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Mannose binding lectin genotype and serum levels in patients with chronic and allergic pulmonary aspergillosis

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Abstract

Background—Several studies suggest mannose binding lectin (MBL) deficiency is associated with various manifestations of aspergillosis. MBL serum levels and function are genetically determined, but levels rise during inflammation.

Objective—We address the relative frequency of deficient genotypes, the relationship between serum level and genotype and both age and disease manifestations in patients with chronic pulmonary (CPA) and allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS).

Method—DNA was extracted from blood samples and *MBL2* genotyping was performed using the INNO-LiPA *MBL2* kit. Serum MBL concentrations were determined by ELISA.

Results—108 patients were evaluated, 70 (65%) with CPA, 38 (35%) with allergic disease (ABPA, SAFS or undefined) and 13 (12%) had both CPA and ABPA. The mean MBL serum level was 1849µg/L and did not differ between groups. 40 subjects (37%) had exon 1 genotypes producing non-functional MBL (A/B, A/C, A/D and O/O), a frequency not different from published normal controls. A/A subjects with CPA had higher levels (2,981µg/L) compared with allergic A/A subjects (2202µg/L) ($p=0.012$). No single haplotype, genotype or allele was significantly related to any aspergillosis phenotype. Worse breathlessness was associated with higher MBL levels among A/A subjects ($p=0.009$) and conversely non-functional genotypes. Mean MBL values were higher in those with an MRC score of 5 compared with those with and MRC score of 1 ($p=0.023$). A/A allergic subjects ($n=27$) in this study were ~11 years younger than allergic A/O subjects ($n=11$, $p=0.02$). Subjects with worse respiratory status or more severe CPA had higher MBL serum levels ($p=0.023$; $p=0.034$). Bronchiectasis was not associated with MBL levels in CPA or allergic aspergillosis.

Conclusion—MBL genotype and serum level modulates progression of aspergillosis.

Keywords

Aspergillosis; *Aspergillus*; innate immunity; mannose binding lectin; MBL

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Introduction

In addition to the much publicised life-threatening invasive infections, *Aspergillus fumigatus* also causes chronic and allergic disease in the upper and lower respiratory tract. Given that most individuals do not suffer any ill effects from *A. fumigatus* exposure, a genetic component of susceptibility is likely. In certain atopic individuals, *A. fumigatus* can cause allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS), as well as other less well-defined allergic disease. It can also cause chronic pulmonary aspergillosis (CPA) in non-immunocompromised people. Several studies as to differential genetic susceptibility to these entities of aspergillosis are published and include IL-4R α (Howard *et al.*, 2002; Woitsch *et al.*, 2004; Zhang *et al.*, 2007), IL-10 (Seo *et al.*, 2005 & Sainz *et al.*, 2007), surfactant A2 (Vaid *et al.*, 2007), *TLR4* and *TLR9* polymorphisms (Carvalho *et al.*, 2008).

In CPA, *Aspergillus* forms cavities in the lung by gradually destroying tissue. These cavities can expand and may contain a fungal ball (aspergilloma) (Soubani *et al.*, 2002). Long term therapy is required to prevent the destruction of an entire lobe or lung (Binder *et al.*, 1982; Denning, 2003). The morbidity and mortality of CPA remains high: patients continue to have some disability, for example fatigue and intermittent secondary infections of remaining pulmonary cavities (Denning *et al.*, 2003). CPA is an unusual complication of other pulmonary disease including tuberculosis, non-tuberculous mycobacterial infection, sarcoidosis, prior pneumothorax, chronic obstructive pulmonary disease (Smith & Denning, 2011). It is uncommon, and there are thought to be under 600 patients in the UK with CPA (Smith & Denning, 2011; Denning, in press). Therefore studies are challenging to undertake.

ABPA usually affects asthmatics and those with cystic fibrosis but can also rarely affect patients without either. The hallmarks of disease are cough, worsening respiratory function with breathlessness, production of sputum plugs and a highly elevated serum total IgE (Ricketti *et al.*, 1983; Patterson *et al.*, 2000; Agarwal, 2009). ABPA is documented to occur in 0.72–3.5% of adult asthmatics attending respiratory clinics (Donnelly *et al.*, 1991 & Eaton *et al.*, 2000). ABPA patients are generally treated with corticosteroids and itraconazole, but some go on to develop CPA. Why some patients develop ABPA is not completely clear, and in particular why some go on to develop CPA has yet to be addressed. Severe asthma with fungal sensitisation (SAFS) is a newly described phenotype of asthma, characterised by severe asthma, positive skin prick or specific IgE tests for any fungus, and a serum IgE <1,000 IU/mL (Denning *et al.*, 2006). Like patients with ABPA, patients with SAFS respond to oral antifungal therapy (Denning *et al.*, 2009; Pasqualotto *et al.*, 2009). The frequency of SAFS is not known, but is a minority of patients with severe asthma.

Mannose-binding lectin (MBL) is a protein of the innate immune system coded for by the *MBL2* gene. MBL can bind to mannose, mannan and *N*-acetylglucosamine in a calcium-dependant manner on a wide variety of micro-organisms including viruses, fungi and bacteria (Kilpatrick, 2002b). MBL circulates in association with MBL-associated serine proteases (MASPs) and upon binding to a pathogen, activates the complement pathway in a manner distinct from the classical and alternative pathways via the cleavage of C2 and C4 to form a C3 convertase or by directly activating C3 (Matsushita & Fujita, 1992; Thiel *et al.*, 1997; Stover *et al.*, 1999; Takahashi *et al.*, 1999). The predominant forms of MBL in humans are dimers, trimers and tetramers, bound together via a collagen-linking region. In humans only the formation of tetramers and higher constitute the presence of functional MBL: higher-order oligomers permit MBL to bind to repetitive carbohydrate patterns with high avidity (Kilpatrick, 2002a).

Six single nucleotide polymorphisms (SNPs) have been characterised in the *MBL2* gene that affect the expression and functionality of MBL (Sumiya *et al.*, 1991; Lipscombe *et al.*, 1992; Madsen *et al.*, 1994; Madsen *et al.*, 1995) (figure 1). Three SNPs are in the promoter/5' untranslated region (UTR) and 3 are located in exon 1, coding for the collagen-linking region. The exon 1 SNPs result in amino acid substitutions at positions 52 (Arg→Cys), 54 (Gly→Asp) and 57 (Gly→Glu) and prevent oligomerisation of MBL subunits. The wild type is coded for by the A allele. The SNPs resulting in amino acid changes at positions 52, 54 or 57 are denoted as the D, B and C alleles respectively and have not been reported to occur simultaneously on the same allele. In European populations heterozygous B, C and D allele carriers occur at a frequency of 22%, 4% and 10% respectively. Collectively, these 3 alleles are termed the O allele: heterozygotes (A/O) produce as little functional MBL as homozygotes (O/O) as the amino acid substitution in the collagen-linking region exhibits a dominant effect over the functional A allele. O/O and A/A homozygotes occur in 4% and 60% of the population respectively (Garred *et al.*, 2006).

The *MBL2* promoter and 5' UTR SNPs occur at nucleotide positions -550 (G→C), -221 (G→C) and +4 (C→T) and are denoted H/L, Y/X and P/Q respectively (the first letter referring to the normal allele in each case) and combinations have been shown to significantly up- and down- regulate MBL expression. The genotypes HYP, LYP and LXP produce high, medium and low levels of MBL protein. All alleles exhibit significant linkage disequilibrium and only 7 haplotypes are regularly seen: HYPA, LYQA, LYPB, LXPA, HYPD, LYQC and LYPB (Dommett *et al.*, 2006).

Multiple studies have investigated MBL serum levels in healthy individuals. Values vary by ethnicity and method used (supplemental table 1). It is difficult to establish what MBL serum level represents a clinically significant deficiency. MBL concentrations below 500 ng/mL were significantly more common in those with invasive aspergillosis (62%) than controls (32%) ($p < 0.001$) (Lambourne *et al.*, 2009). In MBL A and C gene-knockout (KO) mice, there was no increased susceptibility to invasive aspergillosis, and improved survival in KO mice suggesting a detrimental of MBL in invasive aspergillosis (Clemons *et al.*, 2010). However in patients with invasive aspergillosis response to antifungal therapy was similar in those with low MBL levels (69%) and others (52%) ($p = 0.2$) (Lambourne, 2009). Several studies have found varied degrees of association between MBL genotypes and functional protein levels with chronic, allergic and acute aspergillosis (table 1). In light of the association of MBL with aspergillosis, we compared the *MBL2* genotype and MBL serum levels in 108 patients with chronic or allergic aspergillosis to investigate any link between non-functional or circulating levels of MBL and susceptibility to disease and subsequent morbidity.

Patients, materials and methods

Patients were recruited from the infectious disease clinic at the University Hospital of South Manchester (UHSM), which acts as a referral centre for patients with aspergillosis (designated the National *Aspergillosis* Centre in 2009). Patients gave informed written consent and were recruited under the Ethically approved study "Impact of Genetic Risk Factors and Epidemiology of Chronic Necrotising (Fibrosing) Pulmonary Aspergillosis (CNPA) and Aspergilloma". Patients with CPA and ABPA or both conditions and SAFS were recruited, and disease categorisation of these chronic diseases made independently from knowledge of the MBL genotype or serum level. CPA was defined as the presence of at least one pulmonary cavity on thoracic imaging, with or without an aspergilloma, together with symptoms (usually weight loss, fatigue, cough, haemoptysis and breathlessness) for >3 months, and serology (positive *Aspergillus* precipitating IgG antibody in blood) or cultures or histology implicating *Aspergillus* spp (Denning *et al.*, 2003). CPA severity was assessed

in 3 bands of approximate severity and complexity. Band 1: Ambulant and independent; No evidence of antifungal resistance; Treatment with itraconazole capsules or no treatment. Band 2: Significant impairment of respiratory function, sufficient to impair activities of daily living, but ambulant and/or; Failed or developed toxicity to itraconazole capsules and; No evidence of azole antifungal resistance and/or; Evidence of Mycobacterial disease. Band 3: Antifungal azole resistance documented and/or; Long term nebulised or IV antibiotic treatment required (bronchiectasis, *Pseudomonas* colonisation) and/or; Wheelchair bound due to respiratory impairment and/or; Additional severe underlying diseases such as controlled HIV infection, significant renal or hepatic dysfunction. CPA patients may contract other pulmonary infections, but these were controlled at the time of study.

ABPA was defined as asthma (of any severity) or the production of plugs of sputum, with an elevated total IgE of >500 IU/mL (usually >1,000 IU/mL), eosinophilia at some time, and a positive serum *Aspergillus*-specific IgG, or IgE test or *Aspergillus* skin prick test Patterson *et al.*, 2000; Ricketti *et al.*, 1983). Patients with cystic fibrosis and ABPA were not recruited.

SAFS was defined as severe asthma (British Thoracic Society level 4 or 5, positive skin prick or specific IgE tests for any fungus (*A. fumigatus*, *Candida albicans*, *Alternaria alternata* (*tenuis*), *Cladosporium herbarum*, *Penicillium chrysogenum* (*notatum*), *Trichophyton mentagrophytes*) and a serum IgE <1,000 IU/mL (Denning *et al.*, 2009).

Patients with both CPA and ABPA were defined by a combination of both sets of criteria. Age was determined by the date of sample collection since the age at onset of disease is often undefined. Bronchiectasis in ABPA was determined radiologically using standard CT criteria. Patients' breathlessness was assessed on the Medical Research Council (MRC) dyspnoea scale. Briefly, subjects are scored from 1 to 5 on a worsening scale of perceived breathlessness (1 is normal; 5 is severely breathless with trivial activity) (Bestall *et al.*, 1999).

DNA extraction

DNA was extracted using the QIAmp DNA minikit (Qiagen, UK) to the manufacturer's specifications for whole blood. This was diluted 10-fold in PCR-grade water and 1 µL was used as a template for PCR.

MBL2 genotyping

Amplification was performed using the INNO-LiPA *MBL2* Amplification kit (Innogenetics, Belgium) according to the manufacturer's instructions. PCR success was assessed by visualising on a 1.5% agarose gel in Tris Borate EDTA (TBE) buffer before proceeding with genotype analysis using the INNO-LiPA *MBL2* kit to the manufacturer's specification. Briefly, this involved hybridising 10 µL of the PCR product to a labelled membrane followed by stringent washes and a colour development reaction to visualise bands corresponding to each *MBL2* allele.

Serum testing

Serum MBL concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (MBL Oligomer ELISA Kit, BioPorto Diagnostics A/S, DK) with an upper and lower reported detection limit of 4000 and 50 µg/L respectively. Repeat samples were highly consistent.

Statistics

MBL serum levels were compared using unpaired 2-tailed t-tests and analyses of variance as appropriate, with low vs normal levels (<1,000 vs 1,000 µg/L and <500 versus 500 µg/

L) compared using chi-square tests. The distribution of genotype frequencies were tested with Fisher's exact test. Normal control data for British and Danish Caucasians was sourced from Garred *et al.* (2006), as all patients were Caucasians. All statistical analysis was performed using SPSS version 16.0 Testing for deviations from Hardy-Weinberg equilibrium (HWE) was performed using the chi-square test for comparing the observed and expected values at the 0.01% significance level.

Results

Of the 108 patients tested, 35% (38) had allergic disease (ABPA 26%, SAFS 6%, undefined allergic fungal disease 3%) and 65% (70) had CPA (57 (53%) had CPA alone and 13 (12%) had CPA and ABPA). Overall (n=108) mean age was 59.6 years (standard deviation (SD)=11.5, range 26.5 – 86.8 years). For the allergic subjects (n=38) the mean age was 58.8 years; (SD of 13.3, range 26.5 – 79.7 years) and for CPA (n=70) the mean age was 60.1 years; (SD of 10.5, range 29.4 – 86.8 years). The mean serum level was 1698µg/L, and 1931µg/L in allergic and CPA subjects respectively. Of the allergic patients, those with only ABPA had a mean average serum level of 1884µg/L and SAFS was 1193µg/L. Of all the subjects, those with an A/O or O/O genotype had a mean serum level of 512µg/L while those with A/A had a mean level of 2667µg/L ($p<0.0001$). A/A subjects with CPA had a higher mean MBL serum level (2981µg/L) compared with allergic A/A subjects (2202µg/L); a highly significant difference ($p=0.012$) (figure 2). The frequency of subjects with normal or low MBL serum levels was not significantly different for any of the conditions studied. Allergic subjects had MBL serum levels less than 1000µg/L or 500µg/L in 37% and 32% respectively compared to 40% and 24% for CPA subjects.

The frequency of each genotype and associated average MBL serum level is listed in table 2. The allele frequencies of all the promoter SNPs (–550G/C, –221G/C +4C/T) were in HWE, as were the allele frequencies of the coding SNPs 52C/T, 54G/A and 57G/A.

As previously reported, the 5' UTR SNPs with the most profound effect on circulating MBL levels were those at –221 and +4. At the –221 locus, GG genotype (Y) represents normal levels while CC (X) resulted in MBL deficiency (2254 vs 900µg/L respectively, $p=0.19$). At the +4 locus, TT (Q) versus CC genotype (P) results in a higher circulating level (2910 vs 1411µg/L respectively, $p=0.066$). Heterozygous genotypes at either loci result in intermediate MBL serum levels. The exon 1 genotypes producing the lowest MBL serum levels are A/B (n=21), A/C (n=2), A/D (n=12) and O/O (n=5) (figure 3). A/D subjects have significantly higher serum levels than A/B subjects ($p=0.006$). The frequency of A/O and O/O carriers in each population was 43%, and 29% for CPA and allergic subjects respectively (figure 4). No single haplotype, genotype or allele was significantly related to any of the aspergillosis phenotypes ($p>0.05$).

A variety of disease progression/severity markers were analysed to assess associations with MBL. Functional homozygote allergic subjects (n=27) in this study were ~11 years younger than allergic subjects (n=11) carrying exon 1 defects (mean age at the time samples were taken was 55.6 vs 66.4 years old, $p=0.020$). In A/A subjects, the MBL serum level was significantly higher in those without bronchiectasis than those with (mean MBL 2954 vs 2291µg/L, $p=0.032$). For all CPA (combined) subjects there was no significant difference in MBL levels between those with and without bronchiectasis (1852 vs 1980 µg/L; $p=0.74$). A similar non-significant result was obtained for allergic subjects (1493 vs. 1863 µg/L $p=0.42$). Comparison between aspergillosis disease phenotypes reveals that CPA subjects retain average higher MBL serum levels compared to allergic subjects regardless of bronchiectasis (A/A CPA vs A/A allergic: 2981 vs 2202µg/L, $p=0.012$). Furthermore, subjects in CPA band 3 (10/70) had significantly higher circulating MBL levels compared to

those in band 2 (29/70); (all: 2790 vs 1549 μ g/L, $p=0.034$, A/A only: 3700 vs 2992 μ g/L, $p=0.12$) (figure 5a). The relationship between MRC dyspnoea score and MBL levels among A/A subjects is significant ($\rho = 0.33$; $p=0.009$). Mean MBL values were higher in those with an MRC score of 5 compared with those with an MRC score of 1 ($p=0.023$) (Figure 5b). Conversely, 46% of the subjects with non-functional MBL had MRC dyspnoea scores of 4 or greater.

Discussion

In this study we have demonstrated that the frequency of genotypes producing non-functional MBL in patients with chronic or allergic aspergillosis was not different from published normal controls. Non-functional genotypes were more common with worsening breathlessness. ABPA subjects with wild type MBL2 were ~11 years younger than ABPA subjects with non-functional genotypes. We have also shown that amongst wild type genotype patients those with CPA had higher serum MBL levels compared with allergic patients. Subjects with worse respiratory status and more severe CPA had higher MBL serum levels, while those with bronchiectasis had lower MBL levels.

This study contradicts the previous findings of small studies published from our group and collaborators that the *MBL2*D allele is associated with CPA (Crosdale *et al.*, 2001; Vaid *et al.*, 2007) and elevated serum levels are significant in ABPA (Kaur *et al.*, 2005) (table 1). In this larger study, we describe data from 108 subjects of which 70 suffer from CPA (57 with CPA only) and 28 from ABPA. It is likely that this larger dataset has given more accurate results than the small datasets of <15 subjects previously described, but is still a relatively small study. One alternative hypothesis for the disparity in our findings includes referral to our service years ago of more advanced and overt CPA, with an increasing number of milder cases referred more recently; the A/D allele is associated with more severe disease. Another hypothesis relates specifically to *MBL2* A/D heterozygotes. These subjects display a wide variation in serum MBL levels (figure 3) (Minchinton *et al.*, 2002) and demonstrated considerable variability in production of high molecular weight (and functional) MBL heteroligomeric forms (Dean *et al.*, 2006), for uncertain reasons. Therefore within the *MBL2* A/D heterozygote group considerable variation exists, with a possibility that a susceptibility association does exist, but only with those with low production of functional MBL, which is not detected with *MBL2* genotyping. While a low serum

MBL level is associated with invasive aspergillosis (IA) in febrile immunocompromised subjects (Lambourne *et al.*, 2009), we found low MBL only associated with bronchiectasis complicating chronic *Aspergillus* infection in A/A subjects and milder CPA.

The average MBL serum level determined by ELISA in A/A individuals is approximately 1800 μ g/L (calculated using the average healthy levels determined by ELISA for Danish, Inuit, African (Madsen *et al.*, 1994) and Japanese subjects (Terai *et al.*, 2003) (Supplemental table 1)). In our UK aspergillosis population, A/A carriers have a mean average serum level of 2667 μ g/L. Separating these by disease phenotype shows that CPA subjects have significantly higher circulating MBL levels than allergic subjects (2981 vs 2202 μ g/L; $p=0.012$) and substantially higher levels than healthy subjects in the literature (Supplemental table 1). MBL has been shown to carry upstream regulatory elements similar to acute phase reactant proteins such as C-reactive protein and heat shock elements. It also exhibits increased circulating levels of up to three-fold after infection with malaria or hip-replacement surgery regardless of pre-infection or pre-trauma levels (Thiel *et al.*, 1992). In UK children undergoing chemotherapy for various malignancies, the average median MBL level in A/A carriers rose from 2944 μ g/L at the time of diagnosis to 5675 μ g/L at day 7 of the first febrile neutropenic episode (Neth *et al.*, 2001). The higher serum levels in subjects

with CPA may indicate an ongoing immunological response to colonisation with *Aspergillus*; most CPA patients have elevated C-reactive protein and/or plasma viscosity even on treatment, unlike most ABPA and SAFS patients. The true circulating MBL concentration may be significantly higher due to the 4000µg/L cut off imposed by the ELISA method used here. The serum levels of normal HYPA / HYPA individuals assayed by TRIFMA have been shown to reach approximately 10,000µg/L (Steffensen *et al.*, 2000). This is one limitation of our study.

Separate analysis of ABPA and SAFS subjects indicates that individuals with SAFS have a mean MBL serum level of 1193µg/L while those with ABPA have 1884µg/L, regardless of genotype. Conclusions cannot be drawn as to the significance of these differing levels as the SAFS population contained only 7 subjects and the levels are not substantially different from those reported in studies of healthy individuals.

Given the strong relationship between MBL deficiency and bacterial infection, it is possible that MBL could be a disease modifier in the context of CPA and/or allergic aspergillosis as has been suggested in cystic fibrosis (Munleback *et al.*, 2006). Many aspergillosis patients also have recurrent bacterial infections, some in the context of bronchiectasis, others in damaged lung parenchyma. Low MBL levels could predispose these patients to recurrent bacterial infections, leading to worse symptom control, an increased inflammatory state and increased fibrosis. Alternatively, it could be that high functioning MBL levels result in greater complement activation, more neutrophil-mediated damage and resulting fibrosis. Thus our finding that the 27 allergic subjects with functional homozygote MBL2 in this study were ~11 years younger than the 11 allergic subjects carrying exon 1 defects is more consistent with MBL being detrimental. *Aspergillus* has been shown to promote MBL-mediated activation of the complement pathway via a C2 bypass mechanism (Dumestre-Pérard *et al.*, 2008). Deficient patients may be relatively protected from tissue damage resulting from continuous *Aspergillus* exposure in the airways, leading to a later clinical presentation and presumably less bronchiectasis. Given that patients with more advanced CPA (band 3) had slightly higher MBL levels compared with milder disease (band 2), this finding is most consistent with an acute phase response, but could reflect more tissue damage as a result of MBL activity, or be a chance finding of no significance. The banding is somewhat arbitrary, and includes non-pulmonary elements, and so is not a great measure of CPA severity. Case-controlled studies addressing these questions are required, and are complex due to the long time frames, a lack of precision in defining fibrosis (especially if it is localised) and co-morbid conditions such as other genetic defects (i.e. *TLR4*, *IL15*, *IL10*) (Sambatakou *et al.*, 2006; Carvalho *et al.*, 2008)) potentially impacting on disease progression. Respiratory function (as measured by the MRC dyspnoea scale) was worse in patients with higher levels of functional MBL. However, patients with higher MRC scores were more likely to carry non-functional exon 1 alleles (data not shown). These differences probably reflect differing reasons for worsening respiratory status: MBL deficiency may leave patients more susceptible to bacterial infections (with consequent fibrosis and loss of respiratory reserve) whilst in those with functional MBL, worsening breathlessness reflects current or previously uncontrolled disease, possibly reflecting an acute phase response associated with bacterial infection.

This data reinforces previously published work indicating that the genotypes A/O, O/O and X/X are the most important with respect to MBL deficiency. An allelic real-time PCR assay to identify subjects carrying these defects may be a rapid way to diagnose neutropenic patients who might benefit from MBL therapy without bias by varying levels of expression depending on infection status.

In conclusion, this study has found that the known characterised *MBL2* genotype defects are not associated with chronic or allergic aspergillosis. Subjects with chronic infection have significantly higher MBL serum levels than subjects with allergic disease, and those with more severe chronic infection have the highest. MBL presents a dichotomy between high/low functional circulating levels and the susceptibility/protection to infection or allergy and consequent morbidity. Larger and replication studies are required to confirm the findings, with analyses that include disease severity and complications of allergic and chronic pulmonary aspergillosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Diagrammatic representation of the MBL2 gene and final protein conformation, indicating the 3 upstream polymorphisms affection protein expression and serum levels, and the 3 open reading frame SNPs in exon 1 which greatly affect monomer polymerisation and final MBL function.

Figure 2.

MBL serum levels found in A/A subjects with CPA, allergic fungal disease and both CPA and ABPA ($P=0.012$). The bars represent the mean and 95% CI.

Figure 3.

MBL serum level in low producing *MBL2* genotype carriers with aspergillosis. The bars represent the mean and 95% CI ($p=0.006$ for comparison A/D vs A/B). Most levels are very low as expected, but

Figure 4.

The frequency of A/A and O allele carrying subjects with aspergillosis disease phenotype compared to a control population²⁸ ($p = 0.22$ comparing allergic and CPA subjects).

Figure 5. A/A carrier MBL serum levels in a) CPA subjects with increasing morbidity as assessed by CPA band ($p=0.12$) and b) all subjects categorised by respiratory function as assessed by MRC dyspnoea scale ($p=0.009$). The bars represent the mean and 95% CI

Table 1

Summary of the results from previous studies into MBL associations with aspergillosis

Subject type	N	MBL association investigated	p-value	Reference
CNPA	10	D allele	0.015	Crosdale <i>et al.</i> , 2001
CCPA	15	D allele	0.02	Vaid <i>et al.</i> , 2007
ABPA	7	D allele	ns	Vaid <i>et al.</i> , 2007
ABPA	11	Elevated serum level	<0.01	Kaur <i>et al.</i> , 2005
ABPA	11	0 alleles	ns	Kaur <i>et al.</i> , 2006
Febrile immunocompromised with proven or probable invasive aspergillosis	65	Serum level deficiency	<0.001	Lambourne <i>et al.</i> , 2009
CPA	23	B allele	ns	Gomi <i>et al.</i> , 2004

Table 2

The frequency (%) of each individual *MBL2* allele genotype in subjects with CPA and, allergic fungal disease. The mean serum level associated with each individual genotype is also listed.

Locus (substitution)	Genotype	Chronic	Allergic	Mean serum level (µg/L)
H/L -550 rs11003125	GG	14 (20)	7 (18)	2441
	GC	29 (41)	17 (45)	1923
	CC	27 (39)	14 (37)	1462
Y/X -221 rs7096206	GG	43 (61)	20 (53)	2254
	GC	25 (36)	16 (42)	1318
	CC	2 (3)	2 (5)	900
P/Q +4 rs7095891	CC	49 (70)	24 (63)	1411
	CT	16 (23)	14 (37)	2737
	TT	5 (7)	0 (0)	2910
52 (R52C) rs5030737	CC	63 (90)	31 (82)	1984
	CT	7 (10)	6 (16)	1011
	TT	0 (0)	1 (3)	50
54 (G54D) rs1800450	GG	49 (70)	34 (90)	2315
	GA	19 (27)	3 (8)	330
	AA	2 (3)	1 (3)	100
57 (G57E) rs1800451	GG	68 (97)	37 (97)	1899
	GA	1 (1)	1 (3)	125
	AA	1 (1)	0 (0)	50
Allele				
A/A	CC/GG/GG	40 (57)	27 (71)	2667
A/D	CT	7 (10)	5 (13)	1091
	TT	0 (0)	1 (3)	50
A/B	GA	19 (27)	2(5)	344
	AA	2 (3)	1 (3)	100
A/C	GA	1 (1)	1 (3)	125
	AA	1 (1)	0 (0)	50
Other			1 *	

* HYPD LYPB