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EPHX1 polymorphisms, COPD and asthma in 47,000 individuals and in meta-analysis

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ABSTRACT: We tested the hypothesis that two well-characterised functional polymorphisms of the microsomal epoxide hydrolase gene (*EPHX1*), *T113C* and *A139G*, may influence susceptibility to chronic obstructive pulmonary disease (COPD) and asthma.

We genotyped participants from the Copenhagen City Heart Study (n=10,038) and the Copenhagen General Population Study (n=37,022) for the *T113C* and *A139G* variants in the *EPHX1* gene and measured lung function and recorded COPD hospitalisation and asthma and smoking history. Finally, we meta-analysed results from 19 studies including 7,489 COPD cases and 42,970 controls.

The OR for spirometry-defined COPD or COPD hospitalisation did not differ from 1.0 for any of the *EPHX1* genotypes or phenotypes overall, or in smokers or nonsmokers separately (p-value for trend 0.18–0.91). Likewise, *EPHX1* genotypes or phenotypes did not associate with risk of asthma (p-value for trend 0.46–0.98). In meta-analysis, random effects OR for COPD in *T113C* heterozygotes and homozygotes *versus* non-carriers were 1.17 (0.99–1.38) and 1.38 (1.09–1.74), respectively. Corresponding values for *A139G* were 0.93 (0.83–1.05) and 0.89 (0.78–1.02).

Our results indicate that genetically reduced microsomal epoxide hydrolase activity is not a major risk factor for COPD or asthma in the Danish population; however, meta-analysis cannot completely exclude a minor effect on COPD risk.

KEYWORDS: Chronic obstructive pulmonary disease, genetics, lung function, meta-analysis, microsomal epoxide hydrolase, tobacco smoking

Chronic obstructive pulmonary disease (COPD) is a complex disease characterised by airflow limitation that is not fully reversible. This is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases. The clinical manifestations include chronic bronchitis, emphysema and small airway disease with many patients suffering from all three [1]. Tobacco smoking is widely accepted as the most common risk factor for COPD [1, 2]. However, only a fraction of smokers (10–15%) develop the disease [3]. This, together with the familial clustering of early-onset COPD [4] as well as susceptibility to frequent exacerbations in these individuals [5], strongly indicates a genetic aspect in the pathogenesis of COPD.

The microsomal epoxide hydrolase gene, *EPHX1*, is a good candidate for several reasons. First, it is strongly expressed in the lung, but downregulated in COPD [6]. Secondly, the enzyme product has an established role in the detoxification of smoking-induced reactive substances which may cause oxidative stress [7]. Finally, there are two well-characterised variants of the *EPHX1* gene which have been shown to alter enzyme activity considerably [8]. This might account for some of the variety in susceptibility to COPD among smokers.

In the present study, we used data from the Copenhagen City Heart Study, Copenhagen, Denmark (n=10,038) and the Copenhagen General Population Study, Copenhagen, Denmark (n=37,022) to test the hypotheses that genetically altered microsomal epoxide hydrolase activity is associated with risk of COPD and that this relationship could depend on smoking history. Since asthma is another manifestation of chronic pulmonary inflammation, we also tested for association with risk of asthma. Finally, we meta-analysed present and previous studies on risk of COPD.

MATERIAL AND METHODS

Study cohort

In this population-based cross-sectional study, we studied randomly selected white individuals of Danish descent (n=47,060) consisting of participants from two very similar cohorts, the

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Copenhagen City Heart Study (n=10,038) and the Copenhagen General Population Study (n=37,022) [9–11]. In brief, the Copenhagen City Heart Study is a prospective cardiopulmonary study of the Danish general population initiated in 1976–1978, with follow-up examinations in 1981–1983, 1991–1994 and 2001–2003; DNA was taken in 1991–1994 and 2001–2003. The Copenhagen General Population Study is a cross-sectional study of the Danish general population initiated in 2003 and still recruiting. Individuals were randomly selected based on the national Danish Civil Registration System to reflect the Copenhagen general population aged 20–80 yrs and older. Information on diagnoses of COPD (World Health Organization International Classification of Diseases 8th (ICD-8) and 10th (ICD-10) edition, ICD-8 codes 491–492 and ICD-10 codes J41–J44) was collected in the national Danish Patient Registry and the national Danish Causes of Death Registry. All subjects answered similar questionnaires and had objective and clinical parameters measured by the same methods.

The studies were approved by Herlev Hospital (Copenhagen, Denmark) and Danish ethical committees and were conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

Genotyping

Taqman assays analysed on the ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA) were used to genotype two polymorphisms in the *EPHX1* gene (*T113C* (rs1051740) and *A139G* (rs2234922)). These two single nucleotide polymorphisms (SNPs) were chosen because they are functional and have previously been investigated in relation to the end-points under investigation in the current study. Furthermore, these two SNPs tag the entire coding region of the *EPHX1* gene [12]. Since we performed reruns twice, call rates were 99.98% for both polymorphisms. Genotyping was verified by DNA sequencing (MegaBase, Pharmacia, Uppsala, Sweden).

Other covariates

Participants filled out a questionnaire stating information about their current and previous diseases and use of medication. Participants were divided based on their smoking history into ''never-smokers'', ''light smokers'' (<15 pack-yrs), or ''heavy smokers'' (⩾15 pack-yrs); 15 was the median of the cumulated pack-yrs.

End-points

Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were determined using a dry-wedge spirometer (Vitalograph, Maids Moreton, Buckinghamshire, UK) in the Copenhagen City Heart Study and using an EasyOne Spirometer (ndd Medizintechnik, Zürich, Switzerland) in the Copenhagen General Population Study. Triplicates with the two best measurements differing by <5% were obtained, and the best results were used in the analyses. Reference values were derived for males and females separately in the two studies, based on all participants regardless of smoking history [11]. COPD was defined as $FEV_1/FVC < 0.7$ and $FEV_1 < 80\%$ of the predicted value, excluding those with self-reported asthma.

Information about COPD hospitalisation was obtained by linking the participants to the national Danish Patient Registry and the national Danish Causes of Death Registry, using each participant's unique Central Person Register number [9, 10]. The COPD diagnoses were defined according to World Health Organization International Classification of Diseases, 8th and 10th edition (ICD-8: 491–492; ICD-10: J41–J44).

Asthma diagnoses were based on the following three questions. Asthma: ''Do you have asthma?''; asthma medication: ''Do you take medication for asthma/bronchitis daily or almost daily?''; and allergic asthma: ''Do foodstuffs, medicine, grass, flowers, animal hair, or other things give you asthma?''.

Statistical analyses

We used STATA/SE 10.1 (Stata Corporation, College Station, TX, USA) for all statistical analyses, except for the power calculations which were performed with the NCSS/PASS software (NCSS, Kaysville, UT, USA). We used the event rate in a two-sided hypothesis test at $\alpha=0.05$ and $\beta=0.10$ to calculate the minimum effect size that we had 90% power to detect in our study. For trend tests, individual genotypes were coded 0–2 with homozygosity for the common allele as the reference. Based on the predicted microsomal epoxide hydrolase activity as described by HASSETT *et al.* [8], participants were divided into four groups encoded 1–4 (''Fast'' to ''Superslow''; Fast: *113TT/139AG*, *113TT/139GG*, *113TC/139GG*; Normal: *113TT/139AA*, *113TC/139AG*, *113CC/139GG*; Slow: *113TC/139AA*; Superslow: *113CC/139AG*, *113CC/139AA*). We chose to analyse these phenotype groupings to maximise statistical power and to simplify data presentation and interpretation; however, if we examined each of the nine genotype combinations separately, results were similar to those presented for the four phenotype groupings.

We first analysed the relationship of *EPHX1* genotype and phenotype with FEV_1 % predicted. In the subset of individuals from the Copenhagen City Heart Study, we analysed the relationship of *EPHX1* genotype and phenotype with decline in FEV_1 ($mL \cdot yr^{-1}$). Analyses were performed by ANOVA adjusted for age, sex and pack-yrs.

We then analysed the relationship between *EPHX1* genotype and phenotype and risk of COPD or asthma by logistic regression adjusted for age, sex and pack-yrs.

Meta-analyses

We searched PubMed and Embase querying for ''(COPD [MeSH] OR pulmonary emphysema [MeSH] OR COPD OR chronic obstructive pulmonary disease [All fields]) AND (EPHX1 OR microsomal epoxide hydrolase)'' using MeSH/EMTREE terms and free text. 19 studies (the present study and [2, 7, 13–28]) investigating the *EPHX1 T113C* and *A139G* polymorphisms in relation to COPD or emphysema as the primary end-point were included. Two minor studies were excluded from the analyses, because we were unable to retrieve the original papers despite contacting the corresponding authors.

Meta-analyses were performed using random and fixed effect models in STATA/SE 10.1 (Stata Corporation). Weights were calculated under fixed effect models using the inverse variance method. We also calculated summary odds ratios for Caucasians and Asians separately. Funnel plots and Egger's regression test were used to search for publication bias.

TABLE 1 Characteristics of participants by chronic obstructive pulmonary disease (COPD) status

	No event	COPD	
		Spirometry-defined	Hospitalisation
Individuals n	37964	4127	2730
Females %	54	49	53
Age yrs	58 (47–68)	68 (60–75)	69 (62–76)
Smoking %			
Never-smokers	38	13	6
Light smokers	34	27	23
Heavy smokers	29	60	71
Pack-yrs	12 (3–27)	26 (12–42)	30 (15–45)

Data are presented as median (interquartile range), unless otherwise stated.

RESULTS

Clinical characteristics of the participants are presented according to COPD status in table 1. Of the 47,060 participants, 37,964 were free of COPD, 4,127 had COPD according to spirometry and 2,730 were hospitalised due to COPD (967 individuals had both COPD on spirometry and were hospitalised for COPD). As expected, both individuals with spirometry-defined COPD and COPD hospitalisation were older and more likely to be heavy smokers than individuals without COPD. The proportion of females in each group was similar (54% in individuals without COPD *versus* 49% and 53% for spirometry-defined COPD and COPD hospitalisation, respectively). Of the 47,060 subjects, 33,255 were free of asthma, 3,216 reported asthma, 3,312 reported using asthma medication on a daily or almost daily basis, and 3,754 reported allergic asthma (table 1 in the online supplementary material); there were large overlaps among the three asthma groups. Genotype frequencies were in accordance with the Hardy–Weinberg equilibrium and corresponded well with those previously reported for Caucasians [7, 13]. The binding of primers and probes was not influenced by the codon119 polymorphism (sequences reported in table 2 in the online supplementary material) [29]. Since we performed reruns three times, call rates were 99.98% for both polymorphisms. Control sequencing showed 100% concordance with the TaqMan genotyping results.

FEV1 % pred

The *T113C* polymorphism and *EPHX1* phenotype were not associated with FEV1 % pred (p-value for trend was 0.61 and 0.49, respectively; fig. 1a). The p-value for trend for the 139A>G polymorphism reached statistical significance (p=0.03); however, this result could not be confirmed when analysing *EPHX1* phenotype or other COPD-related traits, and we therefore interpret this as likely a spurious result.

EPHX1 phenotype was not associated with FEV1 % pred when stratified by smoking status (p-value for trend 0.53–0.83; fig. 2a).

Decline in lung function

The observed mean values of lung function decline for the reference genotypes and phenotype were 29–30 mL·yr^{-1}. *EPHX1* genotypes or phenotypes were not associated with decline in lung function in the subset of subjects from the Copenhagen City Heart Study (p-value for trend 0.64–0.71; fig. 1b).

On visual inspection, there appeared to be a trend towards increased decline in lung function with predicted decreased microsomal epoxide hydrolase activity among heavy smokers, but the trend was not statistically significant (p-value for trend 0.22; fig. 2b).

Risk of COPD

The OR for spirometry-defined COPD did not differ from 1.0 for any of the *EPHX1* genotypes or phenotypes (p-value for trend 0.34–0.85; fig. 3). Likewise, OR for hospitalisation due to COPD did not differ from 1.0 for any of the *EPHX1* genotypes or phenotypes (p-value for trend 0.24–0.70); we had statistical power to detect a 20–30% increase in risk of COPD for all individual genotypes and phenotypes overall (figs 2 and 3 in the online supplementary material).

After stratification by smoking status, OR for spirometry-defined COPD and COPD hospitalisation did not differ from 1.0 for any of the *EPHX1* phenotypes in either category of

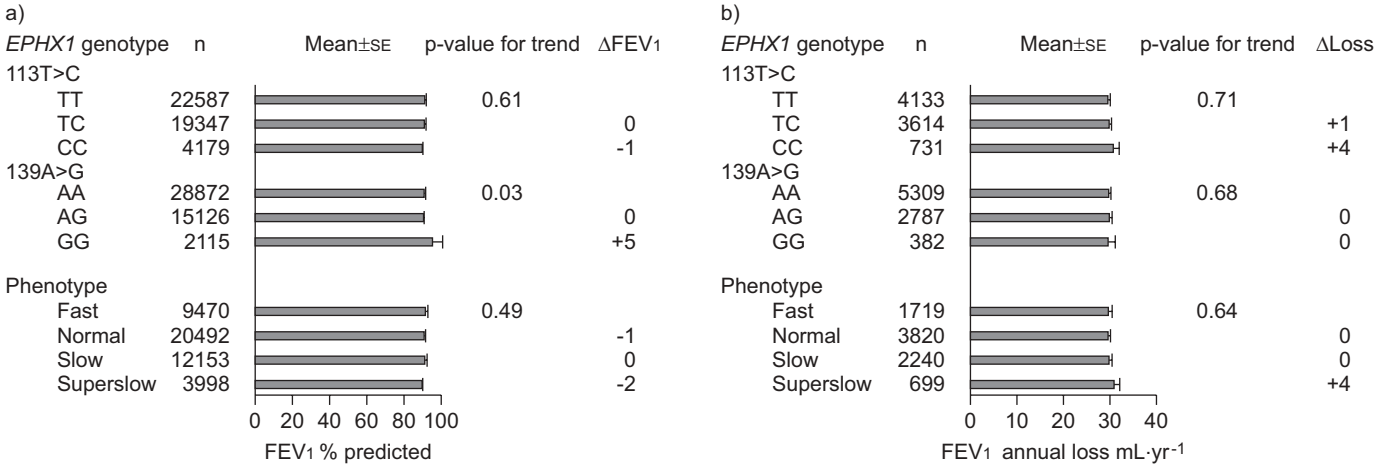

FIGURE 1. Levels of a) forced expiratory volume in 1 s (FEV1) % predicted and b) annual loss in FEV1 according to *EPHX1* genotype and enzyme activity phenotype.

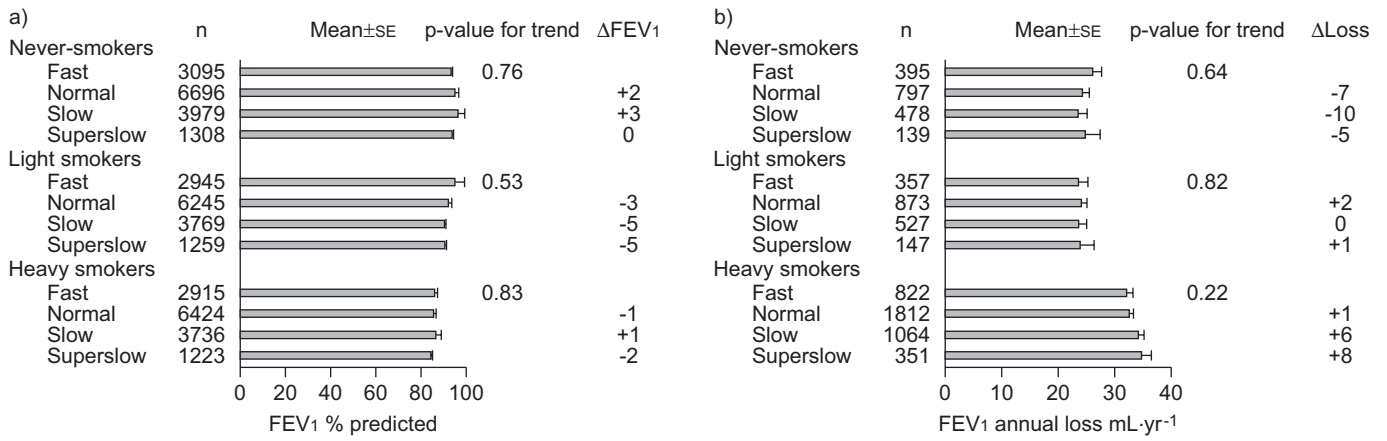

FIGURE 2. Levels of a) forced expiratory volume in 1 s (FEV_1) % predicted and b) annual loss in FEV_1 according to *EPHX1* enzyme activity phenotype, stratified by smoking history. Light smokers: <15 pack-yrs; heavy smokers: ⩾15 pack-yrs.

smokers (p-value for trend 0.18–0.47; fig. 4). Among never-smokers, the OR for hospitalisation due to COPD did deviate from 1.0 (0.60, 95% CI 0.40–0.88); however, this result is not biologically plausible in never-smokers, and the finding could not be confirmed when analysing spirometry-defined COPD. Therefore, this finding likely represents a spurious result.

Meta-analyses

The overall OR (95% CI) for COPD using random effect models were 1.17 (0.99–1.38) in *T113C* heterozygotes and 1.38 (1.09–1.74) in homozygotes *versus* non-carriers (fig. 5). The corresponding ORs were 0.93 (0.83–1.05) in *A139G* heterozygotes and 0.89 (0.78–1.02) in homozygotes (fig. 6). ORs obtained using fixed effect models as well as stratified for ethnicity are also shown in figures 5 and 6.

Risk of asthma

The OR for asthma by definition of asthma medication use was increased for the superslow phenotype (1.21, 95% CI 1.02–1.44; fig. 1 in the online supplementary material). However, this finding was not replicated in the analyses of self-reported asthma or allergic asthma, and it is therefore likely a spurious result. None of the risk estimates for *EPHX1* genotypes or other phenotypes differed from 1.0 in any of the three asthma categories (p-value for trend 0.46–0.98, 0.17–0.98, 0.75–0.82, respectively).

DISCUSSION

With COPD on the rise as a leading cause of morbidity and mortality worldwide, much interest has been focused on uncovering the underlying mechanisms and, thus, potential therapeutic targets. It seems clear that many genetic factors may influence an individual's susceptibility to COPD. A handful of studies have focused on the *EPHX1* gene and especially two common polymorphisms, which reportedly alter enzyme activity [8]. Although the involvement of *EPHX1* in COPD seems plausible, reports on the effect of these polymorphisms on COPD risk have been inconsistent. Our

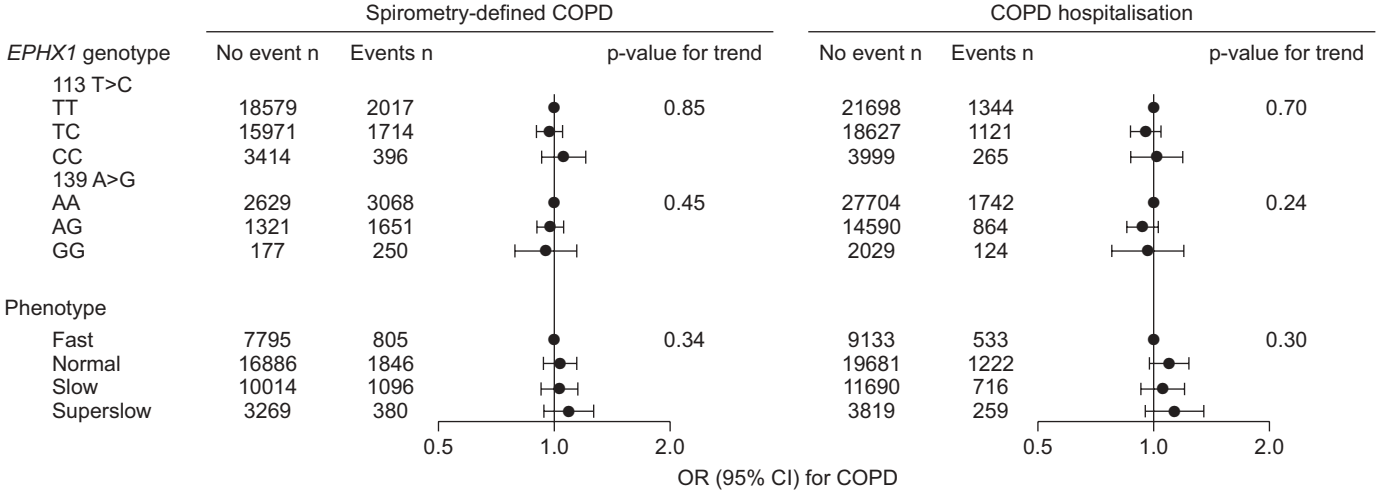

FIGURE 3. Risk of chronic obstructive pulmonary disease (COPD) diagnosed by spirometry or by hospitalisation according to *EPHX1* genotype and enzyme activity phenotype. Spirometry-defined COPD was defined as forced expiratory volume in 1 s (FEV_1)/forced vital capacity <0.7 and FEV_1 <80% of the predicted value, excluding individuals with self-reported asthma and COPD hospitalisation was defined as World Health Organization Classification of Diseases 8th (ICD-8) and 10th (ICD-10) edition, ICD-8: 491–492; ICD-10: J41–J44. Event: individuals with the relevant end-point; no event: disease-free controls by any of the definitions investigated.

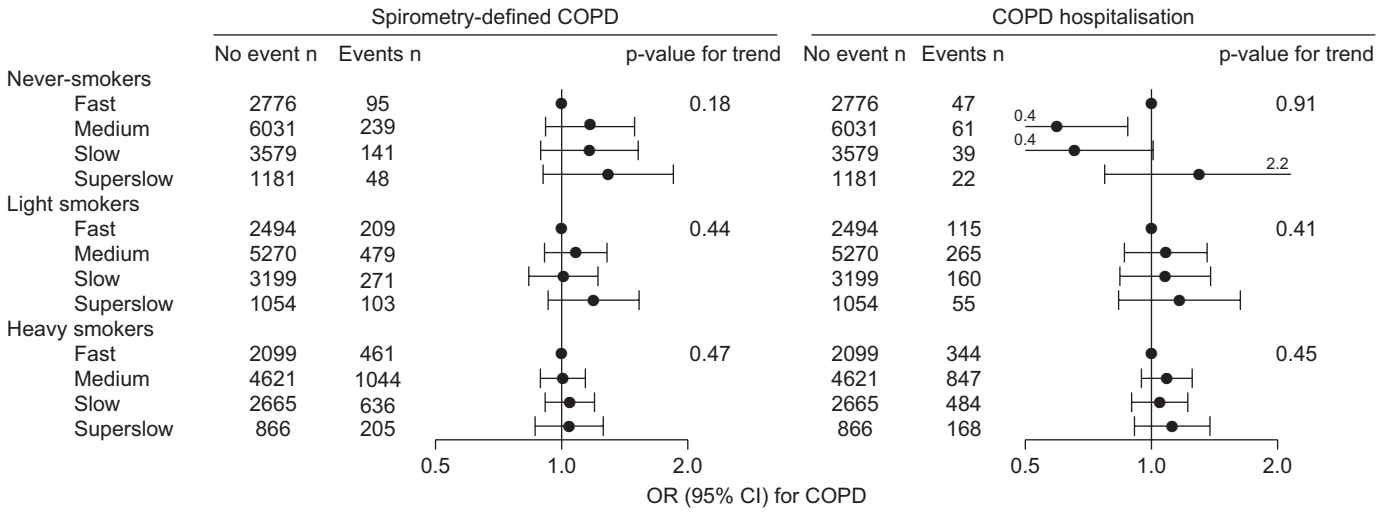

FIGURE 4. Risk of chronic obstructive pulmonary disease (COPD) diagnosed by spirometry or by hospitalisation according to *EPHX1* enzyme activity phenotype, stratified by smoking history. Spirometry-defined COPD was defined as forced expiratory volume in 1 s (FEV_1)/forced vital capacity <0.7 and FEV_1 <80% of the predicted value, excluding individuals with self-reported asthma and COPD hospitalisation was defined as World Health Organization International Classification of Diseases 8th (ICD-8) and 10th (ICD-10) edition, ICD-8: 491–492; ICD-10: J41–J44. Light smokers: <15 pack-yrs; heavy smokers: ⩾15 pack-yrs; event: individuals with the relevant end-point; no event: disease-free controls by any of the definitions investigated.

study contributes results from the hitherto largest population-based investigation of *EPHX1* as a risk factor in COPD. Since we had statistical power to detect even a 20–30% increased risk of COPD for all *EPHX1* genotypes and phenotypes overall, and since we previously found positive associations between risk alleles in other genes and risk of COPD using the same cohorts

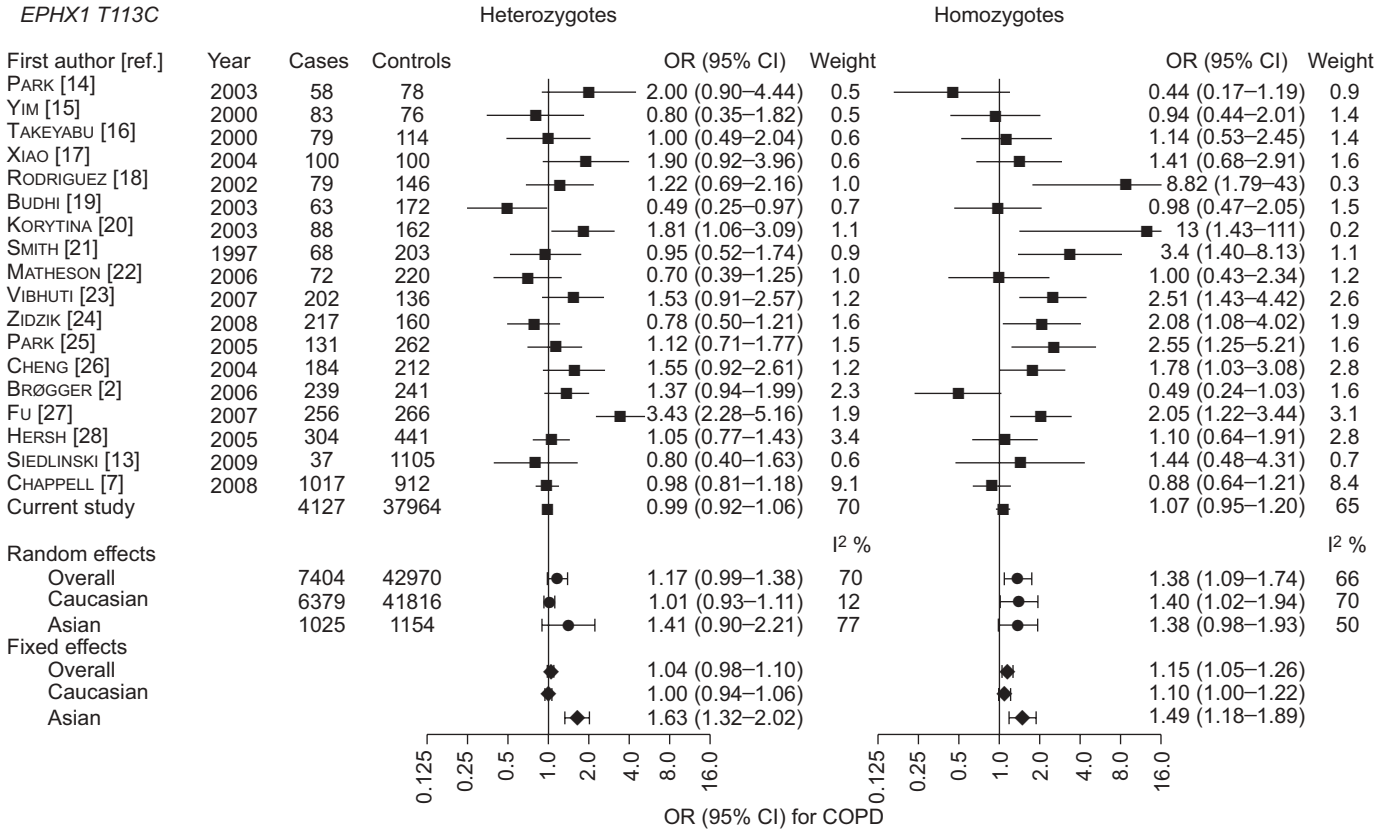

FIGURE 5. Meta-analysis of *EPHX1 T113C* genotype and risk of chronic obstructive pulmonary disease (COPD); overall and stratified analyses using both random and fixed effect models. Weights are from the fixed effect analyses using the inverse variance method. I^2: the variation in OR attributable to heterogeneity.

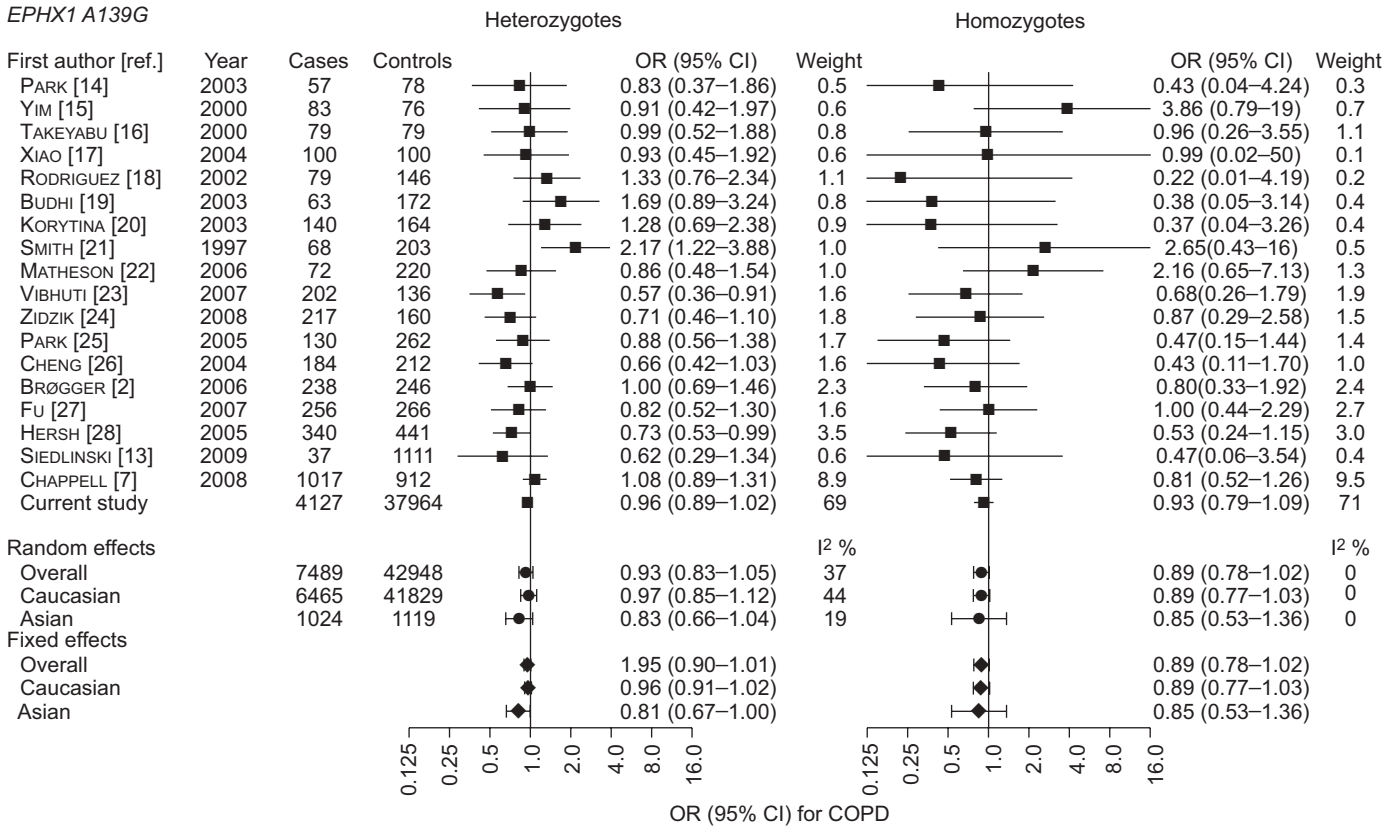

FIGURE 6. Meta-analysis of *EPHX1 A139G* genotype and risk of chronic obstructive pulmonary disease (COPD); overall and stratified analyses using both random and fixed effect models. Weights are from the fixed effect analyses using the inverse variance method. I^2: the variation in OR attributable to heterogeneity.

[11, 30–32], we have confidence in the negative findings of this study. However, the meta-analysis cannot completely exclude a minor effect of *EPHX1* genotype on COPD risk.

FEV1 % pred and decline in lung function

Reports on the relationship between *EPHX1* genotypes and phenotype and FEV1 as a main end-point in Caucasians are scarce and reach varying conclusions. One study found that the *113C/139A* haplotype (~superslow phenotype) is associated with rapid decline in lung function [33]. In accordance with this, another study found that the putative fast allele, *139G* is protective against decline in lung function [28]. However, two recent studies, including the biggest study conducted prior to the present, find no impact of *EPHX1* on FEV1 or decline in FEV1 with time [13, 22]. Our results likewise do not support an association of *EPHX1* with FEV1 or decline in FEV1.

COPD

Several reports exist linking *EPHX1* polymorphisms to altered risk of COPD. Unfortunately, the picture is obscured by lack of consistency in the findings reported. Thus, studies investigating the individual polymorphisms variably find the *T113C* variant to be associated with either increased risk of [21] or protection from [2] COPD. Some suggest that progression rather than susceptibility to COPD is affected by the presence of the *113C* allele [34]. One study found an association of the *113C* allele with functional impairment in COPD [35]. Some studies indicate a protective effect of the putative fast *139G* allele [1, 28]; however, many studies find no effect at all of either polymorphism [7, 22, 36]. Similar disparities are found in studies investigating association of COPD with *EPHX1* phenotype [7, 21, 22, 36]. Overall, there is a tendency that the effect of individual genotypes is observed mostly in Asians, whereas Caucasians are not affected [7, 22, 36, 37]. In concordance with this, our results indicate that *EPHX1* genotype or phenotype is not associated with COPD in the Danish population.

Our main study did not reach statistical significance for the *EPHX1* genotypes while the meta-analysis did for *T113C* homozygotes; however, the 95% CIs overlap, and so these results cannot be ruled contradictory. In accordance with our findings in the main study, meta-analysis did not indicate a significant effect of the *A139G* variant allele. Even though statistical significance was not reached, there is a trend towards a lowered risk of COPD for *A139G* variant allele carriers. This is consistent with the putatively increased microsomal epoxide hydrolase detoxifying activity associated with this allele. Likewise, the putatively lowered enzyme activity associated with the *T113C* variant allele corresponds well with the increased risk of COPD observed for this allele. Previous meta-analyses by Hu *et al.* [36] and Brøgger *et al.* [2] are in agreement with our findings reporting a slightly increased risk of COPD for *113C* homozygotes but no effect of the other genotypes. The most recent meta-analysis by Smolonska *et al.* [37] investigates the effect of variant allele carriers *versus* non-carriers. They do not find any effect on the risk of COPD for either the *T113C* or the *A139G* polymorphisms.

Asthma

Although the clinical manifestations of asthma and COPD are different, some of the same environmental factors may trigger both disease states [6, 38]. Genetic variations in xenobiotic-metabolising enzymes such as *EPHX1* have been found to modify asthma susceptibility [39, 40]. SALAM *et al.* [40] report that high activity phenotypes of *EPHX1* predisposes for asthma. This is in contrast to the findings of DUAN *et al.* [41] who hypothesise that *EPHX1* may have a dual effect on susceptibility to asthma. They report that apart from lowering enzyme activity, the *T113C* variant also upregulates another gene (*ORMDL3*), which has been associated with risk of childhood asthma [42]. A recent pilot study also emphasises the multifactorial nature of bronchial asthma, reporting that gene–gene and gene–environment interactions involving *EPHX1* are important determinants of asthma susceptibility [38]. We were not able to evaluate as many interactions as were in that study. However, our results point to the conclusion that the investigated polymorphisms of the *EPHX1* gene are not major factors in susceptibility to asthma.

Limitations

The size of the effect of *EPHX1* variants on risk of COPD may depend on ethnicity [36, 37]. All participants in the present study were white, Danish subjects, and while this eliminates any blurring due to ethnical heterogeneity of the study population, our results may apply to white subjects only. Our aim was to investigate polymorphisms of the *EPHX1* gene in relation to COPD and other pulmonary outcomes overall. Although we did not find any such association, we cannot rule out the possibility that an association exists in certain subgroups of individuals.

As previously mentioned, the two functional SNPs in exons 3 and 4, respectively, tag the entire coding region of the gene. However, no linkage disequilibrium is observed between the coding region and the promoter region [12]. Therefore, while our results suggest that the two functional SNPs and the corresponding haplotype blocks are not associated with risk of COPD or asthma, nor with level or decrease in lung function, we cannot exclude that variants in the promoter region of the *EPHX1* gene may be associated with these traits.

Summary

Previous studies indicate that common polymorphisms in the *EPHX1* gene are associated with altered risk of COPD and other pulmonary outcomes. Although we have studied the two polymorphisms with the highest predicted effect on microsomal epoxide hydrolase activity in humans, we found no consistent association between these polymorphisms and COPD, COPD-related traits, or asthma. Our results thus indicate with substantial statistical power that genetically reduced microsomal epoxide hydrolase activity *per se* is not a major risk factor for COPD or asthma in the Danish population. However, the meta-analysis cannot completely exclude a minor effect of *EPHX1* genotype on COPD risk.

STATEMENT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

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