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REVIEW ARTICLE

Identification and Function of a Novel Candidate Gene for Asthma:*ADAM 33*

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ABSTRACT

Asthma is a complex disorder of inflammation and remodelling largely restricted to the conducting airways. It is a disorder where there are major genetics and environmental factors that interact together to initiate and propagate the disease into a chronic relapsing disorder. Until recently the genetic factors involved in disease pathogenesis have been restricted to variants in known molecules involved in the inflammatory or remodelling pathways. In this review evidence is presented for a new susceptibility gene for asthma, *ADAM 33*, that was identified by positional cloning. It is suggested that ADAM 33 plays a key role in predisposing to reduced lung function and bronchial hyperresponsiveness characteristic of asthma. Through an understanding of the disease-related SNPs(in *ADAM 33*)it may be possible, not only to identify a gene based diagnostic test, but also to focus attention on developing a new treatment that reverses remodelling changes.

KEY WORDS

a disintegrin and metalloprotease 33, asthma, epithelial mesenchymal tropic unit, genetics

INTRODUCTION

Asthma is a disorder of the conducting airways in which the Th-2 mediated inflammation interacts with structural changes to cause variable airflow obstruction.[1] It is a serious global health problem with over 100 million people affected world wide and the prevalence is increasing, especially among children.[2,3] Fundamental to disordered airway function is the concept of bronchial hyperresponsiveness (BHR) in which the airways constrict too much and too easily to a range of stimuli. In chronic severe asthma, the inflammation and structural changes both become more intense[4] and are paralleled by an increase in BHR that is only partially or non-responsive to treatment with corticosteroids.[5] Explanations for BHR include mucosal and adventitial swelling causing a disproportionate reduction in airway calibre for a given degree of airways smooth muscle (ASM) shortening,[6] excessive ASM shortening,[7] increase in ASM mass causing greater force generation[8] and an excessive velocity of contraction linked to altered cross-bridge cycling.[9] Morphometric studies have shown an increase in smooth muscle mass in proportion to disease severity and computer modelling has revealed that this and altered contractility are the most plausible explanations for BHR.[8-10]

EARLY LIFE ORIGINS OF ASTHMA

While there is a strong genetic predisposition towards asthma (see below), the increase in prevalence of the disease that has occurred in the last 30 years[2] is too rapid to be accounted for by genetic change in the population. Rather, it is more likely that it is due to a shift in environmental influences acting on a pre-existing genetic susceptibility background. For most patients with asthma, the disease begins prior to 6 years of age. Beyond this, there is evidence that processes involved in the development of asthma may begin *in utero*. Atopy is the major risk factor for the development of asthma and it is now well established that sensitisation may begin *in utero*.[11,12] The potential for allergic sensitisation and eventual translation into airways inflammation with wheezing are influenced by many factors in early life, including exposure to tobacco smoke, viral respiratory infections (particularly respiratory syncytial virus), diet, antibiotic use, and domestic (house dust) mite and animal dander sensitisation at 1 to 2 years of age. The regulation of these processes and resulting balance (or im-

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balance) in cytokine production is not well established, but understanding them is likely to be important to the eventual understanding of airway inflammation and the possibility of therapy targeted at injury/repair processes in asthma. Maternal nutrition may also play a part in lung growth and development. Subtle deficiencies in vitamin A can affect airway branching and lung epithelial cell differentiation.[13] There are additional effects on vitamin A depletion on surfactant protein production, which in turn may influence airway host defence.[14] Other maternal dietary and environmental factors also influence asthma susceptibility including the use of medications such as paracetomol[15] and maternal smoking.[16] Furthermore, infants of smoking mothers have been shown to be much more likely to develop both atopy and wheezing illnesses.[17,18]

It is well established that both for the early phenotypes of wheezing in young children, and for later atopic asthma, airway function and bronchial responsiveness measured in the newborn period (and hence dependant on foetal lung development) are significant predictors of asthma.[19] Recent evidence from an Australian cohort has shown that bronchial responsiveness in newborns, independent of allergic sensitisation, is a predictor of the subsequent development of asthma[20] and becomes fully developed by 6 months of age in high risk infants.[21] There may also be an important interaction between atopy and lung development as a recent study has shown between early lung function (sR_{aw} at age 3 years) and a child's risk of atopy.[22]

These early alterations of lung function result from the interaction of environmental factors with the developing lung, which, in genetically susceptible individuals, leads to structural and downstream functional changes in the airways. This hypothesis is supported by the recent observation that thickening of the *lamina reticularis* in bronchial biopsies from young children is present several years before asthma becomes clinically manifest.[23] In addition, studies in non-human primates have shown that prenatal exposure to nicotine causes collagen deposition around the large airways.[24] Morphometry has revealed that thickening of asthmatic airways can account for a large proportion of BHR and excessive airway narrowing observed with established disease. In moderate-severe disease, these structural changes, along with BHR, are poorly responsive to corticosteroids,[25] a finding that was clearly reflected in the ENFUMOSA study where evidence of airway wall remodelling was found.[26] Of considerable interest is that changes, including sub-basement membrane collagen deposition, are present in the airways of asthmatic children[27] and may even precede symptomatic disease.[23] This evidence for remodelling may also account for the lack of effect of inhaled corticosteroids on post-bronchodilator FEV_1 as recently shown in the longitudinal CAMP and START studies.[28,29] The observation that environmental agents impact on the bronchial epithelium and that structural remodelling is associated with tissue injury has led to a study of these processes with the following conclusions.

1. The susceptibility of the asthmatic bronchial epithelium to oxidant stress is greater than normal, as reflected by premature apoptosis, and reflects a fundamental disease-related difference that has potential to translate gene-environmental interactions.[29]

2. In asthma, epithelial repair is prolonged due to impaired epithelial proliferation caused by an imbalance between proliferation and survival signals.[30]

3. The altered epithelium communicates with the underlying mesenchyme to form a trophic unit that propagates and amplifies remodelling events from the epithelial surface to the submucosa through activation of subepithelial effector myofibroblasts.[31]

4. The epithelial mesenchymal trophic unit (EMTU) favours localisation of Th-2 mediated inflammation, enabling IL-4 and IL-13 to work in concert with the EMTU to maintain and amplify the remodelling and inflammatory processes.[32]

This new concept of asthma helps explain why the disease is not linearly linked to atopy ; why, not infrequently, there is incomplete disease resolution with corticosteroids ; why in moderate-severe disease inhaled long acting β-agonists are more effective that increasing the dose of inhaled corticosteroids and even then resolution is often incomplete ; and why, beyond atopy, a range of locally acting environmental factors (passive tobacco smoke exposure, air pollutants, enzymically active allergens and virus infections) are important risk factors for the development and consolidation of this disease.

It follows that Th-2 mediated inflammation and activation of the EMTU may be parallel rather than sequential events with each interacting with the other and each varying between patients and across time. In this context, the asthmatic airway could be considered as a chronic wound where there is injury and impaired repair and, as a consequence, tissue restructuring and ongoing inflammation. If this is accepted, then it would seem more appropriate to develop new therapies that improve epithelial restitution and/or return the EMTU to latency rather than suppressing inflammation alone.

THE GENETIC BASIS OF ASTHMA

Asthma has a high heritability of up to 75%,[33] involving a few genes with moderate effects rather than many genes with small effects.[34] Asthma can be termed a complex genetic disease in that there are multiple genetic effects that interact with the environment to modify both susceptibility and severity of disease.[35] Family-based studies of asthma have identified that in addition to a genetic predisposition to atopy which alters susceptibility to asthma, there are

also genetic effects that relate solely to asthma and possibly regulate susceptibility of the lung to both allergic and other environmental-induced inflammation. Studies in our own laboratory and those of others, have identified candidate regions for asthma genes based on linkage studies, and also polymorphism in candidate genes that are associated with disease phenotypes.[35] While it is clear that asthma results from the interaction between environmental and genetic factors, definition has proved difficult due to the lack of cohorts with extensive environmental exposure data being available for genetic analysis, problems of defining and quantifying exposure and the lack of power of most studies. However, a recent study in a Chinese population which identified an interaction between cigarette smoking and β_2-adrenoceptor genotype in determining susceptibility to asthma[36] indicates the importance of investigating both genetic and environmental factors in the same cohort. An observed interaction between a genetic factor and an environmental factor that leads to increased risk provides support for both observations.

ADAM 33 AS AN ASTHMA SUSCEPTIBILITY GENE

We have recently reported the first novel asthma- related gene identified by positional cloning.[37] In a five year collaboration with Genome Therapeutics Corporation (Waltham, Mass, USA) and Schering Plough (Kenilworth, NJ, USA), a genome wide screen using multi-point linkage analysis and 401 microsatellite markers at a density of 9 cM has been undertaken involving 362 families from Wessex, UK, and 98 from the USA with a least 2 siblings with asthma. For doctor-diagnosed asthma, suggestive evidence of linkage (maximum lod score (MLS) 2.24) was found on chromosome 20 at 9.99 cM. The addition of 13 more markers at 1−2 cM increased the MLS to 2.94 at D20S482 (12.1 cM) which further increased to 3.93 when BHR was included in the definition of asthma, thereby exceeding the threshold for genome wide significance.[38] In contrast, when asthma was condition for serum total IgE and allergen specific IgE, the MLS fell to 2.3 at 11.6 cM and 1.87 at 12.1 cM respectively, including the presence of genes more closely linked to airway altered function than to allergic inflammation *per se*. Confirmation of linkage on 20p13 has come from separate analysis of the UK and US families,[37] and from 2 separate genome-wide studies in other UK and US outbred populations.[39,40]

Physical mapping and direct cDNA selection identified 40 genes in the region under the peak of linkage at 20p13.[37] Single-strand conformation polymorphism (SSCP) analysis and direct sequencing was used to identify SNPs in association studies involving 130 identical-by-descent affected individuals and 217 hypernormal controls. In 23 genes spanning 1 lod support interval around D20S482, 105 SNPs were typed of which 25 localised to a cluster of five genes showing significant association with both asthma and BHR. Fourteen of these lay within a single gene (No. 216) identified as *ADAM 33*, a novel member of the ADAM (A Disintegrin And Metalloprotease) family achieving significance of $p=0.005-0.05$. Both in the combined populations and the UK and US samples when analysed separately, additional SNP typing strengthened the location of the signal to *ADAM 33* and this was further confirmed both by haplotype analysis (5-7 SNP combinations at $p=0.000001-0.005$) and transmission disequilibrium (TDT) testing (10 SNP combinations at $P<0.005$). Because the alleles on the *ADAM 33* gene that conferred increased risk of developing asthma and BHR were so common, their effects translate into a substantial 19−50% population attributable risk for asthma.[37] Replication of the association between *ADAM 33* SNPs and asthma has been seen in studies of both African American, Hispanic and US Caucasian outbred populations[41] as well as two German populations. [42]

ADAM 33 EXPRESSION IN ASTHMA

ADAM 33 is the most recently reported member of the ADAM gene family of Zn^{++}- dependant matrix metalloproteases (MMPs). ADAMs have a complex organisation involving 8 domains, the first 6 encoding signal sequence and pro-, catalytic, disintegrin, cysteine rich and EGF domains[43] which are anchored at the cell surface or Golgi apparatus by a transmembrane domain followed by a cytoplasmic domain with signalling specific sequences. ADAM 33 belongs to the ADAM 12, 15, 19 and 28 subfamily all of which possess proteolytic activity, e.g. sheddase activity for HB-EGF (ADAM 12), insulin-like growth factor binding protein -3 and -5, fibronectin (ADAM 9), and TNFα (ADAM 17).[43,44] Northern blot analysis identified two transcripts of *ADAM 33* at 5.0 and 3.5 kb, but only the latter has been found in cytoplasmic RNA.[37] 17 cDNA libraries have been screened resulting in a 3.5 kb clone containing the entire open reading frame composed of 22 exons with the canonical splice sequence (CT/AG) present at each splice junction. The potential for alternatively spliced transcripts is also indicated by variants lacking exons, or parts of exons. *ADAM 33* is preferentially expressed in smooth muscle, myofibroblasts and fibroblasts, but not in epithelial cells, T-cells or inflammatory leukocytes. In all tissues mRNA transcripts of 5 kb predominated are those of 3.5 kb by 2-5-fold.[45] *In Situ* hybridisation using anti-sense *ADAM 33* probes has confirmed the RNA is expressed in smooth muscle, fibroblasts, and myofibroblasts of asthmatic airways but is not found in the epithelium, endothelium, or inflammatory cells.[45] The significance of *ADAM 33* for asthma is strengthened by the existence of a synthetic region on mouse chromosome 2 at 74 cM that has been linked to BHR[46] overlying an orthologue of that ex-

hibited *ca*. 70% homology with its human counterpart.

The selective expression of *ADAM 33* in mesenchymal cells strongly suggests that alterations in its activity may underlie abnormalities in the function of airway smooth muscle cells and fibroblasts linked to BHR in asthma. These components of the EMTU[47] share a common fibroblastic progenitor, the mature phenotype of which is directed by growth factors including TGF-β,[31] whose release from bronchial epithelial cells are increased in response to damage.[30-48] Asthmatic (myo) fibroblast cells are unusual in that they have the capacity to proliferate in the absence of exogenous growth factors,[31] paralleling the behaviour of asthmatic smooth muscle cells *in vitro*.[49] This shared trait is consistent with the occurrence of a common stem cell population whose numbers are increased in asthma due to an inherent capacity for enhanced cell growth and/or survival.

Members of the ADAM family are proteins with diverse functions that reflect the complex domain structure of these molecules.[46] While certain functions can be attributed to an individual domain (e.g. ectodomain shedding to the metalloproteinase domain[50] and cell adhesion to the disintegrin domain[51]), it is likely that the other domains play important regulatory roles in these functions by conferring specificity and selectivity. ADAM proteins are anchored in the *trans* -Golgi network or plasma membrane, but in some cases, secreted splice variants have been identified. In the case of ADAM 12, an evolutionarily close relative of ADAM 33, ectopic expression of the secreted form of molecule (ADAM 12S) in rhabdomyosarcoma cells results in growth of tumour xenografts which are infiltrated with large numbers of host-derived smooth muscle cells.[52] Although we have identified several alternatively spliced forms of ADAM 33 in lung-derived cDNA,[53] it is not yet known whether a secreted protein variant is produced by airway cells.

While there is limited published literature surrounding the functions of ADAM 33, we have confirmed the selective expression of ADAM 33 mRNA and protein in fibroblasts, myofibroblasts, and smooth muscle cells and have further shown that *ADAM 33* occurs in multiple molecular forms that arise due to alternative mRNA splicing.[53] We have also found that ADAM 33 expression is rapidly and transiently upregulated during TGF-β myofibroblasts differentiation suggesting that it is an early regulator of differentiation.[54]

ADAM 33 AND THE EARLY LIFE ORIGINS OF ASTHMA

ADAM 33 mRNA expression has been shown to occur during murine embryonic development, suggesting that it regulates tissue morphogenesis.[55] In preliminary studies we have found that murine ADAM 33 mRNA expression is induced in embryonic lungs at the start of branching morphogenesis, increases with gestation and remains present into adulthood.[56] This suggests that polymorphism that modulates *ADAM 33* expression and/or function may modulate lung growth and development *in utero* and lead to altered airways responsiveness and increased risk of asthma. In support of the idea that variability in *ADAM 33* may predispose to altered lung function in early infancy, we have recently shown that *ADAM 33* SNPs correlate with lung function measured at age 3 in a cohort of children from Manchester (the MAAS Study).[57] Seventeen single nucleotide polymorphisms (SNPs) spanning 11Kb of the *ADAM 33* gene were analysed in 302 children from a prospective birth cohort in which respiratory questionnaire and measurements of lung function (sR_{aw}) were completed at age 3 years. We have shown a significant association between sR_{aw} and 6 SNPs in *ADAM 33*. In all cases it was the rare allele that was associated with a higher sR_{aw} value and therefore, poor lung function. When compared to children homozygous for the common allele, carriers of the rare allele had higher sR_{aw} values for SNPs : V1 (p=0.024), Q1 (p=0.023), ST+7 (p=0.007) and F+1 (p=0.001). The association with F+1 was of interest as there was a clear gene dosage effect with mean sR_{aw} being 7% higher in heterozygotes and 12% higher in children homozygous for the rare allele than in the wild type. Children homozygous for the rare allele of T1 (p=0.001) and T2 (p=0.003) also had significantly higher sR_{aw} values. There was evidence of linkage disequilibrium between the associated SNPs suggesting that the effects were not independent. Using linear regression analysis, we demonstrated that F+1 was the strongest main effect ; V1, Q 1 and ST+7 had no additional independent effect, whereas the effects of T1 and T2 were independent of F+1. This suggests that there may be more than one functional polymorphism in the *ADAM 33* gene and that disease-related haplotypes are likely to be mechanistically important. These data support the hypothesis that impaired poor early-life lung function is in part a genetically determined trait involving *ADAM 33* that may increase the risk of chronic asthma.

ADAM 33 SNPs AS A PREDICTOR OF SEVERE AND PROGRESSIVE ASTHMA

Although the precise role of ADAM 33 in asthma remains unknown, recent epidemiological studies provide some evidence supporting its role as a susceptibility gene for more severe and progressive disease. In 200 asthmatics in The Netherlands, in whom longitudinal data on asthma and FEV_1 was available over a 20 year period, investigation of 9 SNPs of *ADAM 33* when controlled for other variables, e.g. atopy, smoking, showed that two (S2 and Q-1), were significantly associated with a progressive decline in FEV_1 when compared to normal controls (20.1 *vs.* 6.4 and 22.1 *vs.* 4.1 ml/yr respectively).[58] Mechanistically, this may be linked to the capacity of TGF-β (and related BMP-

proteins) to transiently increase ADAM 33 expression as part of the differentiation trajectory of primitive airway mesenchymal cells to myofibroblasts which are known to be important in chronic wound repair.[54]

CONCLUDING COMMENTS

Applying genetics to a complex disease such as asthma is at last realising its potential. In addition to *ADAM 33* novel candidate genes that have been discovered from positional cloning efforts include PHF11 on chromosome 13q14, the gene product of which contains two PHD zinc fingers that regulate transcription involved in IgE synthesis[59] and DPP10 on chromosome 2Q14 that encodes a homologue of dipeptidyl peptidases capable of cleaving terminal peptides from cytokines and chemokines.[60] How *ADAM 33* and these new genes relate to asthma and allergy in a functional setting will be a challenge. However, what is certain, is that the application of new genetic technologies will reveal further novel genes involved in the onset and persistence of atopy and asthma. As well as those genes already described as interactions of these and others to be discovered with the environment that will undoubtedly reveal new ways to prevent and treat this complex disorder.

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