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Longitudinal Analysis of Bronchodilator Response in Asthmatics and Effect Modification of Age-related trends by Genotype

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Abstract

Background and objectives: Genome Wide Association Studies (GWAS) have identified genetic polymorphisms associated with bronchodilator response (BDR), but it is unknown how these associations change across life stages. We examined the impact of genetic variants on BDR from childhood to adulthood in asthmatics to uncover potential effect modification by age.

Methods: We searched the National Human Genome Research Institute (NHGRI) catalog of published GWAS to obtain a list of genetic associations with BDR, and tested them for effect modification by age in 604 subjects from the Childhood Asthma Management Program (CAMP), a clinical trial with longitudinal measures of BDR (age range 5 to 30 years). We performed longitudinal analyses using linear mixed models and visualized longitudinal changes in BDR using generalized additive models with repeated measures, adjusting for treatment group, sex, and main effects of age and additive genotype.

Results: Increasing age was associated with decreased BDR (−0.24 % per year). Polymorphisms rs295137 near *SPATS2L* and rs2626393 near *ASB3* demonstrated their strongest associations with BDR in early childhood through adolescence, with a large decrease in their magnitude of effect from adolescence onward. The effect estimate for % BDR associated with rs295137 genotype (Beta=1.3; 95% CI 0.6 to 2.1) was diminished by age (interaction term=−0.06, p=0.004). The effect estimate for rs2626393 (Beta=−0.92 (95% CI −1.7 to −0.2) was also modified by age (interaction term= 0.05, p = 0.0004).

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Conclusions: Polymorphisms associated with BDR in childhood may not be relevant for predicting adolescent and adult BDR, which could reflect age-related changes in asthma phenotypes.

Trial Registration: This work uses data from the previous clinical trial of asthma, the Childhood Asthma Management Program (CAMP), registered at [ClinicalTrials.gov](https://clinicaltrials.gov), #

Keywords

Bronchodilator response; age; asthma

INTRODUCTION

Asthma affects over 350 million people world-wide, with high prevalence in both pediatric and adult populations.¹ Responsiveness to asthma medications significantly impacts quality of life for most individuals with asthma. Genome wide association studies (GWAS) have sought to identify genetic variants that predict asthma treatment response, with precision medicine (individualized treatment tailored by genotype)² as the ultimate goal. These studies may combine pediatric and adult populations, with little to no emphasis on the potential role of age in modifying the impact of genetic variants on asthma treatment response. Age may be an important modifier of pharmacogenetic associations, especially since age-related differences in asthma phenotypes may reflect different underlying pathogenic pathways,³ the genetic variation in which may affect also asthma treatment response. In other words, the genetic polymorphisms with utility for predicting asthma treatment responses may change along with age-related changes in asthma phenotype. The identification of age-related asthma phenotypes is not new; clinicians have long understood that childhood asthma tends to be dominated by Th2 mediated responses and eosinophilia, while adult asthma phenotypes are more heterogeneous, and can be either Th2 mediated/eosinophilic, neutrophilic or obesity-related.⁴

One of the most widely used asthma medications in both child and adult populations is albuterol, a short-term β_2 -agonist that serves as a bronchodilator. β_2 -agonists promote bronchodilation by stimulating β_2 adrenergic receptors on airway smooth muscle cells to reduce bronchoconstriction via downstream increases in cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA).⁵ Bronchodilator response (BDR) to albuterol as an asthma phenotype involves inflammation and interactions among multiple airway cell types including the epithelium,⁶ smooth muscle cells⁷ and cells of the autonomic nervous system.⁸ GWAS studies have identified genetic polymorphisms associated with BDR.^{9–11} Thus far, the impact of these genetic variants on longitudinal changes in BDR from childhood to adulthood in individuals with asthma has not been examined. In this study, our aim was to test GWAS polymorphisms associated with BDR for effect modification by age in the Childhood Asthma Management Program (CAMP) clinical trial which has genome-wide polymorphism data and longitudinal bronchodilator response outcomes from early childhood to young adulthood (ages 5 to 30).

METHODS

CAMP Study Design and Participants

CAMP was a randomized, placebo-controlled trial of budesonide, nedocromil, or placebo for mild-to-moderate childhood asthma followed by three phases of observational follow-up; the trial and all follow-up phases included pre-bronchodilator and post-bronchodilator spirometry.¹² A total of 1041 children, 5 to 12 years of age, were enrolled in the trial between December 1993 and September 1995.¹² Inclusion was restricted to children with a history of chronic asthma who, during a 28-day run-in period, had asthma symptoms or a low morning peak flow on 8 or more days; inclusion also required a methacholine challenge with a concentration of 12.5 mg per milliliter or less that resulted in a reduction in FEV₁ by at least 20% (since airway responsiveness is determined by the provocative concentration of methacholine required to reduce the FEV₁ by at least 20% [methacholine PC20], with higher values indicating less airway responsiveness). Approval was obtained from the institutional review boards at each of the CAMP-participating institutions before initiation of the trial. Informed consent was obtained from the parent or guardian of the participant, and the child's assent was obtained before study enrollment.

Participants were randomly assigned to receive budesonide, nedocromil or placebo, all by inhalation. Treatment was continued for a mean of 4.5 years (participants attended 3 clinical trial "visits" per year). The treatment component of the trial ended in 1999, and asthma care was transferred to each participants' health care provider. Over 85% of the original 1041 participants participated in the 4 month transition phase (2 visits) following the trial, and 80% participated in all three phases (the treatment phase, the transition phase, and the continued follow-up phase (1–4 visits per year for 13 years)).¹³ We considered subjects of white race/ethnicity for our longitudinal analysis. Of the original 1041 participants, 711 were white, and 604 white subjects had genotyping data. The 604 subjects included both index children (N=570) and siblings (N=34). Follow-up procedures were identical for both index children and siblings. The age range for the longitudinal follow-up, including the clinical trial period, was 5 to 30 years of age. Longitudinal analyses included 13,997 observations for bronchodilator response in total with a maximum of 28 observations per subject. Follow-up time per subject ranged from 4 to 18 years, with a mean of 16 years $\pm$ standard deviation of 3 years. The number of BDR measurements per subject ranged from 6 to 28, with an average of 23 measurements.

SNP Selection and Genotyping

We used the NHGRI-EBI (National Health Genome Research Institute-European Bioinformatics Institute) catalog of published genome-wide association studies search tool (<https://www.ebi.ac.uk/gwas/>) to search for GWAS associations at a suggestive p value threshold of 1×10^{-5} . We conducted our search with the following search terms "asthma (bronchodilator response)" and "bronchodilator response in asthma". We identified 4 GWAS studies, 3 of which reported associations between genotypes and bronchodilator response. In these studies, four SNPs were associated with BDR^{9–11}. These variants are shown in Table 1, and include SNPs associated with *SPATS2L*, *ASB3*, and *COL22A1*. Of these variants, 3 of the 4 were genotyped in CAMP. One of the SNPs, rs350729 (near the *ASB3* gene), was

not genotyped. Therefore, a proxy SNP (rs2626393) in LD with rs350729 ($R^2 = 0.99$), was identified using the European populations (EUR) in LDlink, a web-based tool from NCBI (<https://analysistools.ncbi.nih.gov/LDlink/>). Genotyping was performed at the Channing Division of Network Medicine using Illumina Quad 610 microarray chips (Illumina, Inc., San Diego, CA). All of the SNPs considered met basic genotype quality control standards (minor allele frequency $>0.05\%$, Hardy Weinberg equilibrium of at least 0.0001).

Spirometry and Bronchodilator Response

Spirometry was performed at least 4 hours after short acting bronchodilator use and 24 hours after long-acting bronchodilator use and met American Thoracic Society (ATS) criteria for acceptability and reproducibility. At least 3 spirometric maneuvers were performed, with at least 2 reproducible maneuvers required for each test. The BDR test was performed at randomization and at subsequent visits during which a methacholine challenge was not administered (twice yearly during the clinical trial and at least annually during the post-trial follow-up). Post-bronchodilator spirometric values were obtained at least 15 minutes after the administration of 2 puffs of albuterol (90 mg per puff). BDR was expressed as the percentage change in FEV₁ as follows: $BDR = 100 \times [(Post-FEV_1 - pre-FEV_1) / pre-FEV_1]$.

Statistical Analysis

To assess the impact of age by genotype interactions on longitudinal BDR response, we used a mixed model approach (PROC MIXED) in SAS Statistical Software. Mixed models included main effects of age and additive genotype, a multiplicative interaction term (age x genotype), covariates (sex and treatment group), and random effects (subject specific intercept and slope). In additional models, we also adjusted for random effect of family (as a small number of children were siblings), but found that this made no difference in the results (see Supplement). Adjustment for the first six genotype principle components also did not change results in a meaningful way (see Supplement). For testing, we used the general linear mixed model with a compound symmetry covariance structure, since our data fit the model assumptions of this approach. (We also considered a first order auto-regressive covariance structure. We found that this model had a higher AIC than the compound symmetry model, therefore we used the compound symmetry covariance structure instead). For visualization, we used a generalized additive mixed model, due to the flexible nature of the linearity assumption.

For the generalized additive mixed model we used the gamm4 package in R, which allows for random effect of age, and a running smoother with no specified knots for the fixed effect of age. (Further details of the smoother can be found in Wood et al 2004).²⁴ We created genotype-specific smooth plots of longitudinal BDR trends with age to visualize trends. Generalized additive mixed models included main effects of age and genotype, as well as covariate adjustment for sex and treatment group.

RESULTS

CAMP Demographics

In CAMP, the mean age of enrollment was 8.95 years (standard deviation (s.d.) $\pm$ 2.13). Characteristics of study subjects are shown in Table E1. The mean age at asthma diagnosis in CAMP subjects was 3.08 years (s.d. $\pm$ 2.44). Sixty percent of subjects were male, and 25% had a maternal history of asthma. Overall, the socioeconomic status of participants was relatively high; only 19% reported a household income level of less than \$30,000 per year. We tested age by genotype interactions in white subjects (N=604) only.

BDR as a function of Age

We examined the main effect of age on BDR response prior to testing for age by genotype interactions. BDR response consistently decreased with age, as is shown in Figure 1. Visual investigation of the generalized additive mixed model (Figure 1) supports the linear assumption between age and BDR. On average, BDR decreased 0.24 % with each increasing year of age. (Table 2) Treatment group and sex did not confound the association between age and BDR over time (Table 2), and were not independent predictors of BDR. Since the CAMP longitudinal follow-up included the typical time period overlapping with the onset of puberty (which is later for males than females), we tested for interactions between age and sex, as a way of potentially accounting for sex-specific shifts in BDR that may occur as following puberty. Test of the interaction term for age x sex were non-significant ($p=0.13$).

Age by Genotype Interactions for Longitudinal BDR

We tested age by genotype interactions for all four identified BDR GWAS variants in mixed models, and found that two of the four variants modified the effect of age on longitudinal BDR response (Table 3). Age interacted with each of these variants to mitigate their effects. With increasing age, the *SPATS2L* variant (T allele) is associated with a smaller increase in BDR, and the *ASB3* variant (C allele) with a smaller decrease in BDR. P values for the interaction terms ranged from $p=0.004$ to 0.0004 , and were both significant at the Bonferroni corrected threshold ($p<0.0125$). These polymorphisms were prevalent in the CAMP population. The minor allele frequency for rs295137 near *SPATS2L* was 41 % (T allele), and the minor allele frequency of rs2626393 near *ASB3* was 34% (C allele). GWAS polymorphisms for BDR identified in the Duan et al ⁽⁹⁾ study (an intronic SNP in *COL22A1* and a downstream variant of *PRKCQ*, *IL2RA*, *IL15RA*, *KLF6*) did not show evidence for effect modification of age related changes in BDR.

After identifying two SNPs as potential effect modifiers of age related BDR response in linear mixed models, we then used generalized additive mixed models to visualize smooths for longitudinal age-related BDR changes by genotype. For each comparison, there were three groups, individuals without the polymorphism, subjects with a heterozygous genotype, and those homozygous for the polymorphism. Longitudinal changes in BDR response by genotype are shown in *SPATS2L* and *ASB3* polymorphisms are shown in figures 2 and 3. Smooths for longitudinal BDR trends with age (centered about the mean) are shown separately for each genotype, with shaded areas indicating the 95% confidence intervals around the smooths (Figure 2a–2c; Figure 3a–3c). For ease of interpretability and

comparison across genotypes, we also plotted an overlay of genotype-specific smooths on a single plot, for changes in percent BDR (expressed as a proportion) with age (Figure 2d and Figure 3d). At the earliest ages, these two variants had opposite directions of effect; the rs295137 variant (T allele) near the *SPATS2L* gene was associated with increased BDR response, while the rs2626393 variant (C allele) near the *ASB3* gene was associated with lower BDR. (These effects are visualized in figures 2 and 3. At the beginning of follow-up, there is a clear distinction by *SPATS2L* rs295137 genotype (figure 2d); those with two copies of the minor allele (TT genotype) had the highest BDR, with heterozygous individuals (CT genotype) showing lower BDR, and those homozygous for the C allele (CC genotype) with the lowest BDR. The *ASB3* variant rs2626393 was also associated with different BDR at the youngest ages (figure 3d); those with two copies of the minor allele (CC genotype) had the lowest BDR, while heterozygous individuals (CT genotype) and those homozygous for the major allele (TT genotype) had higher BDR at the beginning of follow-up.) For both the *SPATS2L* and *ASB3* variants, differential BDR response by genotype diminished with age, such that genotype-specific BDR response curves appeared to converge in adolescence at approximately age 15 years. To test whether these variants had significant main effects or age by genotype interactions after this time point, we performed sensitivity analyses by re-analyzing our mixed models using repeated measures of BDR from age 15 onward (see Supplemental Table E2). In CAMP, the effect of the *SPATS2L* variant (T allele) and its interaction with age were no longer statistically significant, suggesting that *SPATS2L* genotype mainly predicts BDR response in childhood/early adolescence, and that its effects on BDR no longer vary by age once subjects reach adolescence. We also examined the effects of *ASB3* genotype in follow-up data after age 15, to determine if main effects or age related interactions would still be present. The main genetic effect of the *ASB3* variant remained similar, as did the interaction term. These findings suggest that the *ASB3* variant (C allele) may still have some association with BDR response beyond age 15, although its effects are mitigated by increasing age, and graphs of the smooths show convergence for all three genotypes in adolescence. Given the relatively high minor allele frequencies of the *ASB3* and *SPATS2L* SNPs, a large percentage of our study population (41%) had one or more minor alleles for these variants (T for rs295137 near *SPATS2L* and C for rs2626393 near *ASB3*).

DISCUSSION

In this work, we leveraged longitudinal follow-up of over 600 subjects with asthma, each of whom had multiple bronchodilator response measures spanning from early childhood to adulthood, to determine whether associations between genetic polymorphisms and BDR vary by age. Two previously identified BDR GWAS polymorphisms, one in *SPATS2L*¹¹ and the other near *ASB3*,¹⁰ both demonstrated their strongest associations with BDR in early childhood through adolescence, with a large decrease in their magnitude of effects from adolescence into adulthood. These findings indicate that polymorphisms associated with BDR response in childhood may be different from polymorphisms that predict BDR response in adolescence and adulthood. Changes in the associations of these polymorphisms with BDR over time may reflect changes in underlying asthma phenotypes with age.

Age-related changes in asthma phenotypes have been well documented, with marked shifts occurring after puberty.¹⁴ The clinical trajectory of children with asthma typically shows improvement in asthma symptoms over time, a longitudinal trend most likely to occur in subjects with mild to moderate persistent asthma¹⁵. In fact, it is estimated that asthma symptoms persist from childhood to adulthood in approximately one third of cases.¹⁶ Age-related changes in gene expression patterns may underlie these longitudinal changes in asthma phenotypes. Genomic studies in multiple tissue types have identified dramatic shifts in both the transcriptome¹⁷ and the methylome¹⁸ that occur with increasing age; however the dearth of airway tissue samples in these studies makes it difficult to identify age related changes in the lung that may alter asthma phenotypes or treatment response. The relatively few studies to examine lung-specific endophenotypes¹⁹ have related sputum cellularity to asthma treatment response, showing that cell types (i.e. eosinophil vs. neutrophil dominant) are more variable in adults and can be used to guide treatment response,²⁰ whereas cell types in children are not markedly different by severity status, and have yet to be proven a useful guide to treatment.^{21, 22}

The *SPATS2L* and *ASB3* related polymorphisms associated with altered longitudinal bronchodilator responses are both biologically plausible modifiers of this lung function phenotype. In vitro functional experiments, published in conjunction with the BDR GWAS by Himes et al, show that miRNA knockdown on the *SPATS2L* gene leads to increased β_2 adrenergic receptor expression, suggesting that this gene may be important for down regulation of β_2 adrenergic receptors.¹¹ *ASB3* expression appears to be effected by β_2 agonists, and is known to regulate differentiation in muscle cells.²³ GWAS hits in both of these genes emerged in combined cohort studies of both children and adults; however, the primary discovery populations for these polymorphisms contained two clinical trials in childhood subjects (CAMP, CARE), as well as other trials that were a mix of child, adolescent and adult asthmatics (LOCCS, LODO, Sephracor, ACRN). We were unable to detect age modification of GWAS hits from the Duan et al study,⁹ which included polymorphisms in the *COL22A1* gene. Of the genes identified as GWAS hits in our NHGRI search, *COL22A1* is the least biologically plausible hit, with little known about this gene, except that it may encode for a cell adhesion ligand for skin fibroblasts and epithelial cells.

The strongest feature of our study is the longitudinal BDR phenotype data on asthmatics, where the effects of age on BDR trajectory are observed for each individual subject. We observed effect modification of associations by age, even after adjusting for sex and treatment group, and were able to visualize non-linear trends with age through the use of repeated measures generalized additive modeling techniques. Although there were many strengths to our study, a few caveats deserve mention. The CAMP study is unique in its extended, 16 year follow-up of asthmatic subjects and therefore we did not have access to a similar cohort for replication of our findings. We did not have Tanner stage assessment, which may have helped us tease apart age-related effects from those of pubertal onset. To optimize power, we focused only on GWAS hits for testing, but may have missed other important polymorphisms that interact with age to alter BDR. In addition, as with any analysis of BDR phenotypes, it is difficult to disentangle whether BDR shifts with age are due to alterations in treatment response to albuterol, or changes in asthma phenotype that reduce the baseline level of bronchoconstriction. Moreover, by screening only GWAS hits

we may have missed some relevant polymorphisms, especially given that the GWAS studies we drew from combined child and adult populations together (potentially obscuring life-stage specific genetic associations for BDR that we could have tested in our longitudinal models). Nevertheless, focusing our longitudinal analysis on GWAS polymorphisms associated with BDR proved to be an effective method for identifying possible age-related modification of genetic effects.

CONCLUSIONS

In summary, our findings suggest that there are substantial biologic changes that occur in the airway as children age that may vary by genotype. These findings bear relevance for future precision medicine efforts, and suggest that genomic panels for identifying the most efficacious individualized treatment should rely on age-specific genomic makers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BDR	(Bronchodilator Response)
GWAS	(Genome Wide Association Study)

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Figure 1.
Plot of predicted BDR (proportion) with age in CAMP subjects, adjusted for sex and treatment group ($p < 0.001$ for smooth).

Figure 2.

Longitudinal BDR response by SPATS2L (rs295137) genotype. (a-c) Centered smooths adjusted for age, sex, and treatment group, shaded area indicates 95% confidence interval for smooth ($p < 0.001$ for all smooths) (a) Homozygous for Wild-type (CC) (N=206) (b) Heterozygous (CT) (N=297) (c) Homozygous for SNP (TT) (N=101) (d) Smooths for predicted BDR with age for all 3 genotypes overlaid.

Figure 3.

Longitudinal BDR response by ASB3 (rs2626393) genotype. (a-c) Centered smooths for longitudinal BDR with age, adjusted for sex and treatment group, shaded area indicates 95% confidence interval for smooth ($p < 0.001$ for all smooths) (a) Homozygous for Wild-type (TT) (N=259) (b) Heterozygous (CT) (N=274) (c) Homozygous for SNP (CC) (N=71) (d) Smooth for predicted BDR with age for 3 genotypes Overlaid.

Table 1.

BDR GWAS SNPs identified in NHGRI search

GWAS study	SNPs	Minor Allele	Genes	Functional Class	GWAS p value	Minor allele frequency in CAMP
Duan, QL et al., 2013 (PMID: 23508266)	rs11252394	A	PRKCQ, IL2RA, IL15RA, KLF6	Intergenic variant	2×10^{-7}	0.08
	rs6988229	T	COL22A1	Intron variant	9×10^{-6}	0.22
Himes BE et al, 2012 (PMID:22792082)	rs295137	T	SPATS2L	Intergenic variant	3×10^{-6}	0.41
Israel E et al, 2015 (PMID:25562107)	rs2626393 (Proxy SNP for rs350729)	C	ASB3, SOCS, JUND, CEBPB	Intron variant	2×10^{-10}	0.34

Table 2.

Main Effects of Age, Treatment Group and Sex on Longitudinal BDR Outcome in Linear Mixed Models

Characteristic	BDR Outcome (β = BDR % change per level of variable)	
	Univariate Model β (p value)	Adjusted Model β (p value)
Age (years)	-0.24 (<0.0001)	-0.24 (<0.0001)
Male Sex	--	0.12 (0.75)
Treatment Group (vs. Placebo)		
Budesonide	--	-0.42 (0.38)
Nedocromil	--	-0.17 (0.72)

Table 3.

Longitudinal BDR and Age by Genotype Interactions in Linear Mixed Models

	BDR Outcome* (β = BDR % change per level of variable)**							
	<i>SNP rs11252394 (A allele)</i>		<i>SNP rs6988229 (T allele)</i>		<i>SNP rs295137 (T allele)</i>		<i>SNP rs2626393 (C allele)</i>	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI
SNP (Additive)	1.2	−0.2 to 2.6	0.35	−0.5 to 1.2	1.3	0.6 to 2.1	−0.92	−1.7 to −0.2
SNP *Age Interaction	−0.02	−0.08 to 0.04	0.02	−0.02 to 0.05	−0.06	−0.09 to −0.03	0.05	0.02 to 0.08

* Models included both additive genetic effect for SNP and the interaction term; all models adjusted for age, sex and treatment group

** β values with $p < 0.05$ level are indicated in bold.