Hedgehog-interacting protein is a COPD susceptibility gene: the Rotterdam Study

Y.M.T.A. van Durme*,#,**, M. Eijgelsheim#,**, G.F. Joos*, A. Hofman#,¶,
A.G. Uitterlinden#,¶,+, G.G. Brusselle*,# and B.H.Ch. Stricker#,¶,+,§,ƒ

ABSTRACT: The Hedgehog signalling pathway plays an important role in lung morphogenesis and cellular responses to lung injury. A genome-wide association study has demonstrated that two single nucleotide polymorphisms (SNPs) near the Hedgehog-interacting protein (*Hip*) gene, SNP identifiers rs1828591 and rs13118928, are associated with risk of chronic obstructive pulmonary disease (COPD). The aim of the present study was to validate the observed association between genetic variation near the *Hip* gene and COPD, and to investigate whether risk estimates were modified by smoking behaviour.

The association between the *Hip* gene SNPs and COPD was investigated in the Rotterdam Study by logistic regression analyses, adjusted for several covariates. In addition, an association meta-analysis was performed that included data from the genome-wide association study on COPD.

Both SNPs were significantly associated with risk of COPD (OR 0.80; 95% CI 0.72–0.91). Homozygosity for the minor G allele resulted in a decreased risk of COPD of ~40% (95% CI 0.47–0.78). There was a significant interaction with the number of pack-years of smoking (p=0.004). The meta-analysis yielded an odds ratio for COPD of 0.80 per additional G allele (p=3.4×10^{-9}).

Genetic variation near the *Hip* gene was significantly associated with risk of COPD, depending on the number of pack-years of smoking.

KEYWORDS: Chronic obstructive pulmonary disease, epidemiology of chronic obstructive pulmonary disease, genetics, Hedgehog-interacting protein

Chronic obstructive pulmonary disease (COPD) is a major worldwide and still increasing health problem [1]. The disease is characterised by progressive airflow limitation, driven by an abnormal inflammatory response of the airways to inhaled particles and fumes [2, 3]. Smoking is the major aetiological factor for COPD in the Western world [1]. However, only 15–25% of cigarette smokers seem to develop the disease, suggesting the presence of susceptibility genes for COPD [4, 5]. Previous studies have demonstrated familial aggregation of COPD, and some twin studies on pulmonary function have indicated a genetic contribution to clinically relevant parameters, such as forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) [6–8].

The evolutionarily highly conserved Hedgehog (*Hh*) signalling pathway is crucial to several developmental processes, including lung organogenesis, and is also implicated in the response of the airway epithelium to smoking [9–11]. In cells that respond to *Hh*, two proteins are upregulated, *i.e.* the *Hh* receptor Patched protein (*Ptc*) and the vertebrate-specific *Hh*- interacting protein (*Hip*). Both play an important role in the negative feedback regulation of *Hh* signalling. Loss of *Hip* function in mouse has already been associated with specific lung defects and results in neonatal lethality [12]. As in mice, dysfunction of the *Hh* pathway during human fetal life is responsible for severe lung malformations [13–15]. In adult life, the *Hh* pathway plays a central role in the repair and regeneration of several tissues, and aberrant activation of this pathway by epithelial stem cells has been linked to various malignancies, including lung cancer [16, 17].

Two recent genome-wide association (GWA) studies unexpectedly demonstrated that two single nucleotide polymorphisms (SNPs) near the *Hip* locus (SNP identifiers rs13118928 and rs1828591) were associated with COPD and lung

---

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

function, respectively, although genome-wide significance levels were not reached for the COPD phenotype [18, 19].

The objective of the current study was to study the above-mentioned association between SNPs near the *Hip* gene and COPD, and to investigate whether smoking or sex modified this association, in a large prospective population-based cohort study with ~20 yrs of follow-up.

METHODS

Study population

The present study forms part of the Rotterdam Study, a population-based cohort study aimed at assessing the occurrence of and risk factors for chronic diseases in the elderly. The objectives and methods of the Rotterdam Study have been described elsewhere [20, 21]. In short, the Rotterdam Study cohort included 7,983 participants aged ⩾55 yrs living in Ommoord, a well-defined suburb of the city of Rotterdam (the Netherlands). Almost all (99.8%) of the participants are of Caucasian descent. Baseline data were collected during 1989–1993. Participants were visited at home at the start of the study for a standardised interview regarding their health status. Since the start of the Rotterdam Study, each participant has visited the research centre every 2–3 yrs, during which several measurements were performed. In addition, participants were continuously monitored for the onset of major events, which occurred during follow-up through automated linkage with files from general practitioners. Trained research assistants collected information from the medical records of the general practitioners, hospitals and nursing homes. The medical ethics committee of the Erasmus Medical Center (Rotterdam) approved the study. Participants gave written informed consent and permission to retrieve information from treating physicians.

Spirometry

Spirometry was performed by trained paramedical personnel using a SpiroPro® portable spirometer (Erich Jaeger, Hoechberg, Germany) according to American Thoracic Society (ATS)/ European Respiratory Society (ERS) guidelines [22]. FEV_1, FVC and FEV_1/FVC ratio were measured; the spirogram (volume–time curve) and maximal expiratory flow–volume curve were also recorded. Spirometry procedures that yielded results that did not meet ATS/ERS criteria for acceptability and reproducibility were classified as not interpretable [23].

Patient identification and validation

For the validation of the COPD cases, access was available to hospital discharge letters, files from general practitioners, spirometry reports and pharmacy dispensing data for patients participating in the Rotterdam Study, as previously described [23]. Spirometry was performed in 3,550 participants in the context of the Rotterdam cohort study. In addition, throughout the entire study period, spirometry was also performed on clinical indication by respiratory specialists and specialists in internal medicine with a subspeciality in respiratory medicine. In the absence of spirometry, all medical information on subjects who used respiratory medication for ⩾6 months (anatomical therapeutic chemical classification system codes R03AC, R03AK, R03BA, R03BB, R03CC and R03DA) [24] and all hospital discharge letters or mortality reports with a coded diagnosis of COPD (J40–J44 (International Statistical Classification of Diseases and Related Health Problems, 10th revision)) [25] were reviewed. Drug use was only used for potential case finding and not for making the diagnosis of COPD. In the case of confirmed COPD, the day of first drug use was used as an indicator of the date of onset of COPD.

The diagnosis of COPD was classified as definite or probable. Definite COPD was defined by moderate-to-severe obstructive spirometry results (FEV_1/FVC of <0.7 and FEV_1 of <80% of the predicted value) and/or as COPD diagnosed by a specialist in internal medicine (mainly respiratory physicians or those with a subspecialty in respiratory medicine) based upon the combined results of clinical history, physical examination and spirometry. Probable COPD was defined by mild obstructive spirometry results (FEV_1/FVC of <0.7 and FEV_1 of ⩾80% pred) and/or as COPD diagnosed by a physician in another medical speciality (*e.g.* a general practitioner).

The index date was defined as the date of diagnosis of COPD found in the medical reports, the date of a first prescription of COPD medication or the date of the obstructive lung function examination, whichever came first.

Genotyping

The Illumina Infinium II HumanHap550K BeadChip (version 3; Illumina, San Diego, CA, USA) was used for genotyping 6,449 participants with available DNA in the Rotterdam Study, as previously described [26]. Genotyping was performed at the Dept of Internal Medicine of Erasmus Medical Center using BeadStudio software (version 0.3.10.14; Illumina) for genotyping calling. Participants with a call rate of <97.5%, excess autosomal heterozygosity, sex mismatch or outlying identity-by-state clustering estimates were excluded. A total of 5,974 participants were successfully genotyped and passed quality control. Genotype information on two specific SNPs near the *Hip* gene located on chromosome 4 (rs13118928 and rs1828591) was extracted from these data.

Statistical analyses

The demographic and clinical characteristics of the study population are expressed as median with interquartile range (IQR) in the text.

First, the association of rs13118928 and rs1828591 with COPD was studied within the Rotterdam Study. All COPD cases, prevalent and incident, were used for analyses in order to obtain maximal power. Consequently, logistic regression analyses were performed because Cox's proportional hazards analysis was not possible with prevalent cases at baseline. Both SNPs were tested for Hardy–Weinberg equilibrium using Chi-squared statistics. A power calculation using Quanto (University of Southern California, Los Angeles, CA, USA), based on the combined odds ratio (OR) of 0.7 of the Bergen case–control cohort and the National Emphysema Treatment Trial (NETT)/ Normative Aging Study (NAS) population (combined β= -0.332) from the study of PILLAI *et al.* [18], showed that it was possible to demonstrate an OR for COPD of 0.7 with >99% power at a two-sided α of 0.05 [27]. Additive and genotype-based logistic regression models, adjusted for age at baseline, sex, smoking behaviour (ever- and never-smoker) and the total number of pack-years of smoking, were used to calculate ORs and 95% confidence intervals (CIs).

In the additive genetic model, the categorical variable of the number of variant alleles (homozygous wild type (0 variant alleles); heterozygous (1 variant allele); homozygous variant allele (2 variant alleles)) was included as a continuous value in the logistic regression model in order to calculate trends.

The following covariates were considered as potential confounders or effect modifiers: age, sex, smoking behaviour, and the total number of pack-years of smoking. Smoking status was self-reported and categorised as ever- or never-smoker. The category of ever-smoker included both current and former smokers. The number of pack-years was computed from the duration of smoking (in years) multiplied by the number of cigarettes smoked daily divided by 20. The number of pack-years of smoking of each study participant was calculated using prospectively collected data regarding the smoking behaviour of the cohort members, collected during the first, second and third follow-up visits of the Rotterdam Study.

Participants with asthma or a spirometry result suggestive of restrictive lung disease were excluded from the analyses. Interaction terms and subanalyses were introduced to explore potential effect modification by sex and tertiles of pack-years of smoking. A p-value below the conventional level of significance (p<0.05) was considered significant.

Secondly, an inverse-variance-weighted meta-analysis was performed. This analysis combined the results obtained to the recently published data of PILLAI et al. [18]. The regression coefficients and SEM were calculated on the basis of the reported ORs and p-values from the Bergen case–control cohort and NETT/NAS data from the recent GWA study of PILLAI et al. [18].

The results of the association meta-analysis were calculated using Excel version 2007 (Microsoft Office). All other statistical analyses were performed using SPSS for Windows version 15 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics of the study population
Within the Rotterdam Study source population of 7,983 subjects, there were 928 patients with validated prevalent or incident COPD [23]. Both SNPs, rs13118928 and rs1828591, were successfully genotyped in 5,974 out of the 7,983 participants for whom DNA was available, including 742 COPD cases. A total of 256 study participants with asthma or a spirometry result suggestive of restrictive pulmonary disease were excluded (fig. 1). Overall, the median age of the study population at the start of the study was 68.4 yrs (IQR 14 yrs), and 59% were female. Additional clinical characteristics are shown in table 1.

Association between variation near the Hip gene and COPD in the Rotterdam Study
Both SNPs studied, rs13118928 and rs1828591, were in Hardy–Weinberg equilibrium (rs13118928: $\chi^2=1.413$; p=0.235; rs1828591: $\chi^2=1.215$; p=0.270) and in perfect linkage disequilibrium ($r^2=1.00$ in HapMap CEU reference samples (Utah residents of Northern and Western European descent)). Owing to the high linkage between the two SNPs, analyses yielded the same results and thus, for reasons of simplicity, only the results for the rs13118928 SNP are shown in the text and tables.

Under an additive genetic model, there was a significant association between both SNPs and the risk of COPD with an OR of 0.80 per additional guanine (G) allele (95% CI 0.72–0.91; p<0.001). Genotypic analysis showed that homozygosity for the minor G allele was associated with a significantly decreased risk of COPD of ~40% (OR 0.60; 95% CI 0.47–0.78; p<0.001) (table 2). The decrease in risk remained significant when the analyses were restricted to those participants with an available spirometric examination during follow-up in the Rotterdam Study (OR 0.60; 95% CI 0.43–0.89; p=0.011) (data not shown).

There was no interaction with sex (p=0.872), but a significant interaction effect was present with the total number of pack-years of smoking (p=0.004). Additional subanalyses demonstrated that the protective effect of having one or more variant alleles was most pronounced for smoking participants in the highest tertile of pack-years of smoking (>36 pack-yrs), with an OR of 0.80 (95% CI 0.62–0.93; p=0.007) versus an OR of 0.90 (95% CI 0.67–1.09; p=0.209) in those who had never smoked (table 3).

Association between variation near the Hip gene and COPD: meta-analysis with published results
The original p-value, including the Bergen case–control cohort, the International COPD Genetics Network (ICGN) population and the NETT/NAS data, was 1.7×10^{-7}. Since it was not possible to obtain β estimates and SEM for the ICGN population, the p-value for the combination of the Bergen and NETT/NAS data was also calculated. This resulted in a p-value of 1.0×10^{-6}. The combination of the Rotterdam Study results with the Bergen and NETT/NAS results yielded an overall OR of 0.80 per additional G allele (95% CI 0.70–0.84), with a p-value of 3.4×10^{-9}, which is significant at a genome-wide level (table 4; fig. 2).
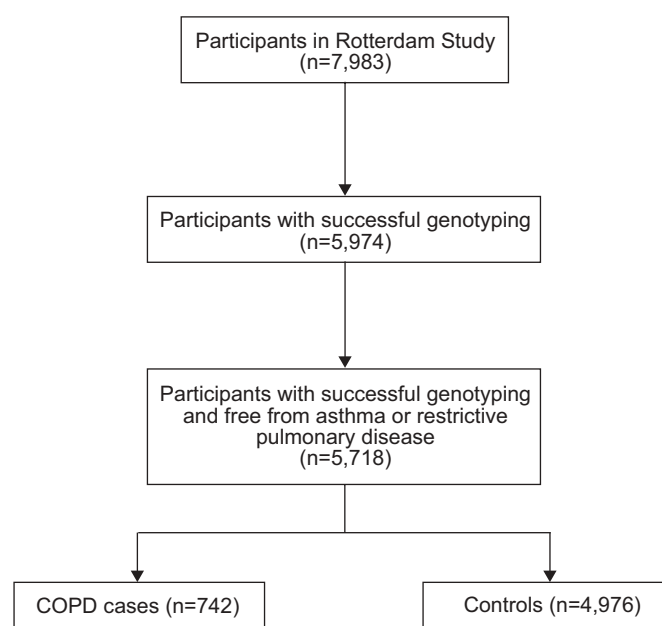

FIGURE 1. Study design. COPD: chronic obstructive pulmonary disease.

TABLE 1	Baseline characteristics of the Rotterdam Study population		
	Total	**Cases**	**Controls**
Subjects n	5718	742	4976
Age yrs	68.4 (14)	67.0 (10)	68.8 (14)
Females	3397 (59)	315 (43)	3082 (62)
Smoking status			
Current smoker	1290 (23)	300 (40)	990 (20)
Former smoker	2311 (40)	325 (44)	1986 (40)
Never-smoker	1960 (34)	111 (15)	1849 (37)
Missing data	157 (3)	6 (1)	151 (3)
Smoking history pack-yrs	9.0 (30)	27.8 (32)	6.8 (28)
rs13118928			
AA	2099 (37)	294 (40)	1805 (36)
AG	2698 (47)	359 (49)	2339 (47)
GG	921 (16)	89 (12)	832 (17)
rs1828591			
AA	2092 (37)	293 (40)	1799 (36)
AG	2703 (47)	360 (49)	2343 (47)
GG	923 (16)	89 (12)	834 (17)

Data are presented as n (%), except for age and smoking history pack-yrs which are presented as median (interquartile range), unless otherwise indicated. A: adenine; G: guanine.

DISCUSSION

The results of the present population-based prospective cohort study confirm the recently observed association between genetic variation around the *Hip* gene and COPD, as described by PILLAI *et al.* [18]. It was found that the minor G allele of the rs13118928 and rs1828591 SNPs is associated with a significantly decreased risk of COPD. The risk seemed to be dependent upon the smoking behaviour of the participants, since the decrease in risk was strongest in subjects who had smoked for >30 pack-yrs at the start of the Rotterdam Study.

The *Hh* signalling pathway is an evolutionarily highly conserved signalling cascade that is crucial to several developmental processes [10, 11]. In mammals, there are three *Hh* proteins: Sonic, Indian, and Desert. Sonic *Hh* specifically targets the lung and is expressed in the lung epithelium during embryogenesis [28]. Signalling is initiated by binding of one of the *Hh* proteins to the 12-pass transmembrane protein receptor *Ptc*, which is required to inhibit the activity of Smoothened (*Smo*), a seven-pass transmembrane protein, which is responsible for regulation of the zinc-finger-containing glioma-associated oncogene homologue (*Gli*) transcriptional effectors, *Gli1*, *Gli2* and *Gli3* [13, 29]. Upon binding of *Hh* to *Ptc*, the *Ptc*-mediated inhibition of *Smo* is relieved [30, 31]. This triggers a cascade that finally results in activation of the *Gli* family and transcription of *Hh* target genes, including *Ptc* and *Gli* itself [32]. The availability of the *Hh* ligands is regulated by expression of *Hip*. The *Hip* protein was initially discovered by screening murine cDNA expression for proteins that bound to Sonic *Hh*. The *Hip* gene, located on chromosome 4 (4q31.21–4q31.3), encodes a transmembrane glycoprotein that binds all three *Hh* proteins with an affinity equal to that of *Ptc* and is upregulated upon *Hh* signalling [28]. *Hip* is expressed in

TABLE 2	Association of the rs13118928 single nucleotide polymorphism with chronic obstructive pulmonary disease in the Rotterdam Study		
Model	**Patients n/N**	**OR (95% CI)**	**p-value**
Additive	742/5718	**0.80 (0.72–0.91)**	2.0×10^{-4}
Genotype-based			
AA	294/2099	1.00 (reference)	
AG	359/2698	0.90 (0.75–1.05)	0.169
GG	89/921	**0.60 (0.47–0.78)**	1.0×10^{-4}

Additive and genotypic logistic regression models, adjusted for age, sex, smoking and the number of pack-years of smoking, were used for the analyses. N: number of subjects in cohort; A: adenine; G: guanine.

all *Hh* target tissues and acts by internalising and degrading the *Hh* protein. Similar to *Ptc*, *Hip* thus serves as a natural antagonist of the *Hh* pathway.

In this way, alterations in the *Hip* gene can result in altered gene expression of a functional protein, or in a nonfunctional protein and upregulation of the *Hh* signalling pathway.

Loss of *Hip* function in mice during fetal life has been associated with specific lung defects resulting in respiratory failure and neonatal lethality [12]. In adult animals, downregulation of *Hip* has been reported in murine lung cancer cell lines [33]. As in rodents, dysfunction of the *Hh* pathway during fetal life in humans is responsible for severe lung malformations [13–15]. In adult life, the *Hh* pathway plays a central role in the repair and regeneration of several human tissues, and aberrant activation of this pathway by epithelial stem cells has been linked to various malignancies, including lung cancer [16, 17]. In addition, *Hip* seemed to be downregulated in human nonsmall cell lung cancer tissues and endothelial cells during angiogenesis [33].

TABLE 3	Association of the rs13118928 single nucleotide polymorphism[#] with chronic obstructive pulmonary disease in the Rotterdam Study, stratified by smoking history		
Smoking history pack-yrs[¶]	**Patients n/N**	**OR (95% CI)**	**p-value**
0	111/1960	0.90 (0.67–1.09)	0.209
>0–≤15	114/1129	0.80 (0.61–1.08)	0.154
>15–≤36	203/1082	0.90 (0.70–1.09)	0.224
>36	277/1095	**0.80 (0.62–0.93)**	**0.007**

Additive logistic regression models, adjusted for age and sex, were used for the analyses. The number of pack-years of smoking was calculated based on information regarding the smoking behaviour of each study participant, collected prospectively at each interview during the three Rotterdam Study follow-up visits. N: number of subjects in cohort. [#]: near the Hedgehog-interacting protein gene; [¶]: categories based on the 33rd percentile of the data in smokers.

TABLE 4	Association of the rs13118928 single nucleotide polymorphism[#] with chronic obstructive pulmonary disease: results of the meta-analysis	
Sample	**OR (95% CI)**	**p-value**
Rotterdam Study	0.81 (0.72–0.91)	<0.001
Bergen cohort[¶]	0.73 (0.62–0.86)	<0.001
NETT/NAS[¶]	0.71 (0.56–0.88)	0.002
Overall	**0.77 (0.70–0.84)**	3.43×10^{-9}

Logistic regression models, adjusted for age, sex, smoking behaviour and the number of pack-years of smoking, were used for the analyses. NETT: National Emphysema Treatment Trial; NAS: Normative Aging Study. [#]: near the Hedgehog-interacting protein gene; [¶]: data from [18].

FIGURE 2. Forest plot demonstrating the odds ratios (ORs) for the association between the rs13118928 single nucleotide polymorphism near the Hedgehog-interacting protein gene with chronic obstructive pulmonary disease in the meta-analysis. Data are presented as adjusted ORs (■ (size reflects weighting)), using a fixed-effects model, and 95% confidence interval (horizontal bars). The centre of the diamond indicates the combined mean effect of the studies (OR 0.77) and its extremities the 95% confidence interval (0.70–0.84) (······: line of no effect). NETT: National Emphysema Treatment Trial; NAS: Normative Aging Study.

The results of the present study and the two previous GWA reports demonstrate that variants near the *Hip* gene influence the risk of COPD. Although it is not clear which regulatory effect explains this association, several hypotheses are possible. First, MADISON *et al.* [34] demonstrated that ectopic expression of *Hip* in mice during fetal life was associated with expanded smooth muscle in the intestinal epithelium [31]. If the same effect were present in the small airways, then it could be speculated that changes in *Hip* influence the thickness of the airway muscle layer and induce more or less airflow limitation. Secondly, a study of POGACH *et al.* [35] demonstrated that several developmental pathways in the lungs of adult mice, including *Hh*, reactivate for tissue repair following hyperoxia. In addition, others have shown that embryogenic signalling pathways, such as *Hh*, are activated in human bronchial epithelial cells exposed to cigarette smoke [36].

The prevalence, morbidity and mortality of COPD in females are increasing [37]. In addition, some reports suggest that females may be at greater risk of developing severe early-onset COPD due to sex differences in the metabolism of cigarette smoke [38]. However, the results of these studies remain controversial since differences in lung development, smoking behaviour, diagnosis and treatment of COPD are possible confounders. Since sex can be of interest in the pathogenesis of COPD, a potential interaction effect of sex on the observed association between variation near the *Hip* gene and COPD was tested for. However, no significant influence was found (p=0.872).

The results of these two studies raise the possibility that the *Hh* pathway is involved in the response of the airway epithelium to tissue injury, and that alterations in *Hh* signalling can lead to susceptibility to COPD, depending upon the severity of the smoking behaviour. Thirdly, since a recent GWA study investigating genetic variants that influence adult height identified several relevant genes in the *Hh* signalling pathway (Indian *Hh*, *Hip* and *Ptc*), height could be considered a potential confounder for the observed association between *Hip* and COPD [39]. However, additional analyses including height as a covariate did not influence the present estimates (data not shown).

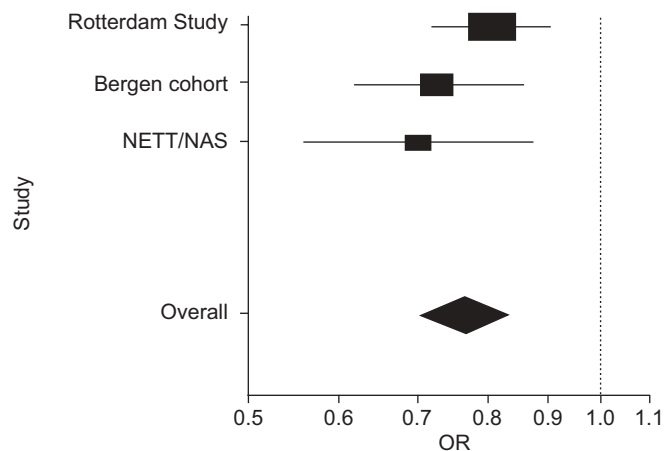The strengths of the present study are the high-quality information available regarding exposures prior to outcome, with prospective data collection, the general population-based setting, the large number of subjects that participated in the Rotterdam Study and the long duration of follow-up. The high response rate and virtually complete follow-up for each participant makes information and selection bias unlikely for these data. A power analysis showed that the present study was of sufficient size to distinguish between the proposed effect and no effect. The study was conducted in a data set independent from the original GWA study.

A similar significant protective risk was demonstrated with the same SNPs, and the combined analysis led to a smaller p-value than in the original report, below the stringent threshold frequently used to define genome-wide significance. Hence the present study satisfies all criteria suggested by the National Cancer Institute/National Human Genome Research Institute Working Group on Replication in Association Studies for establishing positive replication [40].

As for all other association studies, no conclusions regarding causality or the mechanism through which the associated SNPs influence COPD risk can be formed. It is possible that the two SNPs studied are not the causal variants, but are in linkage disequilibrium with other polymorphisms that are responsible for the increased COPD risk. Since the SNPs are not situated within the *Hip* gene and do not seem to exhibit a strong linkage with other SNPs in the *Hip* gene itself, a regulatory function is possible. Additional functional and translational research examining the role of *Hip* in COPD is certainly warranted.

In conclusion, the present study confirms the previously reported association between two SNPs near the *Hip* gene and COPD in a Caucasian population-based cohort study. In addition, a potential gene-by-environment interaction was identified, since the association was most apparent in heavily smoking participants.

STATEMENT OF INTEREST

A statement of interest for G.G. Brusselle can be found at www.erj.ersjournals.com/misc/statements.dtl

REFERENCES

1 Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. *Lancet* 2007; 370: 765–773.

2 Pauwels RA, Rabe KF. Burden and clinical features of chronic obstructive pulmonary disease (COPD). *Lancet* 2004; 364: 613–620.

3 Rabe KF, Beghe B, Luppi F, *et al.* Update in chronic obstructive pulmonary disease 2006. *Am J Respir Crit Care Med* 2007; 175: 1222–1232.

4 Lokke A, Lange P, Scharling H, *et al.* Developing COPD: a 25 year follow up study of the general population. *Thorax* 2006; 61: 935–939.

5 Silverman EK. Progress in chronic obstructive pulmonary disease genetics. *Proc Am Thorac Soc* 2006; 3: 405–408.

6 McCloskey SC, Patel BD, Hinchliffe SJ, *et al.* Siblings of patients with severe chronic obstructive pulmonary disease have a significant risk of airflow obstruction. *Am J Respir Crit Care Med* 2001; 164: 1419–1424.

7 Silverman EK, Chapman HA, Drazen JM, *et al.* Genetic epidemiology of severe, early-onset chronic obstructive pulmonary disease. Risk to relatives for airflow obstruction and chronic bronchitis. *Am J Respir Crit Care Med* 1998; 157: 1770–1778.

8 Chen Y. Genetics and pulmonary medicine.10: Genetic epidemiology of pulmonary function. *Thorax* 1999; 54: 818–824.

9 Chen MH, Wilson CW, Chuang PT. SnapShot: hedgehog signaling pathway. *Cell* 2007; 130: 386.

10 van Tuyl M, Post M. From fruitflies to mammals: mechanisms of signalling *via* the Sonic hedgehog pathway in lung development. *Respir Res* 2000; 1: 30–35.

11 Warburton D, Bellusci S, De Langhe S, *et al.* Molecular mechanisms of early lung specification and branching morphogenesis. *Pediatr Res* 2005; 57: 26R–37R.

12 Chuang PT, Kawcak T, McMahon AP. Feedback control of mammalian Hedgehog signaling by the Hedgehog-binding protein, Hip1, modulates Fgf signaling during branching morphogenesis of the lung. *Genes Dev* 2003; 17: 342–347.

13 Oldak M, Grzela T, Lazarczyk M, *et al.* Clinical aspects of disrupted Hedgehog signaling. *Int J Mol Med* 2001; 8: 445–452.

14 Villavicencio EH, Walterhouse DO, Iannaccone PM. The Sonic hedgehog-Patched-Gli pathway in human development and disease. *Am J Hum Genet* 2000; 67: 1047–1054.

15 Whitsett JA, Wert SE, Trapnell BC. Genetic disorders influencing lung formation and function at birth. *Hum Mol Genet* 2004; 13: R207–R215.

16 Rubin LL, de Sauvage FJ. Targeting the Hedgehog pathway in cancer. *Nat Rev Drug Discov* 2006; 5: 1026–1033.

17 Watkins DN, Berman DM, Burkholder SG, *et al.* Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature* 2003; 422: 313–317.

18 Pillai SG, Ge D, Zhu G, *et al.* A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet* 2009; 5: e1000421.

19 Wilk JB, Chen TH, Gottlieb DJ, *et al.* A genome-wide association study of pulmonary function measures in the Framingham Heart Study. *PLoS Genet* 2009; 5: e1000429.

20 Hofman A, Grobbee DE, Dejong PTVM, *et al.* Determinants of disease and disability in the elderly – the Rotterdam Elderly Study. *Eur J Epidemiol* 1991; 7: 403–422.

21 Hofman A, Breteler MM, van Duijn CM, *et al.* The Rotterdam Study: 2010 objectives and design update. *Eur J Epidemiol* 2009; 24: 553–572.

22 Celli BR, MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* 2004; 23: 932–946.

23 van Durme YM, Verhamme KM, Stijnen T, *et al.* Prevalence, incidence, and lifetime risk for the development of COPD in the elderly: the Rotterdam Study. *Chest* 2009; 135: 368–377.

24 World Health Organization, Anatomical Therapeutic Chemical Classification System. Geneva, World Health Organization, 1996.

25 World Health Organization, International Statistical Classification of Diseases and Related Health Problems, 10th revision. Geneva, World Health Organization, 1992.

26 Newton-Cheh C, Eijgelsheim M, Rice KM, *et al.* Common variants at ten loci influence QT interval duration in the QTGEN Study. *Nat Genet* 2009; 41: 399–406.

27 Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 2003; 19: 149–150.

28 Chuang PT, McMahon AP. Vertebrate Hedgehog signalling modulated by induction of a Hedgehog-binding protein. *Nature* 1999; 397: 617–621.

29 McMahon AP. More surprises in the Hedgehog signaling pathway. *Cell* 2000; 100: 185–188.

30 Ingham PW. Hedgehog signalling. *Curr Biol* 2008; 18: R238–R241.

31 Parkin CA, Ingham PW. The adventures of Sonic hedgehog in development and repair. I. Hedgehog signaling in gastrointestinal development and disease. *Am J Physiol Gastrointest Liver Physiol* 2008; 294: G363–G367.

32 Bak M, Hansen C, Friis HK, *et al.* The human hedgehog-interacting protein gene: structure and chromosome mapping to 4q31.21→q31.3. *Cytogenet Cell Genet* 2001; 92: 300–303.

33 Olsen CL, Hsu PP, Glienke J, *et al.* Hedgehog-interacting protein is highly expressed in endothelial cells but down-regulated during angiogenesis and in several human tumors. *BMC Cancer* 2004; 4: 43.

34 Madison BB, Braunstein K, Kuizon E, *et al.* Epithelial hedgehog signals pattern the intestinal crypt–villus axis. *Development* 2005; 132: 279–289.

35 Pogach MS, Cao Y, Millien G, *et al.* Key developmental regulators change during hyperoxia-induced injury and recovery in adult mouse lung. *J Cell Biochem* 2007; 100: 1415–1429.

36 Lemjabbar-Alaoui H, Dasari V, Sidhu SS, *et al.* Wnt and Hedgehog are critical mediators of cigarette smoke-induced lung cancer. *PLoS One* 2006; 1: e93.

37 Han MK, Postma D, Mannino DM, *et al.* Gender and chronic obstructive pulmonary disease: why it matters. *Am J Respir Crit Care Med* 2007; 176: 1179–1184.

38 Prescott E, Bjerg AM, Andersen PK, *et al.* Gender difference in smoking effects on lung function and risk of hospitalization for COPD: results from a Danish longitudinal population study. *Eur Respir J* 1997; 10: 822–827.

39 Weedon MN, Lango H, Lindgren CM, *et al.* Genome-wide association analysis identifies 20 loci that influence adult height. *Nat Genet* 2008; 40: 575–583.

40 Chanock SJ, Manolio T, Boehnke M, *et al.* Replicating genotype–phenotype associations. *Nature* 2007; 447: 655–660.