

Polymorphisms of the *ADAM33* gene are associated with accelerated lung function decline in asthma

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Summary

Background Asthma is a genetically complex disease characterized by respiratory symptoms, intermittent airway obstruction and airway hyper-responsiveness due to airway inflammation and remodelling. The *ADAM33* gene is associated with asthma and airway hyper-responsiveness and is postulated as a gene for airway remodelling.

Objective To investigate whether polymorphisms of the *ADAM33* gene are associated with accelerated lung function decline in patients with asthma.

Methods In a cohort of 200 asthma patients followed over 20 years, eight single nucleotide polymorphisms of the *ADAM33* gene were analysed to estimate their effect on annual FEV₁ decline.

Results The rare allele of the S₂ polymorphism was significantly associated with excess decline in FEV₁ ($P < 0.05$).

Conclusion These findings suggest that a variant in *ADAM33* is not only important in the development of asthma but also in disease progression, possibly related to enhanced airway remodelling.

Keywords bronchial asthma, disease progression, FEV₁, longitudinal studies, SNP

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Introduction

Asthma is a common respiratory disease characterized by airway hyper-responsiveness accompanied by intermittent airway obstruction and respiratory symptoms. In asthma, the airways show features of acute and chronic inflammation and associated airway remodelling, which include thickening of the airway wall, subepithelial fibrosis and increased smooth muscle mass. These structural changes may contribute to the development of airflow limitation by increasing airway resistance. Although the majority of individuals with asthma have a normal level of lung function over time, a subgroup has accelerated lung function loss [1].

Asthma is a genetically complex disease and multiple genes and environmental factors play a role in its development. Recently, chromosome 20p13 was significantly linked to asthma and airway hyper-responsiveness in families with asthma from the United Kingdom (UK) and the United States (USA). *ADAM33* was identified in this chromosomal region as a candidate gene for asthma and airway hyper-responsiveness [2] and this has been replicated in our Dutch and USA

populations [3]. *ADAM33* is a member of the family of proteins known as ADAMs (A Disintegrin And Metalloproteinase). ADAM proteins play a role in cell fusion, cell adhesion, cell signalling and proteolysis. The latter includes the shedding of cytokines, growth factors or their receptors from the cell surface. Since *ADAM33* is expressed in airway smooth muscle cells and lung fibroblasts, it seems a plausible candidate for airway remodelling [4]. We hypothesise that polymorphisms of the *ADAM33* gene are associated with accelerated lung function decline in patients with asthma.

Materials and methods

Two hundred Dutch Caucasian individuals with a clinical diagnosis of asthma were referred to a local asthma centre in the northern part of the Netherlands between 1963 and 1975 for clinical evaluation and optimization of asthma treatment. All had airways hyper-responsiveness, 81.9% were atopic, geometric mean total serum IgE was 93.0 (range 1.0–2880) kU/L and all were younger than 45 years at that time. Between 1990 and 1999, these individuals were extensively re-examined [5]. Data on smoking history were collected by questionnaire. Data on lung function (forced expiratory volume in 1 s (FEV₁)) and steroid use over the years were collected from available medical records. The study was approved by the Medical Ethics

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Committee of the University Hospital Groningen. Written informed consents were obtained from all participants.

Lung function tests were performed with the same type water sealed spirometer during all successive years (Lode Spirograph D53, Lode Instruments, Groningen, the Netherlands). We genotyped eight single nucleotide polymorphisms (SNPs) in the 3' part of the *ADAM33* gene extending from exon Q to the last exon, exon V (i.e. S_1, S_2, ST_+4, ST_+7, T_1 (M to T), T_2 (P to S), V_-1, V_4). We focused on these SNPs, since they were associated with asthma as described by Van Eerdewegh et al. [2]. Genotyping was performed using the MassARRAY SNP genotyping system (Sequenom, Inc., San Diego, CA, USA). We performed analyses using linear mixed effect (LME) models, since this allowed us to include covariates that change over time. A total number of 4373 FEV₁ values was used in the LME, with age, gender, height, pack years smoking, steroid use and FEV₁ at age 30 years (start of analyses of FEV₁ decline) entered as covariates. Random effects were estimated for the intercept and for the decline in FEV₁. Each SNP was analysed separately; dummy variables for the heterozygous and the homozygous genotype were included in the model to estimate their effects on annual FEV₁ decline compared to the wild-type.

Results

Mean (range) period of longitudinal FEV₁ collection was 22 (2–34) years and median number of FEV₁ measurements as of 30 years of age was 29 (range 2–104) years per individual. Of the 200 individuals with longitudinal lung function data, 152 could be analysed using the LME and had sufficient DNA, 36 could not be analysed due to missing lung function data after the age of 30 years ($n = 29$), missing data on smoking history ($n = 5$) or both ($n = 2$). Twelve individuals were not genotyped for any of the *ADAM33* SNPs due to insufficient or poor quality DNA. All genotyped SNPs were in Hardy Weinberg equilibrium and the T_1 (M to T) was in almost full linkage disequilibrium with the T_2 (P to S) SNP.

The rare alleles of the SNPs S_2, T_1 (M to T) and T_2 (P to S) of the *ADAM33* gene were associated with a significant excess decline in FEV₁ ($P = 0.0061$, 0.0290 and 0.0296 , respectively, Fig. 1). Individuals that were homozygous for the rare alleles had an FEV₁ decline of 46.9–55.2 mL/year, resulting in an excess decline of 23.7–30.0 mL/year compared to the non-risk genotypes. Individuals who were homozygous for T_1 and T_2 ($n = 3$) constituted a subset of the individuals who were homozygous for the S_2 SNP ($n = 9$). The S_2 SNP was the only one that remained significantly associated with excess FEV₁ decline after multiple testing correction for eight tests (Fig. 1). These findings suggest that the S_2 polymorphism is the main locus or in linkage disequilibrium with the main locus determining excess decline in FEV₁. Additional analysis using a recessive model for the S_2 SNP did not change these results. Finally, since the number of individuals who were homozygous for the S_2 SNP was small, a sensitivity analysis on the S_2 SNP was carried out to investigate the influence of possible outliers. Exclusion of each of these subjects one by one did not result in a different estimate for the homozygous S_2 SNP. The characteristics of the individuals by their S_2 genotype are outlined in Table 1, differences were tested by Mann–Whitney U -tests. Since homozygous individuals were significantly older (median age

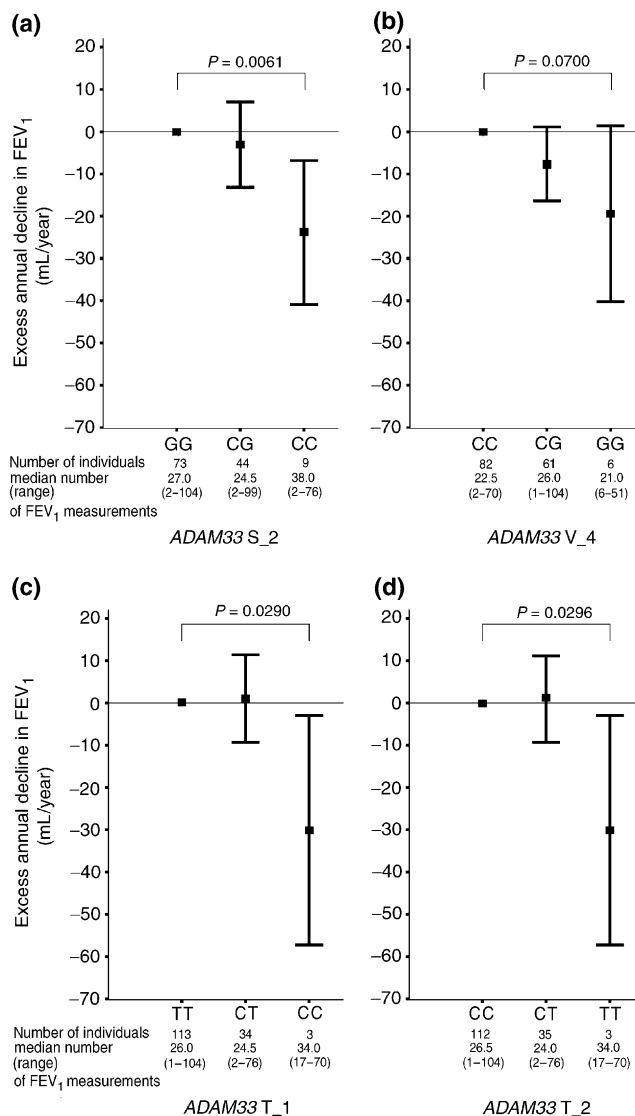


Fig. 1. Excess annual forced expiratory volume in 1 s (FEV₁) decline in mL/year (with 95% confidence interval) for the S_2, V_4, T_1 and T_2 SNPs of the *ADAM33* gene compared to wild-type, corrected for height, gender, pack years smoking, level of FEV₁ at age 30 years and use of steroids. Number of individuals and median number (range) of FEV₁ measurements are presented below the figures. Differences in numbers compared with 152 genotyped individuals are due to missing genotypes.

36.1 years, range (22.5–44.7 years)) at the initial visit than those with the heterozygous variant (26.0 (14.2–44.7 years)) and the wild-type genotype (25.0 (13.7–43.4 years)) ($P = 0.014$ and 0.011 , respectively), and their median age of 36.1 years is beyond the starting point of the analysis (i.e. 30 years), an additional analysis with a starting point at 35 years was performed. This analysis did not change the results and excludes the effect of age differences between groups.

Discussion

ADAM33 is member of the ADAM gene family of Zn²⁺-dependent matrix metalloproteases, which possess proteolytic and adhesion activity. Although the functionality of *ADAM33* has not been disclosed so far, polymorphisms have been associated with asthma [2] and this has been

Table 1. Characteristics of the individuals by their S_2 genotype

	ADAM33 S_2					
	Visit 1962–1975			Visit 1990–1999		
	GG	CG	CC	GG	CG	CC
Number	73	44	9	73	44	9
Male (%)	61.6	70.5	66.7	61.6	70.5	66.7
Age (years)	25.0 (13.7–43.4)	26.0 (14.2–44.7)	36.1* (22.5–44.7)	52.1 (37.9–75.3)	52.7 (37.9–71.8)	62.3† (45.2–69.8)
Pack years smoking	0.4 (0–33.4)	0.4 (0–25.4)	5.5‡ (0–15.6)	3.5 (0–49.9)	4.5 (0–49.3)	14.0 (0–25.9)
FEV ₁ , % predicted (pre-bronchodilation)	60.9 (13.6–122.1)	62.0 (10.6–118.0)	57.9 (20.1–80.0)	68.4 (19.7–111.3)	69.8 (25.5–111.9)	46.7§ (11.6–81.5)
FEV ₁ , % predicted (post-bronchodilation)	89.6 (21.7–136.9)	91.7 (23.0–121.3)	76.8 (41.0–92.2)	78.1 (27.3–119.1)	82.8 (31.0–122.5)	67.8¶ (18.8–99.9)
FEV ₁ /VC % (post-bronchodilation)	69.5 (26.0–91.0)	70.0 (25.0–93.0)	61.0 (38.0–78.0)	64.9 (34.0–86.8)	65.3 (29.6–83.3)	52.5 (21.7–69.1)
Reversibility, % predicted	23.7 (2.9–56.0)	23.4 (3.4–44.8)	20.9 (0.6–35.8)	12.9 (–9.0–37.2)	11.7 (–3.8–27.6)	7.2 (–1.3–24.3)
PC ₂₀ histamine (mg/mL)	4 (0.5–32)	4 (0.5–32)	4 (2–32)	4 (0.5 to ≥32)	4 (0.5 to ≥32)	6 (1.0 to ≥32)

*CC significantly older than CG and GG (respectively, $P=0.014$ and 0.011). †CC significantly older than GG ($P=0.04$) (compared with CG $P=0.08$). ‡CC significantly more pack years than CG ($P=0.03$) (compared with GG $P=0.09$). §CC significantly lower FEV₁% predicted pre-bronchodilation than CG ($P=0.04$) (compared with GG $P=0.06$). ¶CC significantly lower FEV₁% predicted post-bronchodilation than CG ($P=0.04$) (compared with GG $P=0.07$). All values expressed as median (range), unless stated otherwise. Differences were tested by Mann–Whitney U -tests. All individuals were Dutch Caucasians. FEV₁, forced expiratory volume in 1 s.

replicated in two independent studies [3, 6]. This study shows that polymorphisms of the *ADAM33* gene contribute to excess FEV₁ decline in a small subset of asthma patients with a relatively rare genotype (7% of our asthmatics). To our knowledge, this is the first genetic study disclosing an association between polymorphisms in a gene and longitudinal FEV₁ decline in asthmatics. Together with other results [2, 3], our findings suggest that SNPs in *ADAM33* not only confer susceptibility to asthma and airway hyper-responsiveness, but also modify the outcome of asthma. For individuals with asthma this is of clinical importance, since excess decline may lead to irreversible airway obstruction associated with increased symptomatology, including exacerbations and loss of quality of life. Previous association analyses performed in UK, USA, and Dutch populations did not give consistent results as to which SNPs or haplotypes conferred asthma susceptibility [3]. This suggests that multiple SNPs in *ADAM33* or in neighbouring genes may be important. The current study thus does not provide a definite answer as to whether the S_2, T_1 (M to T), and T_2 (P to S) SNPs of the *ADAM33* gene are functionally relevant to disease progression or that they are in linkage disequilibrium with functional variants in *ADAM33* or other genes in the vicinity. Confirmation of this study in other populations is therefore important. In addition, functional data on the *ADAM33* gene have not been published to date but is clearly needed.

Since this gene that encodes ADAM33 in asthma was only discovered in 2002, and we do not have information with respect to its biologic activities, we only can speculate as to its role in lung function loss in asthma. Van Eerdewegh et al. [2] reported that in humans, ADAM33 is expressed by lung fibroblasts and bronchial smooth muscle cells. Therefore, it is conceivable that ADAM33 is associated with airway remodelling [4] when an increase in functional properties or protein production results in proliferation of smooth muscle cells and fibroblasts. Alternatively, loss of ADAM33 function could lead to remodelling if normal ADAM33 function would pro-

tect the airways from increased repair processes. This would result in subepithelial fibrosis, which may contribute to accelerated lung function loss. Since the role of ADAM33 in the airways of asthma patients is yet unknown, we suggest that both options should be further investigated. In a previous study in 200 families in which the asthmatics under study for FEV₁ decline in the current analysis served as probands, the investigated eight SNPs in *ADAM33* conveyed susceptibility to asthma. How can we explain that one gene would serve both as an asthma susceptibility and asthma modifying gene? The current SNP itself in combination with SNPs at different locations in *ADAM33* may result in diverging functional changes of the gene. Alternatively, one SNP in *ADAM33* may only result in accelerated lung function loss in interaction with polymorphisms in different genes, a gene–gene interaction such as we previously found for asthma susceptibility in case of the genes of IL-13 and IL-4RA [7]. Furthermore, interaction of the SNP in ADAM33 that is responsible for asthma susceptibility may interact with genes that are only expressed as a result of environmental exposures and only then result in lung function loss. Future studies in larger cohorts have to establish the plausibility of interactions not only in asthma susceptibility but also in accelerated lung function loss in asthma patients.

Although in a relatively small number of individuals, this study for the first time shows that a gene that confers susceptibility to asthma and airway hyper-responsiveness may also be relevant to its disease progression. Genetic variants in *ADAM33* gene are associated with excess FEV₁ decline over more than 20 years in a cohort of asthma patients.

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