

1 ***NFE2L2* Pathway Polymorphisms and Lung Function Decline in Chronic Obstructive**
2 **Pulmonary Disease**

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33 Abstract

34 An oxidant-antioxidant imbalance in the lung contributes to the development of chronic
35 obstructive pulmonary disease (COPD) that is caused by a complex interaction of genetic and
36 environmental risk factors. Nuclear erythroid 2-related factor 2 (NFE2L2 or NRF2) is a critical
37 molecule in the lung's defense mechanism against oxidants. We investigated whether
38 polymorphisms in the NFE2L2 pathway affected the rate of decline of lung function in smokers
39 from the Lung Health Study (LHS)(n=547) and in a replication set, the Vlagtwedde-Vlaardingen
40 cohort (n=533). We selected polymorphisms in *NFE2L2*, in genes that positively or negatively
41 regulate *NFE2L2* transcriptional activity, and in genes that are regulated by *NFE2L2*.
42 Polymorphisms in eleven genes were significantly associated with rate of lung function decline
43 in the LHS. One of these polymorphisms, rs11085735 in the *KEAPI* gene, was previously shown
44 to be associated with the level of lung function in the Vlagtwedde-Vlaardingen cohort but not
45 with decline of lung function. Of the 23 associated polymorphisms in the LHS, only rs634534 in
46 the *FOSL1* gene showed a significant association in the Vlagtwedde-Vlaardingen cohort with
47 rate of lung function decline but the direction of the association was not consistent with that in
48 the LHS. In summary, despite finding several nominally significant polymorphisms in the LHS,
49 none of these associations were replicated in the Vlagtwedde-Vlaardingen cohort, indicating lack
50 of effect of polymorphisms in the NFE2L2 pathway on the rate of decline of lung function.

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53 Keywords

54 Genetic polymorphism, NRF2, NFE2L2, forced expiratory volume in one second (FEV₁), lung
55 function, chronic obstructive pulmonary disease (COPD)

56

57 **Introduction**

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

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

73 Nuclear erythroid 2-related factor 2 (NFE2L2 or NRF2) is a basic leucine zipper
74 transcription factor that upregulates multiple genes involved in antioxidant and detoxification
75 pathways in response to exposure of the lungs to cigarette smoke (48). Disruption of the *Nfe2l2*
76 gene in an emphysema-resistant mouse model resulted in an early-onset and severe cigarette
77 smoke-induced emphysema suggesting that NFE2L2 is a critical molecule in the lung's defense
78 mechanism against oxidants (48). Oxidative stress causes NFE2L2 to translocate to the nucleus
79 following dissociation from its cytosolic inhibitor, KEAP1 (30). We have shown (39) that the

80 protein levels of NFE2L2 and DJ1 (PARK7), a stabilizer of NFE2L2 (9), are decreased in the
81 lungs of patients with COPD. These data indicate that NFE2L2 plays an important protective
82 role against cigarette smoke-induced COPD.

83 A previous study (66) of four promoter polymorphisms in the *NFE2L2* gene did not
84 demonstrate any associations with COPD in the Japanese population. In contrast, an *NFE2L2*
85 polymorphism (rs2364723) in intron 2 of the gene was associated with level of lung function,
86 although not with its rate of decline, in a European population (54). Most recently, another
87 variant (rs6726395) in intron 2 of the *NFE2L2* gene was associated with rate of decline of lung
88 function in the Japanese population and showed a significant interaction with smoking status
89 (40).

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

98

99 **Materials and Methods**

100 *Study participants*

101 The analyses were performed in a nested case-control design that included participants
102 from the LHS, a clinical trial sponsored by the National Heart, Lung and Blood Institute (1). The
103 LHS was conducted at 10 medical centers in North America, and a total of 5,887 smokers, aged
104 35–60 with spirometric evidence of mild to moderate lung function impairment were recruited
105 (1). Lung function was assessed as forced expiratory volume in one second (FEV₁) % of
106 predicted, i.e., FEV₁ adjusted for age, height, sex, and race. Lung function measurements in the
107 LHS were performed using standardized spirometry in accordance with the American Thoracic
108 Society guidelines (14) and the reference equations were those of Crapo and coworkers (10)
109 based on Caucasian subjects of northern European descent in Salt Lake City.

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

114 Based on the rate of decline of lung function during a 5-year follow-up period, of the
115 3,216 continuing smokers in this study, we selected non-Hispanic whites with a fast decline of
116 FEV₁ (n=262) and with no decline of FEV₁ (n=285). Arbitrary cut off points of FEV₁%
117 predicted/year decrease $\geq 3.0\%$ and increase $\geq 0.4\%$ were used for rapid decliners and non-
118 decliners, respectively. The demographic characteristics of the participants are shown in Table 1.

119 The Vlagtwedde-Vlaardingen cohort was utilized as an independent replication cohort
120 (54). This cohort contains 1,390 subjects with 8,159 FEV₁ measurements completed during eight
121 surveys, that was prospectively followed for 25 years with FEV₁ measurements performed every

122 three years (following European Respiratory Society guidelines) (60). Based on the rate of
123 decline of lung function during this follow-up period, we selected smokers (smoking history > 5
124 pack-years) with a fast decline of FEV₁ (n=233) and with no decline of FEV₁ (n=300). Arbitrary
125 cut off points of FEV₁% predicted/year decrease >0% and increase >7.4% were used for rapid
126 decliners and non-decliners, respectively. The characteristics of these subjects are shown in
127 Table 2.

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

130

131 *Gene / polymorphism selection and genotyping*

132 We selected genes involved in upregulation of *NFE2L2* (*APEX1*, *BRCA1*, *CARM1*, *CREBBP*,
133 *DPP3*, *EP300*, *JUN*, *KAT2B*, *NCOA3*, *PARK7*, *PPARG*, *PRMT1*, and *SQSTM1*) and
134 downregulation of *NFE2L2* (*ATF3*, *BACH1*, *BACH2*, *FOS*, *FOSL1*, *GNAI2*, *KEAP1*, *MAF*,
135 *MAFK*, and *TP53*). In addition, we selected genes known to be regulated by *NFE2L2* (*GPX2*,
136 *GSR*, and *SRXNI*). We also genotyped single nucleotide polymorphisms (SNPs) in three genes:
137 *NFE2L2*, *NFE2L1*, a member of *NFE2L* family shown to act as a repressor of *NFE2L2*, and
138 *NFE2L3*, a member of *NFE2L* family with high homology to *NFE2L2*. Finally, we selected a
139 novel inflammatory gene (*IRG1*) as it was the most highly upregulated gene in the lungs of mice
140 with a deletion of *Nfe2l2* after lipopolysaccharide treatment (59).

141 Tag SNPs and singletons that represent the genetic variation in each gene were selected
142 from resequencing data in the European American Descent populations of the SeattleSNPs
143 Program for Genomic Applications (<http://pga.mbt.washington.edu/>) or HapMap Project

144 (<http://hapmap.ncbi.nlm.nih.gov/>) using the LDselect program (4). LDselect parameter
145 thresholds of $r^2 > 0.8$ and minor allele frequencies (MAFs) greater than 5% were used.

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

154 Of the 619 LHS samples that were genotyped, samples with call rates <95% (n=40) were
155 removed from the analysis. Analyses were further limited to non-Hispanic whites (n=547) of
156 whom 262 were rapid decliners and 285 were non-decliners (Table 1). Of the 349 SNPs that
157 were chosen for genotyping, SNPs with call rates <90% (n=37), SNPs that were monomorphic
158 (n=6), and SNPs that were not in Hardy-Weinberg equilibrium (n=8) were not analyzed. Thus,
159 298 polymorphisms were included in the analyses.

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

165

166

167 *Statistical analysis*

168 For the LHS cohort, Hardy-Weinberg equilibrium tests were performed using the Arlequin
169 population genetics package (52), and linkage disequilibrium (LD) estimation was done using
170 the CubeX, cubic exact solutions program (16). All tests of association were performed under an
171 additive genetic model. The outcome was a dichotomous variable i.e. fast vs. non-decline in lung
172 function (FEV₁ % predicted). The SimHap software (5) was used to perform the multivariate
173 logistic regressions adjusting for confounding factors, i.e., age, sex, pack-years of smoking, and
174 recruitment center.

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

181 In the analysis of the LHS, several of the polymorphisms had small numbers in one or
182 more of the cells and therefore the conventional chi-squared test may not be valid. To address
183 this issue, the p values were reassessed using the permutation procedure implemented in
184 UNPHASED (12), using 10,000 random permutations for each SNP.

185 For the Vlagtwedde-Vlaardingen cohort, an additive genetic model was used to test the
186 association of polymorphisms with the dichotomous outcome of fast vs. non-decline in lung
187 function (FEV₁ % predicted). The SPSS (Version 16) software was used to perform the analyses
188 adjusting for sex and pack-years. Hardy-Weinberg equilibrium tests were performed using
189 Haploview (Version 4.1) (2).

190 **Results**

191 *LHS cohort*

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

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

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

208

209 *Vlagtwedde-Vlaardingen cohort*

210 We attempted to replicate the associations observed in the LHS cohort using the Vlagtwedde-
211 Vlaardingen cohort. Of the 23 associated SNPs, one polymorphism in *KEAPI* (rs11085735) was
212 previously genotyped in this cohort (54) and another in *NFE2L2* (rs10183914) was in LD

213 ($r^2=0.96$) with a previously genotyped SNP (54). The LD between the SNPs in this cohort is
214 shown in Figure 2. All the polymorphisms were in Hardy-Weinberg equilibrium ($p \geq 0.12$).
215 Associations of the SNPs with rate of decline of lung function are shown in Table 4. Only SNP
216 rs634534 in the *FOSL1* gene showed a significant association in the Vlagtwedde-Vlaardingen
217 cohort ($p=0.016$) but the direction of the association was reversed compared to the LHS.

218 Discussion

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

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

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

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

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

255 We were unable to replicate the associations observed in the LHS cohort using the
256 Vlagtwedde-Vlaardingen cohort. Of the 23 associated SNPs, only rs634534 in the *FOSL1* gene
257 showed a significant association in the Vlagtwedde-Vlaardingen cohort but the direction of the
258 association was not consistent with that in the LHS. SNP rs11085735 in the *KEAP1* gene showed
259 significant association in the Vlagtwedde-Vlaardingen cohort as previously reported (54), but
260 this association was with the level lung function and not with decline of FEV₁.

261 The lack of replication may be related to the differences in recruitment between the two
262 studies. The LHS selected mild to moderate COPD patients and the Vlagtwedde-Vlaardingen
263 cohort was from the general population. It is possible that the genetic factors that influence lung

264 function decline in COPD patients could be different than those in the general population. In
265 addition, despite the moderate sample sizes of both of the cohorts lack of replication may be due
266 low power to detect risk alleles of small effect. To address this aspect of the study we have
267 performed power analyses for both cohorts (Figure 3). We have good power to detect
268 associations with odds ratios ≥ 2.0 and reasonable power for common variants with odds ratios
269 ≥ 1.75 in the Lung Health Study. We had higher power to detect associations in the Vlagtwedde-
270 Vlaardingen cohort due to the lower number of comparisons. Nevertheless, odds ratios of genetic
271 associations with COPD are often < 1.5 and therefore lack of power needs to be considered when
272 interpreting these data.

273 Although we did not find replication of the NFE2L2 pathway genes studied in our
274 cohorts, there is evidence of the role of this pathway in the development of COPD. SNPs in
275 classical NFE2L2 targets such as glutathione S-transferase (*GST*) genes, NAD(P)H quinone
276 oxidoreductase (*NQO1*), glutamate-cysteine ligase catalytic subunit (*GCLC*), and heme
277 oxygenase-1 (*HMOX1*) have previously been shown to be associated with COPD (7, 18, 20,
278 21, 29, 33, 44, 55, 56, 65, 69). In contrast, other studies failed to find association of these
279 genes with COPD-related phenotypes (23, 24, 57, 67, 68).

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

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296

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566 **Figure legends**

567 **Figure 1.** Linkage disequilibrium between the polymorphisms associated with lung function in
568 the Lung Health Study

569 **Figure 2.** Linkage disequilibrium between the polymorphisms in the Vlagtwedde-Vlaardingen
570 cohort

571 **Figure 3.** Power of the study design accounting for multiple comparisons in the Lung Health
572 Study ($\alpha= 0.000168$) and Vlagtwedde-Vlaardingen cohort ($\alpha= 0.002174$) for two-sided tests
573 under an additive model of inheritance.

Figure 1.

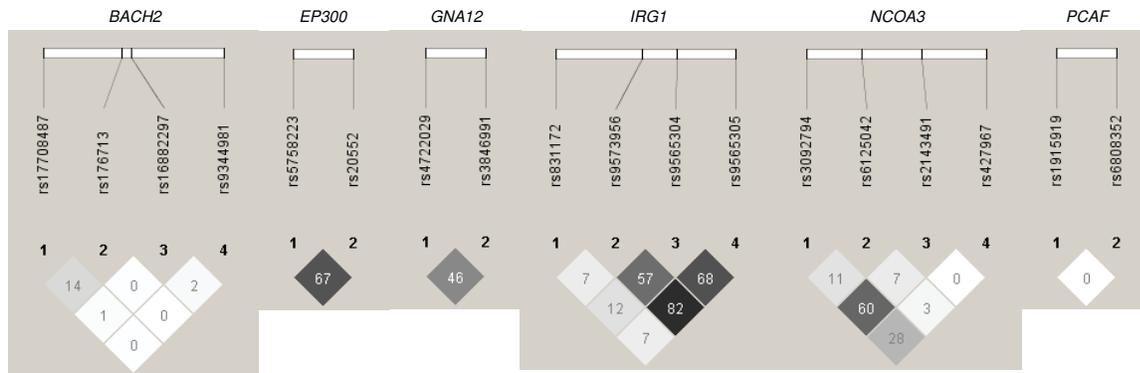


Figure 2.

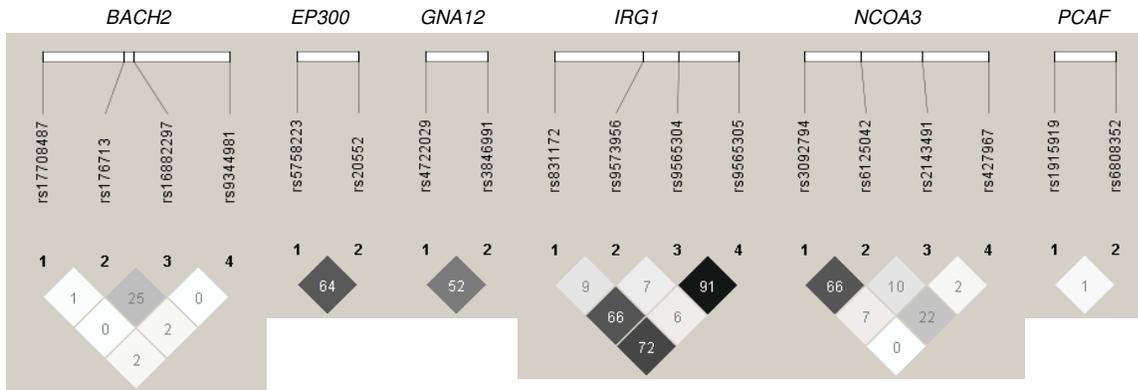


Figure 3.

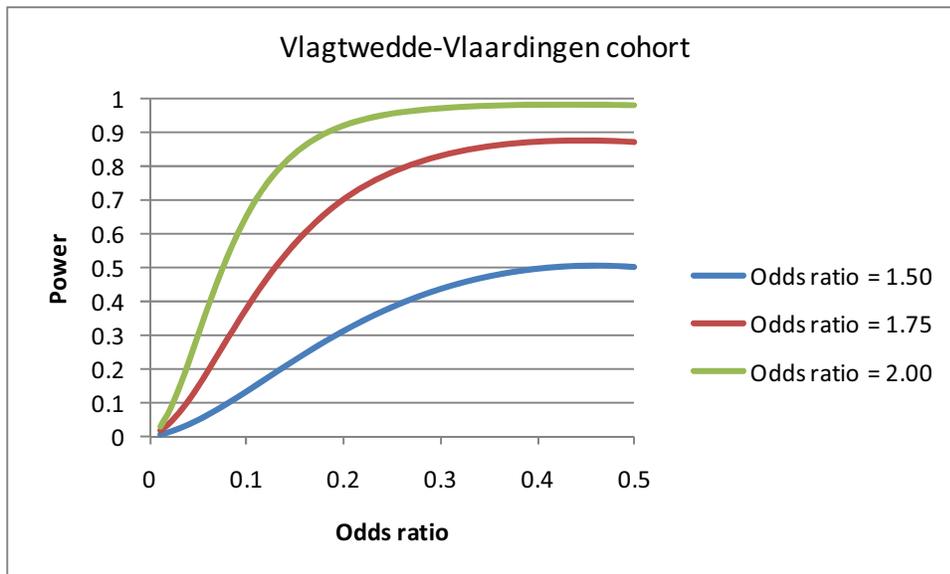
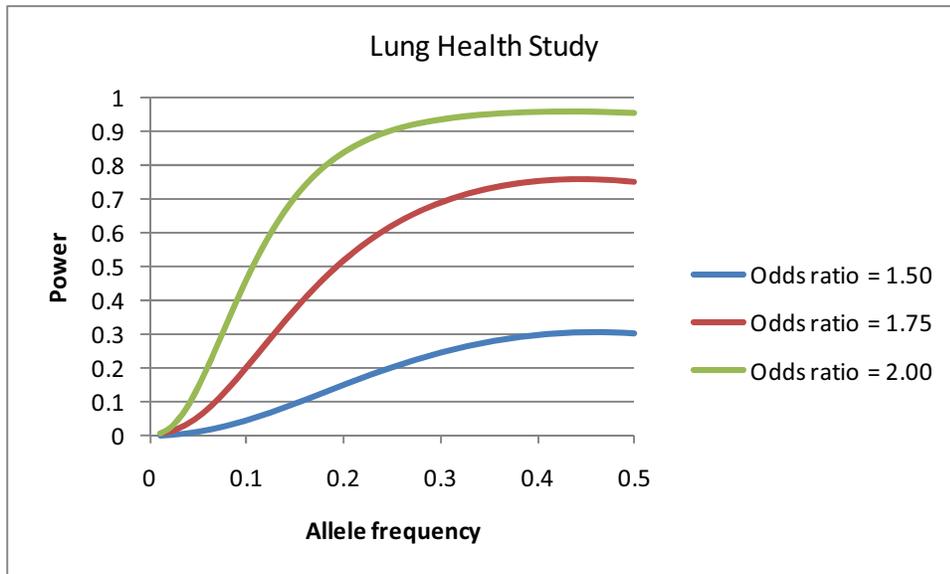


Table 1. The distribution of demographic characteristics for subjects in the Lung Health Study

	Non Decliners (n=285)	Fast Decliners (n=262)	p-value
Men/Women	186/99	152/110	0.0942
Age (years)	47.7 ± 6.9	49.8 ± 6.3	0.0002
Smoking history (pack-years)*	38.5 ± 18.3	43.2 ± 19.4	0.0038
ΔFEV ₁ /year (% predicted pre) [†]	1.1 ± 0.7	-4.2 ± 1.1	<0.0001
ΔFEV ₁ /year (% 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

FEV₁=forced expiratory volume in 1 second

Values are means ± SD for continuous data.

*Number of packs of cigarettes smoked per day / number of years smoking.

[†]Change in lung function over a 5-year period per year as % predicted FEV₁ pre bronchodilator.

[‡]Change in lung function over a 5-year period per year as % predicted FEV₁ post bronchodilator
(3 missing values in fast decliners group and 4 missing values in non decliners group).

[§]Lung function at the start of the Lung Health Study as measured by FEV₁(%) predicted pre bronchodilator.

^{||}Lung function at the start of the Lung Health Study as measured by FEV₁(%) predicted post bronchodilator.

Table 2. The distribution of demographic characteristics for subjects in the Vlagtwedde-Vlaardingen cohort.

	Non Decliners (n=300)	Fast Decliners (n=233)	p-value
Men/Women	215/85	162/71	0.590
Age (years)	49.7 ± 9.6	53.05 ± 9.7	<0.0001
Smoking history (pack-years)*	23.5 ± 16.8	29.4 ± 19.0	<0.0001
ΔFEV ₁ /year (% predicted pre) [†]	0.9 ± 0.7	-0.5 ± 0.5	<0.0001
ΔFEV ₁ /year (% 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	

FEV₁=forced expiratory volume in 1 second

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 FEV₁ pre bronchodilator.

[‡]Change in lung function over the total period someone was in the study per year as % predicted FEV₁ post bronchodilator.

[§]Lung function at the start of the Vlagtwedde/Vlaardingen as measured by FEV₁(%) predicted pre bronchodilator.

^{||}Lung function at the start of the Vlagtwedde/Vlaardingen as measured by FEV₁(%) predicted post bronchodilator.

Table 3. Nominally significant associations of polymorphisms with rate of decline of lung function in the Lung Health Study cohort.

The odds ratios are for a rapid rate of decline and the reference is the wild type homozygote genotype.

SNP	Gene	Genotype	Genotype counts		Unadjusted analysis		Adjusted analysis ^{†††}		
			Non Decliners	Fast decliners	p value [†]	Permuted p-value ^{††}	Odds ratio	95% Confidence interval	p-value
rs9573956	<i>IRGI</i>	AA	6	0	0.0006	0.0009	0.443	0.267-0.735	0.0016
		AG	48	25					
		GG	231	237					
rs3092794	<i>NCOA3</i>	AA	61	41	0.0351	0.0318	0.683	0.525-0.888	0.0044
		AG	153	130					
		GG	71	89					
rs6125042	<i>NCOA3</i>	CC	5	8	0.0517	0.0555	1.646	1.143-2.371	0.0074
		TC	53	68					
		TT	227	184					
rs9565305	<i>IRGI</i>	GG	5	0	0.0043	0.0030	0.522	0.325-0.840	0.0074
		TG	51	31					
		TT	229	231					
rs11085735	<i>KEAP1</i>	GG	261	223	0.0292	0.0496	2.043	1.206-3.461	0.0079
		TG	23	34					
		TT	1	5					
rs17708487	<i>BACH2</i>	AA	168	134	0.1657	0.1628	1.478	1.102-1.983	0.0091
		AG	99	108					
		GG	16	19					
rs8176199	<i>BRCA1</i>	AA	170	140	0.0348	0.0342	1.513	1.097-2.088	0.0116
		AC	82	93					
		CC	8	17					
rs634534	<i>FOSL1</i>	AA	59	45	0.0861	0.0872	0.733	0.567-0.948	0.0179
		AG	145	121					
		GG	79	96					

rs16882297	<i>BACH2</i>	CC	262	226	0.0369	0.0345	1.941	1.105-3.407	0.0209
		GC	23	34					
		GG	0	2					
rs5758223	<i>EP300</i>	AA	160	129	0.0648	0.0610	1.366	1.039-1.796	0.0255
		AG	107	103					
		GG	18	30					
rs4722029	<i>GNAI2</i>	CC	10	14	0.0526	0.0555	1.427	1.043-1.952	0.0262
		TC	77	92					
		TT	197	156					
rs20552	<i>EP300</i>	AA	123	104	0.0634	0.0633	1.333	1.033-1.720	0.0273
		TA	130	110					
		TT	32	48					
rs1915919	<i>PCAF</i>	CC	114	77	0.0281	0.0295	1.338	1.030-1.739	0.0292
		TC	133	139					
		TT	38	46					
rs176713	<i>BACH2</i>	AA	230	192	0.1166	0.1238	1.549	1.042-2.303	0.0305
		AG	53	67					
		GG	2	3					
rs6808352	<i>PCAF</i>	GG	17	33	0.0242	0.0221	1.358	1.029-1.792	0.0307
		TG	129	114					
		TT	139	115					
rs427967	<i>NCOA3</i>	CC	174	184	0.0777	0.0819	0.700	0.504-0.971	0.0325
		TC	100	70					
		TT	11	8					
rs9344981	<i>BACH2</i>	CC	108	83	0.1763	0.1780	1.332	1.023-1.736	0.0334
		TC	140	133					
		TT	37	46					
rs4951627	<i>ATF3</i>	CC	16	7	0.0544	0.0552	0.708	0.514-0.976	0.0349
		CG	92	70					
		GG	177	185					
rs10183914	<i>NFE2L2</i>	CC	115	121	0.3724	0.3840	0.749	0.571-0.982	0.0365
		TC	134	113					

		TT	36	28					
rs9565304	<i>IRG1</i>	AA	215	215	0.0608	0.0625	0.656	0.442-0.974	0.0365
		AG	62	45					
		GG	8	2					
rs3846991	<i>GNAI2</i>	CC	31	37	0.1178	0.1220	1.315	1.013-1.707	0.0394
		CG	120	124					
		GG	134	101					
rs831172	<i>IRG1</i>	AA	93	99	0.2760	0.2760	0.760	0.584-0.990	0.0422
		AG	141	126					
		GG	51	36					
rs2143491	<i>NCOA3</i>	CC	115	127	0.1535	0.1520	0.758	0.579-0.993	0.0444
		TC	134	110					
		TT	35	25					

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

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. The odds ratios are for a rapid rate of decline and the reference is the wild type homozygote genotype.

SNP	Gene	Genotype	Genotype counts		Odds ratio	95% Confidence interval	p-value
			Non Decliners	Fast decliners			
rs9573956	<i>IRG1</i>	AA	0	1	1.279	0.772-2.120	0.340
		AG	35	32			
		GG	260	196			
rs3092794	<i>NCOA3</i>	AA	59	45	0.915	0.703-1.190	0.507
		AG	152	112			
		GG	67	64			
rs6125042	<i>NCOA3</i>	CC	4	2	0.978	0.603-1.584	0.927
		TC	40	25			
		TT	176	120			
rs9565305	<i>IRG1</i>	GG	0	1	1.188	0.723-1.952	0.496
		TG	38	32			
		TT	251	191			
rs11085735	<i>KEAP1</i>	GG	266	205	1.133	0.642-1.999	0.667
		TG	27	24			
		TT	1	0			
rs17708487	<i>BACH2</i>	AA	168	116	1.198	0.903-1.591	0.211
		AG	102	94			
		GG	19	16			
rs8176199	<i>BRCA1</i>	AA	172	126	1.137	0.856-1.511	0.375
		AC	98	82			
		CC	17	18			

rs634534	<i>FOSL1</i>	AA	45	49	1.374	1.060-1.781	0.016
		AG	147	119			
		GG	102	57			
rs16882297	<i>BACH2</i>	CC	261	211	0.629	0.331-1.198	0.158
		GC	28	15			
		GG	1	0			
rs5758223	<i>EP300</i>	AA	157	120	0.850	0.638-1.132	0.266
		AG	99	88			
		GG	29	9			
rs4722029	<i>GNAI2</i>	CC	12	24	1.206	0.908-1.603	0.196
		TC	106	67			
		TT	172	131			
rs20552	<i>EP300</i>	AA	126	90	0.965	0.744-1.252	0.789
		TA	126	114			
		TT	43	23			
rs1915919	<i>PCAF</i>	CC	109	83	0.952	0.736-1.231	0.706
		TC	127	108			
		TT	47	31			
rs176713	<i>BACH2</i>	AA	224	156	1.367	0.950-1.967	0.092
		AG	64	65			
		GG	4	4			
rs6808352	<i>PCAF</i>	GG	31	18	0.920	0.700-1.209	0.549
		TG	115	96			
		TT	138	109			
rs427967	<i>NCOA3</i>	CC	187	160	0.830	0.586-1.173	0.291
		TC	84	57			
		TT	8	5			
rs9344981	<i>BACH2</i>	CC	91	70	0.970	0.758-1.241	0.806
		TC	132	111			
		TT	68	45			
rs4951627	<i>ATF3</i>	CC	14	9	1.093	0.800-1.492	0.577
		CG	80	72			

		GG	194	141			
rs13001694 [†]	<i>NFE2L2</i>	CC	95	82	0.878	0.678-1.137	0.323
		TC	145	116			
		TT	52	32			
rs9565304	<i>IRG1</i>	AA	241	178	1.253	0.819-1.918	0.298
		AG	48	47			
		GG	2	1			
rs3846991	<i>GNAI2</i>	CC	32	42	1.221	0.943-1.582	0.130
		CG	142	98			
		GG	114	84			
rs831172	<i>IRG1</i>	AA	106	78	1.135	0.873-1.476	0.346
		AG	146	114			
		GG	37	37			
rs2143491	<i>NCOA3</i>	CC	100	88	0.978	0.747-1.280	0.871
		TC	156	106			
		TT	35	31			

SNP=single nucleotide polymorphism

[†]In almost complete linkage disequilibrium ($r^2=0.964$) with SNP rs10183914 according to HapMap.