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Genetic variation in *BAFF* and asthma exacerbations among African American individuals

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Capsule Summary

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A BAFF polymorphism is associated with asthma exacerbations and serum BAFF levels. BAFF expression in vivo increases in natural rhinovirus infection. BAFF may play a role in airway antiviral immunity and impact asthma exacerbation rates.

Keywords

BAFF; B-cell activating factor; tumor necrosis factor ligand superfamily; asthma; asthma exacerbations; genetics

To the Editor

African American individuals are disproportionately affected by asthma morbidity and mortality,[1] and socioeconomic factors alone do not completely account for these disparities.[2] Viral illnesses, particularly from rhinovirus, are considered to be the most frequent triggers of asthma exacerbations.[3] As previous studies have shown that the genetic predictors of asthma may differ between African American and white individuals,[4] it stands to reason that the predictors of asthma exacerbation may also differ by race-ethnicity. We evaluated whether single nucleotide polymorphisms (SNPs) in genes thought to modulate innate immune response to respiratory viruses (primarily the RIG-I and MDA5 pathways and BAFF related genes)[5] were associated with exacerbations among African American individuals with asthma.

Complete methods are outlined in the online supplement. Self-identified African American participants with asthma from three independent study cohorts were included in this analysis. This included a discovery cohort, the Chicago Initiative to Raise Asthma Health Equity (CHIRAH) study (n=321), and two replication cohorts, the Study of African Americans, Asthma, Genes & Environments (SAGE) (n=264) and the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE) (n=468) (Table E1). A subset (n=63) of unrelated African American subjects from the SAPPHIRE study also provided serum to determine levels of BAFF by an ELISA kit which specifically recognized BAFF (R&D systems, Minneapolis, MN). To assess whether rhinovirus induces BAFF expression in airways *in vivo*, we utilized samples from a separate study of the common cold in which asthmatic adults were evaluated within 3 days of onset of a rhinovirus upper respiratory tract infection (proven by Virochip[6] in nasal lavage), and again when asymptomatic at baseline (i.e., at least 42 days following cold symptom onset).[6] *BAFF* mRNA was quantified in nasal mucosal samples through a 2-step quantitative real-time RT-PCR.

We used multivariate, negative binomial regression to assess the relationship between genotype and risk of exacerbation in all 3 cohorts. In SAPPHIRE, Cox proportional hazards models were also used to assess the relationship between genotype and the time to exacerbation. Analysis of variance was used to assess differences in the mean serum levels of BAFF by rs17564816 genotype. Analyses of RV induced BAFF expression in vivo compared levels at a point during the acute illness (1–3 days after onset of URI) to baseline levels (42 days after the onset of the URI) using the Wilcoxon signed rank sum test for non-parametric data. All analyses were performed using SAS v9.1 (SAS Institute Inc., Cary, NC).

Of these 16 genes evaluated (see Table E2), only *BAFF* had an intronic SNP, rs17564816, which neared our criterion for a nominal level of significance in our discovery cohort (meeting bonferroni level of significance for the SNPs typed within a gene) with a relative rate [RR]: −2.96 (p=0.003). This borderline protective finding in the CHIRAH cohort was in

contrast to the associations observed in the two other cohorts (Table 1). Specifically, the minor (A) allele of this SNP was associated with an increase in exacerbation rates in SAGE (RR 1.36, p=0.015). In SAPPHIRE, the A allele was also associated with an increase in ED visits (RR 2.32, p=0.001), oral steroid courses (RR 1.68, p=0.034), and overall exacerbation rates (RR 1.96, p=0.001). Similarly, in SAPPHIRE, the A allele was associated with a shorter time to both oral steroid prescriptions (hazard ratio [HR] 2.37, p=0.001) and ED visits (HR 2.70, p=0.001), as well as for exacerbations overall (HR 2.54, p=0.001). In a random subset of SAPPHIRE study participants, serum levels of BAFF varied by BAFF genotype (Online Figure E2). Those with the AA and AG genotypes had higher levels than those with the GG (homozygous wild type) genotypes (AA=859.3 ± 204.5 pg/ml standard deviation [SD]; AG=821.0 ± 242.5 pg/ml SD; GG=725.6 ± 253.8 pg/ml SD; Jonckheere-Terpstra test for trend p=0.022).

In a separate sample of 15 adult asthmatics with a community-acquired upper respiratory tract infection, the copy number for *BAFF* mRNA was almost five fold higher in nasal epithelial cell samples taken at the time of an active rhinovirus upper respiratory tract infection (i.e., 1–3 days from the onset of symptoms mean gene copy number was $1.52x10^7$) when compared with the post-convalescence baseline sample (i.e., at least 6 weeks after symptom onset the mean gene copy number was $3.09x10^6$) ($p<0.003$, Wilcoxon signed rank test) (Figure 1A). Similarly, nasal lavage samples assayed for BAFF protein showed that BAFF was indeed induced by natural rhinovirus infection in these subjects (Figure 1B) (77.2 ± 107.3 pg/ml) when compared with the post convalescent sample (4.6 ± 6.3 pg/ml) (p=0.05, Mann Whitney test).

In summary, an intronic SNP in the BAFF gene, rs17564816 (A allele), was associated with asthma exacerbations in two African American asthmatic cohorts, time to first exacerbation, and higher serum levels of BAFF. BAFF was also up-regulated in response to community acquired rhinovirus infection, the most common viral infection associated with exacerbations in children and adults.[7, 8] BAFF has been shown to be induced by viruses,[9] and be present in elevated levels in the airway epithelium of children with RSV.[10] The ability of the local tissue to mount a rapid and effective local immunoglobulin response against viruses is likely to be important in limiting of the extent and severity of virus-induced tissue damage. Our group has previously shown that dsRNA (a TLR3 ligand and activator produced by RSV and rhinovirus) potently and effectively induces BAFF production in airway epithelial cells, and that expression of BAFF is regulated by TLR3- and IFN-β-dependent signaling.[11] We hypothesize that higher serum levels of BAFF are associated with a more aggressive immune response to virus, and this may be particularly important since the interaction of innate and adaptive immune responses may provoke asthma exacerbations.[12]

This study has limitations. Namely, there were only 5 subjects in CHIRAH with the minor allele and the allelic relationship in this cohort was in the opposite direction to the other two cohorts. Therefore the findings in CHIRAH may have been spurious. Nevertheless, the A allele of rs17564816 was consistently and robustly associated with asthma exacerbations in both SAPPHIRE and SAGE. Given the low frequency of this allele, we did not evaluate the influence of genotype on BAFF induction by rhinovirus, and further studies will be needed.

In conclusion, we evaluated polymorphisms in viral response genes and found an intronic SNP in the BAFF gene associated with asthma exacerbations in African American subjects in two of three cohorts of patients. This SNP was also associated with higher levels of BAFF compared to individuals homozygous for the G allele. Given the importance of asthma exacerbations in minority urban populations, further studies are needed to determine the genetic and environmental contributors to this complex outcome.

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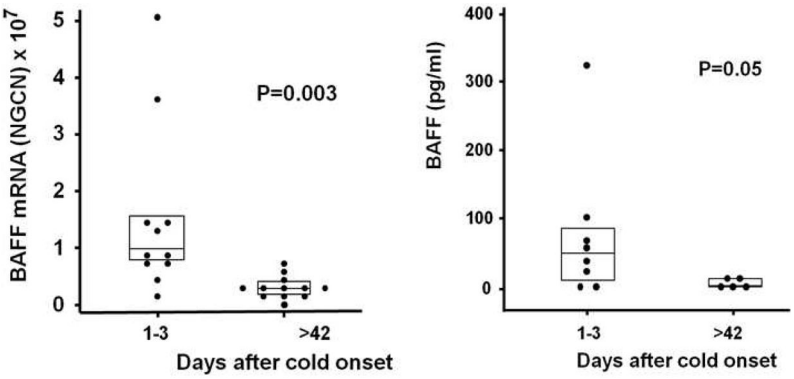

Figure 1. BAFF expression in vivo following Natural Rhinovirus Upper Respiratory Tract Infections

Figure 1A displays the RT-PCR results for BAFF gene copy number in naturally acquired infections. The y-axis represents the BAFF gene copy number. The x-axis represents the timing of the sample in days after cold onset. Figure 1B displays the concentration of BAFF protein in nasal lavage fluids in naturally acquired infections. The y-axis represents the BAFF concentration in pg/ml. The x-axis represents the timing of the sample in days after cold onset.

Table 1

Relationship between *BAFF* polymorphism, rs17564816, and asthma exacerbations in the SAGE and SAPPHIRE cohorts.*

Study cohort	Outcome†	Genetic Model‡	Time-to-event analysis§		Rate of events analysis‖	
			HR	P-value	RR	P-value
SAGE	Composite outcome	Additive	--	--	1.36	0.015
		Dominant			1.40	0.013
		Recessive			1.27	0.40
SAPPHIRE	Oral corticosteroid use	Additive	2.37	0.001	1.68	0.034
		Dominant	2.12	0.003	1.62	0.075
		Recessive	77.41	0.001	6.80	0.058
	Asthma-related ED visits	Additive	2.70	0.001	2.32	0.001
		Dominant	2.75	0.001	2.67	0.002
		Recessive	11.28	0.032	4.29	0.258
	Composite outcome	Additive	2.54	0.001	1.96	0.005
		Dominant	2.36	0.001	2.01	0.007
		Recessive	38.93	0.001	5.06	0.124

BAFF denotes the gene B-cell activating factor; SAGE, Study of African Americans, Asthma, Genes & Environments; SAPPHIRE, the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity; ED, emergency department, HR, hazard ratio; and RR, relative rate.

*Analyses in SAGE were adjusted for patient age, sex, African ancestry, estimated socioeconomic status based on geocoded home address, current smoking status, passive smoke exposure, total serum IgE level, and use of an inhaled corticosteroid. Analyses in SAPPHIRE adjusted for age, sex, African ancestry, educational level, household income, percent of predicted forced expiratory volume at one second, asthma duration, current smoking status, passive smoking exposure, allergic sensitization (defined as =1 allergen-specific IgE value), and baseline corticosteroid use.

†Composite outcome for an asthma exacerbation includes oral corticosteroid use, an asthma-related emergency room visit, or an asthma-related hospitalization.

‡The additive genetic model for rs17564816 was coded as GG=0, AG=1, and AA=2, where A is the minor allele. The dominant model was coded as GG=0 and both AG and AA=1. The Recessive models was coded as both GG and AG=0 and AA=1.

§Time-to-event analyses analyzed with Cox proportional hazard regression models.

‖Rate of event analyses analyzed with negative binomial regression models.

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