

Polymorphism of the mast cell chymase gene (*CMA1*) promoter region: lack of association with asthma but association with serum total immunoglobulin E levels in adult atopic dermatitis

T. Iwanaga*†‡, A. McEuen†, A. F. Walls†, J. B. Clough†, T. P. Keith§, S. Rorke†, S. J. Barton†, S. T. Holgate† and J. W. Holloway*†

Divisions of *Human Genetics and †Infection, Inflammation and Repair, School of Medicine, University of Southampton, Southampton General Hospital, Southampton, UK, ‡Department of Respiratory Medicine and Allergology, School of Medicine, Kinki University, Ohnohigashi, Osakasayama-city Osaka, Japan and §Genome Therapeutics Corporation, Waltham, MA, USA

Summary

Background Mast cell chymase has the potential to be an important mediator of inflammation and remodelling in the asthmatic lung. Previous studies have examined association between promoter polymorphism of the chymase gene (*CMA1*) and allergic phenotypes but the significance of this polymorphism is unclear. We have examined association of a *CMA1* variant in relation to asthma in a large UK Caucasian family cohort.

Methods A polymorphism of the *CMA1* gene promoter (–1903G/A) was genotyped in 341 asthmatic families and in 184 non-asthmatic adults recruited from the UK PCR-RFLP based genotyping. Association with asthma diagnosis, atopy, specific and total IgE, and atopy and asthma severity was examined.

Results Case–control studies did not reveal a significant difference in allele frequency between asthmatics and controls. A significant association was found between *CMA1* genotypes and total IgE levels in subjects with self-reported eczema that remained significant after correction for multiple testing (median total serum IgE GG 297 kU/L, GA 144 kU/L, AA 48.4 kU/L, $P_c = 0.0032$).

Conclusion These data suggest that *CMA1* promoter polymorphism does not contribute to asthma susceptibility or severity but may be involved in regulating IgE levels in patients with eczema.

Keywords asthma, chymase, eczema, IgE, polymorphism (genetic)

Submitted 25 September 2003; revised 15 March 2004; accepted 19 April 2004

Introduction

Mast cells have been implicated, directly or indirectly, in many pathological features of chronic allergic asthma [1]. A range of potent inflammatory mediators may be released from mast cells, but among the most abundant is the chymotryptic protease chymase. There is growing evidence that chymase has the capacity to contribute in important ways to processes of inflammation and tissue remodelling in bronchial asthma [2].

Human chymase is a 226 residue protein with a two amino acid pro sequence, that appears to be encoded by a single gene. The enzyme is selectively stored in mast cell secretory granules and it is released in a fully active form along with histamine, tryptase and other granule contents. A high degree of selectivity for protein substrates has been observed for chymase. However it is particularly effective at cleaving angiotensin I, to generate angiotensin II, a reaction that

chymase can catalyse more efficiently than angiotensin converting enzyme itself [3]. Evidence for potent pro-inflammatory roles for chymase have come from studies involving injection of the human enzyme into rodent models, which have led to increased microvascular permeability [4] and to eosinophil and neutrophil accumulation [5]. Contributions to tissue remodelling have been suggested by observations that human chymase can initiate the formation of collagen fibrils in type I procollagen [6], activate pro-MMP-1 interstitial procollagenase [7], and can release transforming growth factor $\beta 1$ (TGF- $\beta 1$) from the extracellular matrix, a cytokine which can stimulate the formation of connective tissue [8].

Recently, Brightling et al. [9] have reported that the infiltration of mast cells into airway smooth muscle may be a distinguishing feature of asthma and that this may be associated with the disordered airway function. Although the presence of chymase in mast cells in the smooth muscle bundles was little investigated in that study, previous investigations have established that high proportions of mast cells at such sites contain chymase [10].

It is widely accepted that asthma is a heritable disease and a number of studies have shown an increased prevalence of

Correspondence: Dr Takashi Iwanaga, Department of Respiratory Medicine and Allergology, School of Medicine, Kinki University Ohnohigashi, Osakasayama-city, Osaka 589-8511, Japan.

E-mail: iwanaga@med.kindai.ac.jp

asthma, and phenotypes associated with asthma, among the relatives of asthmatic subjects compared with non-asthmatic subjects [11]. An association between atopic dermatitis/eczema (AEDS [12]) and a genetic variant of the gene encoding chymase (*CMAI*), a *Bst*XI polymorphism (–1903G/A), located on the long arm of chromosome 14 (14q 11.2) has previously been reported in a Japanese population [13]. The aim of the present study was to investigate the role of this polymorphism in the *CMAI* gene in conferring susceptibility to the development of atopy and/or asthma in a large population of Caucasian asthmatic families recruited from the Wessex region of southern England.

Materials and methods

Subjects and clinical assessment

Affected sib-pair families Three hundred and forty-one Caucasian affected sib-pair families were collected in the UK, as previously described [14, 15]. The definition of asthma used consisted of a physician's diagnosis of asthma, together with current asthma medication use. Bronchial hyper-responsiveness (BHR) to methacholine, total serum IgE and specific serum IgE were measured [14, 15], and severity scores for atopy and asthma were generated [14, 16]. Eczema was defined as a positive response to the question 'Have you ever had eczema?' on the case report form.

Hyper-normal controls One hundred and eighty-four Caucasian 'hyper-normal' controls were recruited in the UK. These individuals had no diagnosis of asthma, and no family history of asthma [14].

Molecular methods Genomic DNA was extracted from 10 mL peripheral blood leukocytes by means of a Genomic DNA Maxi-Prep Kit (Qiagen, Crawley, UK). Genomic DNA was stored at –20 °C until further use. To optimize genotyping, a nested PCR was used. An initial PCR of the *CMAI* promoter was performed on a PTC-225 DNA Engine Tetrad (MJ Reset, Watertown, MA, USA) in a total volume of 10 µL including 8 ng of genomic DNA, 1.5 mM MgCl₂, 400 nM of primer mix, 0.5 U of *Taq* DNA polymerase (Sigma-Aldrich, Poole, UK), 0.004 U of *Pwo* DNA polymerase (Roche, Mannheim, Germany), and 250 µM of each deoxynucleotide triphosphate (Amersham Biotech, Amersham, UK). The PCR conditions were 2 min at 94 °C, followed by 45 cycles each of 20 s at 94 °C, 30 s at 63 °C, 6 min at 68 °C, and final 20 min at 68 °C. The forward primer was 5'-TTGAG TATCTGCCGCAATCAGAAATC-3' and reverse primer was 5'-GGCTGGGAATCAGGTCACACTATGTG-3'. The nested PCR assays were performed in a total volume of 10 µL including 1 µL initial PCR product diluted to 1/1000, 1.5 mM MgCl₂, 200 nM of each primer, 0.25 U of *Taq* DNA polymerase, and 200 µM of each deoxynucleotide triphosphate. The PCR conditions were 2 min at 94 °C, followed by 30 cycles each of 30 s at 94 °C, 30 s at 58 °C, 30 s at 72 °C, and finally 5 min at 72 °C in 96-well plates. The forward primer was 5'-TACACCCCTTTATCTGATTA-3' and reverse primer was 5'-CTAGTAAGACAGCTCCACAG-3'. Two 2 µL of the nested PCR products were digested with 7.5 U of *Bst*XI (New England Biolabs, Hitchin, UK) for 4 h at 55 °C.

Digested products of 508 bp (–1903A) and 547 bp (–1903G) were separated by MADGE (microplate array diagonal gel electrophoresis) [17] on 5% polyacrylamide gel and visualized by staining with Vistra Green (Amersham Biotech). ABI PRISM[®] BigDye[™] Terminator cycle sequencing (Applied Biosystems, Warrington, UK) was used to validate the genotyping assay.

Statistical methods

Transmission disequilibrium test (TDT) analysis Association of the –1903G/A *CMAI* polymorphisms with asthma and atopy was tested in the trios of mother, father and first affected sibling with available genotype, as previously described [14, 15]. TDT was performed in STATA (Version 6.0, Stata Corporation, College Station, TX, USA) using the programme written by David Clayton (MRC Biostatistics Unit, Cambridge, UK). Any TDT test with a *P*-value of <0.05 was considered significant. Dichotomous variables analysed using TDT were: (i) Asthma positive on questionnaire ('Have you ever had asthma?', 'Was this confirmed by a doctor?' and 'Have you used any medicines to treat asthma, or any breathing problems, at any time in the last 12 months?'); (ii) Asthma positive on questionnaire, with atopy (defined by positive specific IgE (An individual was assigned a positive specific IgE value if his/her level was positive (to grass or tree) or elevated (= 0.35 kU (kilo units)/L for cat, dog, *Dermatophagoides pteronyssinus*, *D. farinae*, *Alternaria*) for at least one such measure) and/or positive skin prick test (weal = 3 mm for house dust mite, cat, grass or tree)); (iii) Asthma positive on questionnaire, with raised total serum IgE (using age corrected cut-offs as previously described [14, 15] (age 5–9 years, >52 kU/L; age 10–14, >63 kU/L; age 15–18, >75 kU/L; age = 19, >81 kU/L)); (iv) Asthma and PC₂₀ methacholine <4 mg/mL (severe asthmatics); (v) Asthma and PC₂₀ methacholine <16 mg/mL and (vi) Asthma and self-reported eczema (defined as a positive response to the question 'Have you ever had eczema?').

Case-control analysis Data were analysed in three separate groups of cases, and participants within each group were considered to be independent. The group of cases were: first affected sibling, second affected sibling, and parents with a diagnosis of asthma. Hardy-Weinberg equilibrium was confirmed using χ^2 with 1 degree of freedom. Genotype frequencies for each group of cases were compared to the 'hyper-normal' control population, and analysed using χ^2 in SPSS Version 11 (SPSS Inc., Chicago, IL, USA).

Disease severity Data were analysed in three separate groups of cases, and participants within each group were considered to be independent. The groups of cases were: first affected sibling, second affected sibling, and parents with a diagnosis of asthma. *t*-tests and non-parametric tests (SPSS Version 11) were performed as appropriate on the following variables: (1) total IgE (age corrected and log transformed to improve normality), (2) forced expiratory volume in 1 s (FEV₁) (% predicted), (3) BHR (slope transformed to [1/(LS slope+30)] × 1000 to improve normality), (4) atopy severity score, (5) asthma severity score, (6) first principal component for weal size (this is computed by taking the first principal component of the mean weal diameters of the skin

prick tests (SPTs) for each allergen to provide a measure of the magnitude of the skin prick response as a whole), and (7) number of positive SPT (analysed by χ^2 test for association). A *P*-value of 0.05 was considered significant. In the parental cohort only, an additional analysis of total IgE in subjects with self-reported eczema was performed.

Results

The phenotypic characteristics of the study population are summarized in Table 1. Table 2 shows the distribution of the genotypes and alleles of the *CMA1* gene polymorphism in 171 asthmatic parents, 306 asthmatic first sibling, 299 asthmatic second sibling and 176 control (non-asthmatic) subjects. The frequencies of promoter polymorphism (–1903G/A) in the *CMA1* gene have previously been reported as G = 0.43, A = 0.57 in Caucasian [18] and G = 0.8, A = 0.2 in Japanese populations [13], respectively. In this study, the frequency of the G allele was 0.48 and A allele was 0.52 in the control population. The genotype frequencies did not deviate significantly from the Hardy–Weinberg expectation in the unrelated asthmatic parents ($\chi^2 = 0.050$, *P* = 0.82), or control population ($\chi^2 = 2.325$, *P* = 0.13). No significant difference in genotype or allele frequency was observed between any of the asthmatic groups and the non-asthmatic control population.

The results of the TDT analysis are shown in Table 3 for; positive asthma, F positive asthma+atopy (SPT positive and/or increased specific IgE), positive asthma+raised total IgE (age corrected), positive asthma+PC₂₀ <4 mg/mL, and asthma+PC₂₀ = 16 mg/mL. There was a trend to an increase in transmission of the –1903G allele to sibs with case report form positive asthma + raised total IgE (age corrected)

Table 1. Phenotypic characteristics in asthmatic families and controls

	Asthmatic parents	Sibling 1	Sibling 2	Controls
Number	189	341	338	184
Mean age (years)	40.2	13.0	9.9	42.3
Gender (% male)	47.1	56.9	53.6	47.0
Asthma (% +ve, doctor diagnosis)	100.0	100.0	100.0	0.0
Atopic eczema (%+ve, questionnaire)	50.8	57.8	62.4	0.0
Hayfever (%+ve, questionnaire)	69.8	64.2	47.0	8.7
FEV ₁ (% predicted)	94.1	94.7	95.6	NT
BHR (1/LS slope+30) × 1000	17.2	14.5	12.0	NT
Median total IgE (kU/L)	107	248.5	311	NT

FEV₁, forced expiratory volume in 1 s; BHR, bronchial hyper-responsiveness; NT, not tested.

(*P* = 0.06), however, this did not reach significance. Within the asthmatic cohorts (first affected sibling, second affected sibling and affected parents) no positive associations were observed between *CMA1* –1903G/A polymorphism and any of the phenotypes examined (Table 4). However, given the previously observed association of the –1903G/A polymorphism with AEDS (especially those with AEDS and low total serum IgE levels (<500 IU/mL)) [19, 20], the analysis was extended to look at the relationship between the –1903G/A genotype and total serum IgE levels in those asthmatic parents with self-reported eczema. A significant association was seen between total serum IgE levels in these subjects (*P* = 0.0004) that remained significant after correction for multiple testing (*P*_c = 0.0032, (*P* = 0.0004 × 8 disease severity analyses)). No association was seen between total serum IgE and the –1903G/A genotype in either of the affected sibling groups with self-reported eczema (first sibling *P* = 0.12, second sibling *P* = 0.61). For the asthmatic parents with eczema, the median total IgE levels for each genotype group were as follows: GG 297 kU/L (*N* = 23), GA 144 kU/L (*N* = 45), AA 48.4 kU/L (*N* = 22).

Discussion

In the current study we have examined the association of the *CMA1* –1903G/A polymorphisms with a number of atopy and asthma phenotypes in a large well-defined Caucasian family cohort. While no significant associations were observed between this polymorphism and any asthma related phenotypes examined, a significant association with total IgE levels in patients with self-reported eczema was observed. A link between IgE levels and eczema has been noted previously in Japanese patients with this condition [19, 20].

It is now widely accepted that asthma is a heritable disease and a number of studies have shown an increased prevalence of asthma, and phenotypes associated with asthma, among the relatives of asthmatic subjects compared with non-asthmatic subjects [21]. Genome screens for asthma and asthma-associated phenotypes have identified a number of regions of linkage. The most consistent and robust linkages have been with regions on chromosomes 5, 6, 12, and 13 [22], although linkage to the *CMA1* locus (14q11.2) has also been reported in a genome scan of US Caucasian families [23]. The *CMA1* gene promoter polymorphism (–1903G/A) has also been previously associated with a several other non-allergic disease phenotypes including heart disease reflecting the wide range of physiological action of mast cell chymase [18, 24].

Given the key role mast cell inflammatory mediators play in the allergic immune response, and in particular the role of the

Table 2. Genotype and allele distribution of *CMA1* –1903G/A in UK asthmatic families and controls

	<i>N</i>	Genotype <i>N</i> (%)			Allele <i>N</i> (%)		χ^2	<i>P</i>
		GG	GA	AA	G	A		
Controls	176	36 (0.20)	98 (0.56)	42 (0.24)	170 (0.48)	182 (0.52)		
Asthmatic parents	171	42 (0.25)	86 (0.50)	43 (0.25)	170 (0.50)	172 (0.50)	0.09	0.77
Sibling 1	306	63 (0.21)	174 (0.57)	69 (0.22)	300 (0.49)	312 (0.51)	0.02	0.88
Sibling 2	299	74 (0.25)	157 (0.53)	68 (0.22)	305 (0.51)	293 (0.49)	0.55	0.46

Table 3. Association of *CMA1* – 1903G/A with asthma and atopy in asthmatic families

Phenotype	Number of genotyped offspring	Allele	Observed transmissions	Expected transmissions	P-value
Asthma positive	332	A	160	147.5	0.15
		G	135	147.5	
Asthma positive and positive atopy	285	A	138	125	0.10
		G	112	125	
Asthma positive and raised total IgE	294	A	149	133.5	0.06
		G	118	133.5	
Asthma positive and PC ₂₀ < 4 mg/mL	240	A	120	107	0.08
		G	94	107	
Asthma positive and PC ₂₀ = 16 mg/mL	289	A	136	128	0.32
		G	120	128	

Table 4. Disease severity analysis; *CMA1* – 1903G/A polymorphism association with atopy and asthma intermediate phenotypes in asthmatic subjects

	Log age-corrected total IgE	FEV ₁ (% predicted)	BHR slope	Atopy severity score	Asthma severity score	First principle component for weal size	Number of positive SPTs
Asthmatic parents, P-value	0.06	0.50	0.24	0.51	0.27	0.60	0.98
First sibling, P-value	0.40	0.40	0.33	0.61	0.89	0.30	0.56
Second sibling, P-value	0.66	0.75	0.20	0.93	0.26	0.50	0.53

FEV₁, forced expiratory volume in 1 s; BHR, bronchial hyper-responsiveness; SPT, skin prick test.

mast cell proteases tryptase and chymase in mediating chronic inflammation of the skin in atopic dermatitis and of the lung in asthma [25, 26], mast cell chymase has been considered a strong candidate gene for atopy and atopic disease. The first study of the potential role of *CMA1* polymorphism in conferring susceptibility to atopic disease was by Mao et al. [13]. In a Japanese cohort, association was seen between AEDS and a *BstXI* polymorphism (– 1903G/A) of the *CMA1* promoter. In a subsequent study of a randomly recruited childhood cohort, the – 1903G/A polymorphism was again associated with AEDS (especially those with AEDS and low total serum IgE levels) [19]. The association with AEDS patients with lower total serum IgE levels (< 500 IU/mL) was also confirmed in a third study of Japanese patients [20]. However, a small study by Kawashima et al. [27] failed to replicate the association. In our study, the observation of association with total serum IgE in patients with reported eczema was found only in the adult cohort. This may reflect differences between the paediatric and adult cohorts (in mean serum IgE levels, the percentage of asthma, etc.), be an age related effect or perhaps reflect that the chymase polymorphism exerts its effect in the context of chronic persistent eczema, rather than at its origin. Two previous studies have examined association of the – 1903G/A polymorphism in Caucasian cohorts. In a study of 50 Australian families [28] and a small case–control study of Italian AEDS patients [29], no association was seen although this may be due to lack of statistical power of these studies given the small size of the cohorts.

While the association of the – 1903G/A polymorphism with atopic eczema has been extensively studied, the contribution of this polymorphism to asthma and BHR remains to be established. The initial studies by Mao et al. [13, 19] failed to find any association with either atopic or non-atopic asthma although no measures of lung function were examined. In contrast, Tanaka et al. [20] found an association

in patients with atopic eczema and asthma between the – 1903G allele and high IgE levels (> 2000 IU/mL). The recent observation that mast cells infiltrate airway smooth muscle and this may be associated with the disordered airway function found in asthma [9] raises the possibility that polymorphism of mast cell mediator genes might alter BHR and susceptibility to asthma. Given only 252 trios would be required to give 80% power to detect a relative risk of 1.5 for asthma with $\alpha = 0.01$ [30], the findings of the present study would suggest that chymase polymorphism is not an important determinant of BHR susceptibility. In the future it would be of interest to examine genetic variability in other mast cell-derived mediators, or in the pathways leading to the generation of these mediators, such as histamine, tryptase, leukotrienes (LT)-D₄ and LT-C₄, Prostaglandin (PG)D₂, and TGF- β which are known to cause smooth muscle proliferation and/or contraction [9].

The functional consequences of the *CMA1* genotype are not clear, and its association with high total IgE levels in subjects with eczema is difficult to explain in terms of the known actions of human chymase. This protease may have a role in controlling the bioavailability of several cytokines, being able to degrade IL-4 [31], convert IL-1 into the active form [32], and release the membrane bound form of stem cell factor [33]. Of these, IL-4 has been implicated as having a key role in the generation of IgE, but its inactivation by human chymase appears paradoxical in the light of the other observations. On the other hand, the addition of a rat chymase (rat mast cell protease I) to a culture of murine spleen cells stimulated with IL-4 and lipopolysaccharide, has been reported to enhance synthesis of IgE [34]. Consistent with this is a report that administration of a synthetic chymase inhibitor (Y-40613) suppressed levels of total IgE in a rat model of atopic dermatitis [35].

Potential limitations of this study should be addressed. As families in this cohort were recruited on the basis of asthma,

there were too few atopic individuals without asthma to study the effect of the polymorphism on atopy alone. In addition, no clinical assessment of eczema was performed on the subjects recruited, resulting in the use of self-reported eczema in analyses. Several related phenotypes of relevance to asthma were analysed in this study and a number of comparisons were made. Although Bonferroni correction is too conservative when phenotypes are not independent, at least for the disease severity analysis, even when adjusting the uncorrected *P* value of 0.0004 for the number of comparisons ($n = 8$), the corrected *P*-value of 0.0032 remains statistically significant. Finally, we cannot exclude that the association observed is due to another causal allele in linkage disequilibrium with the single nucleotide polymorphism studied here.

It is possible that the positive associations seen between the –1903 G/A polymorphism and AEDS and asthma in Japanese patients may reflect the haplotypic background on which the polymorphism lies. In the Japanese population linkage disequilibrium may exist between this polymorphism and other functional variants of chymase that do not exist in the Caucasian population where no association has been seen. In the future, promoter assays and further identification of polymorphism combined with linkage disequilibrium studies will be needed to clearly establish whether the observed association is due to the –1903 G/A polymorphism altering gene expression, or is due to linkage disequilibrium with other functional polymorphisms in the *CMA1* gene.

In summary, we have examined the association of the *CMA1* –1903G/A polymorphisms with a number of atopy and asthma phenotypes in a large well-defined Caucasian family cohort. While no significant associations were observed between this polymorphism and any asthma related phenotypes examined, a significant association with total IgE levels in patients with self-reported eczema was observed in accordance with previous studies [19, 20].

Acknowledgements

We thank the patients and families that participated in this study. The collection of families was funded by Genome Therapeutics Corporation and Schering-Plough. J. W. H. is supported by a Medical Research Council (MRC) Research Training Fellowship.

Contributions of authors

S. T. Holgate, J. B. Clough and T. P. Keith designed the original study in which the subjects were recruited. J. W. Holloway, A. R. McEuen and A. F. Walls initiated this study of the *CMA1* polymorphism. T. Iwanaga performed the genotyping. S. J. Barton and S. Rorke undertook the statistical analysis. T. Iwanaga, A. F. Walls and J. W. Holloway drafted the report. All authors approved the final version of this report.

Contributions of the funding support

Genome Therapeutics Corporation and Schering-Plough were responsible for the design of the original study and

data collection. The funding bodies had no role in the genotyping, data analysis or writing of the report.

Conflicts of interest

None.

References

- 1 Walls AF, He S, Buckley MG, McEuen AR. Roles of the mast cell and basophil in asthma. *Clin Exp Allergy Rev* 2001; 1:68–72.
- 2 Walls AF. The roles of neutral proteases in asthma and rhinitis. In: Busse WW, Holgate ST, eds. *Asthma and rhinitis*, 2nd edn. Boston: Blackwell, 2000; 968–97.
- 3 Urata H, Kinoshita A, Misono KS, Bumpus FM, Husain A. Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart. *J Biol Chem* 1990; 265:22348–57.
- 4 He S, Walls AF. The induction of a prolonged increase in microvascular permeability by human mast cell chymase. *Eur J Pharmacol* 1998; 352:91–8.
- 5 He S, Walls AF. Human mast cell chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells *in vivo*. *Br J Pharmacol* 1998; 125:1491–500.
- 6 Kofford MW, Schwartz LB, Schechter NM, Yager DR, Diegelmann RF, Graham MF. Cleavage of type I procollagen by human mast cell chymase initiates collagen fibril formation and generates a unique carboxyl-terminal propeptide. *J Biol Chem* 1997; 272:7127–31.
- 7 Saarinen J, Kalkkinen N, Welgus HG, Kovanen PT. Activation of human interstitial procollagenase through direct cleavage of the Leu83-Thr84 bond by mast cell chymase. *J Biol Chem* 1994; 269:18134–40.
- 8 Taipale J, Lohi J, Saarinen J, Kovanen PT, Keski-Oja J. Human mast cell chymase and leukocyte elastase release latent transforming growth factor-beta 1 from the extracellular matrix of cultured human epithelial and endothelial cells. *J Biol Chem* 1995; 270:4689–96.
- 9 Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002; 346:1699–705.
- 10 Berger P, Walls AF, Marthan R, Tunon-de-Lara JM. Immunoglobulin E-induced passive sensitization of human airways: an immunohistochemical study. *Am J Respir Crit Care Med* 1998; 157:610–6.
- 11 Holloway JW, Jongepier H, Beghé B, Koppelman GH, Holgate ST, Postma DS. The Genetics of Asthma. *Eur Resp Monogr* 2003; 8:26–56.
- 12 Johansson SG, Hourihane JO, Bousquet J et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001; 56:813–24.
- 13 Mao XQ, Shirakawa T, Yoshikawa T et al. Association between genetic variants of mast-cell chymase and eczema. *Lancet* 1996; 348:581–3.
- 14 Sayers I, Barton S, Rorke S et al. Allelic association and functional studies of promoter polymorphism in the leukotriene C4 synthase gene (LTC4S) in asthma. *Thorax* 2003; 58:417–24.
- 15 Van Eerdewegh P, Little RD, Dupuis J et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; 418:426–30; doi:10.1038/nature00878, *Nature AOP*, published online 10 July 2002.
- 16 Holloway JW, Rorke S, Beghé B, Holgate ST. Genetic influences on asthma severity. In: Szeffer S, Leung D, eds. *Severe asthma*, 2nd Edn. New York: Marcel Dekker, Inc., 2001; 19–38.
- 17 Holloway JW, Beghé B, Turner S, Hinks LJ, Day INM, Howell WM. Comparison of three methods for single nucleotide polymorphism typing for DNA bank studies: sequence-specific

- oligonucleotide probe hybridisation, TaqMan liquid phase hybridisation, and microplate array diagonal gel electrophoresis (MADGE). *Hum Mutat* 1999; 14:340–7.
- 18 Pfeufer A, Osterziel KJ, Urata H et al. Angiotensin-converting enzyme and heart chymase gene polymorphisms in hypertrophic cardiomyopathy. *Am J Cardiol* 1996; 78:362–4.
 - 19 Mao XQ, Shirakawa T, Enomoto T et al. Association between variants of mast cell chymase gene and serum IgE levels in eczema. *Hum Hered* 1998; 48:38–41.
 - 20 Tanaka K, Sugiura H, Uehara M, Sato H, Hashimoto-Tamaoki T, Furuyama J. Association between mast cell chymase genotype and atopic eczema: comparison between patients with atopic eczema alone and those with atopic eczema and atopic respiratory disease. *Clin Exp Allergy* 1999; 29:800–3.
 - 21 Holloway JW, Beghé B, Holgate ST. The genetic basis of atopic asthma. *Clin Exp Allergy* 1999; 29:1023–32.
 - 22 Cookson WO. Asthma genetics. *Chest* 2002; 121 (Suppl.):7S–13S.
 - 23 Collaborative Study on the Genetics of Asthma. A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nat Genet* 1997; 15:389–92.
 - 24 Ortlepp JR, Janssens U, Bleckmann F et al. A chymase gene variant is associated with atherosclerosis in venous coronary artery bypass grafts. *Coron Artery Dis* 2001; 12:493–7.
 - 25 Hart PH. Regulation of the inflammatory response in asthma by mast cell products. *Immunol Cell Biol* 2001; 79:149–53.
 - 26 Walls AF. Mast Cell Proteases in Asthma. In: Holgate ST, Busse WW, eds. *Inflammatory mechanisms in asthma*. New York: Marcel Dekker, 1998; 89–110.
 - 27 Kawashima T, Noguchi E, Arinami T, Kobayashi K, Otsuka F, Hamaguchi H. No evidence for an association between a variant of the mast cell chymase gene and atopic dermatitis based on case-control and haplotype-relative-risk analyses. *Hum Hered* 1998; 48:271–4.
 - 28 Forrest S, Dunn K, Elliott K et al. Identifying genes predisposing to atopic eczema. *J Allergy Clin Immunol* 1999; 104:1066–70.
 - 29 Pascale E, Tarani L, Meglio P et al. Absence of association between a variant of the mast cell chymase gene and atopic dermatitis in an Italian population. *Hum Hered* 2001; 51:177–9.
 - 30 Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996; 273:1516–7.
 - 31 Tunon de Lara JM, Okayama Y, McEuen AR, Heusser CH, Church MK, Walls AF. Release and inactivation of interleukin-4 by mast cells. *Ann N Y Acad Sci* 1994; 725:50–8.
 - 32 Mizutani H, Schechter N, Lazarus G, Black RA, Kupper TS. Rapid and specific conversion of precursor interleukin 1 beta (IL-1 beta) to an active IL-1 species by human mast cell chymase. *J Exp Med* 1991; 174:821–5.
 - 33 Longley BJ, Tyrrell L, Ma Y et al. Chymase cleavage of stem cell factor yields a bioactive, soluble product. *Proc Natl Acad Sci USA* 1997; 94:9017–21.
 - 34 Yoshikawa T, Imada T, Nakakubo H, Nakamura N, Naito K. Rat mast cell protease-I enhances immunoglobulin E production by mouse B cells stimulated with interleukin-4. *Immunology* 2001; 104:333–40.
 - 35 Imada T, Komorita N, Kobayashi F et al. Therapeutic potential of a specific chymase inhibitor in atopic dermatitis. *Jpn J Pharmacol* 2002; 90:214–7.