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Novel strategy to identify genetic risk factors for COPD severity: a genetic isolate

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ABSTRACT: Studies using genetic isolates with limited genetic variation may be useful in chronic obstructive pulmonary disease (COPD) genetics, but are thus far lacking. The associations between single nucleotide polymorphisms (SNPs) in candidate genes and lung function in COPD were studied in a genetic isolate.

In 91 subjects with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage ⩾1 COPD, who were members of an extended pedigree including 6,175 people from the Genetic Research in Isolated Populations study, 32 SNPs were analysed in 13 candidate genes: a disintegrin and metalloprotease domain 33 gene (*ADAM33*), transforming growth factor-β1 gene (*TGFB1*), matrix metalloprotease-1 gene (*MMP1*), *MMP2*, *MMP9*, *MMP12*, tissue inhibitor of metalloprotease-1 gene (*TIMP1*), surfactant protein A1 gene (*SFTPA1*), *SFTPA2*, *SFTPB*, *SFTPD*, glutathione S-transferase P1 gene (*GSTP1*), and haem oxygenase 1 gene (*HMOX1*). Their relation to forced expiratory volume in 1 s (FEV_1), inspiratory vital capacity (IVC) and FEV_1/IVC were studied using restricted maximum likelihood linear mixed modelling, accounting for pedigree structure. Significant associations were replicated in the general Vlagtwedde/Vlaardingen study.

Six SNPs in *TGFB1*, *SFTPA1*, *SFTPA2* and *SFTPD* were significantly associated with FEV_1/IVC in subjects with GOLD stage ⩾1 COPD. Two SNPs in *TGFB1* (C to T substitution at nucleotide -509 and substitution of leucine 10 with proline (Leu10Pro)), Leu50Val in *SFTPA1* and Ala160Thr in *SFTPD* showed evidence suggestive of association with FEV_1/IVC in subjects with GOLD stage ⩾2 COPD. The *TGFB1* associations were replicated in GOLD stage ⩾2 patients from the Vlagtwedde/Vlaardingen population, with similar effect sizes.

It was shown that a genetic isolate can be used to determine the genetics of lung function, which can be replicated in COPD patients from an independent population.

KEYWORDS: Chronic obstructive pulmonary disease, genetically isolated population, lung function, single nucleotide polymorphism

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide, and is expected to increase in prevalence until 2030 [1, 2]. The disease has a large personal, societal, and economic impact. COPD is characterised by chronic airway inflammation, airway remodelling and airflow limitation that is not fully reversible. Since not all smokers develop COPD, genetic susceptibility must play a role in the development of this disease, in addition to environmental factors. The genetic determinants for COPD are difficult to study, since COPD is a disease that becomes clinically manifest only at later ages, when parents of COPD patients have already died and their children are probably too young to manifest airway obstruction. This limits the option of performing family-based genetic research. Moreover, published studies frequently use various definitions of disease status, which makes it difficult to compare their results. Therefore, it makes sense to choose a robust phenotype for definition of COPD, such as the level of lung function, which can be more easily compared between studies. Moreover, a low level of lung function is a predictor of mortality due to COPD [3–5].

Another complicating factor in studies on the genetics of COPD is that COPD is considered a complex genetic trait, *i.e.* multiple, possibly interacting, genetic and environmental factors are involved. Therefore, there are advantages to attempting to identify risk genes in populations that are relatively genetically and environmentally homogeneous, such as genetically isolated populations, in which genetic variation is reduced owing to the small number of founders

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and drift [6]. However, these processes raise the question of whether findings can be extrapolated to the general population. Previous simulation studies suggest that this is the case for common variants with a frequency of >1% [6], but no empirical evidence is available.

A candidate gene study was conducted for level of airflow limitation in patients with COPD who were ascertained as part of the Genetic Research in Isolated Populations (GRIP) study that is being conducted in a young genetically isolated population from the south-western part of the Netherlands. All patients were genotyped using 32 single nucleotide polymorphisms (SNPs) in 13 candidate genes for COPD, chosen based on their previously published association with either COPD, level of lung function or lung function decline, as reported in the general population. Extensive genealogical information was collected, resulting in an extremely large and complex pedigree of 6,175 members. Finally, 1,390 Caucasians from the general Dutch population were studied, including 351 patients with COPD, in order to establish whether or not the present findings could be replicated in the general population. In both studies, it was investigated whether the severity of the disease, as reflected by lung function reduction, is genetically influenced in established COPD.

METHODS

Study populations

The present study forms part of the GRIP programme [7, 8]. The GRIP programme is based in a recently genetically isolated population from the south-western part of the Netherlands, which was founded in the middle of the eighteenth century by ~150 individuals and was genetically isolated until the middle of the twentieth century. The population now includes ~20,000 inhabitants in eight adjacent communities. GRIP programme participants are generally related *via* multiple lines of descent and are inbred *via* multiple consanguineous loops [9, 10].

Subjects with general-practitioner-diagnosed COPD were invited to the research centre to undergo spirometry and complete a questionnaire [11]. Spirometry was performed by trained pulmonary research technicians using a pneumotachograph (Viasys, Houten, the Netherlands; formerly Jaeger spirometry system). Predicted values for forced expiratory volume in 1 s (FEV_1) were calculated using adjusted QUANJER *et al.* [12] equations for Caucasian subjects. DNA was isolated from blood using Puregene® DNA Purification Kits (Gentra, Inc., Minneapolis, MN, USA). All participants gave written informed consent.

In order to verify the findings from the GRIP study in the general population, cross-sectional data from the general-population-based Vlagtwedde/Vlaardingen cohort were used. Questionnaires, spirometric results and DNA were collected [13, 14]. For this study, 351 subjects were selected, according to Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria, with GOLD stage $\geq$1 COPD at the last 1989/1990 survey, of whom 167 had GOLD stage $\geq$2 COPD [15].

Genotyping

SNPs in candidate genes for lung function and COPD, based on their previously published significant associations, were genotyped (table 1). The selected SNPs were either the most significant SNPs in previous studies, tagging SNPs for the gene, or SNPs with a known functional effect on gene expression or function. Genotyping was performed using Applied Biosystems TaqMan® SNP Genotyping Assays (Applied Biosystems, Nieuwerkerk aan de IJssel, the Netherlands). Sequences of primers and probes are available on request.

Statistical analysis

In order to analyse pedigree data, use was made of the measured genotype (MG) approach [33], which models quantitative traits as

$$y_i = \mu + kg_i + \sum_j \beta_j c_{ji} + G_i + e_i$$

where y_i is the phenotype of the ith individual, g the vector of genotypes at the marker under study, k the marker genotype effect, c_{ij} the value of the jth covariate or fixed effect for the individual i, β_j an estimate of the jth fixed effect or covariate and G_i and e_i random additive polygenic and residual effects, respectively. The random effects are assumed to follow multivariate normal distribution with a mean of zero. The variance for the polygenic effects is defined as $\Phi\sigma_G^2$, where Φ is the relationship matrix and σ_G^2 the additive genetic variance due to polygenes. For the residual random effects, the variance is defined as $I\sigma_e^2$, where I is the identity matrix and σ_e^2 the residual variance.

Since the pedigree under analysis was very large, fast genome-wide rapid association using mixed model and regression (GRAMMAR) approximation to the full MG approach was used [34]. The GRAMMAR consists of a fast though conservative test at the screening stage, followed up with full MG analysis of polymorphisms that pass the relaxed screening significance threshold ($p<0.1$). All analyses involving pedigree were performed using ASReml v2.0 [35], a package for linear mixed model analysis using restricted maximum likelihood. This is a joint venture between the biometrics programme of the New South Wales Department of Primary Industries (Orange, Australia) and the Biomathematics University of Rothamsted Research (Harpenden, UK). Statisticians in the UK and Australia have collaborated in its development.

Significant associations were tested using linear regression analyses in the Vlagtwedde/Vlaardingen population. All analyses were adjusted for age, height and sex.

RESULTS

GRIP study population

A total of 157 individuals who were diagnosed with COPD by their general practitioners were ascertained. Spirometric measures confirmed COPD in 91 subjects, *i.e.* subjects with GOLD stage $\geq$1 COPD (defined by an FEV_1/inspiratory vital capacity (IVC) of <70%) [15]. The rest of the subjects could not be defined as having COPD according to their spirometric results and were, therefore, excluded from the analyses. The familial relationship of these 91 subjects was determined in the larger GRIP study database. This resulted in a large extended pedigree structure of 6,175 members. The characteristics of the GRIP COPD population and the Vlagtwedde/Vlaardingen replication cohort are shown in table 2.

TABLE 1 Candidate genes and single nucleotide polymorphisms (SNPs) genotyped in the study population

Gene	Description of gene	SNPs genotyped		Functional SNP	[Refs]
		ID	Alternative name		
ADAM33	A disintegrin and metalloprotease domain 33: exact function unknown; identified by genome-wide screen as susceptibility gene for asthma. Associated with decline in FEV_1 and development of COPD in the general population and severity of inflammation in COPD patients.	rs17548913	ADAM33 F+1		[13, 16]
		rs17548907	ADAM33 Q-1		
		rs3918396	ADAM33 S1		
		rs528557	ADAM33 S2		
		rs597980	ADAM33 ST+5		
		rs2280091	ADAM33 T1		
		rs2280090	ADAM33 T2		
		rs2787094	ADAM33 V4		
TGFB1	TGF-β1: a chemotactic cytokine for fibroblasts, inducing synthesis of matrix proteins and glycoproteins and inhibiting collagen degradation by induction of protease inhibitors and reduction of metalloproteases; TGF-β1 levels are increased in COPD; SNPs have been associated with COPD.	rs1800469	TGFB1 -509C>T	Increased TGF-β1	[14, 17–19]
		rs1982073	TGFB1 Leu10Pro	Increased TGF-β1	
		rs6957	TGFB1 3'UTR		
SFTPA1	SP-A1: SPs are involved in the first response to microorganisms in the lung, regulation of inflammation and structure of alveoli. SPs reduce surface tension at the air–liquid interface and, therefore, prevent alveolar collapse during expiration.	rs1059047	SPA1 Val19Ala		[20–23]
		rs1136450	SPA1 Leu50Val		
		rs4253527	SPA1 Arg219Trp		
SFTPA2	SP-A2: as for SP-A1, homologous gene.	rs1059046	SPA2 Asn9Thr,		
		rs17886395	SPA2 Pro91Ala,		
		rs1965707	SPA2 Ser140Ser		
SFTPB	SP-B: hydrophobic component of pulmonary surfactant.	rs1130866	SPB Ile131Thr	Altered affinity	
SFTPD	SP-D: a C-type lectin present in pulmonary surfactant and several other mucosal surfaces. It modulates innate immunity, allergic response, expression of MMPs, alveolar wall remodelling, emphysema, fibrosis and lipid and macrophage homeostasis. Associated with COPD.	rs721917	SPD Met11Thr	Altered SP-D assembly, function and levels	
		rs2243639	SPD Thr160Ala		
MMP1	MMP-1: an interstitial collagenase involved in tissue remodelling and repair associated with lung development and inflammation. Levels are increased in sputum of COPD patients compared to healthy controls. Associated with lung function decline.	rs1799750	MMP1 -1607G>GG	Additional Ets transcription factor binding site, increased expression	[24, 25]
MMP2	MMP-2: a type-IV collagenase specifically cleaving type IV collagen, the major structural component of basement membranes.	rs243865	MMP2 -1306C>T	loss of SP-1 transcription factor binding site, less expression	[26]
MMP9	MMP-9: a gelatinase B involved in tissue remodelling; smokers with airway obstruction show higher MMP-9 expression than smokers without COPD and nonsmokers.	rs3918278	mmp9_rs3918278	Tagging	[24, 27]
		rs6065912	mmp9_rs6065912	Tagging	
		rs8113877	mmp9_rs8113877	Tagging	
MMP12	MMP-12: a human macrophage elastase involved in degradation of extracellular matrix in lungs of patients with COPD. Associated with lung function decline.	rs2276109	MMP12 -82A>G	AP-1 transcription factor binding site, increased MMP-12	[24]
		rs652438	MMP12 Asn357Ser		
TIMP1	Tissue inhibitor of metalloprotease-1: inhibitor of several MMPs, including MMP-1, MMP-9 and MMP-12. X-chromosomal. Associated with asthma.	rs11551797	timp1 Ile158Ile		[28]
		rs4898	timp1 Phe124Phe		
HMOX1	Haem oxygenase 1: role in oxidant–antioxidant balance in the lung. Genetic variation associated with COPD.	rs2071747	HO1 Asp7His		[29]
GSTP1	Glutathione S-transferase P1: role in oxidant–antioxidant balance in the lung. Associated with COPD.	rs1695	gstp1 Ile105Val	Increased enzyme activity	[30–32]
		rs1138272	gstp1 Ala114Val		

ID: identifier; *ADAM33*: a disintegrin and metalloprotease domain 33 gene; *TGFB1*: transforming growth factor-β1 gene; *SFTPA1*: surfactant protein A1 gene; *MMP1*: matrix metalloprotease-1 gene; *TIMP1*: tissue inhibitor of metalloprotease-1 gene; *HMOX1*: haem oxygenase 1 gene; *GSTP1*: glutathione S-transferase P1 gene; FEV_1: forced expiratory volume in 1 s; COPD: chronic obstructive pulmonary disease; TGF-β: transforming growth factor-β; SP: surfactant protein; MMP: matrix metalloprotease; ADAM: a disintegrin and metalloprotease; -509C>T: cytosine (C) to thymidine (T) substitution at nucleotide -509; G: guanine; A: adenine; Leu10Pro: substitution of leucine 10 with proline; Val: valine; Ala: alanine; Arg: arginine; Trp: tryptophan; Asn: asparagine; Thr: threonine; Ser: serine; Ile: isoleucine; Met: methionine; Phe: phenylalanine; Asp: aspartic acid; His: histidine; UTR: untranslated region; HO: haem oxygenase; Ets: erythroblastosis virus E26 oncogene homologue; AP-1: activator protein-1.

TABLE 2 Characteristics of the Genetic Research in Isolated Populations (GRIP) and Vlagtwedde/Vlaardingen (Vla/Vla) study populations

	Total population			FEV_1<80% pred		
	GRIP	Vla/Vla	p-value#	GRIP	Vla/Vla	p-value#
Subjects n	91	351		67	167	
Age yrs	66.0 (41–84)	58.0 (35–76)	<0.001	66.0 (43–82)	59.0 (35–76)	<0.001
Sex M/F n	47/44	244/107	0.001	36/31	122/45	0.004
Smoking %						
Never-smoker	3.4	18.8	0.001	3.1	16.2	0.026
Ex-smoker	38.6	35.9		38.5	33.5	
Current smoker	58.0	45.3		58.4	50.3	
Smoking history pack-yrs	34.8 (0–120)	21.4 (0–262)	0.001	39.0 (0–120)	26.0 (0–262)	0.015
FEV_1 % pred	69.4 (26.4–110.5)	80.7 (36.0–115.0)	<0.001	63.5 (26.4–79.0)	69.9 (36.0–79.8)	0.001
FEV_1/FVC	56.2 (27.7–68.4)	NA	NA	52.8 (27.7–67.9)	NA	NA
FEV_1/IVC	54.5 (20.7–69.8)	64.9 (29.0–69.9)	<0.001	50.8 (20.7–67.7)	59.2 (29.4–69.8)	<0.001
Chronic cough %	58.2	14.5	<0.001	60.6	22.2	<0.001
Chronic phlegm %	50.5	10.5	<0.001	51.5	15.0	<0.001

Data are presented as median (range) unless otherwise indicated. All of the study subjects had a forced expiratory volume in 1 s (FEV_1)/inspiratory vital capacity (IVC) of <70%. M: male; F: female; FVC: forced vital capacity; NA: not available; % pred: percentage of the predicted value. #: derived from Chi-squared test for comparison of discrete variables and Mann–Whitney U-test for continuous variables.

Association of genes with lung function parameters in GRIP, and replication in Vlagtwedde/Vlaardingen

The effects of SNPs in the studied genes on percentage predicted FEV_1, IVC and FEV_1/IVC were first analysed in the 91 subjects with GOLD stage ⩾1 COPD. None of the SNPs were associated with percentage predicted FEV_1 or IVC. Six SNPs in the transforming growth factor-β1 gene (*TGFB1*), surfactant protein A1 gene (*SFTPA1*), *SFTPA2* and *SFTPD* were significantly associated with FEV_1/IVC (table 3). None of these associations were replicated in subjects from the Vlagtwedde/Vlaardingen cohort with GOLD stage ⩾1 COPD (data not shown).

In addition, the effects of SNPs in the studied genes were analysed using a more stringent definition of COPD, namely GOLD stage ⩾=2 (defined as FEV_1/IVC of <70% and FEV_1 of <80% pred). This resulted in 67 cases in the GRIP population. In these subjects, two SNPs in *TGFB1* (cytosine to thymidine substitution at nucleotide -509 (-509C>T) and substitution of leucine 10 with proline (Leu10Pro)), Leu50Val in *SFTPA1* and Ala160Thr in *SFTPD* showed evidence suggestive of association with FEV_1/IVC ($p<0.10$) (table 3). The *TGFB1* -509C>T and Leu10Pro associations were replicated in GOLD stage ⩾2 subjects from the Vlagtwedde/Vlaardingen population (n=167), with similar effect sizes (see table 3).

DISCUSSION

The present study is the first to use a genetically isolated population to analyse genetic effects on level of lung function in COPD. Interestingly, significant effects of SNPs in COPD candidate genes were found on severity of COPD, assessed by lung function in subjects with COPD, even though the present study population was small. The present results show that levels of FEV_1/IVC, measures of airway obstruction, are genetically influenced in established COPD. This means that, even within patients with phenotypic COPD, genotypes can be identified that are associated with severity of disease. This is of clinical importance since low lung function has been shown to predict mortality in COPD, not only in the general population but also within COPD patients [3–5].

The *TGFB1* SNPs that were associated with FEV_1/IVC in the present populations have previously been associated with development of COPD or with lower FEV_1 and FEV_1/VC in several [17–19], but not all previous studies [14, 36, 37]. The present results (in both the genetically isolated and general population) thus confirm the former studies that implicate a role of *TGFB1* in the severity of airflow limitation. The *SFTPA1* and *SFTPD* SNPs have been associated with COPD previously [20, 38]. It is now shown for the first time that these SNPs may also play a role in severity of COPD. This is plausible since surfactant proteins decrease surface tension at the air–liquid interface and, therefore, reduce the tendency of alveoli to collapse during expiration. The latter contributes to the severity of airway obstruction, as measured by FEV_1/IVC.

No significant associations of a disintegrin and metalloprotease domain 33 gene (*ADAM33*), matrix metalloprotease-1 gene (*MMP1*), *MMP2*, *MMP9*, *MMP12*, tissue inhibitor of metalloprotease-1 gene (*TIMP1*), *SFTPB*, glutathione S-transferase P1 gene (*GSTP1*) and haem oxygenase 1 gene (*HMOX1*) with level of lung function were found in COPD patients. This does not, however, imply that these genes do not play any role whatsoever in COPD. To date, no studies have analysed genetic effects on the severity of airway obstruction within patients with established COPD. The present study shows that SNPs in *TGFB1*, *SFTPA*1 and *SFTPD* may be important in progression of COPD, whereas the SNPs in the other genes, *i.e. ADAM33*, *MMP1*, *MMP2*, *MMP9*, *MMP12*, *TIMP1*, *GSTP1* and *HMOX1*, may simply constitute SNPs that are important in the development of COPD.

TABLE 3 Associations of single nucleotide polymorphisms (SNPs) with forced expiratory volume in 1 s (FEV_1)/inspiratory vital capacity (IVC) in the Genetic Research in Isolated Populations (GRIP) and Vlagtwedde/Vlaardingen (Vla/Vla) study populations

SNP	Comparison#	GRIP						Vla/Vla		
		GOLD ⩾1			GOLD ⩾2			GOLD ⩾2		
		Subjects n	Estimate	p-value	Subjects n	Estimate	p-value	Subjects n	Estimate	p-value
Subjects n			91			67			167	
***ADAM33* ST+5**	wt	15	Ref.		12	Ref.		27	Ref.	
	Het	46	3.8	0.220	31	2.8	0.424	86	2.9	**0.082**
	Hom	29	0.3	0.919	24	1.9	0.594	51	0.8	0.668
***TGFB1* -509C>T**	wt	41	Ref.		29	Ref.		94	Ref.	
	Het	38	-3.8	0.102	30	-3.7	0.146	60	-1.3	0.298
	Hom	11	-6.6	**0.063**	8	-9.4	**0.017**	8	-5.0	**0.070**
***TGFB1* Leu10Pro**	wt	32	Ref.		22	Ref.		68	Ref.	
	Het	40	-4.6	**0.061**	32	-4.7	**0.081**	65	-0.8	0.952
	Hom	13	-5.8	**0.088**	8	-10.8	**0.007**	17	-4.5	**0.028**
***SFTPA1* Leu50Val**	wt	60	Ref.		45	Ref.		123	Ref.	
	Het	19	-2.7	0.329	15	-1.5	0.623	20	2.7	0.159
	Hom	4	13.6	**0.015**	1	18.9	**0.076**	11	1.8	0.474
***SFTPA2* Pro91Ala**	wt	58	Ref.		42	Ref.		117	Ref.	
	Het	29	0.5	0.833	22	-0.1	0.986	41	-1.1	0.423
	Hom	3	-10.2	**0.099**	3	-7.2	0.232	3	0.4	0.923
***SFTPD* Met11Thr**	wt	33	Ref.		22	Ref.		44	Ref.	
	Het	35	-4.4	**0.090**	29	-4.0	0.161	85	-0.9	0.512
	Hom	19	-3.2	0.291	13	-4.3	0.226	31	-0.3	0.888
***SFTPD* Ala160Thr**	wt	33	Ref.		26	Ref.		54	Ref.	
	Het	41	5.6	**0.025**	30	5.4	**0.055**	73	2.1	0.112
	Hom	12	2.0	0.582	8	1.2	0.778	29	-1.6	0.376

A general model of inheritance was used, in which the mutant genotypes were compared to the wild-type (wt). GOLD: Global Initiative for Chronic Obstructive Lung Disease; *ADAM33*: a disintegrin and metalloprotease domain 33 gene; *TGFB1*: transforming growth factor-β1 gene; -509C>T: cytosine to thymidine substitution at nucleotide -509; Leu10Pro: substitution of leucine 10 with proline; *SFTPA1*: surfactant protein A1 gene; Val: valine; Ala: alanine; Met: methionine; Thr: threonine; Het: heterogzygous; Hom: homozygous; Ref.: reference genotype. #: *versus* wild-type.

One important advantage of testing genes in a genetically isolated population is that it provides an opportunity of finding genes associated with disease in a relatively small sample size due to increased homogeneity of the population, as recently demonstrated for multiple sclerosis [39]. Thus, for a lower cost and effort, many genes can be tested regarding their significance in contributing to disease severity, which can subsequently be replicated in a larger sample of the general population. The most important requirement for such studies is that the genetic isolate is representative of the general population or disease-specific study populations. This is indeed the case since it was shown that, in selected subjects with COPD from the general population, the associations found in the young genetic isolate can be replicated in a substantial part. Thus it is possible to translate findings in a genetic isolate to the general population, but correct and comparable phenotyping of the study populations remains crucial to replicate associations between populations.

It was not possible to replicate the results of any of the SNPs in subjects with GOLD stage ⩾1 COPD from the Vlagtwedde/Vlaardingen population. On closer investigation, it appeared that the GRIP patients with GOLD stage ⩾1 COPD had more severe COPD, *i.e.* lower lung function and more symptoms, than COPD patients of similar disease stage in the Vlagtwedde/Vlaardingen population. A more strict definition of COPD (GOLD stage ⩾=2) in the Vlagtwedde/Vlaardingen and GRIP populations gave a phenotypically better comparison. Indeed, when analysing subjects with GOLD stage ⩾2 COPD from the Vlagtwedde/Vlaardingen population, the *TGFB1* SNPs -509C>T and Leu10Pro were significantly associated with FEV_1/IVC, as they were in the GRIP GOLD stage ⩾2 COPD patients.

Since the percentage of subjects with, amongst others chronic cough, was different in both cohorts, the analyses were repeated using straightforward linear regression models with chronic cough in the model to check for stability of the effect estimates. Analyses on FEV_1/IVC in the GRIP GOLD stage ⩾2 population, taking, for example, chronic cough into account, resulted in similar regression estimates for the SNPs in *TGFB1* and *SFTPA1*, but with smaller p-values and slightly higher explained variances, whereas the suggestive associations of the other SNPs disappeared. Additional adjustment for chronic

TABLE 4 Genotype frequencies of significant single nucleotide polymorphisms in the Genetic Research in Isolated Populations (GRIP) compared to the Vlagtwedde/Vlaardingen (Vla/Vla) Global Initiative for Chronic Obstructive Lung Disease ⩾2 population

Genotype	GRIP	Vla/Vla	p-value
Subjects n	67	167	
***ADAM33* ST+5**			
AA	12 (17.9)	27 (16.5)	0. 690
AG	31 (46.3)	86 (52.4)	
GG	24 (35.8)	51 (31.1)	
***TGFB1* -509C>T**			
GG	29 (43.3)	94 (58.0)	0.051
GA	30 (44.8)	60 (37.0)	
AA	8 (11.9)	8 (4.9)	
***TGFB1* Leu10Pro**			
AA	22 (34.9)	68 (45.3)	0.368
AG	33 (52.4)	65 (43.3)	
GG	8 (12.7)	17 (11.3)	
***SFTPA1* Leu50Val**			
GG	45 (73.8)	123 (79.9)	**0.045**
GC	15 (24.6)	20 (13.0)	
CC	1 (3.1)	11 (7.1)	
***SFTPA2* Pro91Ala**			
GG	42 (62.7)	117 (72.7)	0.242
GC	22 (32.8)	41 (25.5)	
CC	3 (4.5)	3 (1.9)	
***SFTPD* Met11Thr**			
TT	22 (34.4)	44 (27.5)	0.522
TC	29 (45.3)	85 53.1)	
CC	13 (20.3)	31 (19.4)	
***SFTPD* Ala160Thr**			
AA	26 (40.6)	54 (34.6)	0.484
AG	30 (46.9)	73 (46.8)	
GG	8 (12.5)	29 (18.6)	

Data are presented as n (%) unless otherwise indicated. *ADAM33*: a disintegrin and metalloprotease domain 33 gene; A: adenine; G: guanine; *TGFB1*: transforming growth factor-β1 gene; -509C>T: cytosine (C) to thymidine (T) substitution at nucleotide -509; Leu10Pro: substitution of leucine 10 with proline; *SFTPA1*: surfactant protein A1 gene; Val: valine; Ala: alanine; Met: methionine; Thr: threonine.

cough in the Vlagtwedde/Vlaardingen GOLD stage ⩾2 population resulted in similar significant regression estimates for the SNPs in *TGFB1* with FEV1/IVC. Therefore, the effect estimates appear to be stable within both GOLD stage ⩾2 groups, irrespective of differences in characteristics between the GRIP and Vlagtwedde/Vlaardingen GOLD stage ⩾2 populations.

Several explanations may exist for the lack of replication for *SFTPA1* and *SFTPD* (Met11Thr) SNP FEV1/IVC results in the Vlagtwedde/Vlaardingen GOLD stage ⩾2 population. First, the original GRIP findings on these genes could be falsely positive. Indeed, multiple (though correlated) outcomes and SNPs were studied in GRIP. Another, more biological, explanation for the lack of replication may be that the prevalence of certain alleles in genetically isolated populations differs from that in a general population as a result of genetic drift and founder effects. Indeed, the genotype frequencies for the *SFTPA1* Leu50Val SNP were significantly different between the two populations, but not for the other SNPs (table 4). A third explanation may be that differences in characteristics exist between the study populations. The GRIP population had more severe COPD and was slightly older than the Vlagtwedde/Vlaardingen COPD population.

In addition, differences in environment may affect the lack of replication of the surfactant protein gene data. The genetically isolated population shares the same environment, similar socioeconomic status and the same general practitioners. The possibility cannot be ruled out that the COPD patients in the GRIP population exhibited a higher prevalence of chronic bronchitis and airway disease, whereas the airway obstruction in the Vlagtwedde/Vlaardingen population may have been caused by emphysema [40–42]. Further research is needed in order to separately assess these phenomena, since computed tomographic scans are necessary, which were not available for any of the present patients.

In conclusion, the present study provides two important messages. First, significant effects of SNPs were found on the severity of COPD, *i.e.* level of lung function in patients with established COPD, in a relatively small genetically isolated population with a large pedigree structure. Secondly, two of these associations were replicated in COPD patients selected from the general population on the condition that they were phenotypically similar. These findings are important since more severe airway obstruction is associated with progression and mortality of COPD. Future studies using this genetic isolate should focus on progression of COPD, since this population seems to be highly suitable for determining genetic risk factors for severity of airway obstruction in established COPD that can be translated to the general population.

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STATEMENT OF INTEREST

None declared.

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