

A Disintegrin and Metalloprotease 33 Polymorphisms and Lung Function Decline in the General Population

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Rationale: A disintegrin and metalloprotease 33 (*ADAM33*) has been identified as a susceptibility gene for asthma and single nucleotide polymorphisms (SNPs) in this gene have been associated with excessive decline of lung function in individuals with asthma. **Objectives:** To assess whether SNPs in *ADAM33* are associated with accelerated lung function loss in the general population and with chronic obstructive pulmonary disease (COPD). **Methods:** DNA was collected from subjects of the Vlagtwedde–Vlaardingen cohort participating in the last survey in 1989–1990 after a follow-up of 25 years. Information was collected every 3 years, including lung function measurements. We defined COPD as GOLD stage 2 or higher at the last survey. A total of 1,390 subjects from the cohort was genotyped for the following SNPs in *ADAM33*: F+1, Q-1, S_1, S_2, T_1, T_2, V_4, and ST+5. Differences in prevalence of SNPs were analyzed with χ^2 tests. Linear mixed effects models were used to analyze FEV₁ decline according to genotype. **Measurements and Main Results:** In the whole population, mean adjusted decline was 18.7 and 12.7 ml/year in females and males, respectively. Individuals homozygous for minor alleles of SNPs S_2 and Q-1 and heterozygous for SNP S_1 had a significantly accelerated decline in FEV₁ of, respectively, 4.9, 9.6, and 3.6 ml/year compared with wild type. We found a significantly higher prevalence of SNPs F+1, S_1, S_2, and T_2 in subjects with COPD. **Conclusions:** We demonstrated that SNPs in *ADAM33* are associated with accelerated lung function decline in the general population. These SNPs are also risk factors for COPD.

Keywords: *ADAM33*; chronic obstructive pulmonary disease; FEV₁; genetics; single nucleotide polymorphism

A disintegrin and metalloprotease 33 (*ADAM33*) has been identified as an asthma susceptibility gene (1). In Dutch, American, German, and Korean asthma populations this finding has been replicated (2–5), in contrast to a Mexican population in which bronchial hyperresponsiveness was not tested (6). We have shown that polymorphisms in the *ADAM33* gene play a role not only in asthma susceptibility, but also in its progression (7). The S_2 polymorphism was associated with accelerated lung function decline in a Dutch asthma population monitored for 23 years. It is yet unknown whether this is specific for asthma or whether polymorphisms in *ADAM33* also affect lung function loss in the general population. A progressive decline in FEV₁ on a population level is partially related with asthma (8). In

addition, lung function decline is a risk factor for the development of chronic obstructive pulmonary disease (COPD) and cardiovascular disease (9). Associations of polymorphisms in *ADAM33* with FEV₁ decline may therefore constitute a risk for the development of COPD as well. In this study, we have investigated the role of eight single nucleotide polymorphisms (SNPs) in the *ADAM33* gene in lung function decline in 1,390 subjects of the Vlagtwedde–Vlaardingen cohort. This large population-based cohort has been monitored for a period up to 25 years, during which time lung function measurements were performed every 3 years. We aimed to establish whether the *ADAM33* gene is associated with accelerated lung function decline in the general population, and whether it is a susceptibility gene for the development of COPD.

METHODS

Subjects

We have used data from the 2,467 subjects of the Vlagtwedde–Vlaardingen cohort participating in the last survey in 1989–1990. This general population-based cohort of exclusively white individuals of Dutch descent started in 1965 and has been followed up for 25 years. The selection of the cohort has been described previously (10, 11). Surveys were performed every 3 years, in which information was collected on respiratory symptoms, smoking status, age, and sex by the Dutch version of the British Medical Council standardized questionnaire (12). A blood sample was taken and spirometry was performed. Details on pulmonary function measurements are provided in the online supplement. In 1989–1990 neutrophil depts from centrifuged blood samples were collected and stored at –20°C. In 2003–2004 DNA was extracted from these samples with a QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) and checked for purity and concentration with a NanoDrop ND-1000 UV–Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE). The study protocol was approved by the local university hospital medical ethics committee and all participants gave their written informed consent.

Genotyping

We genotyped DNA samples of those subjects with more than 1,500 ng of isolated DNA available ($n = 1,390$). There were no differences in characteristics at the last survey between the selected and not-selected groups (Table 1).

Eight SNPs in *ADAM33*, previously described to be associated with asthma, airway hyperresponsiveness, or excessive decline in FEV₁, were genotyped: F+1 (G/A), Q-1 (C/T), S_1 (Val-Iso), S_2 (G/C), ST+5 (A/G), T_1 (Met-Thr), T_2 (Pro-Ser), and V_4 (C/G). The SNP causes an amino acid change when this is indicated between parentheses; otherwise, the base change is shown. See the online supplement for a description of the genotyping protocol; sequences of primers and probes are listed in Table E1 of the online supplement.

Statistics

We identified subjects with COPD on the basis of spirometry according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (13). Subjects were considered to have COPD when they had an FEV₁/VC less than 70% and FEV₁ less than 80% predicted (GOLD stage 2 or higher) at the last survey. Differences in prevalence of rare alleles of SNPs between subjects with and without COPD were tested

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TABLE 1. CHARACTERISTICS OF SUBJECTS IN THE 1989–1990 SURVEY WHO WERE AND WERE NOT GENOTYPED

	Genotyped (n = 1,390)	Not Genotyped (n = 1,077)
Males, n (%)	714 (51.4)	559 (51.9)
Age in years, median (range)	52 (35–79)	54 (35–79)
Pack-years of smoking, median (range)	8.0 (0–161.4)	6.0 (0–158.4)
FEV ₁ %pred, mean (SD)	91.5 (14.6)	91.5 (15.9)
FEV ₁ /VC, mean (SD)	73.9 (8.7)	73.7 (9.1)
GOLD stage 2 or higher, n (%)	186 (13.4)	168 (15.6)

Definition of abbreviation: GOLD = Global Initiative for Chronic Obstructive Lung Disease.

with χ^2 tests. Relative risks and population-attributable risks for the development of COPD were calculated for all SNPs (see the online supplement).

Linear mixed effect (LME) models were used to investigate the effect of polymorphisms in the *ADAM33* gene on the annual decline in FEV₁ (14). Time was defined as the time in years relative to the first FEV₁. FEV₁ measurements were included from the age of 30 years, because an individual's maximal achieved lung function is assumed to have been reached before that age and lung function is considered to be either in the plateau phase or in the decline phase (15). Variables included in the model were age at entry, sex, pack-years of smoking, and the first available FEV₁ after age 30 years and their interaction with time. The outcome of the mean annual decline concerns females of age 30 years entered in the LME, a mean FEV₁ of the population, 0 pack-years of smoking, and with a wild-type genotype. Additional information on LME analyses, Hardy-Weinberg equilibrium, linkage disequilibrium, haplotype analyses, and permutation tests is described in the online supplement. Statistical analyses were performed with SPSS (version 12.0.1 for Windows; SPSS, Chicago, IL), the statistical package R (version 1.9.1) (16), and Arlequin software (version 2.0) (available at: <http://lgb.unige.ch/arlequin>).

RESULTS

For all eight SNPs in *ADAM33*, frequencies for the minor alleles were comparable to those reported previously: F+1, 0.35; Q-1, 0.125; S_1, 0.084; S_2, 0.28; ST+5, 0.58; T_1, 0.21; T_2, 0.17; V_4, 0.26. All SNPs were in Hardy-Weinberg equilibrium and in significant linkage disequilibrium.

In each LME, the mean adjusted annual decline for subjects with the wild-type genotype for the SNPs was determined. The mean of these adjusted annual declines was 18.7 ml/year (range, 18.2–19.9 ml/year). Males declined at 6 ml/year less than females, which could be attributed to the height difference between males and females. Subjects who were homozygous for the rare alleles of Q-1 or S_2 had a significant excessive decline in FEV₁ of,

respectively, 9.6 ml/year ($p = 0.021$) and 4.9 ml/year ($p = 0.033$) compared with wild type. Heterozygous individuals for the S_1 SNP demonstrated a significant excessive decline of 3.6 ml/year ($p = 0.023$) and a nonsignificant excessive decline of 6.4 ml/year for homozygous individuals compared with wild type (Figure 1). None of the other SNPs were significantly associated with excessive decline in FEV₁.

Because it would be of interest to know whether the degree of smoking might interact with SNPs on the decline in FEV₁, we performed LME models with the interaction terms pack-years \times SNP in the model. These interaction terms were not significant.

We did not find an association of level of baseline FEV₁ with any of the eight SNPs in *ADAM33*, which implies that the SNPs have an effect on FEV₁ decline rather than on maximally attained lung function.

We have performed permutation tests to assess whether our results were found due to chance. We performed 3,000 permutations per SNP and ran the analysis on each of these datasets. We found the following significant p values for the observed β -estimates in empiric cumulative distribution: Q-1 CC genotype $p = 0.012$, S_1 AT genotype $p = 0.016$, S_2 TT genotype $p = 0.016$, V_4 TT genotype $p = 0.038$ (Figure E1 in the online supplement). Thus, for all SNPs that were associated with excessive lung function decline in the true data set, the observed β -estimate occurred less than 5% in the empiric cumulative distribution, indicating that our results were not found due to chance. In addition, the V_4 SNP, which was borderline significant in the original data set ($p = 0.064$), appeared to be significant from the permutation test.

Subjects with minor alleles for SNPs F+1, S_1, S_2, and T_2 had COPD (GOLD stage 2 and higher) more frequently than did the remainder (p values of 0.023, 0.020, 0.031, and 0.039, respectively; Table 2). In addition, SNP T_2 was significantly associated with GOLD stage 1 and higher ($p = 0.025$) and SNPs Q-1 and S_1 were significantly associated with GOLD stage 3 and higher ($p = 0.010$ and $p < 0.001$, respectively; see Table E2). Relative risks and population-attributable risks for the development of GOLD stage 2 or higher are shown in the online supplement (see Table E3).

Haplotypes with the highest frequencies are presented in Table 3. We found no significant difference in prevalence of haplotypes between subjects with and without COPD (GOLD stage 2 and higher).

We used haplotypes with frequencies higher than 0.01 to construct genotypes. Only the genotype with at least one minor allele for F+1, Q-1, S_1, S_2, and V_4 was associated with COPD ($p = 0.048$). This likely reflects the individual associations found

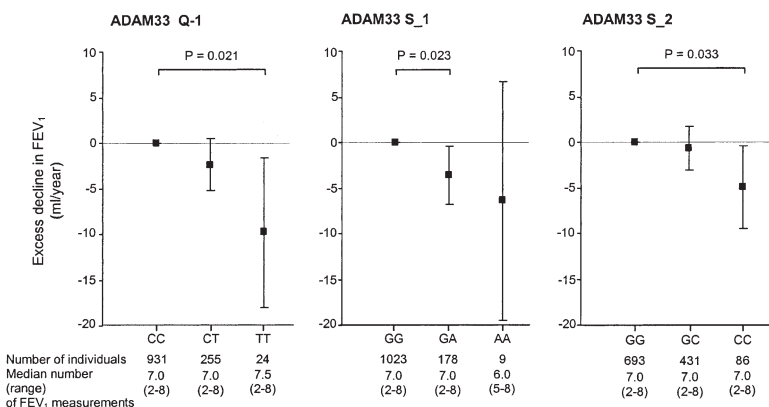


Figure 1. Excess annual decline in FEV₁ in milliliters per year (with 95% confidence interval) for single nucleotide polymorphisms Q-1, S_1, and S_2 compared with wild type; corrected for sex, age, pack-years of smoking, and level of FEV₁ at age 30 years. Mean decline in FEV₁ for wild type in the population is set as a reference at zero. Number of individuals and median number (range) of FEV₁ measurements are presented at the bottom.

TABLE 2. PREVALENCE OF GENOTYPES ACCORDING TO CHRONIC OBSTRUCTIVE PULMONARY DISEASE PHENOTYPE*

SNP	Genotype	No COPD (%)	COPD (%)	p Value (df = 2)	SNP	Genotype	No COPD (%)	COPD (%)	p Value (df = 2)		
F+1	GG	46.7	38.7	0.023	ST+5	AA	17.5	20.0	0.630		
	GA	41.4	41.9			AG	46.2	47.1			
	AA	11.9	19.4			GG	36.3	32.9			
Q-1	TT	78.2	70.9	0.095		T_1	TT	77.0		72.2	0.373
	TC	20.2	25.9				TC	21.3		25.0	
	CC	1.7	3.2		CC		1.7	2.8			
S_1	GG	85.7	77.1	0.020	T_2	GG	76.4	69.4	0.039		
	GA	13.6	21.7			GA	22.1	26.5			
	AA	0.7	1.3			AA	1.5	4.1			
S_2	GG	58.3	48.1	0.031	V_4	CC	58.0	56.1	0.364		
	GC	35.3	41.7			CG	36.5	35.5			
	CC	6.4	10.3			GG	5.5	8.4			

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; df = degrees of freedom; SNP = single nucleotide polymorphism.

* GOLD stage 2 and higher; FEV₁/VC less than 70%, FEV₁ less than 80% predicted.

with SNPs F+1, S_1, and S_2 with COPD. In the whole population, none of the genotypes was associated with significant excessive decline in FEV₁. Thus, haplotype analysis did not provide additional information.

DISCUSSION

We have previously shown that polymorphisms in the *ADAM33* gene are associated with accelerated decline in lung function in an asthma population (7). Interestingly, we now present evidence that polymorphisms in *ADAM33* are associated with excessive lung function decline in the general population as well. This is of great importance because it is well established that low lung function is associated with higher mortality risk, in particular due to COPD and cardiovascular diseases (9, 17–19). Cardiovascular diseases were the most common and COPD the fifth leading cause of death in 2001 worldwide according to the World Health Organization (20). Because the prevalence and mortality due to COPD are expected to increase (21), this disease will become an even larger social and economic burden. COPD is a progressive disease and still poorly manageable with treatment, hence the discovery of new risk factors for this disease is important and may provide better treatments in the future (22).

TABLE 3. ESTIMATED FREQUENCIES OF HAPLOTYPES IN THE WHOLE POPULATION AND IN SUBJECTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE*

Haplotype	Estimated Frequency in Whole Population	Estimated Frequency in COPD
All wild type	0.303	0.266
ST+5	0.210	0.196
ST+5, V_4	0.116	0.089
F+1, S_2, ST+5, T_1, T_2	0.106	0.128
F+1, ST+5	0.075	0.077
F+1, Q-1, S_1, S_2, V_4	0.069	0.101
F+1, Q-1, S_2, ST+5, V_4	0.023	0.023
F+1	0.019	0.021
F+1, Q-1, S_2, ST+5	0.012	0.017
Other	0.068	0.082

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.

* Minor alleles of the SNPs are presented; other alleles in the haplotype are wild type. Haplotypes with frequencies higher than 0.01 are shown.

A study has shown that SNPs in *ADAM33* were associated with reduced levels of lung function in childhood (23). Our data provide evidence that SNPs in *ADAM33* play a role in the development of COPD as well as in accelerated lung function loss in the general population. There are no clear biological explanations for these observations. The *ADAM33* protein is expressed in airway smooth muscle cells and fibroblasts and it has been proposed to contribute to the remodeling process present in asthma (1). *ADAM33* is a member of the *ADAM* family, a family of proteins involved in cell adhesion, cell fusion, cell signaling, and proteolysis (24, 25). The latter can be illustrated by the capacity to shed cytokines, growth factors, or their receptors from the cell surface and the role in remodeling of extracellular matrix components. It is unknown whether the production or activity of *ADAM33* in asthma is increased or reduced, yet overproduction or enhanced activity of *ADAM33* may lead to excessive shedding of inflammatory mediators, compatible with the enhanced airway wall inflammation present in asthma. Shedding and thereby overproduction of growth factors may furthermore induce proliferation of smooth muscle cells and fibroblasts. These features may lead to the remodeling process present in the airways of asthmatic patients. However, our data suggest that this may also reflect a general phenomenon, because polymorphisms in *ADAM33* are associated with accelerated lung function loss in the general population. Moreover, subjects carrying these polymorphisms are susceptible to COPD.

SNPs S_1 and S_2 were associated with FEV₁ decline and COPD. In contrast, F+1 and T_2 were associated with the presence of COPD and not with FEV₁ decline, whereas SNP Q-1 was associated with FEV₁ decline and not with COPD. Our definition of COPD is based on cross-sectional data on FEV₁ and VC, which is different from FEV₁ decline. This may account for the difference in the associations found. The number of subjects with COPD may have been too small to detect the association with COPD for SNP Q-1, because we did find a trend for association ($p = 0.095$).

In our study, three SNPs were associated with excessive annual decline in FEV₁. The strongest association was found with the Q-1 SNP, which was associated with an excessive decline of 9.6 ml/year. SNP Q-1 is located in the intron before exons Q, P, and R, which comprise the epidermal growth factor (EGF) domain (26). EGF signaling is important in lung morphogenesis because mice lacking the EGF receptor (EGFR) demonstrate abnormal branching and poor alveolization. Kheradmand and

coworkers (27) demonstrated that EGFR signaling regulates matrix metalloproteases, which mediate epithelial–mesenchymal interactions during lung morphogenesis. ADAM33 is closely related to matrix metalloproteases but may bind EGF directly. To our knowledge, no studies so far have shown a direct binding of ADAMs to EGF or related EGFR ligands. However, some studies do provide evidence of an interaction of ADAMs with EGFR ligands. Sahin and coworkers investigated the role of ADAMs in the shedding of EGFR ligands in mouse embryonic cells derived from various ADAM knockout mice. They identified ADAM10 as a sheddase of EGF and betacellulin and ADAM17 as a major convertase of epiregulin, transforming growth factor α , amphiregulin, and heparin-binding EGF-like growth factor (HB-EGF) (28). ADAM9 and ADAM12 were also implicated in shedding of HB-EGF. Mochizuki and coworkers (29) found evidence of binding of ADAM28 to insulin-like growth factor-binding protein-3 with subsequent digestion. It is likely that binding of these ligands to the EGF domain of ADAMs precedes shedding by ADAMs. Therefore, if ADAM33 can bind EGF ligands to cleave them, it is also possible that it can bind them for activation. An indication of the importance of the EGF domain is that the number of mRNA transcripts containing a full EGF domain is much higher than the number of transcripts containing a full metalloprotease domain in normal airway fibroblasts (30). However, fibroblasts from subjects with asthma or COPD may display a different splice pattern.

A disturbance in the EGF domain is likely to affect the regulation of ADAM33. Through alternative splicing, exon Q can be spliced out, giving rise to the β -variant of ADAM33. This variant was found in 30% of ADAM33 mRNA transcripts in pulmonary fibroblasts (30). Because the EGF domain is incomplete, it has been suggested that the β -variant prevents maturation of ADAM33 and may exert a dominant-negative effect on its protease activity (31). The intronic SNP Q-1 may influence the splicing of the β -variant (32) and thereby disturb the maturation of ADAM33. Subsequently, the protease activity may be disturbed, resulting in a defect in repair of tissue after damage due to inflammation. This may lead to progressive destruction of alveolar tissue and thereby enhance accelerated decline in lung function. Our data suggest that this process may occur not only in patients with COPD but also to a smaller extent in the general population depending on environmental factors.

SNPs S_1 and S_2 were associated with both excessive FEV₁ decline in the general population and development of COPD. These SNPs are located in exon S, which encodes the transmembrane region. The S_1 SNP causes an amino acid change (valine to isoleucine), but it is unknown whether this also modifies the structure of the protein. If so, the ADAM33 protein may not be anchored correctly in the membrane and therefore may not be able to exert its function. The S_2 SNP is a silent mutation and a biological explanation for the effect of this SNP is that it may be in linkage disequilibrium with the true causative SNP. Further research on ADAM33 should clearly lie in functional studies to elucidate the role of SNPs in ADAM33 in lung function loss, asthma, and COPD.

In conclusion, we found that polymorphisms in the ADAM33 gene are associated with an accelerated decline in FEV₁ in the general population. In addition, we have demonstrated that these polymorphisms are not only risk factors for the development of asthma, but also for COPD. This is the first study implicating one gene to be involved in the development of both asthma and COPD. The SNPs that we have studied are all common, with a frequency of at least 0.084 for the minor allele. Thus, polymorphisms in ADAM33 constitute important risk factors for the development of respiratory diseases in a large subset of the general population.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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