A Disintegrin and Metalloproteinase 33 and Chronic Obstructive Pulmonary Disease Pathophysiology

Margot M.E. Gosman, H. Marike Boezen, Cleo C. van Diemen, Jiska B. Snoeck-Stroband, Thérèse S. Lapperre, Pieter S. Hiemstra, Nick H.T. ten Hacken, Jan Stolk, Dirkje S. Postma, and the GLUCOLD Study Group

Online Data Repository

EXTENDED METHODS

Statistical analysis

We used Arlequin (version 2.000) to test whether SNPs were in Hardy Weinberg equilibrium and linkage disequilibrium (LD). Figure E1 illustrates the LD patterns with D', r and P-values in *ADAM33.* We investigated whether *ADAM33* SNPs are associated with PC_{20} and number and subset of inflammatory cells in sputum and bronchial biopsies. Our primary genetic model for all SNPs was a dominant model (homozygotes and heterozygotes for the minor allele being compared as a group with homozygotes for the major allele). Additionally, SNPs with a minor allele frequency of $\geq$ 0.30 were entered in 1) a codominant model (three genotype groups per SNP separately) and 2) a recessive model (homozygotes and heterozygotes for the major allele being compared as a group with homozygotes for the minor allele). PC_{20}, sputum inflammatory cells, and inflammatory cells in bronchial biopsies were log transformed to obtain a normal distribution. We performed univariate analyses using t-test and ANOVA. Multiple linear regression analyses were performed to investigate the association of polymorphisms in *ADAM33* with PC_{20}, sputum inflammatory cells, and inflammatory cells in biopsies as dependent variables. Independent variables included in the model were gender, smoking status, lung function, genotype, and the interaction of smoking status and genotype. We entered sputum total cell count (centred around the mean of the total population) to the model when analyzing the differential cell count in sputum, and the number of $CD3^+$ cells (centred around the mean of the total population) when analyzing $CD4^+$ and $CD8^+$ cells in bronchial biopsies.

EXTENDED RESULTS

Univariate association of *ADAM33* SNPs with AHR

To provide our readership with all information, we show below the results of the dominant, codominant and recessive genetic models.

Dominant model Subjects with a G-allele for SNP ST+5 had a significantly higher PC_{20} compared to the AA-genotype (geometric mean (gm) 0.74 versus 0.27 mg/ml, p=0.02). SNPs F+1, Q-1, S_1, S_2, T_1, T_2, and V_4 were not significantly associated with AHR.

Codominant model There was a significant difference in PC_{20} methacholine between the three genotypes for SNP ST+5 (AA: gm 0.27 mg/ml; AG: 0.64 mg/ml; GG: 1.04 mg/ml; p=0.04). SNPs F+1 and S_2 were not significantly associated with AHR.

Recessive model Homozygous individuals for the minor allele of SNP S_2 had a significantly lower PC_{20} than subjects with one or more major alleles (gm 0.26 versus 0.72 mg/ml, p=0.008). SNPs F+1 and ST+5 were not significantly associated with AHR.

Univariate association of *ADAM33* SNPs with inflammatory cells in sputum

Dominant model Patients with a G-allele for SNP ST+5 had a significantly lower total sputum cell count compared to the AA-genotype (gm 121 versus $204*10^4$ cells/ml; p=0.04). SNPs F+1, Q-1, S_1, S_2, T_1, T_2, and V_4 were not significantly associated with sputum inflammatory cells.

Codominant model SNPs F+1, S_2, and ST+5 were not significantly associated with inflammatory cells in sputum.

Recessive model SNPs F+1, S_2, and ST+5 were not significantly associated with inflammatory cells in sputum.

Univariate association of *ADAM33* SNPs with inflammatory cells in bronchial biopsies

Dominant model Individuals with a minor allele for SNP S_2 had lower numbers of $CD4^+$ cells than the wild-type for that SNP (gm 41.9 versus 56.1 /0.1 mm^2, p=0.05).

Codominant model There was a significant difference between the three genotypes for SNP ST+5 and SNP S_2 in the number of $CD8^+$ cells in bronchial biopsies (gm ST+5 AA: 27.7/0.1 mm^2; AG: 21.6/0.1 mm^2; GG: 8.2/0.1 mm^2, p=0.003; S_2 GG: 21.8/0.1 mm^2; GC: 20.4/0.1 mm^2; CC: 6.2/0.1 mm^2; p=0.03). No significant associations were found with SNP F+1.

Recessive model Homozygous individuals for the G-allele of SNP ST+5 had a significantly lower number of CD8+ cells and a significantly higher number of eosinophils, compared to individuals with one or more A-alleles for SNP ST+5 (gm 8.2 versus 23.3, p=0.03 and gm 2.4 versus 0.8/0.1 mm^2, p=0.04, respectively).

Multivariate association of *ADAM33* SNPs with AHR and inflammatory cells in sputum and bronchial biopsies, assuming a recessive model

For completeness, we present below the outcome of the multivariate linear regression analyses assuming a recessive model.

SNPs F+1, S_2, and ST+5 were not significantly associated with PC_{20}, total sputum cell count, or sputum differential cell counts. The number of CD8+ cells in bronchial biopsies was significantly lower in individuals with the GG-genotype for SNP ST+5 compared with individuals with one or more A-alleles in SNP ST+5 (gm (95% CI) 7.7 (3.9-13.5) versus 18.7/0.1mm^2 (12.3-28.3), p=0.002), lower in individuals with the CC-genotype for SNP S_2 compared with individuals with one or more G-alleles in SNP S_2 (5.3 (2.4-12.0) versus 16.5/0.1 mm^2 (10.5-26.0), p=

0.007), and lower in individuals with the AA-genotype in SNP F+1 compared with individuals with one or more G-alleles in SNP F+1 (8.3 (4.4-15.4) versus 17.0/0.1 mm^2 (11.1-26.0), p=0.02). The number of eosinophils in bronchial biopsies was significantly higher in individuals with the GG-genotype for SNP ST+5 compared with individuals with one or more A-alleles in SNP ST+5 (3.5 (1.2-9.8) versus 1.2/ 0.1 mm^2 (0.6-2.5), p=0.04)

Differences in SNP prevalence between patients with COPD and controls

Previously, it has been demonstrated by van Diemen *et al.* that SNPs in *ADAM33* are associated with the presence of COPD in a general population.[1] In addition to the data presented in the current manuscript with regard to the association of *ADAM33* with airway hyperresponsiveness and airway inflammation in patients with COPD, we compared the distribution of *ADAM33* SNPs of our COPD patients with that of a population based control group.

As a control group, we selected 1097 Caucasians of Dutch descent without airflow limitation (FEV_1> 80% pred, and FEV_1/forced vital capacity> 70%) from the Vlagtwedde-Vlaardingen cohort.[2][3] Genotyping of the control group has been previously described in detail.[1] Differences in prevalence of rare alleles of SNPs between the COPD patients described in the current manuscript and controls were tested using chi-square tests.

DNA was available from 1097 controls. All genotyped SNPs were in Hardy Weinberg equilibrium and in significant linkage disequilibrium. Clinical characteristics of the controls are presented in table E1. Table E2 shows that the prevalence of the minor allele of SNPs F+1, Q-1, S_1, and S_2 was significantly higher in the COPD group than the control group (p=0.04, p=0.03, p=0.003, and p=0.02, respectively),

whereas the prevalence of the minor allele of SNP ST+5 was lower (p=0.02). The prevalence of SNPs T_1, T_2, and V_4 was not significantly different between both groups. With these findings, we confirm the findings by van Diemen *et al.*[1] i.e. minor alleles for SNPs F+1, S_1, and S_2 are more often prevalent in patients with COPD than in subjects without airflow limitation. Regarding SNP Q-1 we found a significantly higher prevalence of the minor allele in patients with COPD compared with healthy controls, whereas van Diemen *et al.* demonstrated a trend in the same direction. In addition, we found a higher prevalence for the A-allele in SNP ST+5 in patients with COPD.

REFERENCES

1 Van Diemen CC, Postma DS, Vonk JM, *et al*. A disintegrin and metalloprotease 33 polymorphisms and lung function decline in the general population. *Am J Respir Crit Care Med* 2005;**172**:329-33.

2 Rijcken B, Schouten JP, Mensinga TT, *et al*. Factors associated with bronchial responsiveness to histamine in a population sample of adults. *Am Rev Respir Dis* 1993;**147**:1447-53.

3 Van der Lende R, Kok T, Peset R, *et al*. Longterm exposure to air pollution and decline in VC and FEV1. Recent results from a longitudinal epidemiologic study in the Netherlands. *Chest* 1981;**80**:23-6.

FOOTNOTES

Table E1

*Data are presented as mean ± standard deviation or; ‡ median (25th-75th percentile). Definition of abbreviations: FEV_1 = forced expiratory volume in one second; % pred = percentage of predicted value; FEV_1/IVC = forced expiratory volume in one second/inspiratory vital capacity.

Figure E1

Graph of the LD patterns with D', r and P-values in *ADAM33*. Explanation of the color scheme: red indicates a p-value of less than 0.025, orange indicates a p-value between 0.025 and 0.1, and yellow indicates a p-value of greater than 0.1

TABLES

Table E1: Clinical characteristics of the population based control group*

	Controls
Number	1097
Male/female	535/562
Age (years)	50.8 ± 9.5
Current smokers, n (%)	378 (34)
Smoking history (pack-years) ‡	6.6 (0-18.4)
FEV_1 (% pred.)	94.9 ± 11.3
FEV_1/IVC (%)	75.8 ± 6.1

Table E2: Prevalence of genotypes in COPD patients and controls

SNP		COPD % (n)	Controls % (n)	P value Df=2	SNP		COPD % (n)	Controls % (n)	P value Df=2
F+1	GG	34.9 (38)	46.1 (491)	**0.04**	ST+5	AA	23.7 (26)	17.7 (193)	**0.02**
	GA	46.8 (51)	42.1 (455)			AG	53.6 (59)	46.9 (511)	
	AA	18.3 (20)	11.8 (128)			GG	22.7 (25)	35.4 (386)	
Q-1	TT	67.3 (70)	77.9 (844)	**0.03**	T_1	TT	80.3 (86)	77.2(795)	0.70
	TC	28.9 (30)	20.3 (220)			TC	17.8 (19)	21.2 (219)	
	CC	3.8 (4)	1.8 (19)			CC	1.9 (2)	1.6 (17)	
S_1	GG	72.9 (78)	85.3 (934)	**0.003**	T_2	GG	80.8 (88)	76.7 (817)	0.56
	GA	26.2 (28)	14.1 (154)			GA	17.4 (19)	21.8 (232)	
	AA	0.9 (1)	0.6 (7)			AA	1.8 (2)	1.5 (16)	
S_2	GG	44.6 (45)	58.5 (626)	**0.02**	V_4	CC	51.0 (55)	58.2 (631)	0.26
	GC	44.6 (45)	34.9 (374)			CG	44.4 (48)	36.4 (394)	
	CC	10.8 (11)	6.6 (71)			GG	4.6 (5)	5.4 (58)	