MUC7 Polymorphisms Are Associated With a Decreased Risk of a Diagnosis of Asthma in an African American Population

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Purpose: Mucin glycoproteins contribute to lung pathophysiology in asthma. The protein backbone of mucin glycoproteins is encoded by specific *MUC* genes, which exhibit a high degree of polymorphisms that generate a variable number of tandem repeat (VNTR) domains. *MUC7* typically encodes for 6 VNTRs, each with 23 amino acids. In a northern European cohort, a polymorphism encoding *MUC7*5* (5-VNTR) is in 100% linkage disequilibrium with the single nucleotide polymorphism rs9982010 and associated with a decreased risk of being asthmatic and having better lung function. African Americans have a 5- to 10-fold increase in incidence of asthma relative to whites, who are believed to be partially associated with higher genetic susceptibility. Occurrence of the rs9982010 and *MUC7* allelic frequencies was evaluated in inner-city African Americans to test their association with a diagnosis of asthma.

Methods: Genomic DNA, collected from a cohort of African American asthmatic subjects, was used to detect the *MUC7* VNTR polymorphisms and to analyze the rs9982010 single nucleotide polymorphism.

Results: A logistic regression analysis showed that the *MUC7*5*-VNTR allele decreased the likelihood of a diagnosis of asthma (odds ratio, 0.173 [95% confidence interval, 0.041–0.737]; $P < 0.018$) and is not in a strong linkage disequilibrium with the rs9982010 ($r^2 = 0.03$; odds ratio, 66; confidence interval, 5.913–736.72). A novel *MUC7*4*-VNTR polymorphism, identified in an African American nonasthmatic individual, was linked to a structural rearrangement of the VNTR domain.

Conclusions: These data extend the association of *MUC7*5* allelic polymorphisms and asthma to inner-city African Americans.

Key Words: mucin genes, asthma, genetic polymorphism, African American, inner city

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Asthma is a complex, multifactorial disease reflecting genetic and environmental components. Asthma is now regarded as having multiple different subtypes rather than being a single disease entity.[1] The morbidity and mortality associated with asthma are disproportionately high among minority pediatric populations, particularly those who reside in densely populated inner-city areas.[2–6] African Americans are hospitalized for asthma 3 times more often than other Americans, and African Americans living in inner cities are 2 to 6 times more likely to die of asthma.[7] Inner-city and social risk factors likely explain some of the disparities in incidence; however, genotype is an important determinant of host immune responses and contributes to the overall predisposition of individuals to asthma. The contributions of genetic background to the development of asthma in minority populations are understudied.

Mucin glycoproteins (mucins) are the major macromolecular components of the lung mucous layer, which protects the respiratory tract epithelium against infectious agents, allergens, and environmental toxins. Mucins are overproduced in asthma and other lung diseases and contribute to airway pathophysiology and thus to disease morbidity and mortality.[8–12] *MUC* genes encode the protein backbone of mucins, and most of the *MUC* genes have polymorphisms that encode a variable number of tandem repeats (VNTRs)[13] (reviewed by Rose and Voynow[12]) that can result in differences in the length of the protein backbone. Genetic analyses of some *MUC* genes have been carried out in patients with atopy and/or asthma. A longer VNTR length in the *MUC2* gene is associated with a cohort of atopic, nonasthmatic patients, but no associated differences with asthma and VNTR domains of *MUC1*, *MUC4*, *MUC5AC*, or *MUC5B* genes have been found.[14]

However, a study using a northern European cohort showed an association between the risk of having a diagnosis of asthma and a polymorphism in *MUC7*.[15] Typically, *MUC7* encodes 6 nonperfect VNTRs (*MUC7*6*) of 23 amino acids, with the less common *MUC7* polymorphic variant containing 5 VNTRs (*MUC7*5*).[16] A study by Kirkbride et al.[15] in a northern European cohort identified an association of the *MUC7*5* allele with a decreased risk of an asthma diagnosis. A subsequent study identified the rs998210 single nucleotide polymorphism (SNP) in 100% linkage disequilibrium (LD) with the *MUC7*5* polymorphism in the same northern European population.[17] Because African Americans have a higher prevalence of asthma, we hypothesized that they would also have a lower prevalence of the apparently protective *MUC7*5* allele. We therefore investigated the *MUC7* VNTR domain and the occurrence of the rs998210 SNP in the *MUC7* gene to determine its association with asthma in inner-city African American subjects. These 2 polymorphisms were focused on because they are the only *MUC7* polymorphisms that have been associated with asthma to date.

MATERIALS AND METHODS

Human Subjects

The Asthma Severity Modifying Polymorphisms (AsthMaP) project provided asthmatic patient samples for our study. The

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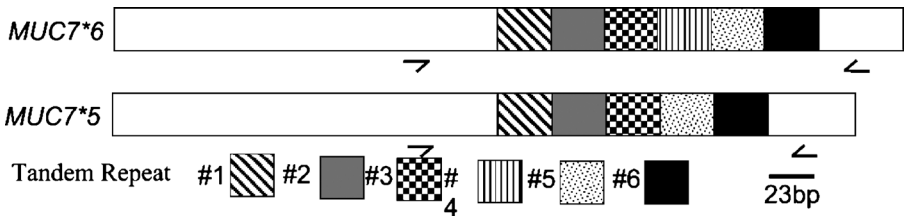

FIGURE 1. Schematic of *MUC7* complementary DNA. Each of the TRs, 69 bp in length, is identified. The arrows on either side of the TR domain indicated the location of primers used for genotyping.

AsthMaP is a study of gene-environment interactions in inner-city pediatric asthma patients treated at Children's National Medical Center, Washington, DC. The nonasthmatic controls were adolescents selected from an ongoing genetic study at Children's National Medical Center on metabolic syndrome in inner-city adolescents.

DNA Isolation

Whole blood or buccal swab samples were collected from asthmatic individuals and nonasthmatic controls under an institutional review board–approved protocol. Genomic DNA was isolated by standard protocols.

MUC7 VNTR Polymorphism Genotyping

The approach used by Rousseau et al.[17] was used to evaluate the VNTR polymorphisms in the genomic *MUC7* gene for each subject. Briefly, polymerase chain reaction (PCR) amplification of genomic DNA was carried out using primers designed to span the entire VNTR domain. The location of the primers is indicated by arrows in Figure 1. The sense primer 5′-cagaatgccaccaccatatcttcaa-3′ and the antisense primer 5′-ggtg caagagtagttggggaagaat-3′ are located at nucleotides 400 to 425 and 959 to 984, respectively, on the genomic *MUC7* DNA in exon 3 (chr 4q13-q21; accession number, L13283).

DNA Sequencing

Polymerase chain reaction products were electrophoresed on a 2% ethidium bromide agarose gel and visualized on a Chemidoc Imager (BioRad, Hercules, CA). Bands identified for DNA sequencing were excised and extracted using QiaQuick gel extraction kit (Qiagen, Valencia, CA), ligated into pCRII-TOPO (Invitrogen, Carlsbad, CA), and sequenced (Davis Sequencing, Davis, CA).

Single Nucleotide Polymorphism Analysis

TaqMan SNP Genotyping Assays analysis of the rs998210 SNP was carried out using specifically designed kits (Applied Biosystems, Foster City, CA) on an ABI 7900HT TaqMan machine. The SNP genotyping assay targeted to *MUC7* determined the C/T transition, located at chr 4-71380925.

Statistical Analysis

The frequency of each *MUC7* polymorphism was imported into a contingency table (Table 1). A χ^2 test was then used to statistically evaluate whether there was an association between the *MUC7* polymorphisms and asthma. To evaluate the associated risk of an asthma diagnosis and the *MUC7* allelic polymorphisms in an African American cohort, logistic regression models (Stata v10; StataCorp, College Station, TX) were used to generate relative odds ratios (ORs) and 95% confidence intervals (CIs). These analyses were repeated to determine the association of the *MUC7*5* polymorphism and the rs998210 SNP in the same population. Hardy-Weinberg equilibrium was tested for each SNP using a 1–degree of freedom χ^2 test.

Because of the limited availability of a control population, a post hoc power analysis was performed to determine the likelihood of finding a significant difference.

RESULTS

Demographics

The sample population was limited by the enrollment of the pediatric population in the AsthMaP study and other non–airway related studies. Our population comprised 84 inner-city patients in the AsthMaP study and 37 nonasthmatic controls. The age and sex of the cohort are reported in Table 2. Overall, the sample population was 46% male and 57% asthmatic patients.

The post hoc power analysis comparing the TR polymorphism in asthmatic patients and nonasthmatic controls, whose sample sizes were of 84 and 37 respectively, showed that we had a 66% power to detect a significant difference ($P < 0.05$).

Allelic Variation in the Number of VNTR Domains

The 2 previously reported *MUC7* VNTR polymorphisms, *MUC7*6* and *MUC7*5*, were identified in our study. They were observed as either homozygous 6/6 (Fig. 2, lane 1: 559 base pairs [bp]) or heterozygous 6/5 (Fig. 2, lane 2: 490 bp) allelic pairings.

We also identified, in a control subject, what looked like a novel polymorphism predicted to encode 4 VNTRs (Fig. 2, lane 3: 421 bp), as the amplicon corresponded to 69 bp (size of a single TR) less than *MUC7*5*. To assess this, DNA from the PCR product (Fig. 2) was sequenced and shown to encode 4 VNTRs. The first encoded VNTR repeat domain contained 2 SNPs that altered the genotype and resulted in changes in the amino acid sequence (Fig. 3). The SNPs resulted in a P169T and a T176S change, indicated in Figure 3 as TR1Δ2 with

TABLE 1. Frequencies of the *MUC7* Polymorphic Alleles

	Control			Asthma	
TR	*6	*5	*4	*6	*5
No. people	67	6	1	165	3
Allelic frequency	0.91	0.08	0.01	0.99	0.01

TABLE 2. Demographic Data on the Human Subject Population

	Total Population	Male	Female	Age Range (Mean), yr
Control	37	13	24	14–20 (18)
Asthma	84	45	39	4–18 (10)

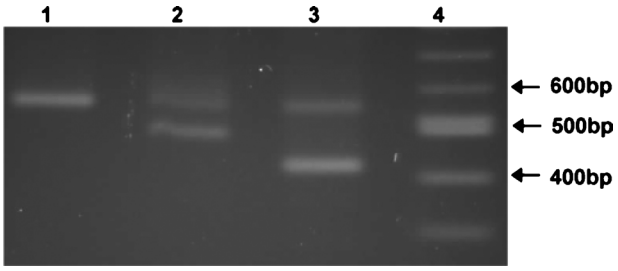

FIGURE 2. Electrophoresis of representative PCR products showing allelic variation in the *MUC7* TR domain. Amplicons were separated on a 2% agarose gel (predicted sizes are 6*, 559 bp; 5*, 490 bp; and 4*, 421 bp). Lane 1, 100 bp ladder; lane 2, 6*/4* alleles; lane 3, 6*/5* alleles; lane 4, 6*/6* alleles.

highlighted changes. Unlike *MUC7*5*, *MUC7*4* showed a rearrangement of the order of its VNTR domains as TR1Δ2 followed by TR2, TR1, and TR2 (Fig. 3). This previously unidentified *MUC7* VNTR is now designated *MUC7*4*.

Frequency Analysis of the *MUC7*6* and *MUC7*5* Allelic Polymorphisms

DNA samples from nonasthmatic and asthmatic patients (Table 2) were analyzed for the frequency of each polymorphism. The frequencies identified in the asthmatic population were *MUC7*6* allele, 0.99 and *MUC7*5* allele, 0.01. In the control population, the frequency results were *MUC7*6* allele, 0.91; *MUC7*5* allele, 0.08; and *MUC7*4* allele, 0.01 (Table 1).

We evaluated the expression of *MUC7* allelic polymorphisms and the associated risk of having a diagnosis of asthma both in the AsthMaP and control cohorts. A logistic regression analysis of the association of *MUC7*5* allelic polymorphism and not having a diagnosis of asthma gave an OR of 0.173 with a 95% CI of 0.041–0.737 and a *P* value of 0.018 (Table 3). These data were supported by a χ^2 analysis showing a significant association of *MUC7*5* ($P = 0.01$) with not having a diagnosis of asthma as a child.

Single Nucleotide Polymorphism Analysis of rs998210

The previously identified SNP rs9982010 in the second intron of the *MUC7* gene was earlier shown to be in 100% LD with the *MUC7*5* allelic polymorphism in a northern European cohort.[17] This SNP was also analyzed in our African American cohort. Our data showed that with the T-to-C conversion, there is a low LD between the C/T SNP and the *MUC7*5* allelic polymorphism. These data show that the African American population only showed an LD measured by $r^2 = 0.03$. These findings are not in concordance with the results in the northern European cohort where a 100% LD with the polymorphic allele is observed. Both the *MUC7*5* and the SNP were in Hardy-Weinberg equilibrium (*MUC7*5* polymorphism, $P = 0.12$; rs998210, $P = 0.29$). A logistic regression analysis performed between the *MUC7*5* allelic polymorphism and the rs9982010 SNP showed an OR of 66.0 ($P < 0.0001$; 95% CI, 5.913-736.72), indicating that the African American individuals with the *MUC7*5* allelic polymorphism were 66 times more likely to have the T-to-C conversion in rs9982010, irrespective of asthma.

TABLE 3. Logistic Regression Analysis of the *MUC7* VNTR Polymorphisms and Their Association With Being Asthmatic

Genotype	Cases	Controls	OR	*P*	95% CI
6/6	81	28	1.0		
6/5	3	6	0.173	0.018	0.041–0.737

DISCUSSION

African Americans have an increased incidence of asthma and are 3 times more likely to be hospitalized with asthma-related symptoms. In addition, African Americans living in inner cities are 2 to 6 times more likely to die of asthma.[7,18] An individual's overall genotype and environment can predispose one to asthma, but the contributions of genetic background to the risk of having a diagnosis of asthma are uncertain.

Mucin overproduction is implicated in asthma.[12] Thus, an association of *MUC* genes with asthma was carried out by Swallow and co-workers. The data showed no association of VNTR numbers in allelic variants in the *MUC1*, *MUC2*, *MUC4*, *MUC5AC*, and *MUC5B* genes.[14] However, this group identified a polymorphism in the *MUC7* gene, *MUC7*5*, with an allelic frequency of 0.10 associated with a decreased risk of having a diagnosis of asthma in a northern European asthmatic cohort.[15]

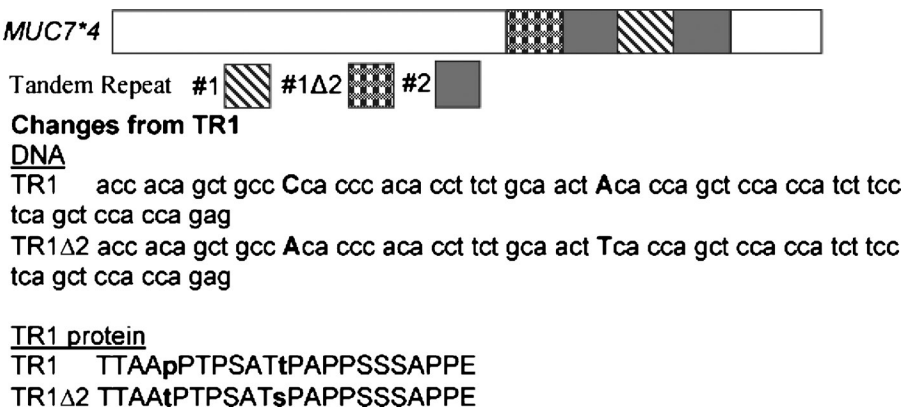

FIGURE 3. Schematic of MUC7*4 complementary DNA/protein showing the changes in the VNTR domain. The order of the repeats is TR1Δ2, TR2, TR1, and TR2. DNA and amino acid sequence of TR1 is compared with the altered TR1 (TR1Δ2) in the *MUC7*4* allelic polymorphism.

This data suggested that the *MUC7*5* allele was protective against asthma, and a subsequent longitudinal study supported the concept that the *MUC7*5* allele had a protective effect on respiratory function.

Because minority pediatric populations in inner cities have a higher prevalence of asthma, we predicted that our cohort would have a lower prevalence of the *MUC7*5* allele if it were protective. Our data showed an allelic frequency of 0.05, which is lower than that observed (0.1) for the *MUC7*5* allele in a northern European cohort.[15] Interestingly, this is supported by a small African cohort (n = 29) that also shows a reduction in the *MUC7*5* allele frequency[15] of 0.052. Although each of the 2 sample sets—northern European cohort and inner-city African Americans—are relatively small, the combined data support the hypothesis of a reduction in the frequency of the protective *MUC7*5* allelic polymorphism in a population that is of greater risk of having a diagnosis of asthma.

In the northern European asthmatic cohort, the rs998210 intronic SNP in the *MUC7* gene has been shown to be in 100% LD[17] with the *MUC7*5*. Our data showed that this SNP is significantly associated with the *MUC7*5* polymorphism (P = 0.0007) in African Americans, but not at 100% LD. Rousseau et al.[17] suggested that this SNP might not be functional, as it does not reside in an identified motif region. These data on *MUC7* allelic polymorphisms highlight one example where a small genetic difference between ethnically diverse populations could impact the susceptibility of having a diagnosis of asthma, especially in a high-risk inner-city population.

Although *MUC7*6* and *MUC7*5* seem to be the 2 most predominant alleles in the human population, unique *MUC7* alleles have been identified, for example, *MUC7*8* in a northern European cohort with atopic asthma.[15] Herein, we identified a novel *MUC7*4* polymorphism in an African American non-asthmatic individual that resulted in a reduction in the number and rearrangement of the encoded TR domains. Two SNPs within the first TR resulted in a change in the amino acid sequence of the first encoded TR (TR1Δ2). The VNTR domains of *MUC7*4* is TR1Δ2, TR2, TR1, and TR2, in contrast to TR1 to 6 of *MUC7*6* and TR1, 2, 3, 5, and 6 of *MUC7*5*.

The role of mucins in the mucosal immune system is only beginning to be understood at the molecular level.[19] Mucins are overproduced in acute and chronic airway diseases and contribute to the disease morbidity and mortality. MUC7 is a small secreted mucin glycoprotein (180 kd) expressed predominantly in the submandibular and sublingual glands[20] and salivary secretions.[21,22] MUC7 has been shown to bind to bacteria, and small recombinant MUC7 peptides exhibit antibacterial, antifungal,[22–24] and antiviral properties.[25] We have recently shown that MUC7 mucin is present in the airway secretions of asthmatic, but not control, pediatric patients,[26] suggesting that MUC7 mucin may have a role in the pathophysiology of asthma. MUC7, like all mucins, is highly *O*-glycosylated, and alterations in the sequence and number of encoded VNTR domains could have a significant impact on its biochemical properties and biological functions and thus its role in diseases. Future studies will be needed to determine mechanisms by which polymorphisms in the *MUC7* gene alter the host innate immune response of MUC7 mucin and its relevance to asthma.

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