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NFE2L2 pathway polymorphisms and lung function decline in chronic obstructive pulmonary disease

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Sandford AJ, Malhotra D, Boezen HM, Siedlinski M, Postma DS, Wong V, Akhabir L, He JQ, Connett JE, Anthonisen NR, Paré PD, Biswal S. *NFE2L2* pathway polymorphisms and lung function decline in chronic obstructive pulmonary disease. *Physiol Genomics* 44: 754–763, 2012. First published June 12, 2012; doi:10.1152/physiolgenomics.00027.2012.—An oxidant-antioxidant imbalance in the lung contributes to the development of chronic obstructive pulmonary disease (COPD) that is caused by a complex interaction of genetic and environmental risk factors. Nuclear erythroid 2-related factor 2 (NFE2L2 or NRF2) is a critical molecule in the lung's defense mechanism against oxidants. We investigated whether polymorphisms in the NFE2L2 pathway affected the rate of decline of lung function in smokers from the Lung Health Study (LHS)(n = 547) and in a replication set, the Vlagtwedde-Vlaardingen cohort (n = 533). We selected polymorphisms in *NFE2L2* in genes that positively or negatively regulate *NFE2L2* transcriptional activity and in genes that are regulated by *NFE2L2*. Polymorphisms in 11 genes were significantly associated with rate of lung function decline in the LHS. One of these polymorphisms, rs11085735 in the *KEAP1* gene, was previously shown to be associated with the level of lung function in the Vlagtwedde-Vlaardingen cohort but not with decline of lung function. Of the 23 associated polymorphisms in the LHS, only rs634534 in the *FOSL1* gene showed a significant association in the Vlagtwedde-Vlaardingen cohort with rate of lung function decline, but the direction of the association was not consistent with that in the LHS. In summary, despite finding several nominally significant polymorphisms in the LHS, none of these associations were replicated in the Vlagtwedde-Vlaardingen cohort, indicating lack of effect of polymorphisms in the NFE2L2 pathway on the rate of decline of lung function.

genetic polymorphism; nuclear erythroid 2-related factor 2; forced expiratory volume in one second

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is the result of a complex interaction of genetic and environmental risk factors (51) and is characterized by irreversible airflow obstruction that results from chronic inflammation and tissue remodeling. Although the main environmental risk factor for COPD is cigarette smoking, longitudinal studies show that only a minority of long-term cigarette smokers develops airflow limitation (15), suggesting that additional environmental and/or genetic factors are important. Family and twin studies have demonstrated that genetic factors play a key role in the etiology of COPD (41, 49). Furthermore, genome-wide association studies of lung function (19, 46, 50, 58, 63), COPD (8, 47), and emphysema (32) have identified several putative loci underlying these traits.

Several lines of evidence suggest that oxidant-antioxidant imbalance in the lung plays a major role in the pathogenesis of COPD. A measure of oxidative stress in the blood (thiobarbituric acid-reactive substances) was shown to correlate inversely with lung function in a population study (53). In addition, reactive oxygen species released by circulating neutrophils play a role in the development of airflow limitation (38). Furthermore, antioxidant nutrients have been associated with preservation of lung function (28, 42).

Nuclear erythroid 2-related factor 2 (NFE2L2 or NRF2) is a basic leucine zipper transcription factor that upregulates multiple genes involved in antioxidant and detoxification pathways in response to exposure of the lungs to cigarette smoke (48). Disruption of the *Nfe2l2* gene in an emphysema-resistant mouse model resulted in an early-onset and severe cigarette smoke-induced emphysema, suggesting that NFE2L2 is a critical molecule in the lung's defense mechanism against oxidants (48). Oxidative stress causes NFE2L2 to translocate to the nucleus following dissociation from its cytosolic inhibitor, KEAP1 (30). We have shown (39) that the protein levels of NFE2L2 and DJ1 (PARK7), a stabilizer of NFE2L2 (9), are decreased in the lungs of patients with COPD. These data indicate that NFE2L2 plays an important protective role against cigarette smoke-induced COPD.

A previous study (66) of four promoter polymorphisms in the *NFE2L2* gene did not demonstrate any associations with COPD in the Japanese population. In contrast, an *NFE2L2* polymorphism (rs2364723) in intron 2 of the gene was associated with level of lung function, although not with its rate of decline, in a European population (54). Most recently, another variant (rs6726395) in intron 2 of the *NFE2L2* gene was associated with rate of decline of lung function in the Japanese

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Table 1. *Distribution of demographic characteristics for subjects in the LHS*

	Nondecliners (n = 285)	Fast Decliners (n = 262)	P Value
Men/Women	186/99	152/110	0.0942
Age, yr	47.7 ± 6.9	49.8 ± 6.3	0.0002
Smoking history, pack-years*	38.5 ± 18.3	43.2 ± 19.4	0.0038
ΔFEV1/yr, % predicted pre†	1.1 ± 0.7	−4.2 ± 1.1	<0.0001
ΔFEV1/yr, % predicted post‡	0.7 ± 0.9	−3.4 ± 1.3	<0.0001
Baseline FEV_1, % predicted pre§	75.5 ± 8.1	72.5 ± 9.0	<0.0001
Baseline FEV_1, % predicted post‖	79.7 ± 7.9	74.7 ± 9.2	<0.0001

Values are means ± SD for continuous data. FEV1, forced expiratory volume in 1 s. *Number of packs of cigarettes smoked per day/number of years smoking. †Change in lung function over a 5 yr period per year as % predicted FEV_1 prebronchodilator. ‡Change in lung function over a 5 yr period per year as % predicted FEV_1 postbronchodilator (3 missing values in fast decliners group and 4 missing values in nondecliners group). §Lung function at the start of the Lung Health Study (LHS) as measured by FEV_1(%) predicted prebronchodilator. ‖Lung function at the start of the LHS as measured by FEV_1(%) predicted postbronchodilator.

population and showed a significant interaction with smoking status (40).

Based on these observations, we hypothesized that the rate of decline of lung function in smokers with mild to moderate airflow obstruction from the Lung Health Study (LHS) (1) would be influenced by polymorphisms in the NFE2L2 pathway. The LHS was a randomized trial of an antismoking intervention and bronchodilator treatment in volunteer smokers (1). We selected polymorphisms in the *NFE2L2* gene in genes that positively or negatively regulate the expression of *NFE2L2* and in genes that are regulated by *NFE2L2*. We sought to determine whether these polymorphisms are associated with decline of lung function in smokers in the LHS and in a replication set, the Vlagtwedde-Vlaardingen cohort.

MATERIALS AND METHODS

Study participants. The analyses were performed in a nested case-control design that included participants from the LHS, a clinical trial sponsored by the National Heart, Lung, and Blood Institute (1). The LHS was conducted at 10 medical centers in North America, and a total of 5,887 smokers, aged 35–60 yr, with spirometric evidence of mild to moderate lung function impairment were recruited (1). Lung function was assessed as forced expiratory volume in 1 s (FEV_1) % of predicted, i.e., FEV_1 adjusted for age, height, sex, and race. Lung function measurements in the LHS were performed using standardized spirometry in accordance with the American Thoracic Society guidelines (14), and the reference equations were those of Crapo and coworkers (10) based on Caucasian subjects of northern European descent in Salt Lake City.

Only participants who self-reported as non-Hispanic white were investigated in this study. Participants of other ethnic groups such as Hispanic white, African American, and Asian accounted for <5% of the total LHS cohort and were excluded to avoid potential problems due to population admixture.

Based on the rate of decline of lung function during a 5 yr follow-up period, of the 3,216 continuing smokers in this study, we selected non-Hispanic whites with a fast decline of FEV_1 (n = 262) and with no decline of FEV_1 (n = 285). Arbitrary cut-off points of FEV_1% predicted/year decrease ≥3.0% and increase ≥0.4% were used for rapid decliners and nondecliners, respectively. The demographic characteristics of the participants are shown in Table 1.

The Vlagtwedde-Vlaardingen cohort was utilized as an independent replication cohort (54). This cohort contains 1,390 subjects with 8,159 FEV_1 measurements completed during eight surveys who were prospectively followed for 25 yr with FEV_1 measurements performed every 3 yr (following European Respiratory Society guidelines) (60). Based on the rate of decline of lung function during this follow-up period, we selected smokers (smoking history > 5 pack-yr) with a fast decline of FEV_1 (n = 233) and with no decline of FEV_1 (n = 300). Arbitrary cut off points of FEV_1% predicted/year decrease >0% and increase >7.4% were used for rapid decliners and nondecliners, respectively. The characteristics of these subjects are shown in Table 2.

Informed consent was obtained from all participants, and this investigation received the approval of the relevant Research Ethics Boards.

Gene/polymorphism selection and genotyping. We selected genes involved in upregulation of *NFE2L2* (*APEX1*, *BRCA1*, *CARM1*, *CREBBP*, *DPP3*, *EP300*, *JUN*, *KAT2B*, *NCOA3*, *PARK7*, *PPARG*, *PRMT1*, and *SQSTM1*) and downregulation of *NFE2L2* (*ATF3*, *BACH1*, *BACH2*, *FOS*, *FOSL1*, *GNA12*, *KEAP1*, *MAF*, *MAFK*, and *TP53*). In addition, we selected genes known to be regulated by NFE2L2 (*GPX2*, *GSR*, and *SRXN1*). We also genotyped single nucleotide polymorphisms (SNPs) in three genes: *NFE2L2*; *NFE2L1*, a member of NFE2L family shown to act as a repressor of NFE2L2; and *NFE2L3*, a member of NFE2L family with high homology to NFE2L2. Finally, we selected a novel inflammatory gene (*IRG1*) as it was the most highly upregulated gene in the lungs of mice with a deletion of *Nfe2l2* after lipopolysaccharide (LPS) treatment (59).

Table 2. *Distribution of demographic characteristics for subjects in the Vlagtwedde-Vlaardingen cohort*

	Nondecliners (n = 300)	Fast Decliners (n = 233)	P Value
Men/Women	215/85	162/71	0.590
Age, yr	49.7 ± 9.6	53.05 ± 9.7	<0.0001
Smoking history, pack-years*	23.5 ± 16.8	29.4 ± 19.0	<0.0001
ΔFEV1/yr, % predicted pre†	0.9 ± 0.7	−0.5 ± 0.5	<0.0001
ΔFEV1/yr, % predicted post‡	NA	NA	
Baseline FEV_1, % predicted pre§	96.2 ± 15.1	100.6 ± 14.7	0.001
Baseline FEV_1, % predicted post‖	NA	NA	

Values are means ± SD for continuous data. *Number of packs of cigarettes smoked per day/number of years smoking. †Change in lung function over the total period someone was in the study per year as % predicted FEV_1 prebronchodilator. ‡Change in lung function over the total period someone was in the study per year as % predicted FEV_1 postbronchodilator. §Lung function at the start of the Vlagtwedde/Vlaardingen as measured by FEV_1(%) predicted prebronchodilator. ‖Lung function at the start of the Vlagtwedde/Vlaardingen as measured by FEV_1(%) predicted postbronchodilator.

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Table 3. *Nominally significant associations of polymorphisms with rate of decline of lung function in the LHS cohort*

			Genotype Counts		Unadjusted Analysis		Adjusted Analysis‡		
SNP	Gene	Genotype	Nondecliners	Fast Decliners	*P* Value*	Permuted *P* Value†	Odds Ratio	95% Confidence Interval	*P* Value
rs9573956	IRG1	AA	6	0	0.0006	0.0009	0.443	0.267–0.735	0.0016
		AG	48	25					
		GG	231	237					
rs3092794	NCOA3	AA	61	41	0.0351	0.0318	0.683	0.525–0.888	0.0044
		AG	153	130					
		GG	71	89					
rs6125042	NCOA3	CC	5	8	0.0517	0.0555	1.646	1.143–2.371	0.0074
		TC	53	68					
		TT	227	184					
rs9565305	IRG1	GG	5	0	0.0043	0.0030	0.522	0.325–0.840	0.0074
		TG	51	31					
		TT	229	231					
rs11085735	KEAP1	GG	261	223	0.0292	0.0496	2.043	1.206–3.461	0.0079
		TG	23	34					
		TT	1	5					
rs17708487	BACH2	AA	168	134	0.1657	0.1628	1.478	1.102–1.983	0.0091
		AG	99	108					
		GG	16	19					
rs8176199	BRCA1	AA	170	140	0.0348	0.0342	1.513	1.097–2.088	0.0116
		AC	82	93					
		CC	8	17					
rs634534	FOSL1	AA	59	45	0.0861	0.0872	0.733	0.567–0.948	0.0179
		AG	145	121					
		GG	79	96					
rs16882297	BACH2	CC	262	226	0.0369	0.0345	1.941	1.105–3.407	0.0209
		GC	23	34					
		GG	0	2					
rs5758223	EP300	AA	160	129	0.0648	0.0610	1.366	1.039–1.796	0.0255
		AG	107	103					
		GG	18	30					
rs4722029	GNA12	CC	10	14	0.0526	0.0555	1.427	1.043–1.952	0.0262
		TC	77	92					
		TT	197	156					
rs20552	EP300	AA	123	104	0.0634	0.0633	1.333	1.033–1.720	0.0273
		TA	130	110					
		TT	32	48					
rs1915919	PCAF	CC	114	77	0.0281	0.0295	1.338	1.030–1.739	0.0292
		TC	133	139					
		TT	38	46					
rs176713	BACH2	AA	230	192	0.1166	0.1238	1.549	1.042–2.303	0.0305
		AG	53	67					
		GG	2	3					
rs6808352	PCAF	GG	17	33	0.0242	0.0221	1.358	1.029–1.792	0.0307
		TG	129	114					
		TT	139	115					
rs427967	NCOA3	CC	174	184	0.0777	0.0819	0.700	0.504–0.971	0.0325
		TC	100	70					
		TT	11	8					
rs9344981	BACH2	CC	108	83	0.1763	0.1780	1.332	1.023–1.736	0.0334
		TC	140	133					
		TT	37	46					
rs4951627	ATF3	CC	16	7	0.0544	0.0552	0.708	0.514–0.976	0.0349
		CG	92	70					
		GG	177	185					
rs10183914	NFE2L2	CC	115	121	0.3724	0.3840	0.749	0.571–0.982	0.0365
		TC	134	113					
		TT	36	28					
rs9565304	IRG1	AA	215	215	0.0608	0.0625	0.656	0.442–0.974	0.0365
		AG	62	45					
		GG	8	2					
rs3846991	GNA12	CC	31	37	0.1178	0.1220	1.315	1.013–1.707	0.0394
		CG	120	124					
		GG	134	101					

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Table 3.—*Continued*

SNP	Gene	Genotype	Genotype Counts		Unadjusted Analysis		Adjusted Analysis‡		
			Nondecliners	Fast Decliners	*P* Value*	Permuted *P* Value†	Odds Ratio	95% Confidence Interval	*P* Value
rs831172	IRG1	AA	93	99	0.2760	0.2760	0.760	0.584–0.990	0.0422
		AG	141	126					
		GG	51	36					
rs2143491	NCOA3	CC	115	127	0.1535	0.1520	0.758	0.579–0.993	0.0444
		TC	134	110					
		TT	35	25					

The odds ratios are for a rapid rate of decline and the reference is the wild-type homozygote genotype. SNP, single nucleotide polymorphism. *Likelihood ratio χ^2-test. †*P* value using 10,000 random permutations. ‡Association under an additive genetic model adjusted for age, sex, pack-years of smoking, and recruitment center.

Tag SNPs and singletons that represent the genetic variation in each gene were selected from resequencing data in the European American Descent populations of the SeattleSNPs Program for Genomic Applications (http://pga.mbt.washington.edu/) or HapMap Project (http://hapmap.ncbi.nlm.nih.gov/) using the LDselect program (4). LDselect parameter thresholds of $r^2 > 0.8$ and minor allele frequencies >5% were used.

Genotyping of the LHS cohort was performed at the McGill University and Génome Québec Innovation Centre (Montreal, Québec, Canada) using Illumina GoldenGate assays. Whole genome amplified DNA was used as a template for the assays. We included polymorphisms in the *IL10* and *IL10RA* genes as quality controls to assess the whole genome amplification, since these polymorphisms have previously been genotyped in the LHS using genomic DNA as a template (22). The genotypes generated from whole genome amplified samples showed good concordance rates (98.1–99.6%) compared with those from genomic samples (data from 9 SNPs in the *IL10* and *IL10RA* genes).

Of the 619 LHS samples that were genotyped, samples with call rates <95% ($n = 40$) were removed from the analysis. Analyses were further limited to non-Hispanic whites ($n = 547$) of whom 262 were rapid decliners and 285 were nondecliners (Table 1). Of the 349 SNPs that were chosen for genotyping, SNPs with call rates <90% ($n = 37$), SNPs that were monomorphic ($n = 6$), and SNPs that were not in Hardy-Weinberg equilibrium ($n = 8$) were not analyzed. Thus, 298 polymorphisms were included in the analyses.

Genotyping of the Vlagtwedde-Vlaardingen cohort was performed at K-Biosciences (Hoddesdon, UK) using their patent-protected KASPar technology. SNPs were chosen for genotyping in this cohort if they were associated with rate of decline of lung function in the LHS ($P < 0.05$). However, SNP rs6125042 (in *NCOA3*) was excluded from the analysis due to a low call rate (71%) and lack of Hardy-Weinberg equilibrium ($P = 0.02$).

Statistical analysis. For the LHS cohort, Hardy-Weinberg equilibrium tests were performed using the Arlequin population genetics package (52), and linkage disequilibrium (LD) estimation was done using the CubeX, cubic exact solutions program (16). All tests of association were performed under an additive genetic model. The outcome was a dichotomous variable i.e., fast vs. nondecline in lung function (FEV_1% predicted). The SimHap software (5) was used to perform the multivariate logistic regressions adjusting for confounding factors, i.e., age, sex, pack-years of smoking, and recruitment center.

A Bonferroni correction for the total number of comparisons ($n = 298$) conducted in the LHS cohort may be overly conservative due to LD between the SNPs. Therefore, we used the SNP Spectral Decomposition (SNP SpD) approach to estimate the effective number of independent marker loci (M_{eff}) (45). With use of the SNP SpD approach and the estimate of M_{eff} provided by Li and Ji (36), the M_{eff} for this experiment was 203.5, and the experiment-wide significance threshold required to keep the type I error rate at 5% was 0.000252.

In the analysis of the LHS, several of the polymorphisms had small numbers in one or more of the cells, and therefore the conventional χ^2-test may not be valid. To address this issue, the *P* values were reassessed by the permutation procedure implemented in UNPHASED (12), using 10,000 random permutations for each SNP.

For the Vlagtwedde-Vlaardingen cohort, an additive genetic model was used to test the association of polymorphisms with the dichotomous outcome of fast vs. nondecline in lung function (FEV_1% predicted). The SPSS (version 16) software was used to perform the analyses adjusting for sex and pack-years. Hardy-Weinberg equilibrium tests were performed with Haploview (version 4.1) (2).

RESULTS

LHS cohort. The most significant associations of the candidate polymorphisms with rate of decline of lung function in the LHS group under the additive model are shown in Table 3. We found previously unreported associations of polymorphisms in 11 genes in the NFE2L2 pathway. The odds ratios for polymorphisms in these genes ranged from 0.44 to 0.76 for protective alleles and from 1.31 to 2.04 for risk alleles. The most significant associations were in the *IRG1*, *NCOA3*, and *KEAP1* genes. Several of these associations were also nominally significant ($P < 0.05$) when analyzed by the permutation procedure implemented in UNPHASED (12) (Table 3).

The majority of polymorphisms (14/23) associated with rate of decline of lung function were tagging SNPs. None of these SNPs were of obvious functional significance although a synonymous polymorphism in the *EP300* gene (rs20552) was in a highly conserved region.

Although 23 polymorphisms showed nominal association with rate of decline of lung function ($P < 0.05$) under the additive model (Table 3), none of these associations remained significant after correction using the effective number of independent marker loci. The estimated effective number of independent SNPs ($n = 203$) is lower than the actual number ($n = 298$) due to the moderate level of LD between the polymorphisms. For example, the LD between the SNPs associated with lung function is shown for the LHS data in Fig. 1.

Vlagtwedde-Vlaardingen cohort. We attempted to replicate the associations observed in the LHS cohort using the Vlagtwedde-Vlaardingen cohort. Of the 23 associated SNPs, one polymorphism in *KEAP1* (rs11085735) was previously genotyped in this cohort (54) and another in *NFE2L2* (rs10183914) was in LD ($r^2 = 0.96$) with a previously geno-

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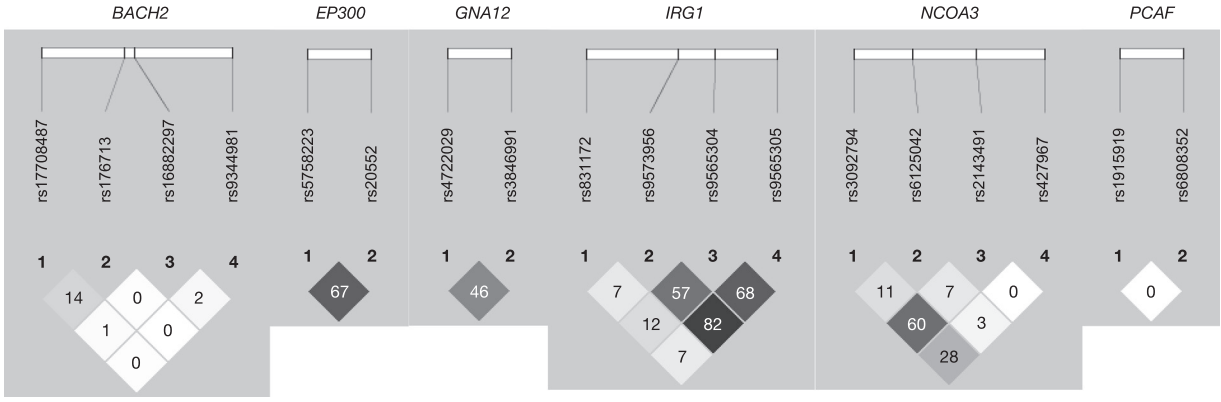

Fig. 1. Linkage disequilibrium between the polymorphisms associated with lung function in the Lung Health Study.

typed SNP (54). The LD between the SNPs in this cohort is shown in Fig. 2. All the polymorphisms were in Hardy-Weinberg equilibrium ($P \geq 0.12$). Associations of the SNPs with rate of decline of lung function are shown in Table 4. Only SNP rs634534 in the *FOSL1* gene showed a significant association in the Vlagtwedde-Vlaardingen cohort ($P = 0.016$), but the direction of the association was reversed compared with the LHS.

DISCUSSION

We investigated whether polymorphisms in NFE2L2 pathway genes were associated with the rate of decline of lung function in the LHS cohort. NFE2L2 is a master regulator of the antioxidant and detoxification pathways, and therefore the genes that we investigated are excellent candidates for COPD susceptibility loci. The four genes that showed the most significant associations in the LHS were *IRG1*, *NCOA3*, *KEAP1,* and *BACH2*. All these associations with rate of decline of lung function are novel, although we previously demonstrated that the polymorphism in the *KEAP1* gene (rs11085735) was associated with cross-sectionally determined level of lung function (54).

Irg1 was most highly upregulated in the lungs of *Nfe2l2*$^{-/-}$ mice following LPS treatment (59). *Irg1* was transcriptionally upregulated in LPS-stimulated macrophages (3, 34) and showed marked differences in expression in *Nfe2l2*$^{+/+}$ and *Nfe2l2*$^{-/-}$ mice after administration of LPS and exposure to cigarette smoke (59). Four polymorphisms in the *IRG1* gene showed significant associations with lung function decline in the LHS cohort. Three of the polymorphisms were in strong LD with each other ($r^2 = 0.68–0.82$), but the other SNP (rs831172) showed an independent association.

Four SNPs in the *NCOA3* gene were nominally associated with rapid decline of lung function. There was strong LD ($r^2 = 0.60$) between two of these variants (rs3092794 and rs2143491), but the remaining two SNPs were likely independent associations. NCOA3 is a member of the p160/steroid receptor coactivator family. NCOA3 associates with the transcription factor CREB binding protein and has histone acetyltransferase activity (6). NCOA3 regulates several transcription factors (17, 35, 62, 64) and acts as a positive regulator of NFE2L2 expression (37).

KEAP1 is a key inhibitor of NFE2L2 (30, 61). NFE2L2 is rapidly ubiquitinated and degraded by the proteasome under basal conditions, and this degradation is promoted by KEAP1. However, binding of KEAP1 to compounds that activate NFE2L2 (oxidants and electrophiles) through its cysteine residues leads to the release and nuclear translocation of NFE2L2 and subsequent induction of NFE2L2-regulated genes (11, 13, 25, 31). We found that a polymorphism in the *KEAP1* gene (rs11085735) was associated with rate of decline of lung function in the LHS in the present study and previously with level of lung function in the Vlagtwedde-Vlaardingen cohort (54). Taken together with the functional role of the protein, these data suggest a role for *KEAP1* as a novel candidate gene for COPD.

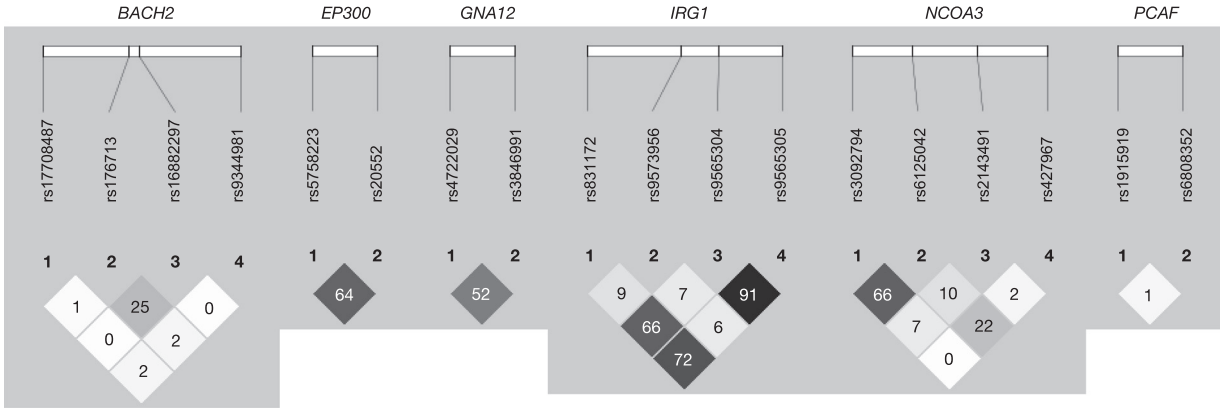

Fig. 2. Linkage disequilibrium between the polymorphisms in the Vlagtwedde-Vlaardingen cohort.

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Table 4. *Associations of polymorphisms with rate of decline of lung function in the Vlagtwedde-Vlaardingen cohort, under an additive genetic model adjusted for sex and pack-years of smoking*

SNP	Gene	Genotype	Genotype Counts		Odds Ratio	95% Confidence Interval	*P* Value
			Nondecliners	Fast Decliners			
rs9573956	IRG1	AA	0	1	1.279	0.772–2.120	0.340
		AG	35	32			
		GG	260	196			
rs3092794	NCOA3	AA	59	45	0.915	0.703–1.190	0.507
		AG	152	112			
		GG	67	64			
rs6125042	NCOA3	CC	4	2	0.978	0.603–1.584	0.927
		TC	40	25			
		TT	176	120			
rs9565305	IRG1	GG	0	1	1.188	0.723–1.952	0.496
		TG	38	32			
		TT	251	191			
rs11085735	KEAP1	GG	266	205	1.133	0.642–1.999	0.667
		TG	27	24			
		TT	1	0			
rs17708487	BACH2	AA	168	116	1.198	0.903–1.591	0.211
		AG	102	94			
		GG	19	16			
rs8176199	BRCA1	AA	172	126	1.137	0.856–1.511	0.375
		AC	98	82			
		CC	17	18			
rs634534	FOSL1	AA	45	49	1.374	1.060–1.781	0.016
		AG	147	119			
		GG	102	57			
rs16882297	BACH2	CC	261	211	0.629	0.331–1.198	0.158
		GC	28	15			
		GG	1	0			
rs5758223	EP300	AA	157	120	0.850	0.638–1.132	0.266
		AG	99	88			
		GG	29	9			
rs4722029	GNA12	CC	12	24	1.206	0.908–1.603	0.196
		TC	106	67			
		TT	172	131			
rs20552	EP300	AA	126	90	0.965	0.744–1.252	0.789
		TA	126	114			
		TT	43	23			
rs1915919	PCAF	CC	109	83	0.952	0.736–1.231	0.706
		TC	127	108			
		TT	47	31			
rs176713	BACH2	AA	224	156	1.367	0.950–1.967	0.092
		AG	64	65			
		GG	4	4			
rs6808352	PCAF	GG	31	18	0.920	0.700–1.209	0.549
		TG	115	96			
		TT	138	109			
rs427967	NCOA3	CC	187	160	0.830	0.586–1.173	0.291
		TC	84	57			
		TT	8	5			
rs9344981	BACH2	CC	91	70	0.970	0.758–1.241	0.806
		TC	132	111			
		TT	68	45			
rs4951627	ATF3	CC	14	9	1.093	0.800–1.492	0.577
		CG	80	72			
		GG	194	141			
rs13001694†	NFE2L2	CC	95	82	0.878	0.678–1.137	0.323
		TC	145	116			
		TT	52	32			
rs9565304	IRG1	AA	241	178	1.253	0.819–1.918	0.298
		AG	48	47			
		GG	2	1			
rs3846991	GNA12	CC	32	42	1.221	0.943–1.582	0.130
		CG	142	98			
		GG	114	84			

Continued

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Table 4.—*Continued*

SNP	Gene	Genotype	Genotype Counts		Odds Ratio	95% Confidence Interval	*P* Value
			Nondecliners	Fast Decliners			
rs831172	IRG1	AA	106	78	1.135	0.873–1.476	0.346
		AG	146	114			
		GG	37	37			
rs2143491	NCOA3	CC	100	88	0.978	0.747–1.280	0.871
		TC	156	106			
		TT	35	31			

The odds ratios are for a rapid rate of decline, and the reference is the wild-type homozygote genotype. †In almost complete linkage disequilibrium ($r^2 = 0.964$) with SNP rs10183914 according to HapMap.

There were four SNPs in the *BACH2* gene that were associated with decline in lung function. Interestingly, there was no strong LD between any of these polymorphisms, suggesting that the associations were independent. BACH2 is a transcription factor that plays a key role in the regulation of nucleic acid-triggered antiviral responses in human cells (26) and is highly expressed in B cells (43). BACH2 acts as a functional antagonist of NFE2L2 (27).

We were unable to replicate the associations observed in the LHS cohort using the Vlagtwedde-Vlaardingen cohort. Of the 23 associated SNPs, only rs634534 in the *FOSL1* gene showed a significant association in the Vlagtwedde-Vlaardingen cohort, but the direction of the association was not consistent with that in the LHS. SNP rs11085735 in the *KEAP1* gene showed significant association in the Vlagtwedde-Vlaardingen cohort as previously reported (54), but this association was with the level lung function and not with decline of FEV_1.

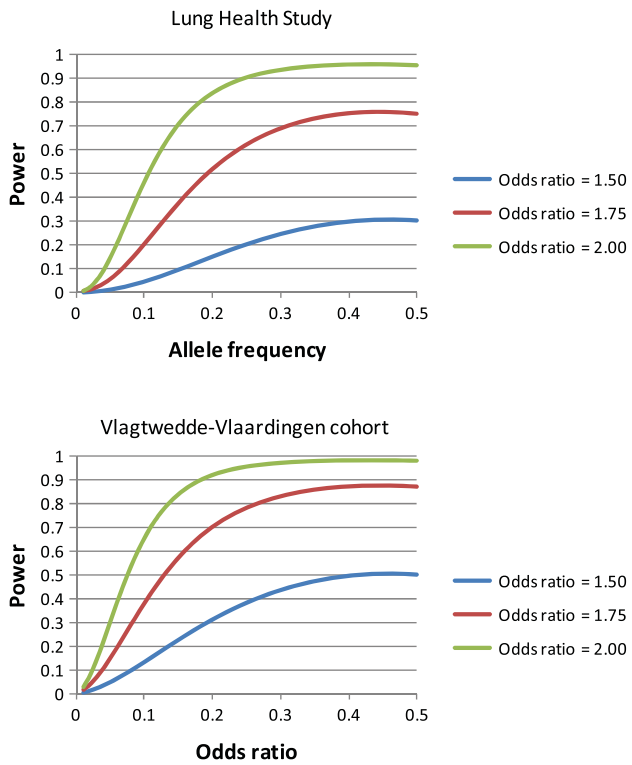

Fig. 3. Power of the study design accounting for multiple comparisons in the Lung Health Study ($\alpha = 0.000168$, *top*) and Vlagtwedde-Vlaardingen cohort ($\alpha = 0.002174$, *bottom*) for 2-sided tests under an additive model of inheritance.

The lack of replication may be related to the differences in recruitment between the two studies. The LHS selected mild to moderate COPD patients and the Vlagtwedde-Vlaardingen cohort was from the general population. It is possible that the genetic factors that influence lung function decline in COPD patients could be different than those in the general population. In addition, despite the moderate sample sizes of both of the cohorts lack of replication may be due low power to detect risk alleles of small effect. To address this aspect of the study we have performed power analyses for both cohorts (Fig. 3). We have good power to detect associations with odds ratios ≥2.0 and reasonable power for common variants with odds ratios ≥1.75 in the LHS. We had higher power to detect associations in the Vlagtwedde-Vlaardingen cohort due to the lower number of comparisons. Nevertheless, odds ratios of genetic associations with COPD are often <1.5, and therefore lack of power needs to be considered when interpreting these data.

Although we did not find replication of the NFE2L2 pathway genes studied in our cohorts, there is evidence of the role of this pathway in the development of COPD. SNPs in classical NFE2L2 targets such as glutathione S-transferase (*GST*) genes, NAD(P)H quinone oxidoreductase (*NQO1*), glutamate-cysteine ligase catalytic subunit (*GCLC*), and heme oxygenase-1 (*HMOX1*) have previously been shown to be associated with COPD (7, 18, 20, 21, 29, 33, 44, 55, 56, 65, 69). In contrast, other studies failed to find association of these genes with COPD-related phenotypes (23, 24, 57, 67, 68).

In summary, despite finding several nominally significant polymorphisms in the LHS, none of these associations were replicated in the Vlagtwedde-Vlaardingen cohort, indicating lack of effect of polymorphisms in the NFE2L2 pathway on the rate of decline of lung function. Alternatively these polymorphisms may have an effect, but our study is underpowered to detect these effects. Combining these data in subsequent meta analyses may be fruitful to more rigorously test their effects.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.J.S., H.B., M.S., J.E.C., N.R.A., P.D.P., and S.B. conception and design of research; A.J.S., H.B., M.S., and V.W. analyzed data; A.J.S., H.B., M.S., and D.S.P. interpreted results of experiments; A.J.S. prepared figures; A.J.S. drafted manuscript; A.J.S., D.M., H.B., M.S., D.S.P., V.W., L.A., J.-Q.H., P.D.P., and S.B. edited and revised manuscript; A.J.S., D.M., H.B., M.S., D.S.P., V.W., L.A., J.-Q.H., J.E.C., N.R.A., P.D.P., and S.B. approved final version of manuscript; L.A. and J.-Q.H. performed experiments.

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