

*Original Research Article***Major Gene Effect and Additive Familial Pattern of Inheritance of Asthma Exist Among Families of Probands with Sickle Cell Anemia and Asthma**KEYDRA L. PHILLIPS,¹ PING AN,² JESSICA H. BOYD,³ ROBERT C. STRUNK,³ JAMES F. CASELLA,⁴ BRUCE A. BARTON,⁵ AND MICHAEL R. DEBAUN^{1,3*}¹*Division of Biostatistics, Washington University School of Medicine, St. Louis, Missouri*²*Division of Statistical Genomics, Department of Genetics, Washington University School of Medicine, St. Louis, Missouri*³*Divisions of Allergy and Pulmonary Medicine and Genetics, Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri*⁴*Division of Pediatric Hematology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland*⁵*Maryland Medical Research Institute, Baltimore, Maryland*

ABSTRACT In the United States, sickle cell anemia (SCA) affects approximately 1 in 400 African-American newborns. Acute episodes of pain and acute chest syndrome (ACS) are the two leading causes of hospitalization. A relationship between the diagnosis of asthma and the incidence of pain and ACS has been established. We tested the hypothesis that a familial pattern of inheritance of asthma exists among first degree relatives of probands with SCA and asthma. Segregation analysis was performed in 104 families ascertained through affected probands. Of these, 19.7% (41/208) of the parents and 31.8% (28/88) of siblings of affected probands reported having been told by a doctor he or she had asthma at any age. Modes of inheritance were tested, using the Pedigree Analysis Package parameterized for the discrete trait of asthma affection status. A major effect was present and significant. Further tests were performed to determine whether transmission probabilities of the major effect followed Mendelian expectations. The additive mode of inheritance was the most parsimonious, while the residual heritability was found negligible. Our results support the hypothesis that a familial pattern of inheritance of asthma exists among first degree relatives of probands with SCA and asthma, suggesting that asthma is a co-morbid condition with SCA rather than a lung disease phenotype mimicking asthma. *Am. J. Hum. Biol.* 00:000–000, 2008. © 2007 Wiley-Liss, Inc.

Sickle cell anemia (SCA) is an autosomal recessive genetic disorder caused by homozygosity for an A-T substitution at codon 6 in the β -globin gene. Phenotypic heterogeneity of SCA is likely the result of multiple modifying genetic factors interacting with the mutation causing SCA (Kutlar, 2005). In the United States, SCA affects approximately 1 in 400 African-American newborns each year (Motulsky, 1973). Acute episodes of pain and ACS are the principal complications of SCA and the two leading causes of hospitalization in this population (Castro et al., 1994; Platt et al., 1991). In addition, ACS affects up to 50% of individuals with SCA at least once in their lifetime and is a significant cause of morbidity and mortality in patients with SCA (Gill et al., 1995; Platt et al., 1994; Vichinsky et al., 1997).

Studies from our group and others indicate that children with SCA and asthma have a higher incidence of ACS and pain when compared with children with SCA without asthma (Boyd et al., 2004, 2006; Knight-Madden and Hambleton, 2003; Nordness et al., 2005). Additionally, abnormal lung function common among individuals with asthma has been described among children with SCA with and without a history of asthma. Koumbourlis et al. (2001) determined that lower airway obstruction occurs in approximately 35% of children with SCD, and airway hyper-responsiveness to bronchodilator occurred in 30% of participants who had an otherwise normal pattern of lung function (Koumbourlis et al., 2001). Leong et al. reported that 64% of children with sickle cell disease without a prior diagnosis of reactive airway disease had significant airway hyperresponsiveness to cold air challenge or a bronchodilator (Koumbourlis et al., 2001).

Asthma is characterized by inflammation and airway hyperresponsiveness and is characterized by recurrent

episodes of wheezing. This chronic disease affects approximately 20% of African-American children (Bloom and Dey, 2006 No. 9261; Fagan et al., 2001 No. 9260; Greaves et al., in press; Mvula et al., 2005 No. 9259; Webber et al., 2002 No. 9258; Yeatts and Shy, 2001) and approximately 15% of children with SCA (Boyd et al., 2004, 2006). Asthma is a complex disorder with genetic, phenotypic, and environmental heterogeneity. Interactions among multiple genes (possibly many genes, each with a modest effect, and a few major genes, collectively with a detectable effect) and environmental factors may increase asthma susceptibility. Multiple genome-wide linkage scans of asthma and asthma-associated intermediate phenotypes, including those in the Collaborative Study on the Genetics of Asthma (CSGA), have identified numerous quantitative trait loci across chromosomes, with difficulty in replicating these reported genomic regions across study cohorts (Blumenthal et al., 2004a,b, 2006; Carroll et al., 2005) 15763445.

We propose to test the hypothesis that a familial pattern of inheritance of asthma exists among first degree relatives of probands with SCA and asthma. Failure to identify a familial pattern of asthma among probands and

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their first degree relatives, would suggest that children with SCA who are identified as having asthma-like symptoms most likely have a lung disease as a result of their SCA rather than asthma.

MATERIALS AND METHODS

Study population

This study was approved by the Institutional Review Board at Washington University School of Medicine. The ascertainment strategy for this study was a single encounter for a child meeting the criteria for the Silent Cerebral Multi Center Clinical Trial (SIT Trial), U01-NS042804 and parental designation of ever having a diagnosis of asthma. The SIT Trial was funded by the National Institutes of Health, National Institute of Neurological Disorders and Stroke and is an international clinical study aimed at evaluating the effectiveness of blood transfusion therapy for silent cerebral infarcts, a common serious complication of SCA. No consideration of family history of asthma was made in recruitment of participants in the trial. Patients were recruited to the trial through referral from pediatric hematologist at the 27 study sites in the United States, Canada, England and France. Informed consent was obtained for all patients.

Questionnaire

A questionnaire was completed for each case of SCA in the SIT Trial, for specific genotype phenotype studies. The questionnaire included specific reference to the phenotypic features of SCA and to identify children who had asthma, we used a positive answer to the question from The Third National Health and Nutrition Examination Survey "Has a doctor ever told you that your child has asthma?" First degree relatives of the proband (parents, siblings) were considered to have asthma if reported "yes" to the same question in the questionnaire [National Center for Health Statistics Plan]. This strategy for ascertainment of a lifetime diagnosis has been used by the Centers for Disease Control and Prevention to assess prevalence of asthma and in multiple clinical research studies (Bloom and Dey, 2006; Fagan et al., 2001; Mvula et al., 2005; Yeatts and Shy, 2001).

Definitions

The proband of the family was defined as the individual with SCA who was initially recruited to the SIT Trial. The proband of the family was considered to meet the inclusion criteria for the SIT Trial if they (1) have SCA (hemoglobin SS) or sickle cell β -thalassemia (hemoglobin SB⁰) as confirmed at the local institution, (2) are 6 through 12 years of age, and (4) provide informed consent by patient's legally authorized guardian acknowledging written consent to join the study. Among this group, we selected only those patients that had a clinical diagnosis of asthma as recorded by the parent.

Statistical analysis of demographics

Statistical analysis was performed using SAS computer software. The χ^2 test and Fisher's exact test were applied to evaluate the significance of differences in proportion. The 95% confidence interval of proportions was calculated

using the method for independent proportions (Newcombe, 1998).

Segregation analysis

The segregation analysis was performed using the Pedigree Analysis Package (PAP) (Hasstedt, 2002). PAP was parameterized for three discrete traits with known prevalence of asthma, through the selection of appropriate sub-routines (Hasstedt, 1993). Using this model, the assumed disease trait was determined by independent contributions of a major effect and a polygenic residual heritability. The major gene effect was assumed to be a result of the segregation of two alleles (A and a) at a single locus in Hardy-Weinberg Equilibrium. Each model in the analysis was described by the following parameters: (1) the displacement in liability of disease between two homozygotes, AA and aa (t); (2) the polygenic residual heritability describing familial similarity not due to the major effect (H); (3) the frequency of the A allele (p); (4) the relative position of the heterozygote Aa in liability respect to the homozygotes, or dominance (d); and (5) the transmission probabilities for the major effect (τ_{AA} , τ_{Aa} , τ_{aa}). These transmission probabilities will describe the pattern of inheritance of the modeled major effect.

In the analysis, two major classes of models were tested: models with Mendelian transmission probabilities and models in which the transmission probabilities were estimated from the data. The first class were Mendelian models, in which $\tau_{AA} = 1$, $\tau_{Aa} = 0.5$, $\tau_{aa} = 0$, included: mixed model (P , d , t , H), sporadic inheritance ($P = 1$, $d = 0$, $t = 0$, $H = 0$), polygenic inheritance without a major effect ($P = 1$, $d = 0$, $t = 0$), no major effect ($P = 1$, $d = 0$, $t = 0$, H), major effect only (P , d , t , $H = 0$), recessive with ($d = 0$) and without ($d = 0$, $H = 0$) polygenic effects, additive with ($d = [1/2]$) and without ($d = [1/2]$, $H = 0$) polygenic effects, dominant with ($d = 1$) and without ($d = 1$, $H = 0$) polygenic effects, and a general model with and without ($H = 0$) a polygenic effect. Hypothesis tests for the Mendelian models were carried out with respect to the mixed model. The second class of models in which the transmission probabilities were estimated, consisted of a nontransmittal model ($P = \tau_{AA} = \tau_{Aa} = \tau_{aa}$) and a general model.

The models tested were evaluated in terms of their goodness-of-fit to the data. The goodness-of-fit was evaluated using the likelihood ratio test (LRT), which is the difference between minus two times the natural log of the likelihood ($-2 \ln L$) obtained under two nested models. The likelihood ratio is approximately distributed as a χ^2 , with the degrees of freedom being equal to the difference in the number of parameters estimated in two nested models. A probability value (or P -value) of $<5\%$ in the LRT was taken to indicate a model that represented a significantly worse fit of the data than the mixed model and was rejected. Models tested were evaluated in terms of their parsimony. The parsimony of each model was evaluated using Akaike's information criterion (AIC), which is $-2 \ln L$ plus twice the number of estimated parameters in a given model. The model with the lowest AIC is the most parsimonious model (Akaike, 1974). Three conditions were considered necessary to infer Mendelian transmission of the disease, (1) rejection of the no major effect hypothesis; (2) nonrejection of Mendelian τ 's; (3) rejection of equal transmission probabilities (Lalouel et al., 1983).

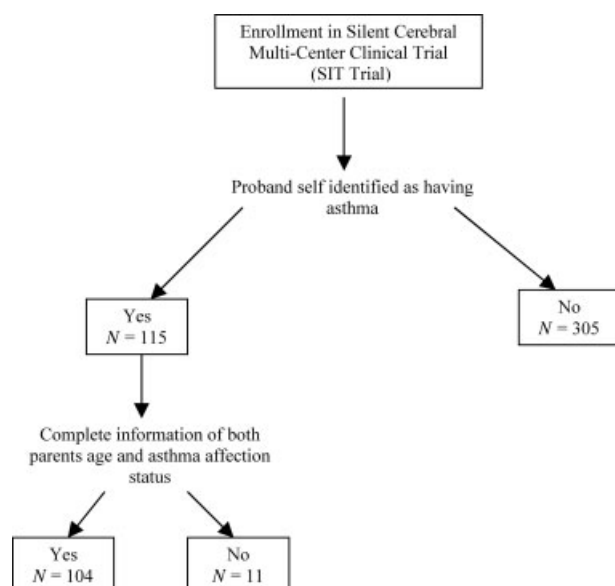


Fig. 1. Flow diagram describing the study participants.

RESULTS

Patient demographics

A total of 420 pedigrees were derived from the SIT Trial during the sampling from December 2004 to June 2006. Among this group, 115 pedigrees (27%) met our criteria for inclusion, a doctor diagnosis of asthma in the child as reported by the parent. Eleven patients were excluded because of either incomplete information on the asthma status of the parents or missing age of one of the parents. Finally, the segregation analysis was performed in 104 nuclear families (all full-siblings were used, with a total of 193 offspring, of which 55% were single offspring families, and 45% were multiple offspring families with sibling size ranging from 2 to 7) Figure 1. Of these 104 families, 19.7% (41/208) of the parents, 23.1% (24/104) of mothers, 16.3% (17/104) of fathers, and 31.8% (28/88) of siblings, were reported as having been told by a doctor he or she had asthma at any age. Of the siblings (28/88) with asthma, 60.7% (17/28) were male. Of these families, 94.2% (98 of 104) were Black or African-American not Hispanic, 1% (1 of 104) were white not Hispanic, and 4.8% (5 of 104) reported "other" ethnicity. A total of 58.7% (61 of 104) of the probands were male. The demographic characteristics of the participants were given in Table 1.

Additive model is most parsimonious Mendelian model

The results of a formal segregation analysis in which models of inheritance were tested suggested that an additive mode of inheritance was the most likely to fit the pedigrees in our cohort. A total of 400 individuals from the 104 nuclear families were included in the analysis. The no major effect model and sporadic inheritance model were both rejected ($\chi^2 = 58.954$, $df = 4$, $P < 0.0001$ and $\chi^2 = 58.954$, $df = 3$, $P < 0.0001$, respectively). The major effect only model was accepted with ($\chi^2 = 2.608$, $df = 1$, $P = 0.1063$). The recessive and dominant modes of inheritance

TABLE 1. Demographic features of the children with sickle cell anemia registered in the silent cerebral infarct multi-center clinical trial

Demographic feature	Asthma cases (N = 115)	Non-asthma cases (N = 305)
Age (mean $\pm$ SD)	10.41 $\pm$ 2.41	10.20 $\pm$ 3.15
Gender	58.04% males 98.26%	47.64% males 98.02%
Ethnicity	Non-Hispanic or Latino 94.78%	Non-Hispanic or Latino 96.37%
Race	African-American or Black	African-American or Black
Number of siblings (mean)	2.72	2.52
Complete family history of asthma	104	289

were both rejected with ($\chi^2 = 24.270$, $df = 2$, $P < 0.0001$) and ($\chi^2 = 58.953$, $df = 2$, $P < 0.0001$, respectively). However, the additive mode of inheritance (with a negligible residual heritability, <5%) was accepted with ($\chi^2 = 3.220$, $df = 2$, $P = 0.1999$) and was most parsimonious with AIC = 671.424.

Asthma inheritance is consistent with Mendelian inheritance under additive mode

Our cohort met the three conditions necessary to infer Mendelian transmission of asthma. The sporadic inheritance model was rejected ($P < 0.0001$). The major effect only and additive models were accepted ($P = 0.1063$ and $P = 0.1999$), and the non-transmittal model was rejected ($P < 0.0001$). The general model was accepted ($P = 0.7356$) when compared to the mixed model and the estimated parameters were consistent with Mendelian Inheritance (Table 2). The parameter estimates for the segregation analysis were listed in Table 3.

DISCUSSION

Distinguishing between whether a child has an acute asthma exacerbation or SCA related lung disease such as acute chest syndrome (ACS) is important for acute treatment and, long-term management, because standard treatments to prevent asthma exacerbations are well established (National Institutes of Health, 1997). Presence of familial history of asthma would support a diagnosis of asthma in a child as opposed to asthma-like symptoms occurring due the SCA. We have documented in this study and in previous work, that asthma is not only present among children with SCA, but it also common, occurring in approximately 25% of this cohort of children selected without regard to asthma and who are at least six years of age. The prevalence of asthma in this cohort is similar to the prevalence of asthma among African-American children without sickle cell disease which is estimated to be, approximately 20% (Bloom and Dey, 2006; Fagan et al., 2001; Greaves et al., 2007; Mvula et al., 2005; Weber et al., 2002; Yeatts and Shy, 2001). Evidence of genetic basis of (7–10) of asthma is well-documented after many years' genetic assessment efforts. More than a dozen of genome-wide linkage scans of asthma and asthma-associated intermediate phenotypes, including those in the CSGA, have been carried out that identified a good number of quantitative trait loci across chromosomes. Our results indicate that a major gene effect was present and

TABLE 2. Segregation analysis of asthma in 104 families with first degree relatives and affected probands

Model	df	2 ln L	χ^2	P	AIC
Tests for major effect					
Mixed model (P, d, t, H)	—	664.204	—	—	672.204
Sporadic model ($P = 1, d = 0, t = 0, H = 0$)	4	723.158	58.954	<0.0001	723.158
No major effect model ($P = 1, d = 0, t = 0, H$)	3	723.158	58.954	<0.0001	725.158
Major effect only model ($P, d, t, H = 0$)	1	666.812	2.608	0.1063	672.812
Tests for mode of inheritance					
Recessive ($P, d = 0, t, H = 0$)	2	688.474	24.270	<0.0001	692.474
Additive ($P, d = 1/2, t, H = 0$)	2	667.424	3.220	0.1999	671.424*
Dominant ($P, d = 1, t, H = 0$)	2	723.157	58.953	<0.0001	727.157
Tests for Non-Mendelian inheritance					
General model ($P, \tau_{AA}, \tau_{Aa}, \tau_{aa}, d = 1/2, t, H = 0$)	1	664.089	0.114	0.7356	674.089
Non-transmittal ($P = \tau_{AA} = \tau_{Aa} = \tau_{aa}, d, t, H = 0$)	3	723.158	59.069	<0.0001	727.158

*Indicates most parsimonious model.

TABLE 3. Parameter estimates for segregation analysis of asthma in 104 families with first degree relatives and affected probands

Model	A allele frequency	Three transmission probabilities			Dominance	Displacement	Heritability
	P	τ_{AA}	τ_{Aa}	τ_{aa}	d	t	H
Mixed model (P, d, t, H)	0.9281	[1]	[1/2]	[0]	0.600	6.000	0.0500
Sporadic model ($P = 1, d = 0, t = 0, H = 0$)	[1]	[1]	[1/2]	[0]	[0]	[0]	[0]
No major effect model ($P = 1, d = 0, t = 0, H$)	[1]	[1]	[1/2]	[0]	[0]	[0]	0.0000
Major effect only model ($P, d, t, H = 0$)	0.9249	[1]	[1/2]	[0]	0.200	23.000	[0]
Recessive ($P, d = 0, t, H = 0$)	0.6166	[1]	[1/2]	[0]	[0]	8.300	[0]
Additive ($P, d = 1/2, t, H = 0$)	0.9300	[1]	[1/2]	[0]	[1/2]	28.000	[0]
Dominant ($P, d = 1, t, H = 0$)	0.0392	[1]	[1/2]	[0]	[1]	10.000	[0]
Non-Mendelian τ 's							
General model ($P, \tau_{AA}, \tau_{Aa}, \tau_{aa}, d = 1/2, t, H = 0$)	0.9283	1	0.470	0.050	[0.500]	6.000	[0]
Non-transmittal ($P = \tau_{AA} = \tau_{Aa} = \tau_{aa}, d, t, H = 0$)	[0.8000]	[0.800]	[0.800]	[0.800]	0.500	14.000	0.0000

[] Denotes that the parameter was set and not estimated.

significant. The major effect followed a Mendelian pattern, suggesting that the major effect was the result of a major gene component. In our analysis, the residual heritability was negligible, which supports the major effect only model being accepted. Thus, the major effect was genetic in origin and was not due to random environmental exposures. Subsequently, the nontransmittal model was rejected, providing more evidence a major gene effect. While there is some evidence that a gene may be segregating in our data, we suggest consideration of the possibility of more than one gene with similar influences, which is consistent with previous studies suggesting oligogenic characteristics of physician-diagnosed asthma (Holberg et al., 1996; Jenkins et al., 1997; Lawrence et al., 1994).

Our results demonstrating a familial pattern of inheritance of asthma among children with SCA and their first degree relatives are similar to the results of children with asthma and without SCA and their first degree family members. On the basis of a similar method of ascertaining asthma status, namely physician diagnosis, multiple studies have consistently demonstrated a familial pattern of inheritance. A previous segregation analysis of physician-diagnosed asthma based on the data from 906 nuclear families in Tucson, Arizona, suggested that a recessive component may influence expression of the physician diagnosed asthma (Holberg et al., 1996). In another study, the inheritance of asthma was mediated by a strong familial component, taking the form of a recessive Mendelian mode of inheritance (Wang et al., 2000). In the Humboldt study, the most parsimonious model was a recessive mode

of inheritance, with and without multifactorial effects (Chen et al., 2001). Other studies suggested a co-dominant mode of inheritance of asthma (European Community of Respiratory Health Survey Group, 1997). Less strong support for the major gene effect of asthma was provided in a study of 7,394 individuals in Australia, where a non-Mendelian and a Mendelian co-dominant model best fit the data and lead to the conclusion that the inheritance of asthma could not be fully described by a single gene (Jenkins et al., 1997). One study showed no evidence for a major gene when the data were tested with and without residual family effect (Martinez and Holberg, 1995). Also, in another study there was lack of convincing evidence of a major gene effect on physician diagnosed asthma (Holberg et al., 1996). Taken together we have provided further evidence that asthma occurs as a co-morbid condition in SCA.

Cautions should be used in interpreting these findings. First, ascertainment correction has not been applied because of experiencing model convergence difficulty possibly due to limited family numbers, small sample size, and discrete phenotype of asthma status, the results thus may not be generalized. Second, the estimate of asthma risk allele frequency of 7% was solely derived from this selected family data, which may not be directly applicable to general populations.

Our study design has some inherent limitations. We relied on self-reported questionnaire data for the asthma status in family members. The questionnaire was generally completed by a parent, who was assumed to have

accurate knowledge of the asthma status of their children and other family members. This strategy may be associated with inaccuracy and could introduce bias associated with spurious dominant patterns, whereas in our data the most likely pattern was that of additive inheritance. Even if children of asthmatics were more likely to receive a physician diagnosis or parental self-report of asthma to a certain degree, such a reporting bias would not explain the major gene effect with negligible residual heritability observed. Any segregation analysis has constraints, and due to the use of a discrete trait and small sample size, standard error estimates could not be calculated.

Further theoretical work will be required to confirm that the assumptions, based on discrete traits, also apply to continuous traits such as IgE measurements. However, our study population of probands with SCA and physician-diagnosed asthma in a population of individuals with a single gene disorder, SCA, is unique and provides support for a genetic basis for modification of sickle cell disease. The results of our segregation analysis provide evidence for the high prevalence of asthma among children with SCA, the presence of a major gene effect and an additive mode of inheritance of asthma among first degree relatives of children with SCA and asthma.

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