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# *MUC7* polymorphisms are associated with a decreased risk of being diagnosed with asthma in an African-American population

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## Abstract

Mucins glycoproteins contribute to lung pathophysiology in asthma. The protein backbone of mucin glycoproteins are 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 VNTR, 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 5 to 10-fold increase in the incidence of asthma relative to Caucasians, 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 being asthmatic. A logistic regression analysis showed that having the *MUC7*5*-VNTR allele decreased the likelihood of being asthmatic (OR=0.173 (CI: 0.041–0.737) and *p*-value of <0.018) and not in a strong linkage disequilibrium with the rs9982010 ($r^2$=0.03; OR=66; CI: 5.913–736.72). A novel *MUC7*4*-VNTR polymorphism, identified in an African-American non-asthmatic individual, was linked to a structural rearrangement of the VNTR domain. These data extend the association of *MUC7*5* allelic polymorphisms and asthma to inner-city African-Americans.

### Keywords

mucin genes; asthma; genetic polymorphism; African-American; inner-city

## INTRODUCTION

Asthma is a complex, multifactorial disease reflecting genetic and environmental components. Asthma is now regarded as having multiple different subtypes rather than being

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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 three times more often than other Americans, and African Americans living in inner-cities are two to six times more likely to die from 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 mucus layer, which protects the respiratory tract epithelium against infectious agents, allergens and environmental toxins. Mucins are overproduced in asthma, as well as 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 (VNTR) [13] [reviewed in [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, non-asthmatic 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 being diagnosed with asthma and a polymorphism in *MUC7* [15]. Typically, *MUC7* encodes 6 non-perfect VNTR (MUC7*6) of 23 amino acids; with the less common *MUC7* polymorphic variant containing 5 VNTR (*MUC7*5*) [16]. A study by Kirkbride et al. (2001) in a Northern European cohort identified an association of the *MUC7*5* allele with a decreased risk of an asthma diagnosis [15]. 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]. Since 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 single nucleotide polymorphism (SNP) in the *MUC7* gene to determine its association with asthma in inner-city African American children. These 2 polymorphisms were focused on as they have 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. AsthMaP is a study of gene-environment interactions in inner-city pediatric asthma patients treated at Children's National Medical Center (CNMC), Washington, DC. The non-asthmatic controls were adolescents selected from an ongoing genetic study at CNMC on metabolic syndrome in inner-city adolescents.

### DNA Isolation

Whole blood or buccal swab samples were collected from asthmatic individuals and non-asthmatic controls under an IRB approved protocol. Genomic DNA was isolated by standard protocols.

### MUC7 VNTR Polymorphism Genotyping

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

### DNA Sequencing

PCR 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 was sequenced (Davis Sequencing, Davis, CA).

### SNP 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 2). A chi-square 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 were used to generate relative odds ratios and 95% confidence intervals (Stata V10, StataCorp, College Station, TX). These analysis 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 chi square test.

Due to 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 contained 84 inner-city children in the AsthMaP study and 37 non-asthmatic controls. The age and gender of the cohort are reported in Table 1. Overall, the sample population was 46% male and 57% asthmatic.

The *post-hoc* power analysis comparing the TR polymorphism in asthmatics and non-asthmatics, samples sizes 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 two previously reported *MUC7* VNTR polymorphisms -- *MUC7*6 and MUC7*5* -- were identified in our study. They were observed as either homozygous 6/6 (Figure2, Lane 1: 559 bp) or heterozygous 6/5 (Figure2, Lane 2: 490 bp) allelic pairings.

We also identified, in a control subject, what looked like a novel polymorphism predicted to encode four VNTR (Figure2, 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 (Figure 2) was sequenced and shown to encode 4 VNTR. The first encoded VNTR repeat domain contained two SNPs that altered the genotype and resulted in changes in the amino acid sequence (Figure 3). The SNPs resulted in a P169T and a T176S change, indicated in Figure 3 as TR1Δ2 with highlighted changes. Unlike *MUC7*5*, *MUC7*4* showed a rearrangement of the order of its VNTR domains, as TR1Δ2 was followed by TR2, TR1 and TR2 (Figure 3). This previously unidentified *MUC7* VNTR is now designated *MUC7*4*.

### Frequency analysis of the MUC7*6 and MUC7*5 allelic polymorphism

DNA samples from non-asthmatic and asthmatic patients (Table 1) 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 2).

We evaluated the expression of *MUC7* allelic polymorphisms and the associated risk of being diagnosed with asthma both in the AsthMaP and control cohorts. A logistic regression analysis of the association of *MUC7*5* allelic polymorphism and not being diagnosed with asthma gave an odds ratio (OR) of 0.173 with a confidence interval (CI) of 0.041 – 0.737 and a *p*-value of 0.018 (Table 3). These data were supported by a $chi^2$ analysis showing a significant association of *MUC7*5* ($p = 0.01$) with not being diagnosed with asthma as a child.

### SNP 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 Americans population only showed an LD measured by an $r^2$ of 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 odd ratio of 66.0 ($p<0.0001$), 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.

## DISCUSSION

African Americans have an increased incidence of asthma and are three times more likely to be hospitalized with asthma related symptoms. Additionally, African Americans living in inner-cities are two to six times more likely to die from 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 being diagnosed with asthma are uncertain.

Mucin overproduction is implicated in asthma (Rose and Voynow, 2006). Thus, an association of *MUC* genes with asthma was carried out by Swallow and co-workers. The data show no association of VNTR numbers in allelic variants in the *MUC1, MUC2, MUC4, MUC5AC, 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 being diagnosed with asthma in a Northern European asthmatic cohort [15]. 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.

Since 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 = 0.052 [15]. Though each of the two sample sets -- Northern European and inner-city African-American -- 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 being diagnosed with asthma.

In the Northern European asthmatic cohort the rs998210 intronic SNP in the *MUC7* gene has been shown to be in 100% LD with the *MUC7*5* [17]. 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 (2006) 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 being diagnosed with asthma, especially in a high risk inner-city population.

While *MUC7*6* and *MUC7*5* appear to be the two most predominant alleles in the human population, unique *MUC7* alleles have been identified, e.g. *MUC7*8* in a Northern European 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 domain of MUC7*4 is TR1Δ2, TR2, TR1, TR2, in contrast to TR1-6 of *MUC7*6* and TR1,2,3, 5,6 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 kDa) 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 anti-bacterial, anti-fungal properties [23;2422], as well as anti-viral 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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## Reference List

1. Martinez FD. Definition of pediatric asthma and associated risk factors. Pediatr Pulmonol Suppl. 1997; 15:9–12. [PubMed: 9316095]
2. Adams PF, Hendershot GE, Marano MA. Current estimates from the National Health Survey, 1996. Vital Health Stat. 1999:10.
3. Wong GW, Chow CM. Childhood asthma epidemiology: insights from comparative studies of rural and urban populations. Pediatr Pulmonol Suppl. 2008; 43:107–116.
4. Eggleston PA. The environment and asthma in US inner cities. Chest. 2007; 132:782S–788S. [PubMed: 17998342]
5. Diette GB, Hansel NN, Buckley TJJ, et al. Home indoor pollutant exposures among inner-city children with and without asthma. Environmental Health Perspectives. 2007; 115:1665–1669. [PubMed: 18008001]
6. Eggleston PA. Environmental causes of asthma in inner city children. The National Cooperative Inner City Asthma Study. Clinical Reviews in Allergy & Immunology. 2000; 18:311–324. [PubMed: 10981263]
7. American Lung Association, E. &. S. U. B. P. a. P. S. NIH Web site. 2002. National Center for Health Statistics, National Health Interview Survey, 1999.
8. Kaliner M, Shelhamer JH, Borson BJ, et al. Human respiratory mucus. Am Rev Respir Dis. 1986; 134:612–621. [PubMed: 3752717]
9. Blyth DI. The homeostatic role of bronchoconstriction. Respiration. 2001; 68:217–223. [PubMed: 11287841]
10. Rogers DF. Mucus pathophysiology in COPD: differences to asthma, and pharmacotherapy. Monaldi Arch Chest Dis. 2000; 55:324–332. [PubMed: 11057087]
11. Holgate ST. The epidemic of allergy and asthma. Nature. 1999; 402:B2–B4. [PubMed: 10586888]
12. Rose MC, Voynow JA. Respiratory Tract Mucin Genes and Mucin Glycoproteins in Health and Disease. Physiol Rev. 2006; 86:245–278. [PubMed: 16371599]
13. Vinall LE, Hill AS, Pigny P, et al. Variable number tandem repeat polymorphism of the mucin genes located in the complex on 11p15.5. Hum Genet. 1998; 102:357–366. [PubMed: 9544852]
14. Vinall LE, Fowler JC, Jones AL, Kirkbride HJ, et al. Polymorphism of human mucin genes in chest disease: possible significance of MUC2. Am J Respir Cell Mol Biol. 2000; 23:678–686. [PubMed: 11062147]
15. Kirkbride HJ, Bolscher JG, Nazmi K, et al. Genetic polymorphism of *MUC7*: Allele frequencies and association with asthma. Eur J Hum Gen. 2001; 9:347–354.
16. Biesbrock AR, Bobek LA, Levine MJ. *MUC7* gene expression and genetic polymorphism. Glycoconjugate J. 1997; 14:415–422.
17. Rousseau K, Vinall LE, Butterworth SL, et al. MUC7 Haplotype Analysis: Results from a Longitudinal Birth Cohort Support Protective Effect of the MUC7*5 Allele on Respiratory Function. Ann Hum Genet. 2006; 70:417–427. [PubMed: 16759176]
18. Beckett WS, Belanger K, Gent JF, et al. Asthma among Puerto Rican Hispanics: a multi-ethnic comparison study of risk factors. Am J Respir Crit Care Med. 1996; 154:894–899. [PubMed: 8887582]
19. Linden SK, Sutton P, Karlsson NG, Korolik V, McGuckin MA. Mucins in the mucosal barrier to infection. Mucosal Immunol. 2008; 1:183–197. [PubMed: 19079178]

20. Piludu M, Rayment SA, Liu B, et al. Electron microscopic immunogold localization of salivary mucins MG1 and MG2 in human submandibular and sublingual glands. J Histochem Cytochem. 2003; 51:69–79. [PubMed: 12502756]

21. Prakobphol A, Levin MJ, Tabak LA, et al. Purification of a low-molecular-weight, mucin-type glycoprotein from human submandibular-sublingual saliva. Carbohydr Res. 1982; 108:111–122. [PubMed: 7139659]

22. Liu B, Rayment S, Oppenheim FG, et al. Isolation of human salivary mucin MG2 by a novel method and characterization of its interactions with oral bacteria. Archives of Biochem Biophys. 1999; 364:286–293.

23. Situ H, Wei G, Smith CJ, et al. Human salivary MUC7 mucin peptides: effect of size, charge and cysteine residues on antifungal activity. Biochem J. 2003; 375:175–182. [PubMed: 12812519]

24. Wei GX, Bobek LA. Human salivary mucin MUC7 12-mer-L and 12-mer-D peptides: antifungal activity in saliva, enhancement of activity with protease inhibitor cocktail or EDTA, and cytotoxicity to human cells. Antimicrobial Agents & Chemotherapy. 2005; 49:2336–2342. [PubMed: 15917530]

25. Habte HH, Mall AS, de Beer C, et al. The role of crude human saliva and purified salivary MUC5B and MUC7 mucins in the inhibition of Human Immunodeficiency Virus type 1 in an inhibition assay. Virol J. 2006; 3:99. [PubMed: 17125499]

26. Watson A, Troxler RF, Pena, et al. MUC7 mucin glycoprotein is present in airway secretions of asthmatic, but not control, patients. Am J Respir Crit Care Med. 2003; 167(A465)

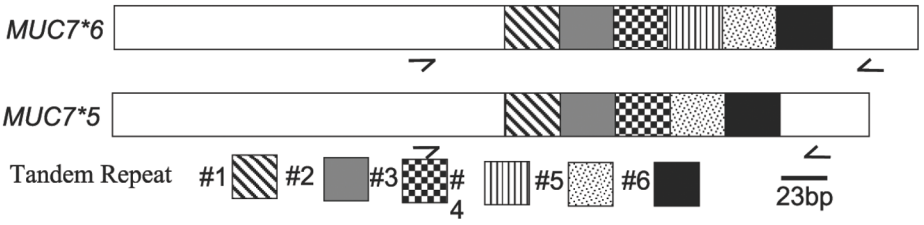


**Figure 1.**
Schematic of MUC7 cDNA. Each of the tandem repeats, 69 bp in length, are identified. The arrows either side of the TR domain indicated location of primers used for genotyping.

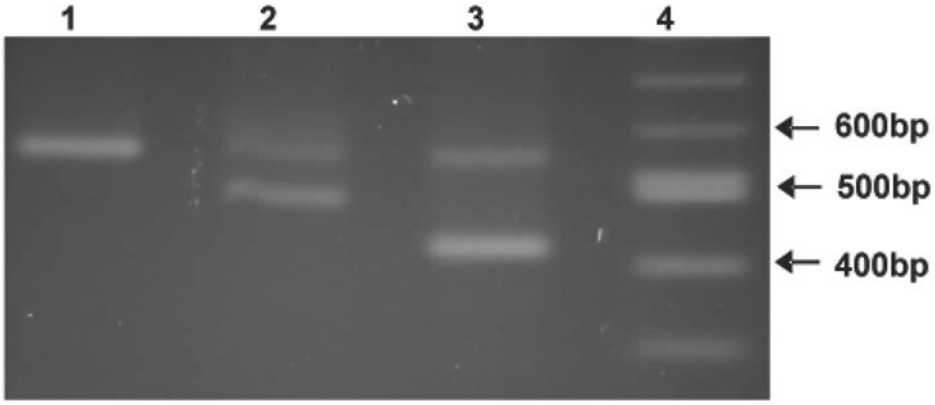


**Figure 2.**
Electrophoresis of representative PCR products showing allelic variation in the *MUC7* tandem repeat domain. Amplicons were separated on a 2% agarose gel. [predicted sizes are: 6* -559bp; 5* - 490bp; 4* - 421bp]. Lane 1, 100bp ladder; Lane 2, 6*/4* alleles; Lane 3, 6*/5* alleles; Lane 4, 6*/6* alleles.

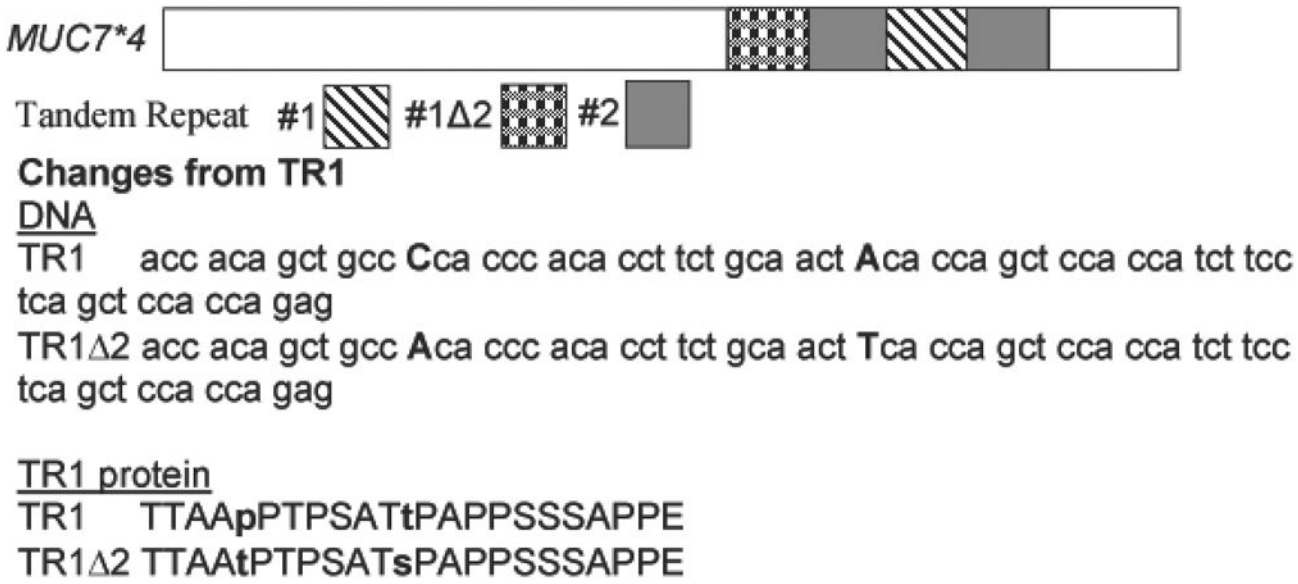


**Figure 3.**
Schematic of MUC7*4 cDNA/protein showing the changes in the VNTR domain. The order of the repeats is: TR1▲2, TR2, TR1 and TR2. DNA and amino acid sequence of TR1 compared to the altered TR1 (TR1▲2), in the *MUC7*4* allelic polymorphism.

**Table 1**

Demographic data on the human subject population.

| | Total Population | Male | Female | Age range yrs. (Mean) |
|---|---|---|---|---|
| **Control** | 37 | 13 | 24 | 14–20 (18) |
| **Asthma** | 84 | 45 | 39 | 4–18 (10) |

**Table 2**

Frequencies of the MUC7 polymorphic alleles.

| | Control | | | Athsma | |
|---|---|---|---|---|---|
| **Tandem Repeat** | ***6** | ***5** | ***4** | ***6** | ***5** |
| Number of people | 67 | 6 | 1 | 165 | 3 |
| Allelic Frequency | 0.91 | 0.08 | 0.01 | 0.99 | 0.01 |

**Table 3**

Logistic regression analysis of the MUC7 VNTR polymorphisms and its association with being asthmatic.

| Genotype | Cases | Controls | Odds Ratio | p-Value | 95% CI |
|---|---|---|---|---|---|
| 6/6 | 81 | 28 | 1.0 | | |
| 6/5 | 3 | 6 | 0.173 | 0.018 | 0.041–0.737 |