Association of COPD candidate genes with computed tomography emphysema and airway phenotypes in severe COPD

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ABSTRACT: The principal determining factors influencing the development of the airway disease and emphysema components of chronic obstructive pulmonary disease (COPD) have not been clearly defined. Genetic variability in COPD patients might influence the varying degrees of involvement of airway disease and emphysema. Therefore, we investigated the genetic association of single nucleotide polymorphisms (SNPs) in COPD candidate genes for association with emphysema severity and airway wall thickness phenotypes.

Polymorphisms in six candidate genes were analysed in 379 subjects of the National Emphysema Treatment Trial (NETT) Genetics Ancillary Study with quantitative chest computed tomography (CT) data. Genetic association with per cent of lung area below -950 HU (LAA950), airway wall thickness, and derived square root wall area (SRWA) of 10-mm internal perimeter airways were investigated.

Three SNPs in *EPHX1*, five SNPs in *SERPINE2* and one SNP in *GSTP1* were significantly associated with LAA950. Five SNPs in *TGFB1*, two SNPs in *EPHX1*, one SNP in *SERPINE2* and two SNPs in *ADRB2* were associated with airway wall phenotypes in NETT.

In conclusion, several COPD candidate genes showed evidence for association with airway wall thickness and emphysema severity using CT in a severe COPD population. Further investigation will be required to replicate these genetic associations for emphysema and airway wall phenotypes.

KEYWORDS: Airway, chronic obstructive pulmonary disease, computed tomography, emphysema, genetic association

Chronic obstructive pulmonary disease (COPD) is characterised by persistent airflow obstruction [1]. Cigarette smoking is a well-known risk factor, but other environmental and genetic factors are likely to be involved. The relative amount of airway and airspace involvement is heterogeneous in COPD subjects, but the key determinants of these different phenotypes, which largely develop from exposure to cigarette smoke, are not known.

Computed tomography (CT) is a useful way of characterising structural changes, including quantitative measurement of emphysema and airway wall phenotypes [2]. A recent paper suggested that genetic components influencing airway wall thickness and emphysema phenotypes were independent [3]. There are several

reports of genetic associations with emphysema severity or emphysema distribution [4–6]. However, combined genetic investigation of both emphysema and airway wall phenotypes in the same population has not been widely reported.

We hypothesised that different genetic variants would influence airway disease and emphysema. We investigated whether single nucleotide polymorphisms (SNPs) in previously reported COPD susceptibility genes were associated with emphysema severity and airway wall phenotypes in a COPD study population. We selected six candidate genes that had been associated with COPD susceptibility in at least two populations.

The National Emphysema Treatment Trial (NETT) is a cohort of emphysema subjects with available chest CT data. We measured airway

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Received:

Nov 01 2009
Accepted after revision:
April 28 2010
First published online:
June 04 2010

This article has supplementary material available from www.erj.ersjournals.com

wall phenotypes using CT scans in a subset of subjects and investigated associations of variants in COPD susceptibility genes with both emphysema severity and airway wall phenotypes in emphysema subjects from NETT.

METHODS

Subjects

The current analysis included 379 non-Hispanic white subjects in the NETT Genetics Ancillary Study with quantitative chest CT data. Subjects enrolled in NETT had severe airflow obstruction (forced expiratory volume in 1 s (FEV1) ≤45% predicted), hyperinflation on pulmonary function testing and bilateral emphysema on high-resolution chest CT [7]. The study was approved by institutional review boards at participating centres. All subjects provided written informed consent.

Chest CT analysis

Emphysema measurements on NETT subjects have been described previously [6]. CT images of the chest were acquired at full inspiration with a minimum of 200 mAs and reconstructed using a high spatial frequency algorithm with a 1–2-mm collimation at 20-mm intervals. Densitometric measures of emphysema were performed using the Pulmonary Analysis Software Suite (PASS, University of Iowa, Iowa City, IA, USA) at a threshold of -950 HU. Airway wall thickness and the square root of wall area (SRWA) were assessed using 3D Slicer (www.slicer.org) and Airway Inspector (www.airwayinspector.org) at Brigham and Women’s Hospital (Boston, MA, USA). The full width at half-maximum method was used to measure the wall thickness and wall area of each airway. From these measures, the airway wall thickness and the SRWA of a 10-mm luminal perimeter airway were calculated [8].

Genotyping

Genotyping in NETT was performed for 122 SNPs in six candidate genes (19 in *EPHX1*, five in *SFTPB*, 21 in *TGFB1*, 64 in *SERPINE2*, seven in *GSTP1*, six in *ADRB2*), including upstream and downstream chromosomal regions. We used pairwise linkage disequilibrium (LD)-tagging in Tagger [9] with a minimum minor allele frequency of 0.10 and an r^2 -threshold of 0.9 based on genotype data in Phase II of the HapMap Project (www.hapmap.org), in order to select a panel of SNPs to capture the LD information in each gene. Additional SNPs were also genotyped, based on previously reported genetic association analyses of COPD-related phenotypes [10–12]. SNPs were genotyped using TaqMan (Applied Biosystems, Foster City, CA, USA) or Sequenom (Sequenom, Inc, San Diego, CA, USA) assays as previously reported [13, 14].

Statistical analysis

Phenotypes tested for association in NETT included per cent of lung area below -950 HU (LAA950), wall thickness and derived SRWA of 10-mm internal perimeter airways. Multivariate analysis was performed using linear regression models with SAS PROC GLM (SAS Institute, Cary, NC, USA), assuming an additive mode of inheritance, and adjusting for age, sex, weight, pack-yr of cigarette smoking, and clinical centre. All statistical analyses were performed using SAS (SAS Institute). LD between SNPs was assessed using the Haploview program [15].

RESULTS

Demographic characteristics

In NETT, the mean age of 379 subjects who had quantitative CT emphysema and/or airway wall measurements was 67.5 yrs, and mean FEV1 % pred was 24.9% (table 1). Emphysema data were available from 358 subjects, and airway data were available from 338 subjects. The mean (range) number of airways analysed per subject was 12 (4–23).

Association analysis with CT phenotypes in NETT

As shown in table 2, three SNPs in *EPHX1* (rs2854450, rs3738042 and rs1009668), five SNPs in *SERPINE2* (rs6734100, rs729631, rs975278, rs6436449 and rs7608941) and one SNP in *GSTP1* (rs11227844) were significantly associated with LAA950. Five SNPs in *TGFB1* (rs1800469, rs1800470, rs1800471, rs11083616 and rs11466321), two SNPs in *EPHX1* (rs2854450 and rs3753658), one SNP in *SERPINE2* (rs6436459) and two SNPs in *ADRB2* (rs1042717 and rs1042718) were associated with airway wall phenotypes (table 3). Statistical analysis showed similar results for airway wall thickness and SRWA. Among the significantly associated SNPs in table 2, rs729631 and rs975278 in *SERPINE2* (pairwise $r^2=97\%$) were in strong LD. Among the significantly associated SNPs in table 3, rs1800470 and rs11083616 (pairwise $r^2=95\%$), rs1800471 and rs11466321 (pairwise $r^2=94\%$) in *TGFB1*, and rs1042717 and rs1042718 (pairwise $r^2=83\%$) in *ADRB2* were in strong LD. A list of genotyped SNPs and LD maps is provided in supplementary table 1 and supplementary figures 1–5, and genetic association results of all SNPs are shown in supplementary table 2 (see online supplementary material).

At the gene level, SNPs in *EPHX1* and *SERPINE2* were associated with both airway wall and emphysema phenotypes; however, only rs2854450 in *EPHX1* was associated with both airway and emphysema phenotypes (fig. 1). The minor allele of rs2854450 was associated with less severe emphysema and thinner airway walls.

TABLE 1 Demographic characteristics of analysed subjects in the National Emphysema Treatment Trial (NETT) Genetics Ancillary Study	
Characteristics	NETT
Subjects n	379
Age yrs	67.5±5.8
Males	241 (64)
Cigarette smoking pack-yr	66.4±30.5
Pre-bronchodilator FEV1 % pred	24.9±6.6
Post-bronchodilator FEV1 % pred	28.1±7.4
LAA950 %	17±11
Wall thickness mm	1.53±0.25
Square root wall area mm	4.6±0.5
Data are presented as mean±sd or n(%), unless otherwise stated. FEV1: forced expiratory volume in 1 s; % pred: % predicted. LAA950: lung area below -950 HU. In NETT, emphysema data were available in 358 subjects and airway wall data (wall thickness and square root wall area) were available in 338 subjects.	

TABLE 2 Genetic association results of quantitative emphysema measurements in subjects in the National Emphysema Treatment Trial Genetics Ancillary Study

Gene	SNP	Location	MAF	LAA950	
				β	p-value
EPHX1	rs2854450	Promoter	0.21	-1.6	0.04
	rs3738042	Promoter	0.32	1.3	0.05
	rs1009668	Exon	0.11	3.3	0.002
SERPINE2	rs6734100	Intron	0.14	-2.5	0.01
	rs729631	Intron	0.19	-2.0	0.02
	rs975278	Intron	0.19	-1.6	0.05
	rs6436449	Intron	0.17	-2.2	0.02
	rs7608941	Intron	0.22	-1.6	0.04
GSTP1	rs11227844	5' flank	0.09	3.2	0.003

SNP: single nucleotide polymorphism; MAF: minor allele frequency; LAA950: per cent of lung area below -950 HU. Each model was analysed assuming an additive mode of inheritance adjusting for age, sex, weight, pack-yrs of smoking and clinical centre. SNPs with p-values ≤ 0.05 are shown.

DISCUSSION

In this study, SNPs in six genes previously associated with COPD susceptibility were investigated for association with emphysema severity and airway wall thickness. In NETT subjects, three genes included SNPs that were associated with emphysema severity, and four genes included SNPs that were associated with airway wall phenotypes.

There have been several previous reports of genetic associations with emphysema or emphysema distribution. One previous study reported that emphysema severity determined by visual emphysema score was associated with a tumour necrosis factor- α promoter polymorphism in 84 subjects with

COPD [4]. Another study revealed that low attenuation area percentage of the lung differed according to *MMP-9* genotype only in the upper lung zones [5]. In NETT subjects, variants in *EPHX1* and *GSTP1* showed significant association with emphysema distribution, suggesting that xenobiotic enzymes may contribute to upper lobe dominant emphysema [6].

In the current study, 358 subjects in NETT were investigated for genetic associations with emphysema. SNPs in *EPHX1*, *GSTP1* and *SERPINE2* were associated with emphysema severity. One of the SNPs associated with emphysema in *EPHX1* (rs1009668) was previously reported by our group to be associated with bronchodilator responsiveness in NETT [16].

Airway wall measurements from chest CT scans are being increasingly used and validated as COPD phenotypes [17, 18]. Airway remodelling is associated with airway narrowing and wall thickening in COPD subjects [19] and this can be evaluated non-invasively using CT [2]. In 338 NETT subjects, we assessed genetic associations with airway wall thickness and airway wall area. SNPs in *EPHX1*, *SERPINE2*, *TGFB1* and *ADRB2* were associated with airway wall phenotypes. *EPHX1* and *SERPINE2* were associated with both emphysema and airway wall phenotypes; *GSTP1* was associated only with emphysema, and *TGFB1* and *ADRB2* were associated only with airway wall phenotypes.

EPHX1 is related to oxidative stress and has been repeatedly associated with COPD susceptibility [10, 20]; however, recently it has also been reported not to be associated with COPD susceptibility in a large number of COPD and control subjects [21]. With CT phenotypes, previously, *EPHX1* was associated with upper lobe predominant emphysema distribution in NETT subjects [6]. In the current analysis, SNPs in *EPHX1* were associated with both emphysema severity and airway wall phenotypes in NETT subjects. At the SNP level, only one SNP in *EPHX1* (rs2854450) was associated with both emphysema and airway wall phenotypes in NETT subjects. This SNP was associated with less emphysema and thinner airway walls,

TABLE 3 Genetic association results of airway wall phenotypes in subjects in the National Emphysema Treatment Trial Genetics Ancillary Study

Gene	SNP	Location	MAF	WT		SRWA	
				β	p-value	β	p-value
TGFB1	rs1800469	5' flank	0.30	-0.04	0.05	-0.03	0.36
	rs1800470	Exon	0.38	-0.05	0.004	-0.1	0.008
	rs1800471	Exon	0.09	-0.06	0.06	-0.2	0.005
	rs11083616	Intron	0.38	-0.04	0.05	-0.1	0.04
	rs11466321	Intron	0.07	-0.06	0.10	-0.2	0.02
EPHX1	rs2854450	Promoter	0.21	-0.05	0.02	-0.1	0.006
	rs3753658	Promoter	0.12	0.06	0.02	0.13	0.02
SERPINE2	rs6436459	Intron	0.23	0.05	0.03	0.06	0.17
ADRB2	rs1042717	Exon	0.20	-0.05	0.01	-0.08	0.06
	rs1042718	Exon	0.17	-0.06	0.003	-0.11	0.01

SNP: single nucleotide polymorphism; MAF: minor allele frequency; WT: wall thickness of a hypothetical 10-mm luminal perimeter airway; SRWA: square root of wall area of a hypothetical 10-mm luminal perimeter airway. Each model was analysed assuming an additive mode of inheritance and adjusting for age, sex, weight, pack-yrs of smoking and clinical centre.

FIGURE 1. Square root of wall area (SRWA) of a hypothetical 10-mm internal perimeter airway and % of lung area below -950 HU (LAA950) by genotype at rs285540 single nucleotide polymorphism (SNP) in *EPHX1* in National Emphysema Treatment Trial subjects. Mean $\pm$ SEM for SRWA (■) and LAA950 (▒) are shown. CC, CT and TT represent the genotypes at this SNP. CC: n=206; CT: n=101; TT: n=17.

although there was negative overall correlation between emphysema severity and airway wall thickness in NETT subjects [8]. It is not clear whether that promoter SNP has a direct functional effect; it is more likely that it is in LD with a functional variant or variants in *EPHX1*.

Transforming growth factor (TGF)- β 1 was previously reported to be associated with COPD by our research group [11]. TGF- β 1 has been suggested to be involved in airway remodelling in COPD [22, 23]. In 85 asthma subjects, serum TGF- β 1 levels were associated with a promoter polymorphism in *TGFB1*, but there was no association between airway wall thickness and *TGFB1* genetic polymorphisms [24]. However, in 27 subjects with asthma, sputum TGF- β 1 levels were associated with airway wall area corrected by body surface area [25]. In the present study, several SNPs in *TGFB1* were associated with airway wall thickness in the NETT cohort. Among SNPs with significance, rs1800469 and rs1800470 were previously reported to be associated with COPD [11] and respiratory symptom severity phenotypes [13] by our group.

One SNP (rs1042713) of *ADRB2* was recently reported to be associated with airway lumen area in Korean COPD subjects [26]; however, *ADRB2* SNPs rs1042717 and rs1042718 were associated with airway wall thickness in the current study. The optimal airway phenotypes to reflect airway remodelling or COPD severity have not been determined.

It is interesting that some xenobiotic enzymes (*EPHX1*) and protease-related genes (*SERPINE2*) were associated with both emphysema and airway phenotypes, while possible airway remodelling-related genes (*TGFB1* and *ADRB2*) were associated only with airway wall phenotypes. There may be different mechanisms of pathogenesis for emphysema and airway remodelling. Although we recognise that our association results will need to be replicated in larger populations and different ethnic groups, genetic studies may provide insight into these pathogenetic mechanisms. For example, TGF- β 1 is known to have a role in immune response, cell growth and

tissue repair [11]. Although, in animal models, *TGFB1* was important in the development of emphysema [27], in our study, this gene was associated with airway phenotypes in a severe COPD cohort suggesting that *TGFB1* may be associated with airway remodelling. *ADRB2* was reported to be associated with COPD susceptibility and β_2 -agonist responsiveness [8]. Although this gene may be related to bronchodilation, it may also have a role in airway remodelling.

The statistical significance of the genetic associations with CT phenotypes in this study was not stronger than previous reports from our group using functional impairment [13] and emphysema distribution [6] phenotypes. It is possible that the currently available emphysema and airway phenotypes do not optimally capture these disease processes. We analysed SNPs in six candidate genes, and it is likely that many other genes also contribute to emphysema and airway disease pathogenesis.

There are several limitations to this study. Subjects were recruited from multiple clinical centres with different CT scanners that may affect the CT parameters. Although we adjusted for clinical centre in our association analysis, residual bias could still exist. We analysed SNPs in six genes, and multiple statistical comparisons are a potential concern. In this initial analysis of genetic determinants of CT-related phenotypes, we did not correct association p-values for multiple statistical testing. We acknowledge that many of the SNPs which showed modest significance with p-values less than a nominal threshold of 0.05 may be false positives. Although the optimal approach to adjust for multiple testing is not clear, multiple statistical testing may be less of a problem since most of these genes have already been associated with COPD susceptibility in multiple populations. In addition, replication of our results in other populations will be required.

The NETT population is a US-based cohort of patients with advanced COPD and a clinical and subjective imaging diagnosis of emphysema. These subjects were screened specifically for participation in a clinical trial of lung volume reduction surgery. They are a predominantly emphysematous subgroup of COPD cases. This may have limited the detection power of the genetic data for the airway phenotypes. However, we previously found that lung function was associated with airway wall thickness in NETT subjects [8], and we now report genetic associations with airway wall thickness in this same population. Thus, airway wall phenotypes may contribute to airflow obstruction even in subjects with severe emphysema.

In conclusion, one SNP in *EPHX1* was associated with both emphysema and airway wall thickness using CT in a severe COPD population. Significant associations with emphysema and airway wall area individually were found for SNPs in several other candidate genes. Different genetic determinants may influence airway remodelling and emphysema development. Further work will be required to confirm the genetic determinants of emphysema and airway disease.

SUPPORT STATEMENT

This manuscript is subject to the NIH Public Access Policy (<http://publicaccess.nih.gov/>). This study was funded by the following grants. NIH R01 HL075478, P01 HL083069, R01 HL084323, U01 HL089856, K23HL089353, and the Parker B. Francis Foundation. Funding was also provided by GlaxoSmithKline.

STATEMENT OF INTEREST

Statements of interest for E. Hoffman and E.K. Silverman, and for the study itself can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

ACKNOWLEDGEMENTS

The authors would like to sincerely thank the manuscript reviewers for helpful suggestions.

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