Alpha Globin Gene Copy Number and Exhaled Nitric Oxide in Healthy Black Adults

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ABSTRACT

The genetic determinants of fractional exhalation of nitric oxide (FeNO), a marker of lung inflammation, are understudied in Black populations. Alpha globin (*HBA*), which is polymorphic in Black populations, restricts nitric oxide signaling in arterial endothelial cells; however, its role in regulating the release of NO from respiratory epithelium is less well understood. We hypothesized that *HBA* deletions would be associated with higher FeNO. In a cross-sectional study of 643 healthy Black adults, we found no association between *HBA* copy number and FeNO using a prespecified additive genetic model; however, a post hoc recessive genetic model found FeNO to be higher among subjects homozygous for the HBA deletion.

INTRODUCTION

Nitric oxide (NO) has numerous biological activities in the lung, serving as a bronchodilator, vasodilator, neurotransmitter, and inflammatory mediator. In asthmatic individuals, the fractional exhalation of nitric oxide (FeNO) is a validated measure of airway inflammation;[1,2] however, little is known about genetic factors influencing FeNO, particularly among healthy Black individuals.

Recently, a new paradigm of NO regulation in the vasculature has emerged in which endothelial alpha globin binds directly to endothelial NO synthase (eNOS) and limits the release of NO.[3] Genetic deletion of alpha globin, common among Black Americans, is associated with improved NO-mediated vascular perfusion and with protection from kidney disease.[4–6] Alpha globin, beta globin, and eNOS are also expressed in airway epithelium, and while beta globin has been found to interact directly with eNOS to regulate the oxidation of NO, the role of alpha globin in healthy respiratory epithelium remains undefined.[7–10]

To address this gap, we sought to examine whether genomic deletion of the alpha globin gene would be associated with changes in exhaled nitric oxide. We hypothesized that non-asthmatic, non-allergic, healthy Black individuals carrying the *HBA* deletion would have increased respiratory epithelial NO signaling, measured as higher FeNO levels.

METHODS

Healthy black individuals aged 18-40 years were enrolled in a multi-center, cross-sectional cohort at four university sites near Durham, North Carolina from 2005 to 2008.[11] The protocol was approved by the Duke University Institutional Review Board (#Pro00004947). Age, sex, race, and ethnicity were self-reported. Only non-Hispanic African American participants were enrolled. Participants were asked to confirm they were healthy (i.e., no chronic illnesses or chronic use of any medication except oral contraceptives); had no history of asthma, allergic rhinitis, hay fever, or atopic dermatitis; and were nonsmokers. Blood samples were obtained. *HBA* copy number was measured by droplet digital PCR (ddPCR) on genomic DNA. Total

serum immunoglobulin-E (IgE) was measured using the Pharmacia CAP system. FeNO was measured in triplicate with a Sievers 280i Nitric Oxide Analyzer (GE Analytical Instruments, Boulder, Colo) according to American Thoracic Society recommendations.[1] A 50 mL/s flow rate was established against resistance to maintain 5 cmH_2O oropharyngeal pressure.[11] Additional exclusion criteria for this analysis were: not consenting to future research and serum cotinine level $\geq$25 ng/mL signifying active tobacco use.

For continuous measures, medians and 25th and 75th percentiles were calculated by *HBA* genotype. Group differences were assessed by Kruskal-Wallis test. Categorical variables were calculated as percentages and differences were assessed by Fisher's exact test. IgE and FeNO were log transformed due to skewness. The association of *HBA* genotype with FeNO was evaluated using multivariable linear regression employing a linear effect of *HBA* gene copy number with adjustment for age, sex, and total serum IgE levels. BMI was previously found not to be associated with FeNO in this cohort and was not included in the model.[11] Two post-hoc sensitivity analyses were performed: one evaluated *HBA* copy number as a categorical variable and one evaluated a recessive mode of inheritance in which the homozygous deletion genotype (-a/-a) was compared against all other genotypes (-a/aa, aa/aa, and aa/aaa).

RESULTS

Of 895 original study participants, 720 consented for future research and had DNA available for genotyping. Sixty-four participants were excluded due to high cotinine levels and 13 were excluded due to indeterminate *HBA* genotype. The remaining 643 participants were 35% male and had a median (25th,75th) age of 20 (19, 22) years, serum IgE level of 58.3 (22, 160) kU/L, and FeNO value of 20 (13, 31) ppb (Table 1).

HBA deletion was common with 30 (4.7%) -a/-a, 197 (30.6%) -a/aa, 405 (63%) aa/aa, and 8 (1.2%) aa/aaa genotypes. Median (25th, 75th) FeNO was 25 (18, 39) ppm in the -a/-a group, 20 (12, 27) ppm in the -a/aa group, 20 (13, 32) ppm in the aa/aa group, and 37 (9, 52)

ppm in the aa/aaa group (Table 1 and Figure 1). In an unadjusted linear regression analysis using the pre-specified additive genetic model, the coefficient for *HBA* copy number with FeNO was 0.001 (95% CI: -0.039, 0.040; p=0.978). After adjustment for sex, age, and serum IgE, the coefficient for *HBA* copy number with FeNO was -0.005 (95% CI: -0.042, 0.033; p=0.811; Table 2). In post hoc sensitivity analyses, the adjusted association between the homozygous genotype -a/-a and FeNO was 0.099 (95% CI -0.007, 0.206; p = 0.066) when analyzed as a categorical variable and 0.107 (95% CI 0.003, 0.212; p = 0.045; Table 2) when analyzed using a recessive mode of inheritance.

DISCUSSION

Alpha and beta globin have recently emerged as regulators of NO signaling in the vascular endothelium and respiratory epithelium, respectively;[3,10] however, there are few studies evaluating the impact of globin gene variants on NO signaling in vivo. In this study, we characterized a common *HBA* deletion in healthy Black individuals and examined the relationship between *HBA* copy number and FeNO. We found no association between *HBA* genotype and FeNO using a pre-specified additive genetic model; however, a post hoc analysis using a recessive mode of inheritance identified homozygosity for the *HBA* gene deletion to be associated with higher FeNO levels. This latter finding is consistent with the proposed mechanism that alpha globin limits the release of nitric oxide and suggests that lower alpha globin expression allows greater release of NO from respiratory epithelium in healthy, non-asthmatic individuals. More work is needed to understand the role of epithelial alpha globin in the setting of inflammatory lung disease and to determine whether alpha globin interacts with iNOS, which is structurally similar to eNOS, and is expressed under allergic or inflammatory conditions.[12,13]

Study strengths included the large cohort size, representation of an understudied minority population, high frequency of the *HBA* gene deletion, and a well-defined quantitative outcome measure. Adjustment for total serum IgE, which is associated with FeNO, was a strength of this study; however, the absence of evaluation for subclinical IgE sensitization was a limitation. Other limitations included post hoc testing of different genetic inheritance models, performing FeNO measurement at a single flow rate that does not distinguish alveolar from bronchial NO sources,[14] and data on the potential confounders of recent upper respiratory tract infection and eosinophilic cationic protein were unavailable.[11] In conclusion, homozygosity for a common *HBA* gene deletion appears to be associated with higher fractional exhalation of nitric oxide among healthy Black adults.

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DISCLOSURES

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. This article was previously published in abstract form. We certify that the submission is original work and is not under review at any other publication.

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Table 1. Participant characteristics grouped by alpha globin genotype

			HBA genotype								
	All participants		**-a/-a**		**-a/aa**		**aa/aa**		**aa/aaa**		***P* value†**
No. participants*	643		30 (4.7%)		197 (30.6%)		408 (63%)		8 (1.2%)		
Male Sex, No. (%)	222	(35)	12	(40)	66	(34)	142	(35)	4	(50)	0.678
Age, years	20	(19,22)	20	(19,22)	20	(19,22)	20	(19,22)	19	(19,24)	0.833
Mean FeNO‡, ppb	20	(13,31)	25	(18,39)	20	(12,27)	20	(13,32)	37	(9,52)	0.107
Total IgE, IU/mL	58	(22,160)	28	(16,96)	66	(25,176)	57	(21,158)	36	(24,46)	0.125
Body mass index, kg/m2	27	(24,32)	28	(26,33)	27	(23,33)	26	(23,32)	27	(25,34)	0.778
Height, inches	168	(162,175)	170	(161,177)	168	(163,173)	168	(162,175)	168	(163,176)	0.961
Weight, kilograms	77	(65,92)	81	(69,93)	77	(67,93)	76	(65,91)	85	(74,101)	0.716
Systolic blood pressure, mmHg	117	(109,124)	117	(108,130)	117	(110,123)	117	(109,125)	127	(126,133)	0.242
Diastolic blood pressure, mmHg	68	(63,74)	68	(63,73)	67	(62,74)	68	(63,75)	79	(70,85)	0.703
Mean arterial pressure, mmHg	84	(79,90)	85	(80,88)	82	(79,89)	84	(80,91)	99	(91,100)	0.352

No. = number; FeNO = fractional exhaled nitric oxide; ppb = parts per billion; IgE = Immunoglobulin E; IU = international unit; mL = milliliter; mmHg = millimeters of mercury; MAP =

mean arterial pressure. Values are median (25^{th}, 75^{th} percentile) except where otherwise indicated.

* Total number of participants (n=643). Missing data are as follows: Sex (n=2, <1%); Age (n=13, 2%); Mean FeNO (n=4, <1%); Total IgE (n=25, 3.8%}; Body mass index and weight (n=4, <1%); Systolic blood pressure, diastolic blood pressure, and mean arterial pressure (n=292, 45.4%).

† P values calculated for differences between groups by Kruskal-Wallis non-parametric analysis of variance and for categorical variables p values were calculated as percentages within each category and differences were assessed by Fischer's exact test.

‡ Mean FeNO levels measured according to ATS recommendations (reported here as median [25^{th}, 75^{th} percentile] of the mean recorded FeNO)

Table 2. Multivariable regression analysis of *HBA* genotype and fractional exhaled nitric oxide*

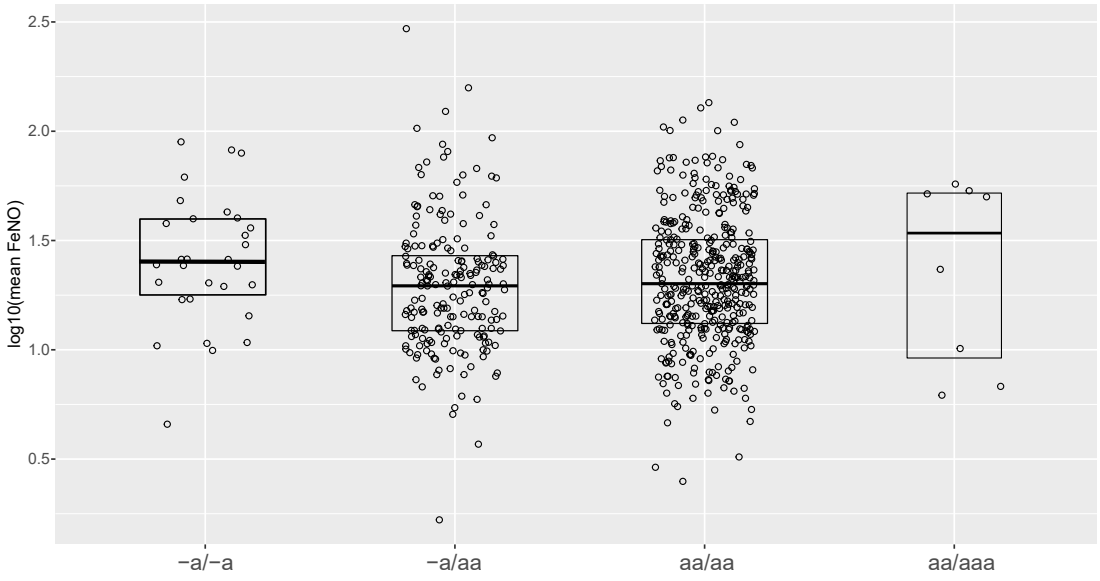
Multivariable linear regression model employing *HBA* genotype as an integer gene copy number			
	Beta Coefficient	95% Confidence Interval	*P* value
HBA copy number, per copy	-0.005	(-0.042, 0.033)	0.811
Age, per year	-0.001	(-0.006, 0.003)	0.587
Male sex	0.122	(0.074, 0.170)	< 0.001
Log_{10} IgE	0.137	(0.098, 0.176)	< 0.001
Post hoc multivariable linear regression model employing *HBA* genotype with a recessive mode of inheritance			
	Beta Coefficient	95% Confidence Interval	*P* value
HBA genotype, -a/-a	0.107	(0.003, 0.212)	0.045
Age, per year	-0.001	(-0.006, 0.003)	0.541
Male sex	0.120	(0.071, 0.166)	< 0.001
Log_{10} IgE	0.140	(0.102, 0.181)	<0.001

HBA = alpha globin gene; No. = number; Log_{10} = log base 10; IgE = immunoglobulin E

* Out of n=643 participants contributing data to the multivariable regression models n=44 participants were excluded due to missing data and n=599 participants were included in the multivariable regression models. Missing data by variable are as follows: Age (n=13, 2%); Sex (n=2, <1%); Total IgE (n=25, 3.8%); Mean FeNO (n=4, <1%).

Figure 1. Fractional exhaled nitric oxide levels in 634 Black individuals grouped by alpha globin genotype.

Log_{10} = log base 10; FeNO = fractional exhaled nitric oxide; aa/aa = alpha globin genotypes; ddPCR = droplet digital polymerase chain reaction. Bold lines represent 50th percentile and upper and lower box lines represent 25th and 75th percentiles for each genotype.



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