



# The protease inhibitor PI\*S allele and COPD: a meta-analysis

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**ABSTRACT:** In many countries, the protease inhibitor (SERPINA1) PI\*S allele is more common than PI\*Z, the allele responsible for most cases of chronic obstructive pulmonary disease (COPD) due to severe alpha 1-antitrypsin deficiency. However, the risk of COPD due to the PI\*S allele is not clear.

The current authors located studies that addressed the risk of COPD or measured lung function in individuals with the PI SZ, PI MS and PI SS genotypes. A separate meta-analysis for each genotype was performed.

Aggregating data from six studies, the odds ratio (OR) for COPD in PI SZ compound heterozygotes compared with PI MM (normal) individuals was significantly increased at 3.26 (95% confidence intervals (CI): 1.24–8.57). In 17 cross-sectional and case-control studies, the OR for COPD in PI MS heterozygotes was 1.19 (95%CI: 1.02–1.38). However, PI MS genotype was not associated with COPD risk after correcting for smoking. Furthermore, mean forced expiratory volume in one second, a measure of airflow obstruction and a defining feature of COPD, did not differ between PI MS and PI MM individuals. There were not enough cases to summarise the risk of COPD in PI SS homozygotes.

In conclusion, the results show that the PI SZ genotype is a significant risk factor for chronic obstructive pulmonary disease. The risk of chronic obstructive pulmonary disease due to the PI MS genotype is not substantially elevated.

**KEYWORDS:** Alpha 1-antitrypsin, chronic obstructive pulmonary disease, emphysema, heterozygote, meta-analysis

Severe alpha 1-antitrypsin (AAT) deficiency (OMIM 107400) is the most important known genetic risk factor for developing chronic obstructive pulmonary disease (COPD). AAT is a circulating serine protease inhibitor (PI) that protects lung parenchyma from the damaging effects of proteases, particularly neutrophil elastase. AAT is encoded by the PI locus (HUGO symbol; SERPINA1), located on chromosome 14q as part of a cluster of other serine protease inhibitor genes [1].

Severe AAT deficiency is usually caused by the presence of two copies of the mutant Z allele. In most populations in which allele frequencies have been measured, the S allele is the more common variant [2, 3]. The S mutation is thought to have arisen on the Iberian Peninsula, and has a different geographic distribution than the Z allele [4, 5]. In the USA, the S allele frequency is 2–4%, compared with 1–2% for the Z allele [2]. Only in northern European nations is the Z allele the more common variant, reflecting the historical origin of the mutation [3].

Initial studies on the PI SZ genotype reported an increased risk of COPD associated with this variant [6]. Other studies have suggested that the increased risk is confined to smokers only [7–10]. However, these studies were all lacking in control subjects (PI MM), making an accurate risk estimate difficult.

The S allele (Glu264Val) leads to a reduction in plasma AAT concentration, although not to the same degree as the Z allele [11, 12]. Consequently, the PI MS genotype is usually not considered to carry an elevated risk for COPD. The recent American Thoracic Society (ATS) statement on AAT deficiency does not mention PI MS heterozygosity as a possible risk factor for COPD [13].

The current authors recently published a meta-analysis in PI MZ heterozygous individuals, finding an increased risk of COPD (odds ratio (OR): 2.31; 95% confidence intervals (CI): 1.60–3.35) [14]. However, there was no difference in pulmonary function, measured as the forced

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expiratory volume in one second (FEV<sub>1</sub>), between PI MM and PI MZ individuals. The current authors now report a parallel analysis to examine the risk of COPD due to the PI\*S allele. The present study attempted to address the following questions: 1) Is there an increased risk of COPD in individuals with genotypes PI SZ, PI MS, or PI SS? and, if so, 2) What are the magnitudes of the increased risk?

## MATERIAL AND METHODS

### Study selection

Methods of the search strategy, study assessment and data analysis were similar to the present authors' previously published meta-analysis of PI MZ heterozygotes [14]. For the analysis of COPD risk due to the PI\*S allele, studies were identified through a search of MEDLINE, from January 1966 to May 2003, using the medical subject headings: alpha 1-antitrypsin, alpha 1-antitrypsin deficiency, protease inhibitors, obstructive lung diseases, chronic obstructive pulmonary disease, pulmonary emphysema, forced expiratory volume, respiratory function tests and spirometry. Bibliographies of identified articles and reviews were searched for additional references. M. Dahl and C.P. Hersh independently assessed studies for inclusion. Discussion with E.K. Silverman was used to resolve disagreements.

Case-control and cross-sectional studies using the categorical outcome of COPD, based on pulmonary function tests or a physician's diagnosis, and studies reporting FEV<sub>1</sub> per cent predicted as a continuous outcome measure were included. It was stipulated that PI type had to be determined using isoelectric focusing, acid starch gel with crossed immunoelectrophoresis, or a molecular genotyping method. In case-control designs, studies that used controls from a previously published report were included, as long as the cases and controls were from the same country and the same technique was used for assessment of PI type.

Exclusion criteria were as follows: 1) studies that inferred PI type from serum AAT levels, since genotypes could not be accurately determined; 2) family studies and studies of children only; 3) for the continuous outcome, studies that did not present FEV<sub>1</sub> as a per cent of the predicted value; and 4) duplicate analyses of the same population of cases.

### Study quality

The quality of each study was assessed using the following questions. 1) Was the phenotype of COPD defined by spirometry? 2) Were cases and controls in case-control studies or individuals with different genotypes in population-based studies matched on ethnicity? 3) Did the study control for cigarette smoking? 4) Did the authors test for Hardy-Weinberg equilibrium (HWE)?

The quality of a study was not a factor for inclusion, except when cases and controls were derived from different countries, as mentioned above. The quality questions were used to define subgroups of studies for further analysis.

### Data analysis

For studies that defined COPD using both spirometry and physician diagnosis, the spirometric definition was used. Using the categorical outcome of COPD, separate analyses

were performed for the PI SZ and PI MS genotypes. In each analysis, the summary effect estimate was calculated using the random effects method of DERSIMONIAN and LAIRD [15]. The Q-statistic was used to test for heterogeneity among studies.

Funnel plots and weighted regression were used to assess publication bias [16]. For the PI MS genotype, subgroup analyses were performed based on study design, use of spirometric criteria to define COPD, and adjustment for cigarette smoking. In a sensitivity analysis, each study was individually removed and the OR recalculated to determine the stability of the summary effect estimate.

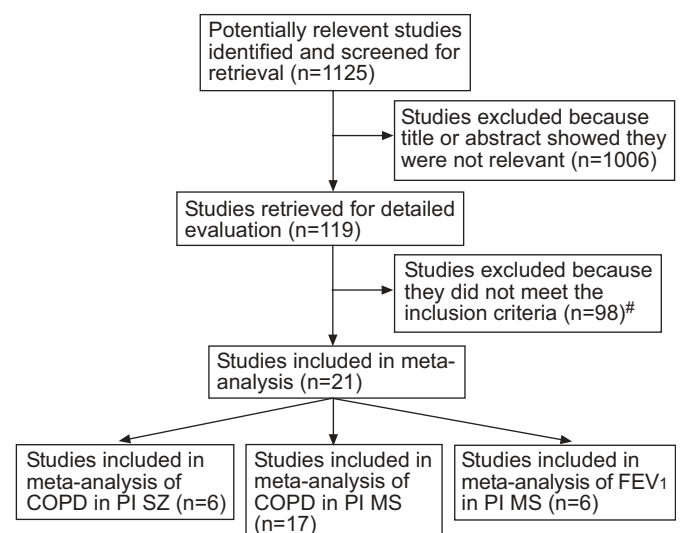
Studies that measured FEV<sub>1</sub> (% predicted) as a continuous outcome were analysed separately. A summary difference in mean FEV<sub>1</sub> between PI MM individuals and PI MS individuals was calculated, using the random-effects model [15].

## RESULTS

### Study eligibility

The literature search yielded 1,125 references, of which 119 were reviewed in detail (fig. 1). The 21 studies included in the meta-analysis are listed in tables 1–3 [12, 17–36]. Seventeen case-control or cross-sectional studies reported the categorical outcome of COPD in PI MS *versus* PI MM individuals; of these, six also included PI SZ individuals in the study. Seven studies included a total of 23 PI SS individuals, yet a total of only four affected PI SS individuals was found [12, 22, 24, 27–29, 34]. Due to the small numbers, an analysis of PI SS individuals was not performed. Six studies measured FEV<sub>1</sub> (% pred) as a continuous outcome in PI MS heterozygotes. Only one study measured FEV<sub>1</sub> in PI SZ and PI SS individuals, so a meta-analysis of the continuous outcome in PI SZ and PI SS subjects could not be performed [12].

Of the 119 papers retrieved for detailed evaluation, the most frequently excluded studies were those that examined COPD risk in PI MZ heterozygotes only (n=30; fig. 1) [37–66]. Other



**FIGURE 1.** Flow diagram of study selection for the meta-analyses. COPD: chronic obstructive pulmonary disease; PI: protease inhibitor; FEV<sub>1</sub>: forced expiratory volume in one second. #: see text for details of excluded studies.

**TABLE 1** Case-control studies of chronic obstructive pulmonary disease risk in protease inhibitor (PI) MS and PI SZ individuals

1st Author [ref.]	PFT diagnosis	Matched ethnicity	Adjusted for smoking	Cases		Controls	
				PI MS	PI SZ	PI MM	PI MM
FAGERHOL [22]	N	#	N	7/1/135	14/1/268		
TALAMO [35]	Y	N	N	8/--/84	8/--/94		
KUEPPERS [26]	Y	#	N	12/--/138	6/--/193		
BARNETT [18]	Y	Y	N	6/--/87	5/--/81		
COX [21]	N	Y	N	7/--/101	57/--/644		
KUEPPERS [27]	Y	N	Y	5/--/97	8/--/98		
LOCHON [29]	N	#	N	2/--/21	25/--/231		
ABBOUD [17]	Y	N	N	5/1/42	0/0/26		
BARTMANN [19]	N	#	N	34/18/429	42/1/583		
LIEBERMAN [28]	N	Y	N	75/2/595	110/5/1213		
POLLER [33]	N	#	N	9/--/137	10/--/130		
SANDFORD [34]	Y	Y	Y	16/--/163	7/--/66		

Data presented as n, unless otherwise stated. PFT: pulmonary function test; N: no; Y: yes. #: studies conducted in European nations where ethnic homogeneity is likely.

excluded studies lacked an appropriate control group (n=13) [6–10, 67–74]; measured PI type by a suboptimal method (n=13) [74–86]; were reviews, editorials or conference proceedings (n=10) [79, 87–95]; measured AAT level as a proxy for genotype (n=9) [85, 87, 96–102]; grouped together multiple variant genotypes (n=8) [103–110]; used radiographic outcomes or pulmonary function tests other than FEV<sub>1</sub> (n=6) [111–116]; did not report FEV<sub>1</sub> as a per cent of predicted (n=4) [103, 107, 117, 118]; defined COPD based on self-report or based on autopsy findings (n=4) [117, 119–121]; studied families or children only (n=4) [106, 122–124]; assessed other rare alleles (n=3) [94, 125, 126]; analysed the same cases as another study (n=2) [127, 128]; or reported allele frequencies rather than genotypes (n=1) [129]. Some studies were excluded for more than one reason. Of the four studies that reported FEV<sub>1</sub> in absolute volumes, rather than as a per cent predicted, only one would not have been excluded by other criteria [118]. Therefore, a separate analysis of these papers could not be performed.

**TABLE 2** Cross-sectional studies of chronic obstructive pulmonary disease (COPD) risk in protease inhibitor (PI) MS and PI SZ individuals

1st Author [ref.]	PFT diagnosis	Matched ethnicity	Adjusted for smoking	PI MS COPD/ Total PI MS	PI SZ COPD/ Total PI SZ	PI MM COPD/ Total PI MM
Matzen [30]	N	N	N	6/60		27/427
Chan-Yeung [20]	Y	Y	Y	7/89		98/1006
Ostrow [32]	Y	N	Y	1/26		13/330
Gulsvik [24]	N	#	N	14/60	1/3	123/1054
Dahl [12]	Y	#	Y	73/456	4/10	1053/7018

Data presented as n, unless otherwise stated. PFT: pulmonary function test; N: no; Y: yes. #: studies conducted in European nations where ethnic homogeneity is likely.

### Quality determination

Tables 1–3 list the characteristics of the 21 included studies. In nine studies, COPD was defined by explicit pulmonary function criteria [12, 17, 18, 20, 26, 27, 32, 34, 35]. A physician's diagnosis was used in the other eight categorical studies. The clinical assessment may have included spirometry, but the authors did not provide specific diagnostic criteria.

Of the 21 studies in the analyses, 13 were conducted in the USA and Canada, two nations with ethnically diverse populations. To control for this, five of these studies restricted their analyses to Caucasians only [20, 21, 28, 31, 34], and one study matched cases and controls by ethnicity [18]. Eight studies were done in Europe [12, 19, 22–24, 26, 29, 33]. Ethnic diversity is presumed to be less in these populations, although none of the authors specifically addressed this issue.

Seven studies adjusted for cigarette smoking, using a variety of epidemiological methods [12, 20, 23, 25, 27, 32, 34]. KUEPPERS *et al.* [27] matched cases and controls by their smoking histories, *i.e.* nonsmokers, moderate smokers and heavy smokers. GIRARD *et al.* [23] matched by smoking status as well. The study by HALL *et al.* [25] was restricted to nonsmokers. OSTROW *et al.* [32] stratified subjects by smoking status (smokers *versus* nonsmokers), while CHAN-YEUNG *et al.* [20] used three strata (nonsmokers, ex-smokers and current smokers). The studies by SANDFORD *et al.* [34] and DAHL *et al.* [12] used logistic regression to adjust for smoking history.

In only one paper, the authors reported testing for HWE [12]. In this study, the observed genotype frequencies were consistent with HWE. The majority of papers fulfilled two or fewer of the four pre-determined quality criteria. The studies by CHAN-YEUNG *et al.* [20] and SANDFORD *et al.* [34] each met three criteria, and the study by Dahl *et al.* [12] was the only one to contain all four quality measures.

### PI SZ compound heterozygotes

The six case-control and cross-sectional studies that examined COPD risk in PI SZ individuals are shown in tables 1 and 2. In the pooled analysis (fig. 2), there were a total of 42 PI SZ compound heterozygotes, of whom 27 had COPD. The summary OR for COPD in PI SZ individuals compared with PI MM was significantly elevated at 3.26 (95% CI: 1.24–8.57). Although formal testing was not statistically significant (Q test: Chi-squared (5 degrees of freedom): 6.87; p=0.23), clinical heterogeneity was still possible among these observational

**TABLE 3** Studies measuring forced expiratory volume in one second (FEV<sub>1</sub>) as a percentage of predicted in protease inhibitor (PI) MS heterozygotes

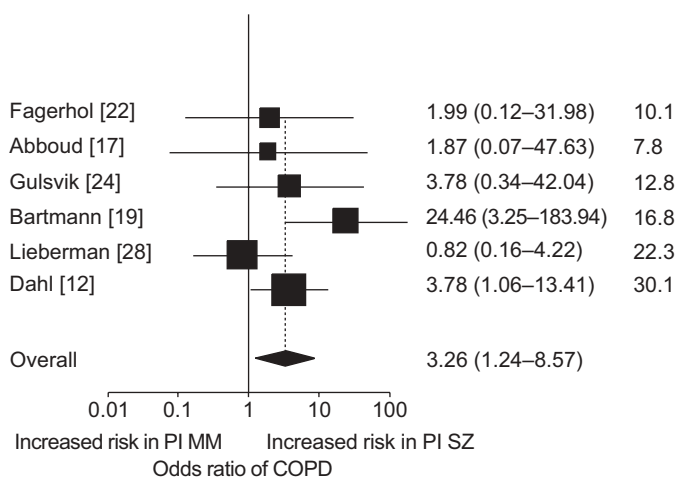
1st Author [ref.]	Matched ethnicity	Adjusted for smoking	PI MS	Mean FEV <sub>1</sub> % pred	PI MM	Mean FEV <sub>1</sub> % pred
Webb [36]	N	N	30	101	395	92
Hall [25]	N	Y	14	105	14	107
Morse [31]	Y	N	208	98.2	2637	96.0
Girard [23]	#	Y	39	95.1	39	92.2
Ostrow [32]	N	Y	14 <sup>+</sup>	99.5	104 <sup>+</sup>	98.9
			12 <sup>§</sup>	97.7	226 <sup>§</sup>	92.2
Dahl [12]	#	N <sup>†</sup>	459	90	7037	90

Data presented as n, unless otherwise stated. N: no; Y: yes. #: studies conducted in European nations where ethnic homogeneity is likely. †: FEV<sub>1</sub> data not adjusted for smoking; +: nonsmokers; §: smokers.

studies. Therefore, a random effects model was used for the analysis. The funnel plot of OR *versus* standard error was symmetric, and Egger's test was negative for publication bias (p=0.98). In the sensitivity analysis, removing the BARTMANN *et al.* [19] study from the meta-analysis reduced the OR to a value that was not significantly >1. However, the new OR remained within the 95% CI of the overall effect estimate shown in figure 2. Removing any other study did not substantially change the summary OR. Due to the small number of individuals and studies, no subgroup analyses were performed.

**PI MS heterozygotes**

Twelve case-control and five cross-sectional studies examined risk of COPD in PI MS heterozygotes compared with PI MM individuals (tables 1 and 2). Only three studies showed a significantly increased risk for PI MS heterozygotes [24, 26, 28]. The pooled analysis (fig. 3) found a small, but significant elevation in risk (OR: 1.19, 95% CI: 1.02–1.38). Heterogeneity among studies was unlikely (Q test: Chi-squared (16 degrees of freedom): 14.82; p=0.54).

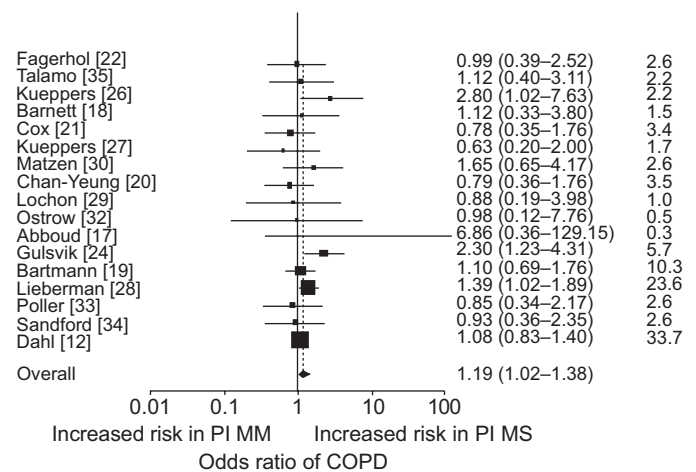


**FIGURE 2.** Cross-sectional and case-control studies reporting risk of chronic obstructive pulmonary disease (COPD) in protease inhibitor (PI) SZ compound heterozygotes. Box sizes are proportional to inverse-variance weights. Lines represent 95% confidence intervals (CI). Three columns of data represent study, OR (95% CI) and % weight, respectively.

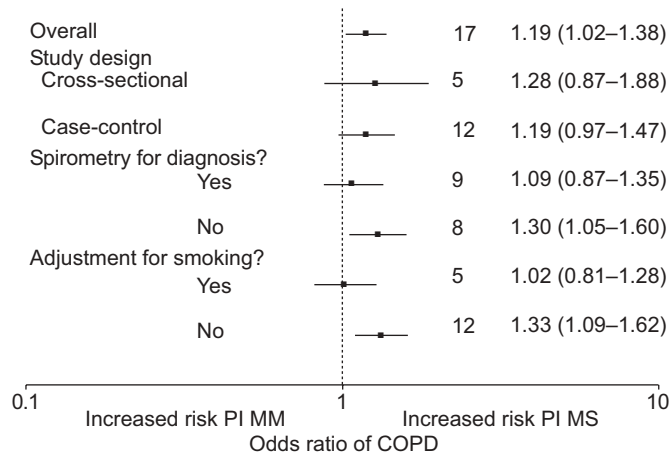
Subgroup analyses of the PI MS categorical studies are shown in figure 4. The five cross-sectional studies had a summary OR of 1.28 (95% CI: 0.87–1.88), similar to the summary OR of 1.19 (95% CI: 0.97–1.47) in the 12 case-control studies. The effect estimate (OR) for the studies that used spirometry to define COPD was 1.09 (95% CI: 0.87–1.35) and for the studies that adjusted for cigarette smoking was 1.02 (95% CI: 0.81–1.28).

The funnel plot of OR *versus* standard error showed fewer studies than expected with an OR higher than the summary measure. Publication bias would be expected to have the opposite effect, studies with higher OR might be more frequently published. Egger's test showed no statistical evidence of publication bias (p=1.0). The summary OR was not significantly changed when each study was individually removed in the sensitivity analysis.

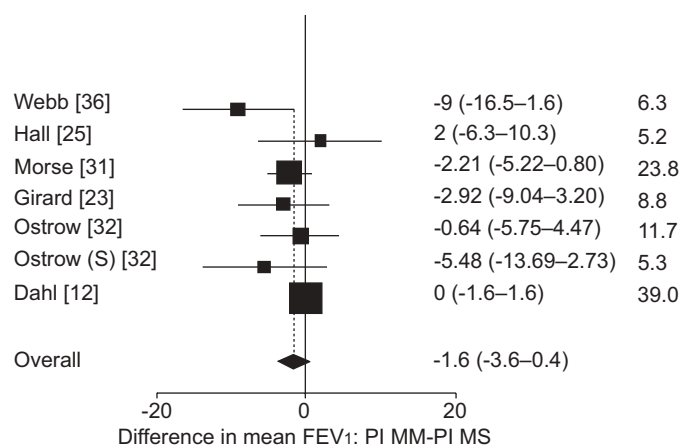
Table 3 details the six studies that measured FEV<sub>1</sub> (% pred) in both PI MS and PI MM individuals. The study by OSTROW *et al.* [32] reported mean FEV<sub>1</sub> values stratified by smoking status.



**FIGURE 3.** Cross-sectional and case-control studies of chronic obstructive pulmonary disease (COPD) risk in protease inhibitor (PI) MS heterozygotes. Box sizes are proportional to inverse-variance weights. Lines represent 95% confidence intervals (CI). Three columns of data represent study, OR (95% CI) and % weight, respectively.



**FIGURE 4.** Subgroup analysis of cross-sectional and case-control studies reporting risk of chronic obstructive pulmonary disease (COPD) in protease inhibitor (PI) MS heterozygotes. Three columns of data represent subgroup, studies (n) and odds ratio (95% confidence intervals), respectively.



**FIGURE 5.** Studies reporting spirometry in protease inhibitor (PI) MS heterozygotes. Mean difference is calculated by subtracting the mean forced expiratory volume in one second (FEV<sub>1</sub>; % predicted) in protease inhibitor (PI) MS individuals from the mean FEV<sub>1</sub> in PI MM individuals. Box sizes are proportional to inverse-variance weights. Lines represent 95% confidence intervals (CI). Three columns of data represent study, difference in mean FEV<sub>1</sub> (95% CI) and % weight, respectively.

Each stratum was entered separately into the pooled analysis. The meta-analysis of difference in mean FEV<sub>1</sub> (% pred) between PI MM and PI MS individuals is shown in figure 5. The mean FEV<sub>1</sub> (% pred) was lower in the PI MS heterozygote group in only one of these studies [25]. Overall, there was no difference in mean FEV<sub>1</sub> (% pred) between PI MM and PI MS individuals (difference in mean FEV<sub>1</sub>: PI MM-PI MS=-1.61 % pred; 95% CI: -3.59–0.38). Heterogeneity was possible among these studies (Q test: Chi-squared (6 degrees of freedom): 8.56; p=0.20).

The funnel plot of difference in mean FEV<sub>1</sub> versus standard error revealed fewer than expected studies finding a greater mean FEV<sub>1</sub> in PI MM individuals. Egger's test did not reveal

significant evidence of publication bias (p=0.14). In the sensitivity analysis, removing the study by DAHL *et al.* [12] led to a lower mean FEV<sub>1</sub> in PI MM as compared with PI MS individuals.

## DISCUSSION

In a meta-analysis of studies examining COPD in PI SZ compound heterozygotes, a three-fold elevation in COPD risk due to the PI SZ genotype was found. The CI was broad, but the increased OR was statistically significant. Due to the lack of available data, an analysis of mean FEV<sub>1</sub> in PI SZ individuals or an analysis of PI SS homozygotes could not be performed. In a meta-analysis of cross-sectional and case-control studies, a small, but statistically significant increase in risk for COPD amongst PI MS heterozygotes was found. In subgroup analyses aggregating studies that adjusted for smoking or defined COPD based on spirometry, the risk due to the PI MS genotype was not significantly elevated. In addition, studies measuring pulmonary function did not reveal a difference in FEV<sub>1</sub> between PI MS and PI MM individuals.

The finding of a three-fold elevated risk in the PI SZ individuals is not surprising, since the PI SZ genotype has been considered a risk factor for COPD, based on case series and registry data. However, the recent ATS statement on AAT deficiency concludes that the PI SZ genotype only confers an elevated risk in cigarette smokers, and that the risk in nonsmokers is not increased [13]. Due to the limited number of subjects with smoking information, it was not possible to calculate separate OR's for PI SZ smokers and nonsmokers. Additional research is warranted with careful characterisation of phenotype and smoking history in the PI SZ study subjects.

After correction for smoking, PI MS was not associated with COPD risk. Nevertheless, the discrepancy in the results found in the analyses of overall COPD risk and FEV<sub>1</sub> in PI MS individuals requires further investigation. The potential risk increase found in the categorical studies was not corroborated by the FEV<sub>1</sub> results. If all PI MS individuals were at an increased risk for COPD, then a lower lung function among these individuals as a group would be expected. Airflow obstruction, defined as a reduction in FEV<sub>1</sub>, is a hallmark of COPD and is central to most diagnostic criteria [130]. The different results for the analyses of COPD risk and of FEV<sub>1</sub> could be consistent with an increased risk only in a subgroup of PI MS individuals. Due to the different methods used to control for smoking in the various studies, this hypothesis could not be formally tested. Alternatively, the discrepancy in results could reflect a small risk increase in all PI MS individuals, which was not detected in the spirometry studies, due to differences in subject ascertainment or sampling, incomplete adjustment for smoking status, or biased estimates in the case-control studies, due to such factors as population stratification.

Although statistical testing did not reveal evidence of publication bias, the funnel plots in both PI MS analyses showed asymmetry, with a deficiency of studies with a stronger effect estimate than the pooled OR. The small numbers of studies in these analyses most likely explains this finding. A study that found an increased risk of COPD or lower lung

function in PI MS heterozygotes would not be expected to go unreported.

The small number of studies reporting FEV<sub>1</sub> in PI MS individuals is partly a result of the current authors' inclusion criterion that required studies to report FEV<sub>1</sub> as a percentage of the predicted value. This requirement was intended to control for potentially important covariates in the measurement of lung function, since commonly used prediction equations account for age, sex, and height [131]. There were not enough available studies reporting FEV<sub>1</sub> in absolute volumes to perform a separate meta-analysis. It is under speculation as to whether differences in FEV<sub>1</sub> should be adjusted for COPD diagnosis. Unfortunately, none of the authors presented the FEV<sub>1</sub> levels stratified by COPD diagnosis, which would be necessary to perform an adjusted analysis. However, as most of the studies were community-based studies (evidenced by the normal mean FEV<sub>1</sub> levels in these studies) it is unlikely that removing the minority of individuals with COPD will change the overall results.

The overall study quality was found to be quite variable. Although this could be a potential limitation to the present analysis, it is an inevitable consequence of pooled analyses of observational studies. All meta-analyses are subject to limitations due to the methods used in the original studies. Without data on cigarette smoking in each enrolled individual, it could not be assured that the studies adequately addressed this important risk factor. Consequently, the pooled analysis may be subject to residual confounding or an unmeasured genotype-by-environment interaction. None of the studies explicitly addressed population stratification, so the meta-analysis could not be protected from spurious results arising from stratification within the individual studies.

In the analyses of the PI MS genotype, the COPD risk was not increased in the minority of studies that controlled for smoking, but this does not completely exclude the possibility that there may be an increased risk of COPD in at least a subset of PI MS individuals. With an estimated 16 million PI MS carriers in the USA and 26 million in Europe [132], the overall impact on COPD may not be trivial. Further study is warranted to confirm whether this increased risk may be present.

It was found that the risk of COPD is approximately tripled in PI SZ compound heterozygotes. The risk increase in PI SZ individuals was higher than the risk previously estimated for PI MZ heterozygotes (OR: 2.31) [14], although the confidence intervals overlapped. However, because of the rarity of the PI SZ genotype, its overall effect on COPD risk in the population is likely to be small.

In conclusion, due to the small numbers of protease inhibitor SZ compound heterozygotes sampled in individual studies, further research to determine risk in subgroups (e.g. cigarette smokers) will require more focused sampling of protease inhibitor SZ subjects. Protease inhibitor SZ individuals who come to attention through symptoms or screening should be advised that they are at an increased risk for the development of chronic obstructive disease, although not to the same degree as protease inhibitor ZZ individuals.

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