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## Decorin and $TGF-\beta_1$ polymorphisms and development of COPD in a general population

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### Abstract

**Background:** Decorin, an extracellular matrix (ECM) proteoglycan, and  $TGF-\beta_1$  are both involved in lung ECM turnover. Decorin and  $TGF-\beta_1$  expression are decreased respectively increased in COPD lung tissue. Interestingly, they act as each other's feedback regulator. We investigated whether single nucleotide polymorphisms (SNPs) in *decorin* and  $TGF-\beta_1$  underlie accelerated decline in FEV<sub>1</sub> and development of COPD in the general population.

**Methods:** We genotyped 1390 subjects from the Vlagtwedde/Vlaardingen cohort. Lung function was measured every 3 years for a period of 25 years. We tested whether five SNPs in *decorin* (3'UTR and four intron SNPs) and three SNPs in  $TGF-\beta_1$  (3'UTR rs6957, C-509T rs1800469 and Leu10Pro rs1982073), and their haplotypes, were associated with COPD (last survey GOLD stage = II). Linear mixed effects models were used to analyze genotype associations with FEV<sub>1</sub> decline.

**Results:** We found a significantly higher prevalence of carriers of the minor allele of the  $TGF-\beta_1$  rs6957 SNP ( $p = 0.001$ ) in subjects with COPD. Additionally, we found a significantly lower prevalence of the haplotype with the major allele of rs6957 and minor alleles for rs1800469 and rs1982073 SNPs in  $TGF-\beta_1$  in subjects with COPD ( $p = 0.030$ ), indicating that this association is due to the rs6957 SNP.  $TGF-\beta_1$  SNPs were not associated with FEV<sub>1</sub> decline. SNPs in *decorin*, and haplotypes constructed of both  $TGF-\beta_1$  and *decorin* SNPs were not associated with development of COPD or with FEV<sub>1</sub> decline.

**Conclusion:** Our study shows for the first time that SNPs in *decorin* on its own or in interaction with SNPs in  $TGF-\beta_1$  do not underlie the disturbed balance in expression between these genes in COPD.  $TGF-\beta_1$  SNPs are associated with COPD, yet not with accelerated FEV<sub>1</sub> decline in the general population.

## Background

Chronic obstructive pulmonary disease (COPD) is characterized by irreversible airway obstruction and persistent airway inflammation. Transforming growth factor- $\beta_1$  ( $TGF-\beta_1$ ) is one of the important cytokines involved in this inflammatory process, which has been associated with cell proliferation and differentiation. It is furthermore involved in repair of the extracellular matrix (ECM) after inflammation and tissue injury amongst others by promoting synthesis of elastin and collagen. Studies have shown that  $TGF-\beta_1$  expression is increased in the airways of COPD patients [1,2] In contrast, a recent article from Pons *et al* showed that alveolar macrophages from COPD patients release less  $TGF-\beta_1$  in response to lipopolysaccharide than smokers with normal lung function and non-smokers[3] This may lead to a reduced anti-inflammatory and anti-elastolytic response in COPD patients, subsequently contributing to progressive ECM destruction.

Decorin is a component of the ECM that regulates collagen fibrillogenesis. [4-6] In addition, it can interact with a wide variety of growth factors, cytokines and adhesion molecules through its extensive binding area, thereby not only playing a role in ECM assembly but also in control of cell proliferation and tissue morphogenesis.[7] $TGF-\beta_1$  has been shown to downregulate synthesis of decorin in fibroblasts and decorin can in turn inhibit  $TGF-\beta_1$ . [8] Decorin may thus act as a negative feedback regulator of  $TGF-\beta_1$  mediated repair responses. Conversely,  $TGF-\beta_1$  can downregulate expression of decorin in fibroblasts from emphysema patients.[9] We have shown previously that decorin expression is diminished in the peribronchiolar area of lung tissue from patients with severe emphysema, while  $TGF-\beta_1$  production from fibroblasts of these patients is increased.[10] Noordhoek *et al* showed that  $TGF-\beta_1$  and basic fibroblast growth factor give a stronger reduction of decorin production in the culture supernatant of fibroblasts from patients with severe emphysema than from patients with mild emphysema. [9] It thus appears that the regulation of decorin production is disturbed in lung tissue from patients with severe emphysema. This will lead to diminished binding and neutralization of  $TGF-\beta_1$  by decorin followed by higher  $TGF-\beta_1$  concentrations and activity with lower decorin production as a result.

We hypothesized that the reciprocal regulation of the  $TGF-\beta_1$  and *decorin* genes is disturbed in COPD due to a genetic mutation in one or both of these genes. We have tested this hypothesis by investigating three single nucleotide polymorphisms (SNPs) in  $TGF-\beta_1$  and five SNPs in *decorin* on the development of COPD and on lung function decline in a large cohort derived from the general population (the Vlagtwedde/Vlaardingen cohort).

## Methods

### Subjects

We used data from 2467 subjects of the Vlagtwedde/Vlaardingen cohort participating in the last survey in 1989/1990. This general population-based cohort of Caucasians of Dutch descent started in 1965. Surveys, during which pulmonary function measurements were performed, were held every three years. The selection of the cohort has been described previously. [11-13] Surveys were performed every 3 years during which information was collected on respiratory symptoms, smoking status, age and gender by the Dutch version of the British Medical Council standardized questionnaire. A blood sample was taken and spirometry was performed. Details on pulmonary function measurements are provided in the additional file 1. The methodology for standardization and equipment used for lung function measurements was the same throughout the study. In 1989/1990 neutrophil depot of centrifuged blood was collected and stored at  $-20^{\circ}\text{C}$ . In 2003/2004 DNA was extracted from these samples with the QiaAmp<sup>®</sup> DNA Blood Mini Kit and checked for purity and concentration with the NanoDrop<sup>®</sup> ND-1000 UV-Vis Spectrophotometer. The study protocol was approved by the local university hospital's medical ethics committee and participants gave written informed consent.

### Genotyping

We genotyped DNA of those subjects with more than 1500 ng isolated DNA available ( $N = 1390$ ). Three SNPs, previously associated with COPD or level of lung function were genotyped in  $TGF-\beta_1$ : rs6957 in the 3'UTR, rs1800469 in the promoter region (C-509T) and a coding SNP rs1982073 (Leu10Pro, G/T). [14-16] Coding SNPs in *decorin* have been identified in the NCBI and Celera databases, but are only prevalent in African populations (frequency 0.05–0.12) but not in Caucasian populations (frequency 0.00). According to the HapMap database there are two large LD blocks in the *decorin* gene, and a region including the 3'UTR that forms no LD block. [17]. There are 4 haplotype tagging SNPs located in introns, resulting in 3 major haplotypes, which cover the information of the gene. Therefore, we genotyped one SNP in the 3'UTR (rs1803343), and the 4 haplotype-tagging SNPs: rs11106030, rs741212, rs566806, rs516115 and rs3138241. The genotyping protocol is described in the additional file 1; the characteristics of the genotyped SNPs in additional file 2. To determine whether the SNPs were in Hardy Weinberg equilibrium and whether they were in linkage disequilibrium, tests were performed with the statistical package R (version 1.9.1).

### Statistics

We identified subjects with COPD using the GOLD criteria (GOLD stage II or higher, i.e.  $FEV_1/VC < 70\%$  and

**Table 1: Characteristics of genotyped subjects in the 1989/1990 survey**

	No COPD (N = 1156)	COPD (N = 188)
Males, n (%)	554 (47.9)	137 (72.9)
Age in years, median (IQR)	50 (35–79)	59 (35–76)
Pack-years of smoking, median (IQR)	7.5 (0–21.6)	25.5 (6.6–35.7)
FEV <sub>1</sub> %pred, median (IQR)	95.8 (87.9–104.5)	71.1 (61.1–77.1)
FEV <sub>1</sub> /VC, median (IQR)	76.6 (62.1–80.5)	60.0 (54.5–65.7)

Abbreviations: FEV<sub>1</sub>, forced expiratory volume in 1 second; VC, vital capacity

FEV<sub>1</sub><80% predicted) at the last survey[18] Characteristics of subjects with and without COPD at the last survey are presented in table 1. Differences in allele frequencies and haplotype frequencies between subjects with and without COPD were tested using Chi-square tests. We used ANOVA and linear regression models to study the effect of SNPs on first and last available FEV<sub>1</sub> and FEV<sub>1</sub>/VC (adjusted for gender, age, pack-years, and height in regression models).

Linear Mixed Effect (LME) models were used to investigate the effect of SNPs in *TGF-β<sub>1</sub>* and *decorin* on annual FEV<sub>1</sub> decline in the general population, like published previously.[19,20] Time was defined as time in years relative to the first FEV<sub>1</sub>, starting from the age of 30.[21] Variables included in the model were age at entry, gender, pack-years, the first FEV<sub>1</sub> after age 30, and their interaction with time. Since including the level of the first FEV<sub>1</sub> after age 30 and its interaction with time could introduce bias due to regression to the mean, these variables were also included in the model as random effect variables. The results of these analyses showed no change in estimates of the variables in the model or a better fit of the model, which indicates that there was no bias due to regression-to-the-mean. Therefore, the results are presented without these random effects. To test whether SNPs were associated with FEV<sub>1</sub> decline within subjects with COPD, we performed LME analyses on these subjects only. Since Celedón *et al* found stronger linkage results of *TGF-β<sub>1</sub>* SNPs and lung function in smokers only, we additionally performed LME models stratified to smoking status. [14] We also included interaction terms of *TGF-β<sub>1</sub>* SNPs and *decorin* SNPs to test for genetic interaction of these SNPs.

Instead of performing pre- or post-hoc power analysis and correction for multiple testing, we performed permutation tests to assess whether our results might have been found due to chance. Genotypes were randomly shuffled among individuals to produce 3000 datasets. The LME models were rerun on each of these datasets to generate a distribution of the beta estimates for additional FEV<sub>1</sub> decline of the homozygous minor allele genotype compared to FEV<sub>1</sub> decline of the homozygous wild type allele genotype under the null hypothesis, being no association

of the SNPs under study and FEV<sub>1</sub> decline. If the observed beta estimate from the true data is found in the lower 5% percentile of the empiric cumulative distribution ( $p < 0.05$ ), one can assume that the observed beta estimate is not found due to chance.

We also estimated *TGF-β<sub>1</sub>* haplotype frequencies in the whole population and in subjects with a COPD phenotype. Estimated haplotype frequencies for *TGF-β<sub>1</sub>* higher than 1% in the general population were used to construct phased multi-locus genotypes of *TGF-β<sub>1</sub>*. For *decorin*, we constructed the phased multi-locus genotypes as known from the HapMap database. With Chi-square tests we determined for each haplotype whether there was a difference in prevalence of carriers between subjects with and without COPD. Also, the excess decline in FEV<sub>1</sub> in the whole population was determined for each phased multi-locus genotype in the LME.

Statistical analyses were performed using SPSS (version 12.0.1 for Windows), the statistical package R (version 1.9.1) and Arlequin [22].

## Results

Allelic frequencies for the minor alleles of the *TGF-β<sub>1</sub>* and *decorin* SNPs in this population were comparable to those reported in the Celera and/or in the NCBI dbSNP database: *TGF-β<sub>1</sub>* rs6957 0.18, rs1800469 0.28, rs1982073 0.38, *decorin* rs1803343 0.02, rs11106030 0.06, rs741212 0.12, rs566806 0.26, rs516115 0.22 and rs3138241 0.06. All SNPs were in Hardy Weinberg equilibrium. The *TGF-β<sub>1</sub>* rs1800469 SNP was in significant LD with rs1982073 and rs6957. Rs6957 was in almost significant LD with rs1982072 ( $p = 0.06$ ). The *decorin* SNPs were in significant LD. Graphs of the LD patterns with  $D'$ ,  $r$  and  $P$ -values in both genes are presented in the additional file 3.

### Prevalence of SNPs and haplotypes in *TGF-β<sub>1</sub>* and *decorin* in COPD and control subjects

The distribution of the *TGF-β<sub>1</sub>* rs6957 genotypes was significantly different between subjects with and without COPD ( $p = 0.001$ , table 2). The other *TGF-β<sub>1</sub>* SNPs were not associated with COPD. We also found no association of SNPs in *decorin* with the prevalence of COPD.

**Table 2: Prevalence of genotypes according to COPD phenotype (GOLD stage II or higher; FEV<sub>1</sub>/VC<70%, FEV<sub>1</sub><80% predicted).**

SNP		No COPD N (%)	COPD N (%)	P value df = 2	SNP		No COPD N (%)	COPD N (%)	P value df = 2
TGF-β <sub>1</sub> rs1800469	GG	584 (52)	106 (58)	0.541	Decorin rs741212	AA	878 (76)	131 (76)	0.913
	GA	474 (40)	67 (36)			AG	242 (22)	43 (22)	
	AA	87 (8)	10 (6)			GG	15 (2)	4 (2)	
TGF-β <sub>1</sub> rs1982073	AA	382 (36)	75 (44)	0.297	Decorin rs516115	AA	614 (55)	102 (55)	0.949
	AG	533 (49)	72 (42)			AG	431 (38)	65 (38)	
	GG	156 (15)	23 (14)			GG	79 (7)	15 (7)	
TGF-β <sub>1</sub> rs6957	GG	771 (69)	103 (56)	0.001	Decorin rs3138241	GG	863 (88)	136 (89)	0.733
	GA	327 (29)	71 (39)			GA	114 (12)	10 (11)	
	AA	30 (2)	10 (5)			AA	3 (0)	1 (1)	
Decorin rs11106030	CC	996 (87)	170 (91)	0.217	Decorin rs1803343	AA	1079 (94)	173 (93)	0.507
	CA	142 (12)	8 (8)			AG	69 (6)	13 (7)	
	AA	4 (1)	1 (1)			GG	0 (0)	0 (0)	

Abbreviations: COPD, Chronic Obstructive Pulmonary Disease; FEV<sub>1</sub>, forced expiratory volume in 1 second; VC, vital capacity; TGF-β<sub>1</sub>, transforming growth factor-β<sub>1</sub>; df, degrees of freedom

We used estimated haplotype frequencies higher than 0.01 to construct phased multi-locus genotypes for TGF-β<sub>1</sub>. The haplotype consisting of the minor allele for TGF-β<sub>1</sub> rs6957 and the wild type alleles for TGF-β<sub>1</sub> rs1800469 and rs1982073 was more prevalent in subjects with COPD (p = 0.014). Because the prevalence of carriers of other haplotypes containing the minor allele at TGF-β<sub>1</sub> rs6957 was also increased in subjects with COPD, this finding only reflects the individual association of the TGF-β<sub>1</sub> rs6957 SNP with COPD. Carriers of at least one haplotype with the minor alleles for TGF-β<sub>1</sub> rs1800469 and rs1982073 and the wild-type allele for rs6957 were less prevalent in COPD (p = 0.030). We found no significant associations of phased multi-locus genotypes in *decorin* with the prevalence of COPD (table 3). We also did not find associations of haplotypes containing SNPs of both TGF-β<sub>1</sub> and *decorin* with COPD (data not shown).

### Lung function

We found no significant associations (i.e. cross-sectional) between the SNPs tested and FEV<sub>1</sub> and FEV<sub>1</sub>/VC at the first or at the last survey in linear regression models (data not shown). The mean adjusted annual decline in lung function (expressed as decrease in FEV<sub>1</sub> in ml/yr) was determined for subjects with the wild-type genotype for the SNPs in TGF-β<sub>1</sub> and *decorin* using LME models. The outcome of the mean annual decline concerns females with age 30 when entered in the LME, a mean first FEV<sub>1</sub> of the population, and zero pack-years. The mean of these adjusted annual declines was 19.2 ml/yr (range 18.7–19.6). We did not find any significant association of SNPs in either TGF-β<sub>1</sub> or *decorin* with accelerated lung function decline (table 4). We added interaction terms of TGF-β<sub>1</sub> and *decorin* SNPs in the model, but found no significant inter-

actions. In addition, we did not find any significant association of haplotypes of either TGF-β<sub>1</sub> or *decorin* with accelerated lung function decline (results not shown). We also tested whether SNPs were associated with lung function decline within subjects with COPD or within smokers, but found no significant associations (table 4 and additional file 4). To test whether results were not missed due to chance, we performed permutation tests. We ran 3000 permutations on our sample of 1390 subjects and performed LME analyses on each of these permutations. The lack of associations with lung function decline was confirmed in these analyses.

### Discussion

Decorin and TGF-β<sub>1</sub> can act as each other's feed back regulators in ECM turnover and their expression is respectively decreased and increased in lung tissue of COPD patients. We assessed whether polymorphisms in *decorin* and TGF-β<sub>1</sub> are associated with the development of COPD and accelerated lung function decline in the general population. This is the first study assessing SNPs in *decorin* and we did not find any association with COPD or lung function loss. Contrary to our hypothesis, the observed disturbed balance between decorin and TGF-β<sub>1</sub> in COPD is not caused by a combination of SNPs in their genes, since we found no significant interaction terms of *decorin* and TGF-β<sub>1</sub> SNPs with respect to FEV<sub>1</sub> decline. Moreover, we found no associations of phased multi-locus genotypes containing SNPs of both TGF-β<sub>1</sub> and *decorin* with the presence of GOLD stage II and III COPD in our population. This disturbed balance may be affected by SNPs in TGF-β<sub>1</sub> alone since the 3'UTR SNP in TGF-β<sub>1</sub> is predictive of COPD (stage GOLD II). We found, however, no association of SNPs in TGF-β<sub>1</sub> with longitudinal decline in lung

**Table 3: Prevalence of *TGF-β<sub>1</sub>* and decorin haplotypes in subjects with and without COPD (GOLD stage II or higher; FEV<sub>1</sub>/VC<70%, FEV<sub>1</sub><80% predicted).**

Carrier of Haplotype*					No COPD N (%)	COPD N (%)	P value #
TGF-β <sub>1</sub>	rs1800469	rs1982073	rs6957				
	0	0	0		239 (23)	34 (22)	0.686
	0	I	0		106 (11)	11 (7.6)	0.264
	0	I	I		27 (3)	6 (4)	0.417
	I	I	0		288 (29)	31 (20)	<b>0.030</b>
	0	0	I		95 (9)	25 (16)	<b>0.014</b>
	I	I	I		160 (16)	34 (22)	0.086

  

Decorin	rs3138241	rs516115	rs714212	rs11106030	No COPD N (%)	COPD N (%)	P value
	0	0	0	0	1009 (93)	175 (92)	0.515
	0	I	I	0	234 (22)	47 (27)	0.715
	I	I	0	I	133 (12)	15 (9)	0.950

Abbreviations: COPD, Chronic Obstructive Pulmonary Disease; FEV<sub>1</sub>, forced expiratory volume in 1 second; VC, vital capacity; *TGF-β<sub>1</sub>*, transforming growth factor-β1

\* 0 means wild-type; I means minor allele

# P value of Chi-square test for difference in prevalence of haplotype between subjects with and without COPD

function. In addition, no associations were observed of SNPs in *TGF-β<sub>1</sub>* with level of FEV<sub>1</sub> or FEV<sub>1</sub>/VC cross-sectionally.

It is puzzling that we observed that the *TGF-β<sub>1</sub>* rs6957 SNP and a haplotype in *TGF-β<sub>1</sub>* were associated with COPD, but not with excess decline in FEV<sub>1</sub> or with level of FEV<sub>1</sub> and FEV<sub>1</sub>/VC at the last survey. We have tested whether there were differences in first available FEV<sub>1</sub> (which might suggest a relation to maximal attained lung function level) between the genotypes that could explain the lack of association with FEV<sub>1</sub> decline but this was not the case. Another possibility would be that the FEV<sub>1</sub> decline is only affected by SNPs in certain subgroups, such as smokers. Our stratified analyses showed no such effect.

Although the functionality of the *TGF-β<sub>1</sub>* rs6957 SNP is not known yet, it has previously been associated with lower pre- and post-bronchodilator FEV<sub>1</sub> and with lower FEV<sub>1</sub>/FVC.[14] Similarly, we have shown here that this SNP is associated with development of COPD. Various studies have indicated that the rs1800469 and rs1982073 SNPs are functional and result in higher levels of circulating *TGF-β<sub>1</sub>*. [23-26] Since *TGF-β<sub>1</sub>* has anti-inflammatory and pro-repair activities, these SNPs are thought to be protective against the development of COPD. Indeed, we and others have found that (carriers of haplotypes of) the minor alleles of these SNPs are significantly less prevalent in COPD patients compared to controls.[14,16]. Similar to Celedón *et al*, we found an association of a haplotype with at least one minor allele of the rs1800469 and rs1982073 *TGF-β<sub>1</sub>* SNPs and COPD, while they also found associations with these SNPs separately. [14,16]

The differences in study populations may explain these dissimilarities, e.g. our subjects had milder COPD (FEV<sub>1</sub><80% predicted) than the COPD patients in the Celedón study (FEV<sub>1</sub><45% predicted). Despite the differences in associations, it is still conceivable that carrying both of the SNPs decreases the risk to develop COPD. The two other studies linking *TGF-β<sub>1</sub>* SNPs and COPD have also demonstrated that these SNPs are less prevalent in COPD, though these studies did not test haplotypes[15,16]

Many SNPs have been described in the *TGF-β<sub>1</sub>* gene, but only a few have been intensively studied in genetic association studies. Cross-sectional studies have found associations of SNPs in *TGF-β<sub>1</sub>* with the presence of COPD, and with lower levels of FEV<sub>1</sub> and FEV<sub>1</sub>/FVC in several populations. [14-16] We did not analyze every SNP in the *TGF-β<sub>1</sub>* gene that was previously reported to be associated with COPD. However, since Celedón *et al* found strong LD (r<sup>2</sup> = 0.98) between promoter SNPs and 3'UTR SNPs in a Caucasian population, we are confident that any association that might exist would have been revealed by the SNPs or by their haplotypes.[14]

This is the first study on SNPs in *decorin* in a general population or in COPD patients. We were interested in polymorphisms in this gene, since decorin expression in COPD patients is diminished.[9,10] Decorin plays a direct role in the repair processes after inflammation through its regulation of matrix metalloproteases and tissue inhibitors of metalloproteases.[27,28] Furthermore, decorin is the natural inhibitor of *TGF-β<sub>1</sub>* and may therefore influence the repair process in the lung indirectly. We

**Table 4: Annual decline in FEV<sub>1</sub> according to genotypes of TGF- $\beta_1$  and decorin. Changes in decline between genotypes in the total population and in subjects who developed COPD (GOLD stage II or higher; FEV<sub>1</sub>/VC<70%, FEV<sub>1</sub><80% predicted) are presented.**

		Total population				COPD			
Genotype		N	Decline in FEV <sub>1</sub> (ml/yr)*	$\Delta$ FEV <sub>1</sub> compared to WT	P value†	N	Decline in FEV <sub>1</sub> (ml/yr)*	$\Delta$ FEV <sub>1</sub> compared to WT	P value†
<b>TGF-<math>\beta_1</math></b>	rs6957	AA	918	-19.2		103	-37.1		
		AG	399	-18.3	+0.9	71	-33.5	+3.6	0.297
		GG	40	-18.2	+1.0	10	-28.8	+8.3	0.239
	rs1800469	GG	716	-18.9		106	-34.3		
		GA	555	-17.6	+1.2	67	-36.2	-1.9	0.587
		AA	103	-20.3	-1.5	10	-31.9	+2.4	0.698
	rs1982073	GG	477	-19.1		75	-34.8		
		GA	623	-17.9	+1.2	72	+0.9	0.876	
		AA	185	-17.9	+1.2	23	-35.1	-0.3	0.959
<b>Decorin</b>	rs1803343	GG	1293	-18.7		173	-35.9		
		GA	85	-18.3	+0.4	13	-33.6	+2.3	0.698
	rs11106030	CC	1206	-18.9		170	-35.2		
		CA	162	-19.6	-0.7	8	-38.3	-3.1	0.577
		AA	6	-30.5	-11.6	1	-39.9	-4.7	0.797
	rs741212	AA	1039	-18.6		131	-35.1		
		AG	198	-20.1	-1.5	43	-38.2	-3.1	0.439
		GG	20	-14.1	+4.5	4	-23.2	+11.9	0.282
	rs516115	AA	737	-18.8		102	-34.4		
		AG	519	-18.5	+0.3	65	-35.9	-1.5	0.669
		GG	96	-18.9	+0.1	15	-35.0	-0.6	0.930
	rs3138241	GG	1187	-18.8		136	-35.7		
		GA	157	-19.5	-0.7	10	-38.7	-3.0	0.588
		AA	5	-25.7	-6.8	1	-31.6	+4.1	0.888

Abbreviations: FEV<sub>1</sub>, forced expiratory volume in 1 second; TGF- $\beta_1$ , transforming growth factor- $\beta_1$ ; COPD, Chronic Obstructive Pulmonary Disease; WT, wild-type

\*decline in FEV<sub>1</sub> adjusted for gender, first FEV<sub>1</sub> after age 30 years, pack-years, and age; † P value indicates significance of the effect of the genotype on decline in FEV<sub>1</sub> compared to wild-type

hypothesized that these processes may be genetically influenced. Since the coding SNPs in decorin described in the NCBI and Celera databases were not prevalent in Caucasians (but only in African populations), we genotyped four tagging SNPs, located in introns, and additionally a 3'UTR SNP. Although we found no significant associations of these SNPs with COPD or lung function decline, we can not rule out completely that there is no genetic defect in *decorin* that increases the risk to develop COPD. However, since we selected tagging SNPs that cover the genetic information of the decorin gene according to Hap-Map and given the large population under study, we assume that we would have observed an association of SNPs or haplotypes in *decorin* if there existed one in this population.

The lack of a genetic association of SNPs in the *decorin* gene does not rule out an important role of the decorin protein in COPD development. Decorin is a member of the proteoglycan family, a family of macromolecules composed of a protein core with glycosaminoglycan side chains which are produced post-translationally. It is possible that the function or activation of decorin is disrupted through an altered posttranslational modification of this glycosaminoglycan chain. In this case, modifications in the protein core, which might be caused by SNPs, may not be important and will not be detected. Decorin can be expressed in six splice variants, but the function of these splice variants is not known yet. Nevertheless, a shift in prevalence of one of these splice variants may affect the biological role that decorin exerts in TGF- $\beta_1$  regulation, thereby influencing the pathology within the lung.

## Conclusion

Contrary to our hypothesis, we were not able to identify the *decorin* gene as a genetic risk factor for the development of COPD. Consequently, SNPs in *decorin* do not seem to underlie a disturbed regulation of this gene and  $TGF-\beta_1$  resulting in COPD, nor can they be held responsible for the development of COPD and decline in  $FEV_1$  in the general population. We found that  $TGF-\beta_1$  SNPs are associated with the development of COPD but not with accelerated lung function decline or other lung function measures in the general population. Together with previous findings, this study establishes the  $TGF-\beta_1$  gene as a risk factor for the development of COPD.

## Competing interest statement

The author(s) declare that they have no competing interests.

## Authors' contributions

Every author contributed to reviewing of the paper. CCD performed the lab work, statistical analyses and drafted the manuscript. DSP is co principal investigator of the project, obtained funding of and supervised the project, and helped draft the manuscript. JMV contributed to the statistical analyses. MB contributed to the lab work. IMN contributed to the statistical analyses. HMB is co principal investigator of the project, obtained funding of and supervised the project, and helped draft the manuscript. All authors read and approved the final manuscript.

## Additional material

### Additional File 1

*Methods. Detailed description of the pulmonary function protocol and the genotyping protocol*

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### Additional File 2

*Characteristics of genotyped SNPs. Table with specifications of the genotyped SNPs, i.e. location, characteristics and sequences of primers and probes.*

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### Additional File 3

*Linkage Disequilibrium of SNPs in decorin and  $TGF-\beta_1$ .*

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## Additional File 4

*Annual decline in  $FEV_1$  according to genotypes of  $TGF-\beta_1$  and decorin. Changes in decline between genotypes in never smokers and current and past smokers are presented.*

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