Role and diagnostic value of gene variants in assessing the risk of chronic obstructive pulmonary disease

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ABSTRACT. Meta-analyses have revealed many positive associations between gene variants and susceptibility to chronic obstructive pulmonary disease (COPD). However, some of those positive results may be false positives. Therefore, we investigated the genetic polymorphisms associated with COPD risk and determined their diagnostic value. We extracted the odds ratio (OR) and 95% confidence interval for each polymorphism from published meta-analyses concerning gene variants and COPD susceptibility in October 2014, subsequently we calculated false-positive report probabilities (FPRPs) for statistically significant associations (P value < 0.05). We determined the diagnostic value of the true positive polymorphisms of COPD using the Meta-DiSc software. Twenty-five gene polymorphisms were significantly associated with COPD risk. The FPRP test results were as follows: 1) when the

prior probability was 0.001 and the OR was 1.5, *ADAM33* rs612709, *CHRNA3/5* rs1051730, *CHRNA3/5* rs8034191, *CHRNA3/5* rs16969968, and *TGFB1* rs1800470 were truly associated with COPD risk (FPRP < 0.2); 2) when the prior probability was 0.000001 and the OR was 1.5, all the variants except *TGFB1* rs1800470 remained noteworthy; and 3) when the probability was 0.000001 and the OR was 1.2, *ADAM33* rs612709 and *CHRNA3/5* rs1051730 remained true positives. Unfortunately, the results of the diagnostic accuracy meta-analyses suggested that none of the variants had high value for COPD diagnosis.

Key words: COPD; Polymorphism; Susceptibility; FPRP

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is considered to be both preventable and treatable. COPD involves continual and progressive air current limitation and intensive chronic inflammatory response owing to toxic particles and gases in the respiratory tract and lungs. According to Halbert et al. (2006), the prevalence of COPD is approximately 10% in people older than 40 worldwide. In the Chinese population, 8.2% suffer from COPD [95% confidence interval (CI) = 7.9-8.6] (men, 12.4%; women, 5.1%) (Zhong et al., 2007). In 2010, COPD led to the death of approximately three million individuals, and was ranked third as the cause of death globally (Lozano et al., 2012). In 2006, the cost of COPD per patient ($1963.80) demanded 40% of the family's economic income ($4849.80) in China (Fang et al., 2011). The etiology of COPD involves complicated genetic and environmental factors, the most crucial of which is tobacco consumption. However, although 90% of COPD cases are attributed to long-term smoking, only one quarter of smokers suffer from COPD. Also, smokers with comparable smoking histories demonstrate marked heterogeneity in lung function (Løkke et al., 2006).

Furthermore, COPD generally occurs in smokers, who have a family history of chronic obstructive respiratory tract diseases, may also be associated with the risk of COPD. Each year, more studies indicate certain candidate genes that are involved in COPD risk, such as the *ADAM* family of genes (A disintegrin and metalloprotease), *TGFB1* (transforming growth factor-b1), and *CHRNA3/5* (a-neuronal nicotinic acetylcholine receptor subunit).

Unfortunately, each separate study has a distinct sample size, which may induce conflicting outcomes with lower statistical powers. Another deficiency of genetic epidemiology studies is their lack of reproducibility. A few studies have attempted to find a formerly published statistically significant result on a genetic mutation, but have failed owing to "false-positive" reports (Ioannidis et al., 2001; Morgan et al., 2007). Hence, meta- and pooled analyses have been employed to combine both positive and negative results from separate studies and weigh these results by their precision.

Previous meta-analyses have reported over 30 single nucleotide polymorphisms (SNPs) associated with COPD susceptibility. However, for molecular epidemiology studies, Wacholder et al. (2004) suggested that use of the P value alone is inadequate for evaluating statistical significance. The prior probability of the association between the genetic variant and the disease is real, and the statistical power of the test is also required. They invented a method to assess the false-positive report probability (FPRP), which determines whether a result is a true positive (noteworthy) or not. Because three parameters are required to adequately

evaluate a medical diagnostic test (sensitivity, specificity, and the predictive value of a positive test), three analogous parameters are required to adequately evaluate statistical tests on the associations between genetic variants and COPD. The P value is analogous to 1 minus specificity. The study power is analogous to sensitivity. Nevertheless, in medical diagnostics, the predictive values of positive tests can still be low, while specificity and sensitivity are high, because positive diagnostic tests mainly produce false-positive results when the condition is rare. This situation is also crucial in evaluating statistical tests of exposure-disease hypotheses, because when the prior probability that a hypothesized association is true is small, a statistically significant result is probably a false positive. The FPRP, defined as "the probability of no association given a statistically significant finding", is another analogous parameter to 1 minus the predictive value of a positive test. Therefore, it is actually the FPRP that illustrates how probable the hypothesis is, instead of the P value. In brief, meta- and pooled analyses can also contain false-positive results. However, there has been no FPRP analysis on the associations between genetic variants and COPD. Moreover, among the true-positive results the diagnostic value is unclear. We performed this study to evaluate the validity of published associations and the diagnostic value of the relevant SNPs.

MATERIAL AND METHODS

All potential meta- and pooled analyses that had investigated the association between genetic variants and COPD risks were identified in October 12, 2014. We used the PubMed, Embase, China National Knowledge Internet, and Wanfang databases. The following search terms were used for the literature search: "COPD" or "chronic obstructive pulmonary disease", "polymorphism" or "polymorphisms" or "variants" or "variant" or "mutation" or "mutations", and "meta" or "meta-analysis" or "system review". The articles included in our study had to meet three criteria: 1) they must have been evaluations of genetic polymorphisms of COPD using meta-analyses or pooled analyses; 2) they must have evaluated COPD susceptibility as the outcome (analyses of emphysema, chronic bronchitis, spirometry, etc. were excluded); and 3) they must have been published in English or Chinese. If there were two or more meta- or pooled analysis concerning an identical association, only the one with the most cases was included. We extracted the following data from each study: gene, genetic polymorphism, odds ratio (OR) and 95%CI, number of studies, and number of cases and controls.

Associations were considered statistically significant when the reported 95%CI of OR excluded 1.0. For each statistically significant association we included, we calculated the FPRP using the Excel spreadsheet shared by Wacholder et al. (2004). The FPRP value was determined by the P value, the given prior probability for the association, and the statistical power of the test. We calculated FPRP values for two levels of prior probabilities: at the level of 0.001, which would be similar to that expected for a candidate gene; and at the level of 0.000001, which would be similar to that expected for a random SNP. Therefore, a reader can evaluate the association according to their own judgment. Wacholder et al. (2004) suggested that the statistical power be estimated based on the ability to detect an OR of 1.5, when the alpha level was equal to the observed P value. Dong et al. (2008) found that for small ORs, an OR of 1.5 could be too conservative; so both ORs of 1.5 and 1.2 were used. FPRP values less than 0.2 indicate that the association is still significant for a given level of prior probability.

For true-positive polymorphisms, we further investigated the diagnostic value including sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio

(NLR), diagnostic OR (DOR), and calculated area under the curve (AUC) to COPD using the Meta-DiSc software.

RESULTS

We identified 152 studies after initial research, among which 7 were not in English or Chinese and 31 were not about associations between genetic variants and COPD risk. One hundred and fourteen studies were left for full-text view. Among those, 47 were not meta- or pooled analyses and 6 did not take COPD risk as outcomes. Another 40 had fewer subjects than analyses concerning the same variant (Figure 1). Ultimately, we included 21 published meta- and pooled analyses, encompassing 23 different genes and 44 variant-COPD associations. Among the summary ORs of these 44 variants, 25 were reported to be significantly associated with the risk of COPD. Each included variant is shown in Table 1.

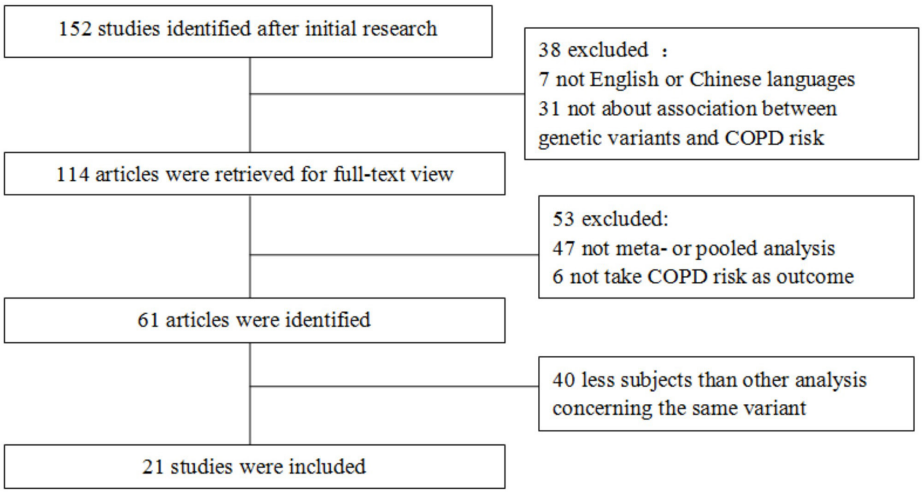

Figure 1. Flow diagram of selection process.

The FPRP values were calculated at two levels of prior probabilities. As shown in Table 2, among the 25 associations, the FPRP values of 20 gene-variant/cancer associations were higher than 0.2 at both prior probabilities (0.001 and 0.000001), and these results are not regarded as noteworthy. At a prior probability level of 0.001 and a statistical power to detect an OR of 1.5, five associations were noteworthy (FPRP ≤ 0.2) for: 1) *ADAM33* rs612709 (OR = 0.60; P value < 0.001); 2) *CHRNA3/5* rs1051730 (OR = 1.14; P-value < 0.001); 3) *CHRNA3/5* rs8034191 (OR = 1.29; P value < 0.001); 4) *CHRNA3/5* rs16969968 (OR = 1.27; P value < 0.001); and 5) *TGFB1* rs1800470 (OR = 0.73; P value = 0.002). At a very low prior probability (0.000001), four associations remained noteworthy: *ADAM33* rs612709, *CHRNA3/5* rs1051730, *CHRNA3/5* rs8034191, and *CHRNA3/5* rs16969968. The number was reduced to two (*ADAM33* rs612709 and *CHRNA3/5* rs1051730) when we calculated at a statistical power based on an OR of 1.2. Consistent with FPRP, those associations that were noteworthy at a very low level of prior probability were highly statistically significant.

Table 1. Gene variants included from the meta-analyses and pooled analyses.

Author	Year	Country	Gene	SNP
Xiao et al.	2014	China	*IL1B*	31T/C
Zhou et al.	2014	China	*ADAM33*	rs2280091, rs612709, rs3918396, rs511898, rs597980, rs2787094, rs2280090, rs2280089, rs528557
Hu et al.	2008	China	*GSTM1*	NULL
Zhong et al.	2010	China	*GSTP1*	rs1695
Van Durme et al.	2010	Belgium	*HHIP*	rs13118928
Chen et al.	2013	China	*IL13*	1112C/T
Dahl et al.	2005	Denmark	*SERPINA1*	PI*SZ, PI*MS
Castaldi et al.	2010	USA	*SOD3*	rs1799896
			TGFB1	rs1800470
			TNF	rs1800629
Cui et al.	2014	China	*CHRNA3/5*	rs1051730, rs6495309, rs8034191, rs16969968
Zhang et al.	2011	China	*TGFB1*	rs1982073
Yu et al.	2014	China	*COX2*	rs20417, rs689466
			MMP12	rs652438
Li et al.	2012	China	*ACE*	I/D
Niu et al.	2012	China	*ADRB2*	Arg16Gly, Glu27Gln
Li et al.	2013	China	*EPHX1*	A139G, T113C
Xue et al.	2012	China	*GSTT1*	NULL
Xie et al.	2014	China	*IL1B*	rs16944, rs1143634
Duan et al.	2014	China	*IL1RN*	VNTR,
			IL13	2044G/A
Zhou et al.	2013	China	*MMP1*	rs1799750
			MMP3	rs35068180
			MMP9	rs3918242
			MMP12	rs2276109
Ma et al.	2013	China	*SFTPB*	NA*
			SFTPD	NA
			SFTPA	NA
Cui et al.	2013	China	*TNF*	rs1800630
Gong et al.	2011	China	*TGFB1*	rs1800469, rs361525

*Not available. SNP = single nucleotide polymorphism.

As shown in Table 3, the pooled sensitivity, specificity, PLR, NLR, DOR, and AUC for the five true-positive variants were: 1) *ADAM33* rs612709, 0.28 (95%CI = 0.26-0.31), 0.72 (95%CI = 0.70-0.74), 0.90 (95%CI = 0.66-1.22), 1.10 (95%CI = 0.95-1.28), 0.81 (95%CI = 0.50-1.32), and 0.374; 2) *CHRNA3/5* rs1051730, 0.49 (95%CI = 0.48-0.50), 0.48 (95%CI = 0.48-0.49), 1.07 (95%CI = 1.05-1.09), 0.93 (95%CI = 0.88-0.98), 1.16 (95%CI = 1.11-1.21), and 0.538; 3) *CHRNA3/5* rs8034191, 0.52 (95%CI = 0.50-0.54), 0.60 (95%CI = 0.58-0.62), 1.16 (95%CI = 1.10-1.23), 0.84 (95%CI = 0.69-1.04), 1.41 (95%CI = 1.26-1.58), and 0.556; 4) *CHRNA3/5* rs16969968, 0.47 (95%CI = 0.45-0.49), 0.51 (95%CI = 0.50-0.52), 1.12 (95%CI = 1.07-1.18), 0.92 (95%CI = 0.77-1.09), 1.29 (95%CI = 1.16-1.45), and 0.543; and 5) *TGFB1* rs1800470, 0.62 (95%CI = 0.58-0.67), 0.47 (95%CI = 0.45-0.50), 0.99 (95%CI = 0.82-1.21), 1.06 (95%CI = 0.72-1.55), 0.94 (95%CI = 0.52-1.72), and 0.515.

Table 2. Overall statistically significant gene-variant chronic obstructive pulmonary disease (COPD) associations and false-positive report probabilities (FPRPs).

Gene	SNP	N	Cases	Controls	OR (95%CI)	P	Power		FPRP			
							OR = 1.20	OR = 1.50	P = 0.001		P = 0.000001	
									OR = 1.20	OR = 1.50	OR = 1.20	OR = 1.50
IL1B	31T/C	6	764	879	0.77 (0.63-0.94)	0.010	0.219	0.922	0.979	0.917	1.000	1.000
ADAM33	rs2280091	8	2112	3952	1.76 (1.27-2.43)	< 0.001	0.010	0.166	0.983	0.781	1.000	1.000
ADAM33	rs3918396	7	1436	3460	1.69 (1.23-2.32)	0.001	0.017	0.230	0.986	0.835	1.000	1.000
ADAM33	rs612709	6	1341	3359	0.6.(0.52-0.68)	< 0.001	< 0.001	0.050	< 0.001	< 0.001	< 0.001	< 0.001
ADAM33	rs511898	8	1600	3602	1.18 (1.02-1.38)	0.038	0.583	0.999	0.985	0.975	1.000	1.000
ADAM33	rs597980	2	296	2294	1.25 (1.05-1.48)	0.009	0.318	0.983	0.968	0.907	1.000	1.000
EPHX1	T113C	25	8259	42883	1.33 (1.06-1.69)	0.020	0.200	0.837	0.990	0.959	1.000	1.000
GSTM1	null	12	1697	1867	1.46 (1.16-1.83)	0.001	0.044	0.593	0.958	0.633	1.000	0.999
GSTP1	rs1695	15	2214	2666	1.56 (1.23-1.97)	< 0.001	0.014	0.371	0.932	0.336	1.000	0.998
HIP1	rs13118928	1	742	4976	0.60 (0.47-0.78)	< 0.001	0.007	0.216	0.950	0.386	1.000	0.998
IL1RN	VNTR	5	322	362	2.59 (1.02-6.58)	0.045	0.053	0.125	0.999	0.997	1.000	1.000
IL13	1112 C/T	8	1319	831	1.82 (1.14-2.92)	0.013	0.042	0.211	0.997	0.984	1.000	1.000
MMP12	rs652438	4	710	1652	1.62 (1.08-2.42)	0.018	0.071	0.354	0.996	0.981	1.000	1.000
SOD3	rs1799896	2	978	7604	1.97 (1.24-3.13)	0.004	0.018	0.124	0.996	0.971	1.000	1.000
SFTPA	NA*	3	383	369	1.65 (1.02-2.69)	0.045	0.101	0.351	0.998	0.992	1.000	1.000
TNF	rs1800630	4	606	622	0.73 (0.56-0.96)	0.024	0.172	0.742	0.993	0.970	1.000	1.000
TNF	rs1800629	27	4474	7338	1.19 (1.01-1.40)	0.036	0.540	0.997	0.985	0.973	1.000	1.000
CHRNA3/5	rs1051730	9	10466	39054	1.14 (1.10-1.18)	< 0.001	0.998	1.000	< 0.001	< 0.001	< 0.001	< 0.001
CHRNA3/5	rs8034191	5	2652	2565	1.29 (1.18-1.41)	< 0.001	0.056	1.000	< 0.001	< 0.001	0.266	< 0.001
CHRNA3/5	rs6495309	4	1977	2131	1.26 (1.09-1.45)	0.001	0.248	0.993	0.835	0.559	1.000	0.999
CHRNA3/5	rs16969968	3	1996	6463	1.27 (1.17-1.39)	< 0.001	0.109	1.000	0.002	< 0.001	0.660	0.175
TGFB1	rs1800470	5	1482	3774	0.73 (0.64-0.83)	< 0.001	0.022	0.917	0.067	0.002	0.986	0.628
TGFB1	rs1982073	10	1507	2542	0.82 (0.70-0.96)	0.014	0.421	0.995	0.970	0.932	1.000	1.000
COX2	rs20417	6	930	721	1.33 (1.06-1.67)	0.014	0.188	0.850	0.987	0.943	1.000	1.000
COX2	rs689466	2	230	275	1.48 (1.03-2.14)	0.037	0.132	0.528	0.996	0.986	1.000	1.000

*Not available. SNP = single nucleotide polymorphism.

Table 3. Diagnostic value of noteworthy variants after calculating false-positive report probabilities (FPRPs).

	Gene polymorphisms				
	ADAM33 rs612709	*CHRNA3/5* rs1051730	*CHRNA3/5* rs8034191	*CHRNA3/5* rs16969968	*TGFB1* rs1800470
Power					
OR = 1.20	<0.001	0.998	0.056	0.109	0.022
OR = 1.50	0.050	1.000	1.000	1.000	0.917
FPRP					
P = 0.001					
OR = 1.20	<0.001	< 0.001	<0.001	0.002	0.067
OR = 1.50	<0.001	< 0.001	<0.001	<0.001	0.002
P = 0.000001					
OR = 1.20	<0.001	0.266	1.000	0.660	0.986
OR = 1.50	<0.001	<0.001	<0.001	0.175	0.628
Sen	0.28 (0.26-0.31)	0.49 (0.48-0.50)	0.52 (0.50-0.54)	0.47 (0.45-0.49)	0.62 (0.58-0.67)
Spe	0.72 (0.70-0.74)	0.48 (0.48-0.49)	0.60 (0.58-0.62)	0.51 (0.50-0.52)	0.47 (0.45-0.50)
+LR	0.90 (0.66-1.22)	1.07 (1.05-1.09)	1.16 (1.10-1.23)	1.12 (1.07-1.18)	0.99 (0.82-1.21)
-LR	1.10 (0.95-1.28)	0.93 (0.88-0.98)	0.84 (0.69-1.04)	0.92 (0.77-1.09)	1.06 (0.72-1.55)
DOR	0.81 (0.50-1.32)	1.16 (1.11-1.21)	1.41 (1.26-1.58)	1.29 (1.16-1.45)	0.94 (0.52-1.72)
AUC	0.374	0.538	0.556	0.543	0.515

DOR = diagnostic odds ratio; AUC = area under the curve.

DISCUSSION

Increasing numbers of studies have suggested that certain candidate genes are involved in COPD risk, but individual studies have been limited by sample size and low reproducibility. Therefore, numerous meta- and pooled analyses have been published to assess the overall results of the association between the risk of COPD and genetic variants. Unfortunately, the results of meta- and pooled analyses can also contain false-positive findings. Liao et al. (2014) applied the FPRP methodology to evaluate nine positive associations between genetic variants and oral cancer risk included from meta- and pooled analyses, but none was noteworthy after calculation. However, no analogous study concerning COPD has been published. Therefore, we conducted the present FPRP analysis, and further investigated its diagnostic value. To our knowledge, this is the first and most comprehensive FPRP analysis conducted to assess the association between gene polymorphisms and COPD susceptibility.

In the present study, we found 32 variants reported to be positive in meta- and pooled analyses. Among those 32 mutations, 8 variants were found to be associated with decreased risk of COPD, while the other 24 variants were associated with an increased risk of COPD, and had ORs above 1.0. Five of the 32 polymorphisms were true-positives after we had calculated the FPRP: *ADAM33* rs612709 (case + control: 1341 + 3359), *CHRNA3/5* rs1051730 (case + control: 10,466 + 39,054), *CHRNA3/5* rs803419 (case + control: 2652 + 2565), *CHRNA3/5* rs16969968 (case + control: 1996 + 6463), and *TGFB1* rs1800470 (case + control: 1482 + 3774). Among those, *CHRNA3/5* rs1051730, *CHRNA3/5* rs8034191, and *CHRNA3/5* rs16969968 were associated with an increased risk of COPD, while *ADAM33* rs612709 and *TGFB1* rs1800470 were associated with decreased risk of COPD. ADAM proteins play a role in cell adhesion, cell signaling, cell fusion, and proteolysis (Primakoff and Myles, 2000). They abound in smooth muscle cells and may play a part in airway hyper-responsiveness. Thus, variants in this gene may be involved in COPD risk. *TGF-b1* plays a role in the deposition of extracellular matrix proteins such as collagens and fibronectin, as

well as other facets of fibrosis. Researchers have discovered that the airway epithelium tissues of smokers with COPD express more TGF-b1 than smokers without COPD (Vignola et al., 1997). Polymorphisms in the *TGFB1* gene may, therefore, be related to COPD development. Moreover, the chromosome 15q25 region includes the *CHRNA5-CHRNA3-CHRNB4* cluster, which encodes the subunits of alpha-nicotinic acetylcholine receptor (nAChR). These polymorphisms function by regulating the inflammatory response to smoking, and have an effect on nicotine dependence and smoking-related traits (Gwilt et al., 2007).

For the five true-positives, the results of diagnostic accuracy meta-analyses suggested that these polymorphisms do not have a high diagnostic value, since all the PLRs were less than 2.0, and the AUCs were less than 0.7. The explanations for this might be as follows. First, the number of articles included in our study was limited, which may have influenced the diagnostic value of the test results. Second, the pathogenesis of COPD remains unclear, with both genetic and environmental determinants. There may be interaction between the environment and genetic susceptibility, and mutual interaction between different SNPs as opposed to one single polymorphism alone. Third, the role one SNP plays may differ in different ethnicities, genders, etc. Fourth, heterogeneity is an important issue when explaining the results of the present study. Although the results suggested that the diagnostic value of candidate gene associations was not high, we could not completely rule out the possibility that these variants are not risk factors for COPD.

Several issues should be considered when interpreting the results. First, although we made a concerted effort, we could not rule out the possibility that we left out some studies owing to language limitations and other reasons. Second, our data were extracted from published meta- and pooled analyses. Some original studies might have been missed, and selection bias cannot be completely avoided. Third, only published studies were included, so publication bias may have occurred.

In conclusion, we found only five gene polymorphisms that were truly associated with COPD risk after evaluation of the FPRP value. Although we did not find practical diagnostic biomarkers from candidate gene association studies, the method is still valuable, and the results may guide future studies on COPD. Future studies are needed to clarify the etiology and the role genetic factors play in COPD.

Conflicts of interest

The authors declare no conflict of interest.

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