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Gene-by-environment effect of house dust mite on purinergic receptor P2Y12 (*P2RY12*) and lung function in children with asthma

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Abstract

Background—Distinct receptors likely exist for leukotriene(LT)E4, a potent mediator of airway inflammation. Purinergic receptor P2Y12 is needed for LTE4-induced airways inflammation, and P2Y12 antagonism attenuates house dust mite-induced pulmonary eosinophilia in mice. Although experimental data support a role for P2Y12 in airway inflammation, its role in human asthma has never been studied.

Objective—To test for association between variants in the P2Y12 gene (*P2RY12*) and lung function in human subjects with asthma, and to examine for gene-by-environment interaction with house dust mite exposure.

Methods—19 single nucleotide polymorphisms (SNPs) in *P2RY12* were genotyped in 422 children with asthma and their parents (n=1266). Using family-based methods, we tested for associations between these SNPs and five lung function measures. We performed haplotype association analyses and tested for gene-by-environment interactions using house dust mite exposure. We used the false discovery rate to account for multiple comparisons.

Results—Five SNPs in *P2RY12* were associated with multiple lung function measures (P values 0.006–0.025). Haplotypes in *P2RY12* were also associated with lung function (P values 0.0055–0.046). House dust mite exposure modulated associations between *P2RY12* and lung function, with minor allele homozygotes exposed to house dust mite demonstrating worse lung function than those unexposed (significant interaction P values 0.0028–0.040).

Conclusions and clinical relevance—*P2RY12* variants were associated with lung function in a large family-based asthma cohort. House dust mite exposure caused significant gene-by-environment effects. Our findings add the first human evidence to experimental data supporting a role for P2Y12 in lung function. P2Y12 could represent a novel target for asthma treatment.

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Keywords

Purinergic receptor; leukotriene; asthma; house dust mite; lung function

INTRODUCTION

Leukotrienes are eicosanoid lipid mediators that contribute to inflammation in asthma and atopic disorders. The cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4 cause inflammation, smooth muscle contraction, and bronchoconstriction that characterize asthma [1].

In contrast to LTC_4 and LTD_4, LTE_4 is stable and abundant in biologic fluid such as bronchoalveolar fluid and urine of asthmatic individuals [2]. Although LTE_4 is a weak agonist at the classical receptors for cysteinyl leukotrienes ($CysLT_1$ and $CysLT_2$), LTE_4 potently induces airways hyperresponsiveness and mucosal eosinophilia [3], and urinary levels of LTE_4 are used to gauge therapeutic response in asthmatic patients [4]. Asthmatic airways exhibit selective responsiveness to LTE_4, in contrast to LTC_4 and LTD_4 [5]. Although evidence points to LTE_4 playing a key role in airway inflammation, our understanding of its potential receptor(s) and their biology remain limited [1]. The modest effect of current leukotriene receptor antagonists, which block only $CysLT_1$, could be due to uninhibited effects of LTE_4 in asthmatics [6].

Purinergic receptor P2Y12 is a G-protein coupled receptor that may play a key role in LTE_4-induced airway inflammation. Better known for its role as the receptor for adenosine diphosphate that is targeted by thienopyridine antiplatelet agents such as clopidogrel and prasugrel, P2Y12 has mostly been studied in cardiovascular and cerebrovascular disease [7–11]. However, *in silico* data highlighted P2Y12 as a candidate LTE_4 receptor [12], with subsequent *in vitro* and murine data supporting a role for P2Y12 in LTE_4-induced mast cell activation, goblet cell metaplasia, eosinophilia, and IL13 production [3]. However, the role of P2Y12 in human asthma has not been explored, and there have been no genetic studies of the P2Y12 gene (*P2RY12*) in asthmatic subjects.

In this study, we report a genetic epidemiologic analysis of *P2RY12* in a large family-based cohort of 422 children with asthma and their parents (1266 total subjects). We found that genetic variants in *P2RY12* are associated with lung function. Given the likelihood that asthma and lung function are governed by both genetic and environmental forces, we were also interested in examining gene-by-environment effects for *P2RY12*. As experimental data demonstrate house dust mite allergen to be a potent stimulator of cysteinyl leukotriene production [13], and P2Y12 antagonism attenuates pulmonary inflammation induced by intranasal house dust mite allergen [3], we specifically examined for interactions with house dust mite exposure. We found that the effects of *P2RY12* variants on lung function are modulated by subjects' house dust mite exposure.

METHODS

Study Population

The Childhood Asthma Management Program (CAMP) is a multicenter North American clinical trial designed to investigate the long-term effects of inhaled anti-inflammatory medications in children with mild to moderate asthma [14]. Participating children had asthma defined by symptoms greater than 2 times per week, use of an inhaled bronchodilator at least twice weekly or use of daily medication for asthma, and increased airway responsiveness to methacholine (PC_{20} 12.5 mg/ml). Children with severe asthma or other clinically significant medical conditions were excluded. Of the 1041 children enrolled in the

original clinical trial, 968 children and 1518 of their parents contributed DNA samples to the CAMP Genetics Ancillary Study [15]. Selection criteria for genotyping were (a) self-described non-Hispanic white ethnicity and (b) availability of sufficient DNA for microarray hybridization; 422 children and their parents (n=1266) met these criteria. Written informed consent was obtained from study participants. The institutional review boards of Brigham & Women's Hospital and the eight CAMP Study Centers approved the study protocols.

Measures

Phenotypic data were collected from each child in CAMP at randomization. A complete description of the trial design and methodology of CAMP has been previously published [14]. Participating children stopped all asthma medications except for as needed albuterol 6 weeks prior to randomization. Methacholine challenge was performed 2 weeks prior to randomization, and spirometry was performed at randomization. Relevant to this study, spirometry (including measurements for forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), bronchodilator response (BDR)), methacholine challenge, and skin prick tests were performed on all children. BDR was expressed as the percentage change in FEV_1 as follows: BDR = (Post-FEV_1 - pre-FEV_1)/pre-FEV_1. House dust samples were collected by vacuuming the patient's mattress, bedroom floor, family room floor, kitchen floor, and upholstered furniture.

Genotyping

SNP genotyping for CAMP subjects and their parents was performed on Illumina Human-Hap550 Genotyping BeadChip (Illumina, Inc., San Diego, CA). Duplicate genotyping was performed on 5% of the sample to assess genotype reproducibility. Genotype quality control was assessed by <1% discordance rate, <5 Mendelian inconsistencies, and genotype completion rates >98% for all loci.

19 SNPs in *P2RY12* and its 20kb flanks with minor allele frequency >5% were available and selected for analysis. All SNPs were in Hardy-Weinberg equilibrium ($p > 0.01$). Of the 422 nuclear families in CAMP, 25 were excluded from this analysis because of Mendelian inconsistencies (n= 6) or missing >5% of genotypic data (n = 19), leaving 397 families for analysis. Linkage disequilibrium (LD) patterns were plotted using Haploview [16].

Statistical Analyses

Statistical calculations for the baseline characteristics of subjects were performed using R v. 2.9.0 [17]. We performed family-based testing for association between SNPs in *P2RY12* and five lung function measures (BDR, FEV_1, FVC, FEV_1/FVC, PC_{20}) under a recessive genetic model using the Pedigree-Based Association Test (PBAT) [18] implemented in Helix Tree v6.4.3 (Golden Helix, Bozeman, MT). Analyses were adjusted for age, sex, and height. Standard phenotypic residuals were used as the offset to maximize power. An advantage of family-based association testing is that it is robust against population stratification and population admixture [19]. To account for potential false positives from multiple testing, we used the false discovery rate method [20], a strategy considered to be more powerful than Bonferroni correction [21].

Haplotypes in *P2RY12* were defined using the confidence interval method [22]. Family-based haplotype association analyses were conducted in PBAT [18] using the FBAT Generalized Estimating Equations test parameter [23].

We tested for gene-by-environment interaction between house dust mite exposure and *P2RY12* associations with lung function measures using the family-based association test of interaction (FBAT-I) implemented in PBAT [18]. Exposure to house dust mite allergen

(*Dermatophagoides pteronyssinus*) was defined in two ways: dichotomized at the 4th quartile of measured *Der p* level (1.85 μg/g) and dichotomized at 10 μg/g or greater. Previous studies have shown that 10μg/g of house dust mite exposure influences asthma exacerbations [24,25], and this level has been used in recent studies of gene-by-environment interactions [26,27]. However, given the skewed distribution of measured *Der p* levels in our cohort and the relatively small number of subjects exposed to 10μg/g of *Der p* or greater, we also employed the quartile definition to augment power.

RESULTS

The baseline characteristics of the index children in CAMP are shown in Table 1. Consistent with the known gender distribution of asthma in children, there were more boys than girls. CAMP children were highly atopic, with 88% of subjects exhibiting a positive skin test reaction to at least one allergen, and 47% positive to house dust mite in particular. There were no significant differences in physical characteristics or lung function between subjects exposed and unexposed to house dust mite using either definition of house dust mite exposure. As would be expected, there was a higher prevalence of skin test reaction to *D. pteronyssinus* and *D. farinae* among subjects exposed to house dust mite.

The linkage disequilibrium (LD) patterns for the *P2RY12* SNPs genotyped are shown in Fig. 1a. There was high LD between a few SNP pairs, but no large LD blocks within the gene and its 20kb flanks.

The results for association testing between *P2RY12* SNPs and lung function measures BDR, FEV_1, and FEV_1/FVC are shown in Table 2. The region between 152541653 base pairs and 152571916 base pairs contained five SNPs (rs9848789, rs17282940, rs7615865, rs1491978, rs7637803) that were each associated with lung function. Minor allele homozygotes for these SNPs had better lung function, with lower BDR and higher FEV_1 and FEV_1/FVC. There was high LD only between the first two SNPs in this region (rs9848789 and rs17282940, $r^2 = 1.0$) and between the second two SNPs (rs7615865 and rs14919878, $r^2 = 0.98$) (Fig. 1a). The Q values for these associations ranged from 0.11 to 0.12, suggesting that about one in ten of these associations could be false discoveries due to multiple comparisons. Additionally, there was a suggestion for association between rs7637803 and PC_{20} (P value 0.04) and between rs9653953 and FVC (P value = 0.03). Association tests for PC_{20}, FVC and other SNPs were otherwise nonsignificant.

Haplotype blocks for *P2RY12* are shown in Fig. 1b. Table 3 shows the results for association testing between the haplotype blocks and lung function measures. There were significant associations between both haplotype blocks and BDR, FEV_1, and FEV_1/FVC.

Table 4 shows the results for gene-by-environment analyses. The effect of house dust mite exposure at the fourth quartile or greater on the association between *P2RY12* SNPs and lung function measures was significant for six SNPs (P values 0.0028–0.044). This level of house dust mite exposure modified the relationship between four SNPs in *P2RY12* (rs8180086, rs3732765, rs10935840, rs11708767) and airway hyperresponsiveness (PC_{20}), and between two SNPs in *P2RY12* (rs7615865, rs1491978) and BDR. The effect of house dust mite exposure dichotomized at 10μg/g on the association between *P2RY12* SNPs and lung function measures was significant for five SNPs (P values 0.018–0.040). House dust mite exposure modified the relationship between three SNPs in *P2RY12* (rs3732765, rs10935840, rs11708767) and airway hyperresponsiveness (PC_{20}), and between two SNPs in P2RY12 (rs10935844, rs8180086) and FVC. The association between 3 SNPs (rs3732765, rs10935840, rs11708767) and airway hyperresponsiveness was modified by house dust mite exposure regardless of house dust mite definition exposure used. The overall and exposure

stratified results for association testing are additionally shown in Table 4. For ease of viewing, SNPs and phenotypes without significant interaction P values are not listed in this table.

To illustrate the gene-by-environment interactions we found, Fig. 2 shows the relationship between *P2RY12* and airway responsiveness stratified by house dust mite exposure. Fig. 2A shows the results when house dust mite exposure was dichotomized at the fourth quartile of measured *Der p* level, and Fig. 2B shows the results when house dust mite exposure was dichotomized at 10 μg/g. PC_{20} was low for those exposed to house dust mite *and* carrying two copies of the minor allele of each of these SNPs. Minor allele homozygotes not exposed to house dust mite had similar PC_{20} values to subjects with one or two copies of the major allele and variable house dust mite exposure. BDR and FVC were higher for those exposed to house dust mite and carrying two copies of the minor allele of each of the identified SNPs, while minor allele homozygous not exposed to house dust mite had similar BDR and FVC to subjects with one or two copies of the major allele and variable house dust mite exposure.

DISCUSSION

This is the first study to examine *P2RY12* in human subjects with asthma. We found evidence for association between variants in *P2RY12* and lung function in a large cohort of families with asthma, and evidence that house dust mite modulates these associations through gene-by-environment effects. We used family based methods, thus avoiding bias from population stratification [19]. Our work establishes a human context for previous i*n silico, in vitro,* and murine data suggesting a role for P2Y12 in LTE_4-modulated airway inflammation.

Our findings add to a growing literature on potential receptors and receptor modulators of LTE_4 [3, 28]. As the most abundant and stable cysteinyl leukotriene, LTE_4 is more potent than other leukotrienes in causing airways hyperresponsiveness to histamine in asthmatics and tracheal constriction in guinea pigs [2]. It has received relatively little attention since it binds poorly to $CysLT_1$ (the target of leukotriene receptor antagonist medications such as montelukast) [29] and $CysLT_2$ (the other well-characterized cysteinyl leukotriene receptor) [30]. Interest in a separate LTE_4 receptor recently rekindled based on murine studies in which LTE_4 caused 64-fold more vascular permeability in mice lacking $CysLT_1$ and $CysLT_2$ [28]. The identification and characterization of receptors and modulators of LTE_4 could lead to new therapeutic targets for asthma and atopic disorders.

The *P2RY12* SNPs that we found to be associated with BDR, FEV_1, and FEV_1/FVC were in proximity to one another (Table 2, Fig. 1a), suggesting that this region of P2RY12 may be of particular functional interest. The fact that many of these SNP were associated with bronchodilator response as well as FEV_1 and FEV_1/FVC would be consistent with these SNPs influencing not only baseline lung volumes, but also airway reversibility. The associated SNPs are intronic [31], with rs7615865 marking a potential splice site [32]. Polymorphic variation at these loci could lead to altered splicing and RNA production, resulting in aberrant P2Y12 protein structure and causing downstream effects on receptor function, inflammation, and lung function. Alternatively, these regions could have regulatory variants that are in transcription factor binding sites.

Meta-analyses of genome-wide studies have been performed to identify genes associated with pulmonary function [33, 34]. *P2RY12* was not among the top genes reported, but these studies were performed in general populations unselected for asthma. It is likely that findings would be different if the contributing genome-wide studies had been performed in

populations of asthmatic subjects, as there may be genes important in determining lung function in subjects with asthma that may be distinct from those influencing lung function in subjects without respiratory disease. A subsequent study replicated some of the gene associations from the general population meta-analyses in subjects with asthma [35]. However, this study focused on candidate genes drawn from the general population study results, and thus never tested for *P2RY12*. Again, it is likely findings would be distinct had the initial genome-wide studies for lung function been performed in populations selected for asthma. A large, consortium-based genome wide association study of asthma was also recently performed, but the study focused on asthma phenotypes and IgE, and it did not test for associations with lung function measures [36].

Our finding that several SNPs and haplotypes in *P2RY12* are associated with lung function in children with asthma is supported by *in vitro* and murine data that provide potential mechanisms for this effect. Experiments using shRNA-mediated knockdown of P2Y12 in LAD2 cells, a well-differentiated human mast cell sarcoma line, show that P2Y12 is needed for LTE_4-mediated mast cell activation [3]. Mast cells populate the upper and lower respiratory tract epithelia, unequivocally play a role in allergic reactions, and their hyperplasia is observed in the inflamed mucosal epithelium and adjacent smooth muscle of asthmatic subjects [37]. P2Y12 knock out mice do not exhibit LTE_4-induced goblet cell metaplasia, IL13 production, and eosinophilia [3], phenomena that are also observed in asthmatic subjects. Further, treatment of mice with the P2Y12 antagonist clopidogrel leads to decreased airways inflammation and decreased goblet cell hyperplasia [3].

P2Y12 and its gene had not been studied in asthmatic subjects prior to this investigation. Given the known role of P2Y12 as the receptor for ADP-mediated platelet aggregation, previous studies of *P2RY12* focused on cardiovascular and cerebrovascular disease. These studies identified haplotype variants in *P2RY12* that were inconsistently associated with platelet aggregation, peripheral arterial disease, venous thromboembolism, myocardial infarction, and cerebrovascular accidents [7–11]. One study identified a haplotype in *P2RY12*, H2, as a gain-of-function haplotype with regards to platelet aggregation [7]. Our first haplotype block includes this H2 haplotype. We tested for association between the previously defined H2 haplotype and lung function, and these results did not differ significantly from the results for our first haplotype block. The *P2RY12* haplotypes that we tested were associated with lung function, consistent with our results from single SNP association testing.

House dust mite exposure modulated the effect of *P2RY12* SNPs on lung function. With house dust mite exposure, subjects recessive for the minor allele of identified SNPs exhibited worse lung function measures. Specifically, homozygotes for the minor allele of the identified SNPs who were also exposed to house dust mite had lower PC_{20} levels (Fig. 2). These gene-by-environment effects for airway responsiveness were significant with either definition of house dust mite exposure. The risk for worse lung function with recessive status and house dust mite exposure was further supported by our gene-by-environment tests with BDR, where we observed higher BDR in subjects recessive for the minor allele of identified SNP and exposed to dust. The interaction effect with BDR was significant with exposure to the highest quartile of house dust mite and not significant when the more stringent threshold of 10μg/g *Der p* was employed. This may have been due to limited power with the more stringent threshold of *Der p* exposure, given only 44 index subjects met this strict exposure criterion. Conversely, subjects recessive for the minor allele of identified SNPs and exposed to house dust mite demonstrated higher FVC volumes, with interaction effects significant with the 10μg/g *Der p* exposure model but not with the more liberal 4th quartile house dust mite exposure model. It is possible that gene-by-environment interactions for lung volumes are more sensitive to high levels of environmental exposure,

thus resulting in the significant effects seen only with high levels of house dust mite exposure. Children with asthma have been observed to have higher FVC volumes than children without asthma [38, 39]. The SNPs contributing to the gene-by-environment interactions we observed were distinct for the lung function measure being tested, suggesting variable gene-by-environment effects dependent on both SNP and phenotype being tested. Gene-by-environment effects were most pronounced in subjects recessive for the minor alleles of each SNP. Given the relative high minor allele frequencies for these variants (33–49%), a substantial proportion of this population (10–24%) would be recessive for these variants and influenced by these gene-by-environment effects.

There were some variants in *P2RY12* that were associated with lung function measures without gene-by-environment effects (Table 2), and other variants that exhibited associations with lung function measures only if gene-by-environment effects were included (Table 4). The former tended to be for BDR and lung volume phenotypes, while the latter for airway hyperresponsiveness as well as BDR. This could reflect a greater tendency for airway hyperresponsiveness to be influenced by environmental exposures. The fact that there was no overall association between many of these SNPs and lung function if house dust mite exposure was not considered corroborates a role for house dust mite in gene-by-environment interactions for these *P2RY12* SNPs. Since house dust mite allergen has been observed to stimulate rapid and robust production of cysteinyl leukotrienes [13], it is possible that P2Y12 receptors mediate the LTE_4-driven components of this effector response *in vivo*.

As the first study of P2RY12 in human subjects with asthma, our findings add an important human dimension to existing *in silico, in vitro*, and murine studies of P2Y12 and airway inflammation. We genotyped multiple markers in the gene and found associations with lung function measures. Our study additionally offers the strengths of family-based association findings free from population stratification bias, examination of single SNP as well as haplotype associations, and formal tests of gene-by-environment interactions for a common environmental allergen that impacts asthma severity in the population at large. Despite the strengths of our study, we acknowledge several limitations. Our analyses were limited to CAMP, a single multi-center study. While we have identified polymorphisms and haplotypes in *P2RY12* that are associated with lung function, we have not defined precise mechanisms for their effect, although existing experimental studies of P2Y12 lend clues to potential mechanisms as discussed. It is also possible that more comprehensive genotyping within the *P2RY12* gene could further define the functional variants involved. Finally, some of our findings could be due to false positive associations from multiple testing, but our adjustments for multiple comparisons suggested only a one in ten chance of false discovery among the many associations we identified.

In summary, we found that SNPs and haplotypes in *P2RY12* were associated with lung function in a large cohort of children with asthma and their parents. Further, house dust mite exposure modulated the effect of these genetic variants on airway responsiveness. Our data contribute to a growing literature on gene-by-environment interactions in asthma [40]. Our findings add human evidence to existing *in silico, in vitro,* and murine data supporting a role for P2Y12 in LTE_4-mediated airway inflammation and asthma.

Acknowledgments

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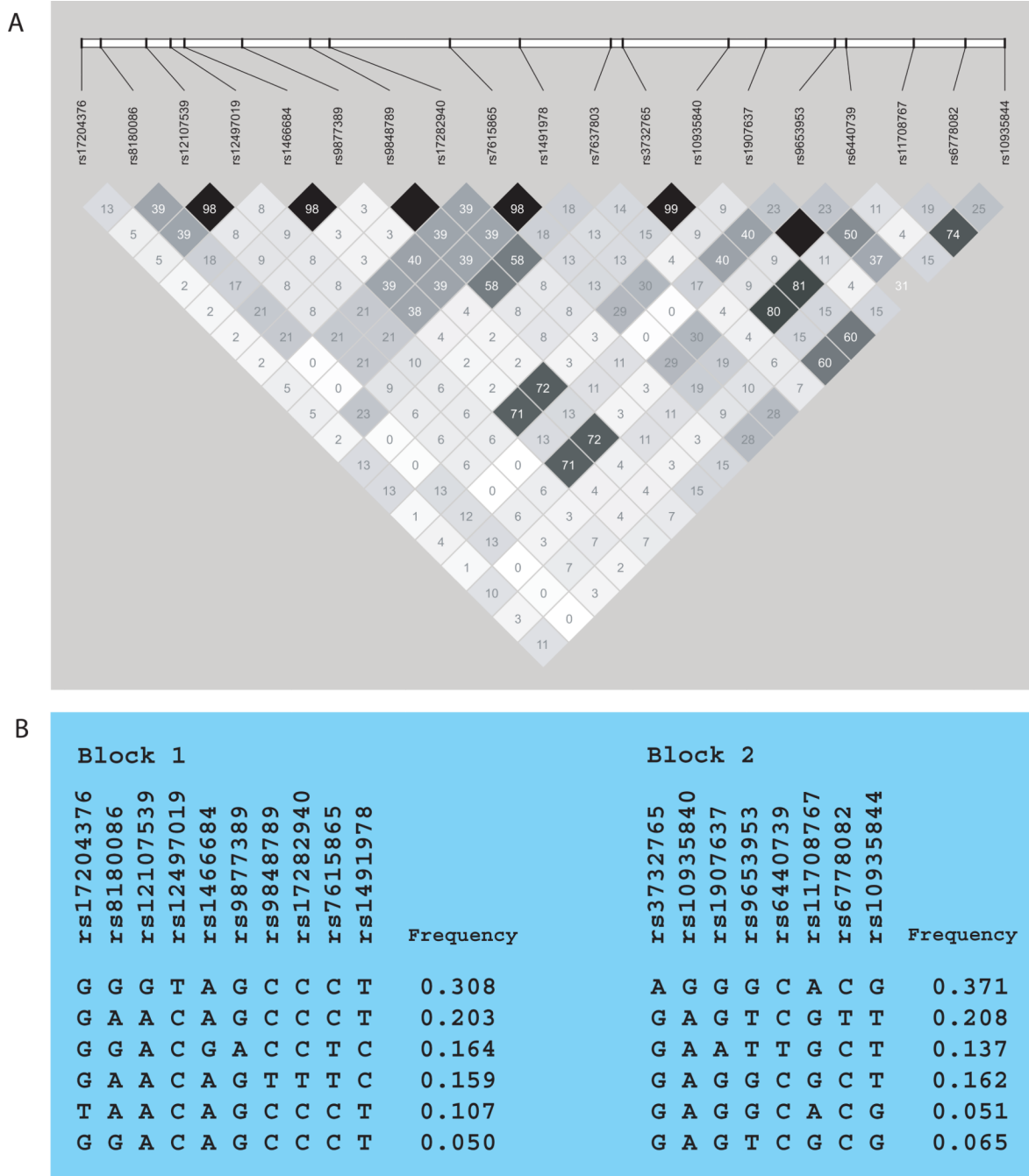
Fig. 1.
(**A**) Linkage disequilibrium plot for single nucleotide polymorphisms (SNPs) in *P2RY12* and its 20kb flanks. r^2 values for pairwise correlation are shown. (**B**) Haplotype blocks for *P2RY12*. Two blocks were defined using the confidence interval method. Haplotypes for each block are shown in order of frequency.
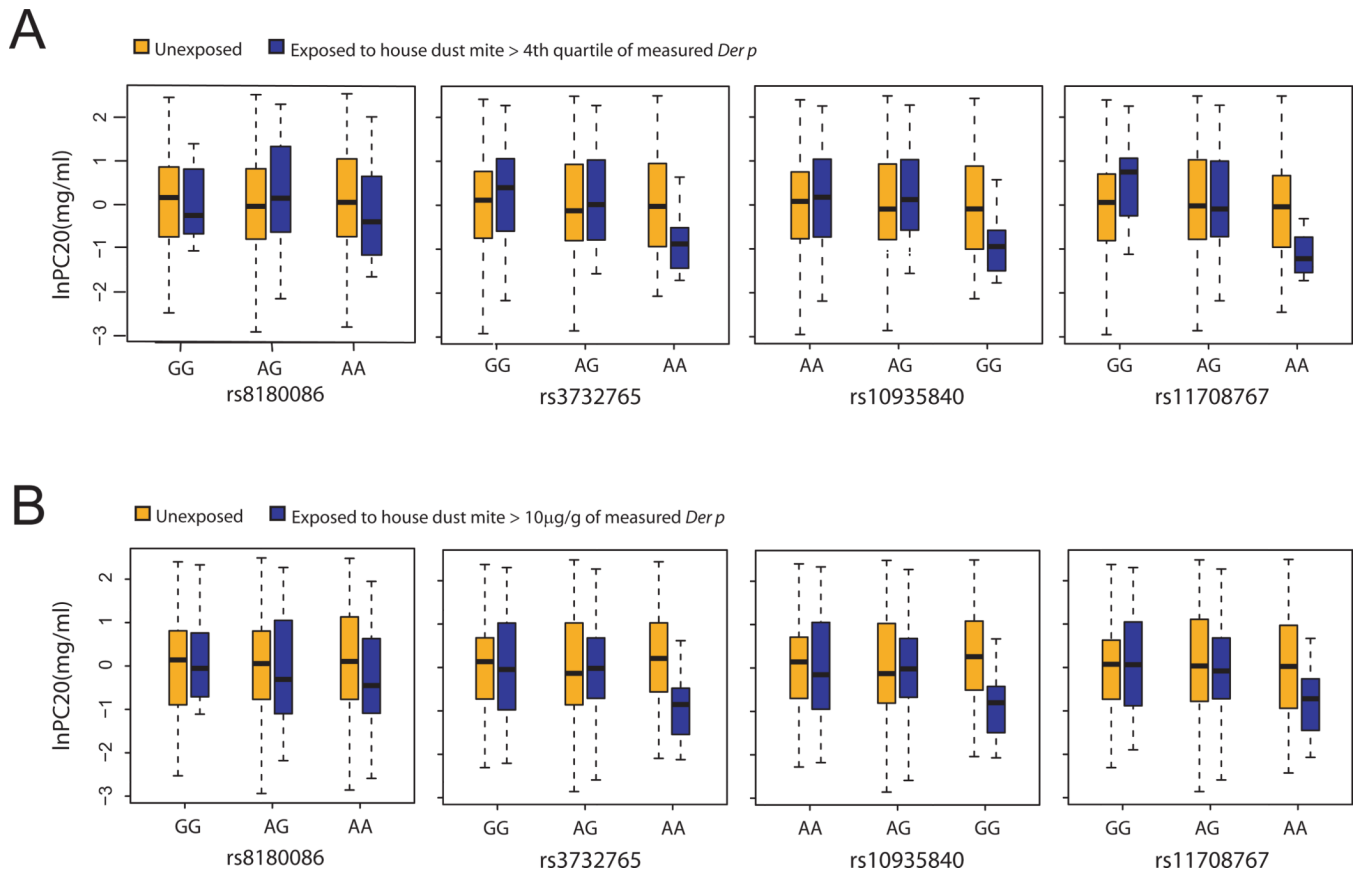
Fig. 2.
Relationship between *P2RY12* SNPs and airways responsiveness stratified by house dust mite exposure. **(A)** House dust mite exposure dichotomized at 4th quartile of measured *Der p* level, and **(B)** House dust mite exposure dichotomized at 10μg/g of measured *Der p* level. With both definitions of house dust mite exposure, subjects with two copies of the minor allele and dust exposure had greater airway responsiveness (lower PC_{20}) than minor allele homozygotes without dust exposure. Tests for gene-by-environment interaction were significant.

Table 1

Baseline characteristics of index children in the Childhood Asthma Management Program

	All subjects (n=422)	Quartile definition of Der p exposure*			10µg/g definition of Der p exposure*		
		Exposed (n=100)	Unexposed (n=300)	P value^	Exposed (n=44)	Unexposed (n=356)	P value^
Age (years)	8.7 (2.1)	8.6 (2.0)	8.8 (2.1)	0.37	8.7 (2.0)	8.8 (2.1)	0.87
Sex (female)	156 (37%)	37 (0.37)	113 (0.38)	0.91	16 (36%)	134 (38%)	0.87
Height (cm)	132.5 (13.5)	131.4 (14.0)	132.9 (13.6)	0.34	131.7 (13.5)	132.7 (13.7)	0.65
Weight (cm)	31.9 (10.6)	31.7 (11.0)	32.0 (10.5)	0.79	30.9 (9.1)	32.0 (10.8)	0.45
FEV_1 (L)	1.63 (0.46)	1.63 (0.48)	1.63 (0.46)	0.99	1.58 (0.44)	1.64 (0.47)	0.42
FVC (L)	2.07 (0.61)	2.08 (0.66)	2.08 (0.60)	0.96	2.00 (0.64)	2.09 (0.62)	0.38
FEV_1/FVC	79.4 (8.5)	79.9 (8.4)	79.3 (8.4)	0.50	80.7 (8.8)	79.3 (8.4)	0.32
$lnPC_{20}$ (mg/ml)	0.025 (1.15)	−0.053 (1.14)	0.057 (1.15)	0.41	−0.016 (1.11)	0.035 (1.15)	0.78
BDR (%)	10.8 (10.4)	9.7 (8.6)	11.1 (10.7)	0.18	10.6 (8.9)	10.8 (10.4)	0.88
Physician visit for asthma in past year	394 (98%)	97 (0.97)	279 (0.93)	0.08	43 (98%)	333 (94%)	0.11
Any positive STR	370 (88%)	83 (0.83)	268 (0.89)	0.13	38 (86%)	313 (88%)	0.78
Der p STR	197 (47%)	68 (0.68)	122 (0.41)	1.5×10^{-6}	33 (75%)	157 (44%)	5.7×10^{-5}
Der f STR	195 (46%)	64 (0.64)	124 (0.41)	7.8×10^{-5}	31 (70%)	157 (44%)	8.1×10^{-4}
Der p level in house dust (µg/g)	3.67 (9.31)	13.69 (14.63)	0.32 (0.36)	8.4×10^{-15}	25.15 (15.69)	1.01 (1.85)	4.6×10^{-13}

Numbers are mean (standard deviation) or number (percent)

*
House dust mite exposure dichotomized at 4th quartile of measured Der p levels (1.85 µg/g) for quartile definition of exposure. House dust mite exposure dichotomized at Der p level of 10µg/g for 10µg/g definition. Levels not available for 22 subjects.

^
P value for comparison between house dust mite exposed and unexposed

STR = skin test reaction

Table 2

Association between *P2RY12* SNPs and lung function measures

Marker	Allele	Frequency	BDR β (SE)	P value	FEV₁ β (SE)	P value	FEV₁/FVC β (SE)	P value
rs17204376	T	0.09	−0.015 (0.017)	0.82	0.047 (0.045)	0.74	−0.37 (1.589)	0.24
rs8180086	A	0.46	−0.011 (0.014)	0.90	0.024 (0.022)	0.47	0.63 (0.975)	0.81
rs12107539	G	0.32	0.011 (0.013)	0.07	−0.001 (0.021)	0.07	−0.97 (0.867)	**0.018**
rs12497019	T	0.31	0.011 (0.013)	0.07	−0.003 (0.021)	0.07	−0.89 (0.871)	**0.021**
rs1466684	G	0.18	0.005 (0.013)	0.92	−0.036 (0.028)	0.13	0.16 (0.974)	0.97
rs9877389	A	0.17	0.004 (0.013)	0.92	−0.034 (0.028)	0.14	0.23 (0.984)	0.96
rs9848789	T	0.16	−0.044 (0.024)	**0.006**	0.071 (0.039)	**0.025**	2.68 (1.675)	**0.012**
rs17282940	T	0.16	−0.044 (0.024)	**0.006**	0.070 (0.038)	**0.025**	2.68 (1.656)	**0.012**
rs7615865	T	0.33	−0.020 (0.013)	**0.019**	0.017 (0.023)	0.64	1.53 (0.934)	**0.009**
rs1491978	C	0.33	−0.019 (0.013)	**0.023**	0.019 (0.023)	0.71	1.59 (0.939)	**0.007**
rs7637803	T	0.21	−0.020 (0.018)	**0.008**	0.039 (0.032)	**0.017**	1.23 (1.274)	0.058
rs3732765	A	0.36	−0.002 (0.009)	0.91	0.017 (0.020)	0.96	−0.42 (0.768)	0.33
rs10935840	G	0.36	−0.002 (0.009)	0.92	0.018 (0.020)	0.96	−0.30 (0.768)	0.36
rs1907637	A	0.14	0.006 (0.012)	0.75	−0.015 (0.031)	0.22	0.37 (0.998)	0.91
rs9653953	T	0.42	0.011 (0.016)	0.29	−0.029 (0.027)	**0.047**	−0.13 (0.932)	0.36
rs6440739	T	0.14	0.007 (0.012)	0.75	−0.016 (0.031)	0.22	0.32 (0.997)	0.91
rs11708767	A	0.41	−0.002 (0.009)	0.89	0.014 (0.020)	0.86	−0.45 (0.739)	0.69
rs6778082	T	0.20	0.007 (0.017)	0.96	−0.023 (0.030)	0.33	0.42 (1.277)	0.99
rs10935844	G	0.49	0.007 (0.010)	0.56	−0.003 (0.020)	0.58	−1.32 (0.714)	0.27

Significant associations are bolded

SE = Standard Error

Table 3

Association between *P2RY12* haplotypes and lung function measures

Haplotype	BDR		FEV$_1$		FEV$_1$/FVC	
	GSS	P value	GSS	P value	GSS	P value
Block 1	7.53	**0.030**	4.78	**0.0055**	4.78	**0.046**
Block 2	2.90	**0.017**	3.44	**0.032**	3.44	**0.037**

Significant associations are bolded

GSS = Global Score Statistic

Table 4

Gene-by-environment effect of house dust mite exposure on associations between *P2RY12* SNPs and lung function measures

Measure	Marker	Allele	Quartile definition of house dust mite exposure ^					10 µg/g definition of house dust mite exposure			
			Association test P values			Interaction		Association test P values		Interaction	
			All	Exposed	Unexposed	β(SE)	P value	Exposed	Unexposed	β (SE)	P value
Airway responsiveness *	rs8180086	A	0.50	**0.012**	0.49	0.89(0.33)	**0.0088**	0.16	0.93	0.59 (0.40)	0.15
Airway responsiveness	rs3732765	A	0.57	**0.0091**	0.32	1.24(0.45)	**0.0035**	0.058	0.89	1.39 (0.71)	**0.033**
Airway responsiveness	rs10935840	G	0.54	**0.0058**	0.35	1.25 (0.45)	**0.0028**	**0.028**	0.94	1.47 (0.70)	**0.022**
Airway responsiveness	rs11708767	A	0.22	**0.019**	0.83	0.75 (0.39)	**0.042**	**0.038**	0.44	1.07 (0.62)	**0.039**
BDR	rs7615865	T	**0.019**	0.88	**0.018**	0.07 (0.03)	**0.042**	0.44	0.07	0.002 (0.02)	0.94
BDR	rs1491978	C	**0.023**	0.88	**0.021**	0.07 (0.03)	**0.044**	0.44	0.08	0.02 (0.88)	0.14
FVC	rs10935844	G	0.61	0.83	0.14	0.09 (0.07)	0.21	**0.011**	0.86	0.20 (0.07)	**0.018**
FVC	rs8180086	A	0.15	0.07	0.92	0.05 (0.10)	0.56	0.17	**0.024**	0.23 (0.11)	**0.040**

^ House dust mite exposure dichotomized at 4th quartile of measured *Der p* levels (1.85 µg/g) for quartile definition of exposure. House dust mite exposure dichotomized at *Der p* level of 10µg/g for 10µg/g definition.

* As reflected by methacholine challenge response (PC20)

Significant associations are bolded

SE = Standard Error