table 1) and very high

reference LD based on 1000 Genomes Project CEU samples (R^2=0.974, Supplementary Table 2). The interaction signal was observed in both cohorts ($P_{interaction\text{-}GACRS}=7.18\times10^{-6}$ & $P_{interaction\text{-}CAMP}=5.43\times10^{-4}$, respectively, Table 2). Furthermore, we confirmed that the association signals around our top region were not affected by mismapped reads from the X or Y chromosome.

As pubertal transitions may impact asthma and atopy, we separated all subjects into presumed prepubertal or postpubertal children by parsing at age 11. We used this threshold because the sex-divergence in airway responsiveness was previously reported at age 11, the mean age for Tanner stage 2 in CAMP cohort [27]. In the prepubertal group (N=921 and male= 61.02%), the significant gene-by-sex interaction was still observed ($P_{interaction}=9.63\times10^{-4}$) while the postpubertal group (N=252 and male= 60.71%) had a *P* value of 0.0123. Also, effect direction wasthe same in both groups. Therefore, the sex-interaction was not likely driven by different proportions of pubescent children amongst boys vs. girls and there is no clear puberty effect using our previously defined age cutoff in CAMP.

FBAT subgroup analysis for SNP rs1255383

The atopy subgroup analyses (atopy vs. no atopy) in the merged GACRS and CAMP trio data show no significant transmissions (P=0.711 and P=0.370 respectively, Table 3), reflecting the non-significant result in the atopy main effect analysis. However, subgroup analyses stratified by sex and atopy status reveal an opposite transmission behavior. We observe a significant over-transmission of the minor allele (cytosine) in males with atopy and a significant under-transmission in females with atopy ($P=4.83\times10^{-4}$ and $P=3.82\times10^{-7}$, respectively). This is in line with the highly significant fbatge result. Furthermore, the subgroup with no atopy shows the opposite transmission behavior compared to the atopy subgroup: under-transmission in males and over-transmission in females (P=0.027 and P=0.127, respectively, Table 3). This extends the observed sex-specific association to the subgroup with no atopy and demonstrates that the gene-by-sex interaction is related to atopy and not asthma affection status, since all offspring in our analysis, with atopy or no atopy, are asthmatics. A logistic regression analysis of SNP rs1255383 with atopy as the outcome, also confirmed that cytosine is risk decreasing in females and risk increasing in males (Supplementary Table 3 and Figure 2A).

Analysis of SNP rs1255383 with STR for specific allergens and atopy markers

We examined the sex-specific FBAT association for high IgE, high eosinophil count, and specific allergens (Table 2). For all specific allergens, we observed significant interactions between rs1255383 and sex. Moreover, both high eosinophil count and high IgE as atopy markers reproduce a significant sex-specific association for SNP rs1255383 ($P_{interaction}=6.20\times10^{-7}$ and $P_{interaction}=1.37\times10^{-7}$ respectively, Table 2). As quantitative analysis, log10 transformed eosinophil count or IgE showed lesser significance ($P_{interaction}=7.18\times10^{-3}$ and $P_{interaction}=0.0267$, respectively).

The sex-differential association between SNP rs1255383 and elevated eosinophil count was also observed with the same direction of the estimated effect in the UK Biobank

participants with childhood asthma, demonstrating decreased risk in females and increased risk in males (sex interaction with male reference; OR=0.670, P=0.0058). This interaction was not significant in the control group without any history of asthma (OR=1.037, P=0.419). We also confirmed that the gene-by-sex interaction for asthma affection status was not significant ($P_{interaction}$=0.899) in the UK Biobank. The demographic characteristics in cases (N=9,701) and controls (N=295,918) from the UK Biobank are presented in detail in Supplementary Table 4.

The eQTL analysis and sex-specific differential expression for atopy-related markers

Our top SNPs in strong LD are associated with increased expression of *ZNF33B* in whole blood RNA in the GTEx database (Supplementary Table 2). However, the eQTL associations were not reported to be sex-specific [28]. In GACRS samples with whole-blood RNA gene expression available, we did not observe a significant eQTL association between our top variant and *ZNF33B* gene expression (P=0.176) nor a sex-specific eQTL association, potentially due to small sample sizes (N=242). However, *ZNF33B* expression is suggested to be higher in samples with the CC genotype for SNP rs1255383 (Figure 2B). Decreased *ZNF33B* expression in females was moderately associated with atopy, while it was not observed in males (P=0.0775 vs. 0.760, respectively, Figure 2C). These data suggest that the novel sex-divergent locus associated with atopy in childhood asthma might involve *ZNF33B* (alias *ZNF11B*, *KOX31*, and *KOX2*).

Discussion

In this study, we discovered novel sex-divergent associations with atopy in children with asthma on 10q11.21, including rs1255383, rs2483695, rs2484297, and rs2484304 in/near the *ZNF33B* gene. Zinc-finger proteins (ZNFs), representing nearly half of all transcription factors, are implicated in numerous cellular processes (e.g. proliferation, differentiation, and apoptosis) through transcriptional regulation, ubiquitin-mediated protein degradation, signal transduction, actin targeting, DNA repair, and cell migration [29]. Most of the ZNFs in the Cys2His2 family are key transcriptional regulators in immune cell development and function but the dysregulation of zinc ion levels and ZNFs in the skin also plays an important role in antigen-specific immune reactions [30]. ZNFs in mouse influence allergen-induced airway inflammation and airway hyper-responsiveness by Th2 cell differentiation and cytokine expression, suggesting that intracellular zinc homeostasis by ZNFs is an important modulator of adaptive immune responses [31, 32]. Prior clinical studies have also demonstrated that ZNFs are associated with bronchodilator response and lung function in children [33, 34]. In detail, SNP rs3752120 in the *ZNF432* gene was involved in the bronchodilator response which was modified with inhaled steroids in the CAMP cohort [33]. Another ZNF gene region including *ZNF579*, *ZNF865*, and *FIZ1* in 19q13.42 was not associated with asthma but lung function in Peruvian children [34]. Furthermore, SNP rs4802207 in 19q13.31 (*ZNF224*, *ZNF225*, and *ZNF284*) was reported as a risk locus for childhood asthma in the population of Hispanic descent [35].

The identified locus on 10q11.21 includes a previously reported cluster of expressed *ZNF* genes [36, 37], but this region has not been reported for asthma or sex-interactions in

previous asthma studies. Recently, down-regulation of *ZNF33B* was also implicated in leukocyte differentiation, and T cell activation and regulation [38]. Our observation of sex-specific associations in 10q11.21 for atopy and relatively reduced expression of *ZNF33B* in females with atopy suggests a role of *ZNF33B* in autoimmune response but functional follow-up is required.

The strengths of this analysis include the two well-phenotyped childhood asthma studies, CAMP and GACRS, that have WGS data for admixed population. As the genetic studies of CAMP and GACRS are family-based, the impact of substructure is mitigated. Also, the ascertainment of both cohorts on the basis of asthma was very stringent, suggesting that misclassification of atopy in childhood asthma, while possible, is highly unlikely. Our study has several limitations. First, the significant gene-by-sex interaction of SNP rs1255383 was not replicated with the exact same phenotype in the UK Biobank. We used the population-based cohort with deeply phenotyped adults and high eosinophil counts as an atopy marker. As UK Biobank and GACRS/CAMP were based on different study designs, i.e. unrelated case-control and trios, respectively, the analytical methods here are different. The potential confounding factor such as population stratification in data from the UK Biobank is much harder to control, compared to the robust family studies. Despite these limitations, our sex-specific association finding in GACRS/CAMP was also observed in the UK Biobank subjects with self-reported childhood asthma but not in those without childhood asthma. Second, X/Y chromosome genes were not analyzed but autosomal genes have been demonstrated to have sex-specific phenotypic effects in humans [39]. Third, we did not identify a significant sex-specific eQTL association for *ZNF33B* expression, neither in CRA nor in GTEx. Potential explanations include the small sample size in CRA, the partly different data basis in GTEx that includes healthy adults, or that the sex-specific mechanism occurs downstream from gene expression on the protein level. Also, a candidate causal gene in the locus on 10q11.21 should be comprehensively considered with cell-type-specific effects in immune cells related atopic asthma. Last, rare variant based analysis in WGS was not performed due to sample size and the need to extend the FBAT framework to implement set-based interaction tests that can be used for gene-by-sex interaction analyses with rare variants.

In conclusion, our study identified genetic variations on 10q11.21 demonstrating sex-specific associations with atopy among asthmatic children of different ethnicities, supporting further investigation of *ZNF33B* as a potential driver of sex-divergent features in childhood atopic asthma. Larger studies addressing the genetic and genomic bases for sex-specific architecture in immune responses are needed for a more detailed understanding of sex differences in childhood asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement

Our summary statistics will be available through accession phs001726 (NHLBI TOPMed Childhood Asthma Management Program) in dbGaP. This deposition is in process.

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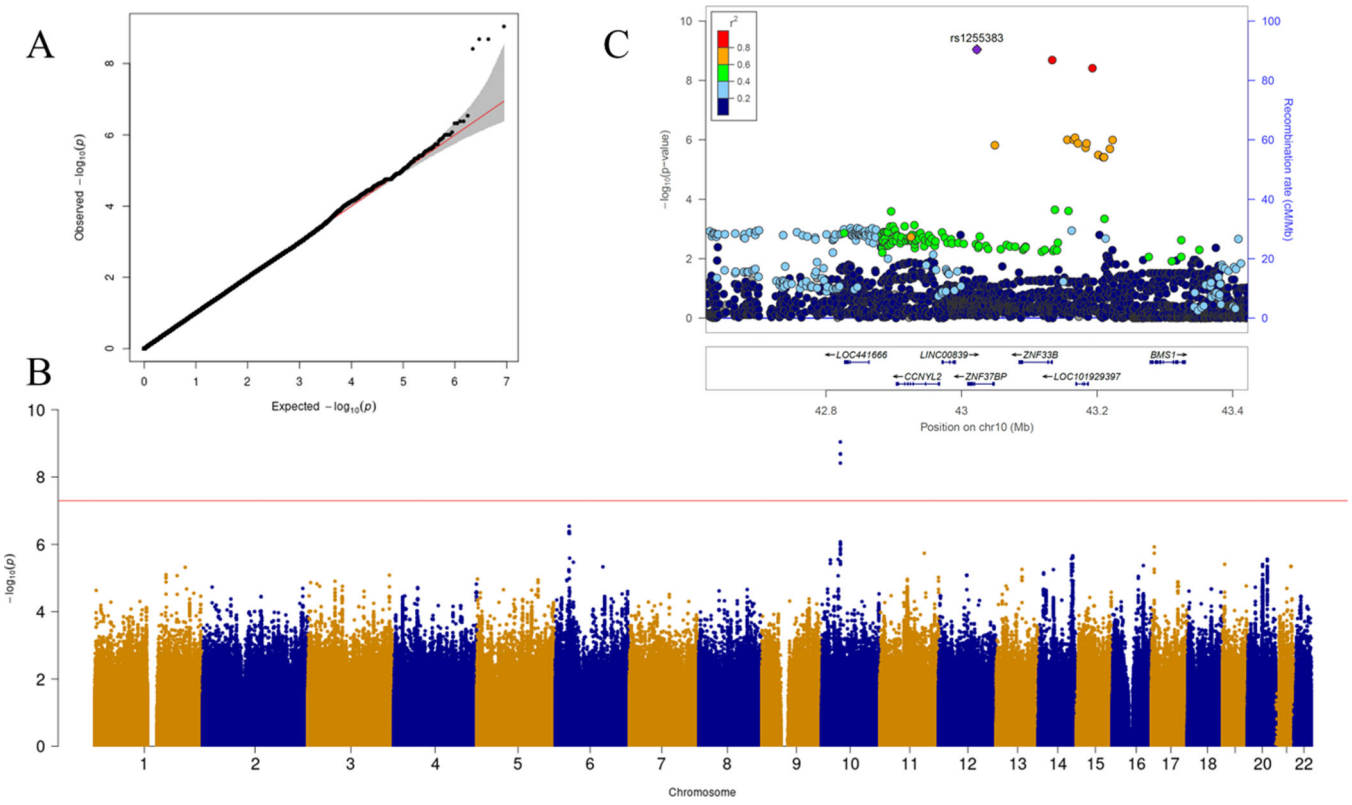

Figure 1.

Quantile-quantile plot (A) and Manhattan plot (B) for the genome-wide fbatge test results for atopy. The red line corresponds to a genome-wide significant p value of 5×10^{-8}. Regional plot (C) for the sex-specific atopy associated locus in 400 kb upstream and downstream regions. Purple diamond represents the top-ranked SNP, rs1255383 (chromosome 10) and other SNPs are colored according to their R^2 value in relation to that SNP. LD information was estimated using the 1000 Genomes Project EUR datasets.

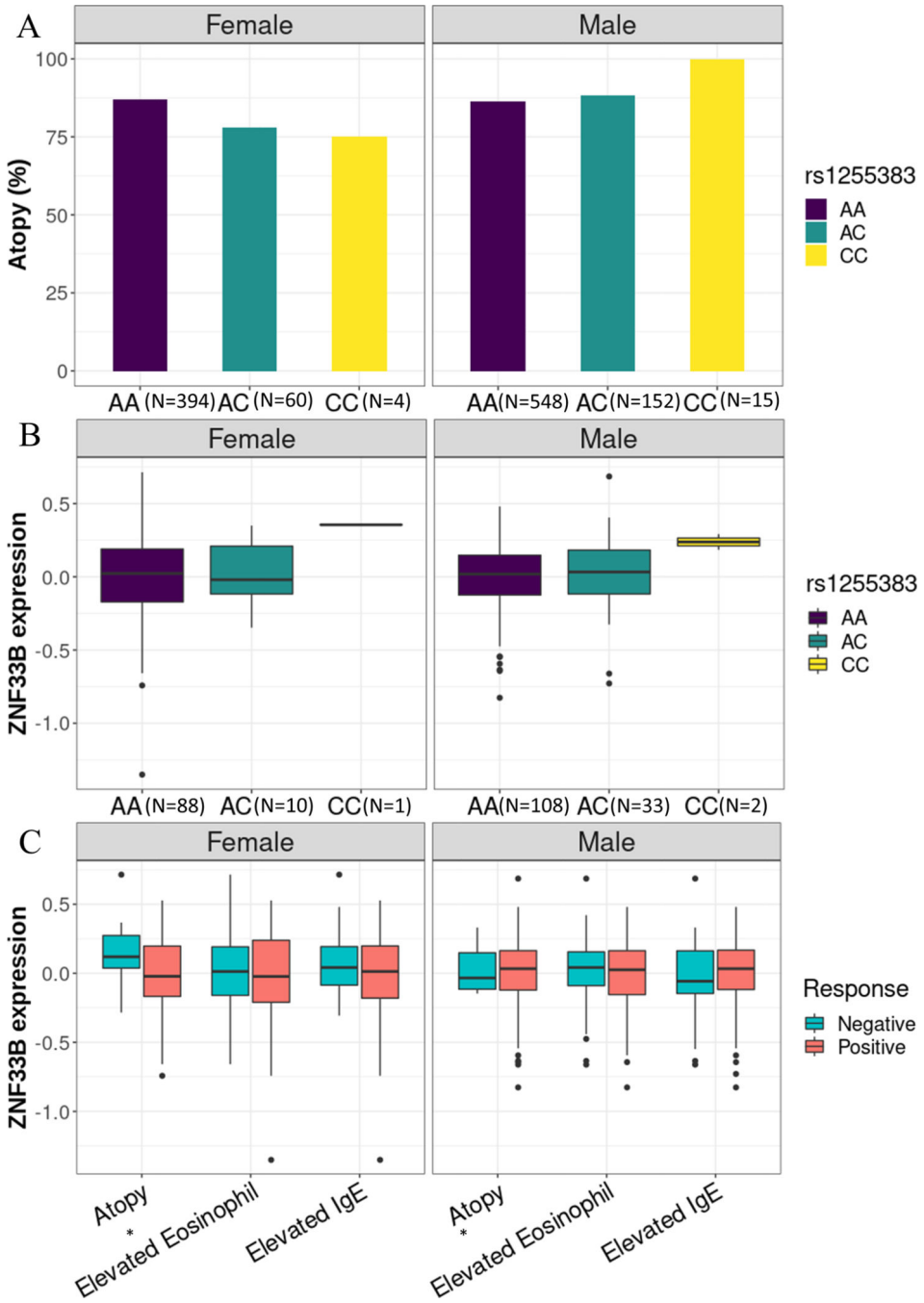

Figure 2.

The sex-differential effect of SNP rs1255383 on atopy in 1,173 asthmatic trios, showing decreased risk in females (left, panel A) and increase risk in males (right, panel A), the eQTL association between the genotype for SNP rs1255383 and *ZNF33B* expression in samples from GACRS (N = 242, left: female vs. right: male), showing higher expression for the homozygous CC genotype (panel B), and gene expression of *ZNF33B* stratified by sex,

showing the decreased expression of *ZNF33B* in females with atopy (panel C, **P* value of atopy = 0.0775 vs. 0.760).

Table 1.

Demographic characteristics and clinical features of study subjects (N=1,173). Mean ± SD or Number (%) is shown.

	GACRS probands	CAMP probands	GACRS + CAMP	Sex		*P*-value*
				Male	Female	
Number	889	284	1,173	715 (60.95)	458 (39.05)	
Male	523 (58.83)	192 (67.61)	715 (60.95)	715 (100)	0 (0)	
Age (year)	9.28 ± 1.85	8.89 ± 2.17	9.18 ± 1.94	9.16 ± 1.96	9.22 ± 1.91	0.659
Self-reported ethnicity						0.238
African		28 (9.86)	28 (2.39)	16 (2.24)	12 (2.62)	
European		216 (76.06)	216 (18.41)	144 (20.14)	72 (15.72)	
Hispanic	889 (100.0)	18 (6.34)	907 (77.32)	539 (75.38)	368 (80.35)	
Other		22 (7.75)	22 (1.88)	16 (2.24)	6 (1.31)	
Onset age of asthma	2.25 ± 2.20	2.93 ± 2.38	2.41 ± 2.26	2.36 ± 2.18	2.49 ± 2.38	0.335
Body mass index (kg/m^2)	18.44 ± 3.87	18.19 ± 3.15	18.38 ± 3.71	18.38 ± 3.76	18.37 ± 3.64	0.979
Spirometric measure						
FEV_1 (% of predicted)	98.93 ± 16.64	93.77 ± 13.61	97.67 ± 16.10	98.26 ± 15.32	96.76 ± 17.22	0.130
FEV_1/FVC (%)	84.41 ± 7.65	79.56 ± 7.80	83.23 ± 7.96	82.58 ± 7.72	84.24 ± 8.23	6.28×10^{-4}
Blood tests						
log_{10} (total serum IgE, IU/mL)	2.50 ± 0.66	2.24 ± 0.67	2.44 ± 0.68	2.48 ± 0.64	2.37 ± 0.73	8.76×10^{-3}
Elevated total serum IgE (≥ 100 IU/mL)	697 (78.40)	195 (68.66)	892 (76.04)	571 (79.20)	328 (71.46)	2.06×10^{-3}
log_{10} (eosinophil, count/mm^3)	2.60 ± 0.42	2.57 ± 0.41	2.59 ± 0.42	2.60 ± 0.40	2.57 ± 0.45	0.199
Elevated eosinophil (≥500 count/mm^3)	429 (48.26)	110 (38.73)	539 (45.95)	341 (47.30)	201 (43.79)	0.269
Skin reactivity to:						
At least one allergen (atopy)	742 (83.46)	251 (88.38)	993 (84.65)	609 (85.17)	384 (83.84)	0.552
D. pteronyssinus	683 (76.83)	139 (48.94)	822 (70.08)	502 (70.21)	320 (69.87)	0.940
D. farinae	658 (74.02)	134 (47.18)	792 (67.52)	480 (67.13)	312 (68.12)	0.778
P. americana	427 (48.03)	63 (22.18)	490 (41.77)	313 (43.78)	177 (38.65)	0.0893
B. germanica	447 (50.28)	82 (28.87)	529 (45.10)	317 (44.34)	212 (46.29)	0.568
cat hair	142 (15.97)	146 (51.41)	288 (24.55)	186 (26.01)	102 (22.27)	0.163
dog hair	63 (7.09)	69 (24.30)	132 (11.25)	84 (11.75)	48 (10.48)	0.562
A. tenuis	49 (5.51)	105 (36.97)	154 (13.13)	113 (15.80)	41 (8.95)	8.15×10^{-4}
mixed grass pollen	87 (9.79)	110 (38.73)	197 (16.79)	139 (19.44)	58 (12.66)	3.08×10^{-3}
mixed tree pollen	93 (10.46)	10 (3.52)	103 (8.78)	67 (9.37)	36 (7.86)	0.235
Severity						
Ever hospitalized for asthma (yes)	385 (43.31)	87 (30.63)	472 (40.24)	294 (41.12)	178 (38.86)	0.486
Hospitalized for asthma in last year (yes)	44 (4.95)	18 (6.34)	62 (5.26)	39 (5.45)	23 (5.02)	0.852

	GACRS probands	CAMP probands	GACRS + CAMP	Sex		
				Male	Female	*P*-value*
Emergency room for asthma in last year (yes)	740 (83.24)	77 (27.11)	817 (69.65)	496 (69.37)	321 (70.09)	0.845

FEV1: forced expiratory volume in one second, FVC: forced vital capacity

*P-value refers to the comparison between male and female with respect to each phenotype.

Table 2.

The robust gene-by-sex tests (fbatge in fbati package) for atopy, asthma, total IgE, eosinophil count, and skin test reactivity to specific allergens with respect to SNP rs1255383.

Affection status		GACRS probands (N=889)		CAMP probands (N=284)		Merged probands (N=1,173)		
		Informative families*	$P_{interaction}$	Informative families*	$P_{interaction}$	Informative families*	Correlation with atopy (φ)	$P_{interaction}$
Atopy		85	7.18×10^{-6}	36	5.43×10^{-4}	121	-	9.08×10^{-10}
Asthma		101	4.56×10^{-4}	42	1.05×10^{-3}	143	-	5.86×10^{-7}
Atopy markers	Elevated eosinophil count (≥ 500 cells/ mm^3)	45	4.31×10^{-5}	15	4.25×10^{-2}	60	0.291	6.20×10^{-7}
	Elevated total IgE (≥ 100 IU/mL)	72	5.46×10^{-6}	27	2.47×10^{-2}	99	0.469	1.37×10^{-7}
Allergens	*D. pteronyssinus*	77	3.91×10^{-5}	21	7.98×10^{-4}	98	0.626	1.37×10^{-8}
	D. farinae	72	1.10×10^{-5}	23	1.68×10^{-3}	95	0.588	9.10×10^{-9}
	P. americana	51	1.06×10^{-4}	6	8.29×10^{-2}	57	0.340	1.03×10^{-6}
	B. germanica	53	3.42×10^{-4}	10	3.72×10^{-2}	63	0.365	6.39×10^{-6}
	cat hair	21	1.92×10^{-3}	23	5.13×10^{-3}	44	0.228	1.07×10^{-5}
	dog hair	9	1.59×10^{-1}	11	2.26×10^{-3}	20	0.142	0.000379
	A. tenuis	6	3.95×10^{-1}	12	1.04×10^{-2}	18	0.157	0.004875
	mixed grass pollen	6	4.81×10^{-2}	12	5.61×10^{-2}	18	0.179	0.009275
	mixed tree pollen	14	2.31×10^{-2}	0	1	14	0.146	0.018289

*Informative families as provided by fbatge.

Table 3.

The subgroup analyses using FBAT with respect to atopy and sex (male-only vs. female-only) for rs1255383.

Cohort	Phenotypes	SNP	A1	Allele frequency	FBAT				
					Informative families*	S-E (S)	Var (S)	Z	*P*-Value
Merged data (N=1,173, atopy: 84.65%)	atopy (N=993)	rs1255383	C	0.108	320	−3.500	89.250	−0.370	0.711
	no atopy (N=154)	rs1255383	C	0.108	57	−3.500	15.250	−0.896	0.370
Male only trios (N=715, atopy: 85.17%)	atopy (N=609)	rs1255383	C	0.112	199	26.000	55.500	3.490	4.83×10^{-4}
	no atopy (N=90)	rs1255383	C	0.112	38	−7.000	10.000	−2.214	0.0269
Female only trios (N=458, atopy: 83.84%)	atopy (N=384)	rs1255383	C	0.104	121	−29.500	33.750	−5.078	3.82×10^{-7}
	no atopy (N=64)	rs1255383	C	0.104	19	3.500	5.250	1.528	0.127

*trios with at least one heterozygous parent.