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From the authors:

I thank B.C. Creagh-Brown and C. Shee for their complimentary comments. Furthermore, I completely agree with the principles described in their letter regarding the use of noninvasive ventilation (NIV) in selected patients who have chosen to forego, or are not offered, endotracheal intubation and invasive mechanical ventilation. In fact, I had the privilege of co-authoring a recent statement published by the Society of Critical Care Medicine [1] regarding the use of noninvasive ventilation for patients with “do not intubate” orders that I think is entirely consistent with the comments by B.C. Creagh-Brown and C. Shee. In that statement, we argued that the use of NIV for patients with acute respiratory failure can be classified into three categories. 1) NIV as life support with no preset limitations on life-sustaining treatments. 2) NIV as life support when patients and families have decided to forego endotracheal intubation. 3) NIV as a “purely palliative” measure when patients and families have chosen to forego all life support and are receiving comfort measures only.

As pointed out by B.C. Creagh-Brown and C. Shee, there is compelling evidence that some patients in our “second category” (those for whom invasive ventilation is not desired or indicated but who do want life-sustaining treatments) will benefit from NIV, especially those patients with acute respiratory failure from chronic obstructive pulmonary disease or congestive heart failure.

As for those in the “third category” (patients for whom NIV is used purely for the palliation of symptoms), I also agree with B.C. Creagh-Brown and C. Shee that highly selected patients at the end-of-life may receive some palliative benefit from NIV, provided their clinicians are experienced with NIV. This is an important area in need of further research, but one in which some indirect evidence of benefit exists [1]. However, I suspect I may differ from B.C. Creagh-Brown and C. Shee with regard to my area of greatest concern regarding NIV in this purely palliative setting. In my clinical experience, I see many more patients at the end-of-life exposed to NIV that is unlikely to provide any benefit than for whom this potentially beneficial therapy is withheld.

Perhaps B.C. Creagh-Brown, C. Shee and I agree that both of these concerns about the misapplication of noninvasive ventilation are problematic and we should work to limit them both.

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STATEMENT OF INTEREST

None declared.

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No effects of EPHX1 polymorphisms on the level or change of FEV₁ in the general population

To the Editors:

CHAPPELL *et al.* [1] recently found that several single nucleotide polymorphisms (SNPs) in the glutamate cysteine ligase (catalytic subunit; GCLC) and epoxide hydrolase 1 (EPHX1) do not associate with the presence or severity of chronic obstructive pulmonary disease (COPD). We have previously shown in two independent population-based cohorts that the functional polymorphism rs17883901 and the trinucleotide GAG repeat in GCLC form unique genotype combinations that are associated with lower lung function in interaction with smoking [2]. CHAPPELL *et al.* [1] did not study the latter variation in GCLC, nor the interaction between SNPs and smoking in relation to COPD severity, which is of special

interest given the *in vivo* role of GCLC. They additionally studied SNPs in EPHX1, including nonsynonymous SNPs (nsSNPs) Tyr113His and His139Arg that previously provided both positive and negative associations with COPD across studies and races [1, 3–7]. We aimed to extend these findings by showing the effects of EPHX1 SNPs on the level and change of forced expiratory volume in one second (FEV₁) in the general population, and additionally investigated whether EPHX1 SNPs smoking interactions are associated with both outcomes, as was the case with GCLC.

We genotyped five SNPs in EPHX1 (three SNPs tagging the 5 kb promoter region: rs3753658, rs10753410 and rs2854450; and two nsSNPs: rs1051740 (Tyr113His) and rs2234922

TABLE 1 Doetinchem cohort characteristics

Time of the visit yrs	1999–2003
Total duration of follow-up yrs/number of visits[#]	10/3
Males	541 (47.0)
Age yrs	49.9 (31.2–70.9)
Height cm	172.6±9.1
Smokers/ex-smokers	308 (26.7)/473 (41.1)
Pack-yrs smoked	
Total cohort	5.0 (0.0–84.0)
Ever-smokers	12.5 (0.004–84.0)
FEV₁ L	3.39±0.79
FEV₁ change mL·yr⁻¹	-26.2±33.4
Total EPHX1 SNPs call rate %	98.5

Data are presented as n (%), median (range) or mean±SD, unless otherwise stated. FEV₁: forced expiratory volume in one second; EPHX1: epoxide hydrolase 1; SNP: single nucleotide polymorphisms. [#]: applicable for the 70.4% of subjects who additionally participated in the third survey (2003–2007); the remainder of the cohort participated in the first (1993–1997) and the second (1999–2003) surveys only.

(His139Arg)) in a random sub-sample (n=1,152; tables 1 and 2) of the prospective, population-based Doetinchem cohort with DNA and spirometry available (n=3,224) [2]. Taqman assays (Applied Biosystems, Nieuwekerk aan de IJssel, the Netherlands) were used to genotype two nsSNPs [2]. Promoter SNPs were genotyped by K-Bioscience Ltd (Hoddesdon, UK) with patent protected KASPar technology. SNPs and haplotypes were analysed in additive, recessive and dominant models for their effect on: 1) FEV₁ level at the survey of 1998–2002 (table 1) [2]; and 2) FEV₁ change during 10-yr follow-up (surveys 1993–1997 (n=1,152), 1998–2002 (n=1,152) and 2003–2007 (n=811)), with linear mixed effect models (S-PLUS, version 7.0; TIBCO, Palo Alto, CA, USA) for SNPs [2]. For data of the promoter and exon regions, haplotypes (>5% frequency) were compared with the remaining pooled haplotypes using linear regression analysis (haplo.stats, version 1.3.1) [8] in the R environment [9], with FEV₁ change defined using first and last FEV₁.

Since EPHX1 is a phase II detoxification enzyme and nsSNPs in EPHX1 are functional [10], these were tested statistically with an interaction term, *i.e.* pack-yrs × genotype/genotype combination/haplotype (defined as: slow (His113-His139+His113-Arg139 pooled); normal (Tyr113-His139); and fast (Tyr113-Arg139)). Interaction analysis of haplotype or genotype combinations contained two dummy variables and haplotype or genotype combinations were compared with each other.

The study provided 80% power for detecting 158 mL difference in FEV₁ level and 7 mL·yr⁻¹ in FEV₁ change for the lowest prevalent SNP (*i.e.* rs10753410) or haplotype in an additive or dominant model ($\alpha=0.05$, tested two-sided).

In our study, all EPHX1 SNPs were noncorrelated ($r^2<0.05$ for any SNP pair). Both FEV₁ level and FEV₁ change were not significantly associated with any of the SNPs in any model (table 3) nor were they different between slow, normal and fast combinations of EPHX1 genotypes. Four prevalent haplotypes were observed within the promoter (frequencies 55.2, 19.8, 13.3% and 11.5%) and coding region (slow: 27.8% (21.9 + 5.9); normal: 56.8%; fast: 15.4%), and none were significantly associated with FEV₁ level or change.

We observed no significant interactions between pack-yrs and nsSNPs or their combinations for either level or change in FEV₁, nor was there a significant interaction with EPHX1 haplotypes and FEV₁ level. The normal haplotype interacted with pack-yrs, providing 0.5 ± 0.2 mL·yr⁻¹ less FEV₁ decline per pack-yr ($p=0.01$) than the slow haplotypes pool in a dominant model ($p>0.31$ for recessive or additive model).

We found no effect of genetic variations in EPHX1 on the level or change of FEV₁ in the general population. Furthermore, there was no significant interaction between functional nsSNPs and pack-yrs in either outcome studied. Given the high number of tests performed (n=108), the single significant association of the EPHX1 haplotype with FEV₁ decline in interaction with smoking may be a spurious observation, and this needs replication.

Our observations support and extend the previously reported negative results of CHAPPELL *et al.* [1]. Since a lower forced

TABLE 2 Epoxide hydrolase 1 genotype characteristics

Genotype characteristics	Heterozygotes	Homozygotes mutant	Minor allele frequency	HWE test p-value [#]
rs10753410	240 (21.2)	13 (1.2)	11.8	0.45
rs2854450	360 (32.0)	42 (3.7)	19.8	0.73
rs3753658	265 (23.4)	22 (1.9)	13.6	0.82
rs1051740 (Tyr113His)	472 (41.3)	83 (7.3)	27.9	0.37
rs2234922 (His139Arg)	377 (32.8)	55 (4.8)	21.2	0.55
Slow [†]			402 (35.3)	
Normal [†]			513 (45.0)	
Fast [†]			225 (19.7)	

Data are presented as n (%), unless otherwise stated. HWE: Hardy Weinberg equilibrium. [#]: for 1 degree of freedom Chi-squared test comparing the observed genotypes distribution with the expected as derived from the allele frequencies; [†]: Tyr113His and His139Arg genotype combinations defined as previously described [1, 5, 6].

TABLE 3 Effects of epoxide hydrolase 1 genotypes on the level and change of forced expiratory volume in one second (FEV₁) in the Doetinchem cohort[#]

SNP	FEV ₁ level [†] mL				FEV ₁ change ⁺ mL·yr ⁻¹			
	Heterozygote		Homozygote mutant		Heterozygote		Homozygote mutant	
	Regression coefficient	95% CI	Regression coefficient	95% CI	Parameter estimate	95% CI	Parameter estimate	95% CI
rs10753410	42.9	-24.4–110.2	11.4	-244.3–267.0	0.7	-3.9–5.3	-4.2	-21.1–12.8
rs2854450	43.1	-16.1–102.4	-80.1	-224.5–64.4	3.0	-1.1–7.0	-0.9	-10.7–8.9
rs3753658	-22.2	-86.9–42.5	-47.2	-253.6–159.2	-0.8	-5.3–3.7	-9.8	-23.8–4.3
rs1051740 (Tyr113His)	-13.7	-70.6–43.1	-64.7	-172.2–42.8	-0.4	-4.3–3.5	-1.2	-8.7–6.2
rs2234922 (His139Arg)	-20.8	-79.6–38.1	-22.0	-150.0–105.9	2.5	-1.5–6.5	2.7	-6.0–11.5

SNP: single nucleotide polymorphism; CI: confidence interval. All between-genotype differences, including comparison of heterozygotes and homozygote mutant groups, were not significant ($p > 0.15$ for both outcomes). [#]: $n = 1,152$; [†]: estimated for heterozygous and homozygous mutant genotypes as compared with wild-types using linear regression adjusted for age, sex, height and pack-yr smoked; ⁺: estimated for heterozygous and homozygous mutant genotypes as compared with wild-types, using linear mixed effect model adjusted for age, sex, initial FEV₁ level, pack-yr smoked and their interaction with time.

expiratory volume in one second level and an accelerated forced expiratory volume in one second decline are both important indices of chronic obstructive pulmonary disease, epoxide hydrolase 1 does not likely play a role in the development of chronic obstructive pulmonary disease in Caucasians.

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STATEMENT OF INTEREST

None declared.

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Pulmonary hypertension therapy and COPD: still many questions to be answered

To the Editors:

After great development in the knowledge of pulmonary arterial hypertension (PAH) during the last decades, there is now an increasing interest in the pathophysiology of other forms of pulmonary hypertension, such as chronic obstructive pulmonary disease (COPD). The results obtained in the study by STOLZ *et al.* [1] have shown that for an average COPD Global Initiative for Chronic Obstructive Lung Disease stage III-IV population, the use of bosentan is not related to a significant improvement in exercise capacity and is associated with worsening of ventilation-perfusion mismatch. Although it is the first randomised study to address the use of bosentan, a proven PAH specific therapy, in the setting of COPD, the study design may lead to misinterpretation of the results. The study was not designed to treat COPD associated pulmonary hypertension. Furthermore, the absence of invasive measurements did not allow a proper subgroup analysis that could provide further support for the use of specific PAH therapy in the treatment of “disproportional” pulmonary hypertension associated with COPD. It is well known that most patients who present with pulmonary hypertension in the setting of COPD and other diseases actually have other associated causes for pulmonary hypertension development rather than an idiopathic PAH-like disease [2, 3], for instance, frequently presenting with some degree of left ventricle dysfunction. Since the study did not perform invasive haemodynamic assessment of the COPD patients, no assumption about a possible presence of post-capillary impairment, a situation in which bosentan has shown no effect so far [4], could be made.

Another matter of debate is the use of an echocardiogram to assess the presence of pulmonary hypertension in patients with severe COPD, since the technical difficulties in this specific clinical presentation have shown to impair the accuracy of this methodology.

In summary, we believe the authors have addressed an important question, but due to the chosen study design we remain with more questions than answers about the use of specific pulmonary arterial hypertension therapy in the setting of chronic obstructive pulmonary disease.

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STATEMENT OF INTEREST

None declared.

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To the Editors:

In the paper by STOLZ *et al.* [1], bosentan deteriorated not only exercise capacity but also hypoxaemia and quality of life among subjects with severe or very severe chronic obstructive pulmonary disease (COPD). While the concept of a new drug category which could improve functional status among these patients is challenging, the theoretical background and clinical data to support the rationale for this trial [1] is rather weak.

In the study by GUNTHER *et al.* [2], the use of bosentan in 12 subjects with idiopathic pulmonary fibrosis was generally well