Microvascular hyperpermeability in COPD airways

Chronic obstructive pulmonary disease (COPD) is characterised by an abnormal inflammatory response of the lungs. An increase in the albumin concentration in the sputum of COPD patients has previously been reported. This may suggest that the airway microvascular permeability is increased in COPD airways because the albumin comes from the vasculature via endothelial contraction at post-capillary venule lesions. However, measurement of sputum samples has some limitations such as contamination by saliva. We have measured the albumin concentration of the airway lumen in patients with COPD using a new direct technique for collecting airway epithelial lining fluid.

Eighteen untreated patients with peripheral lung cancer undergoing a bronchoscopic examination for the diagnosis were recruited to the study. Approval was obtained from the Wakayama Medical University ethics committee and the patients gave their written informed consent. The mean (SE) age of the patients was 70.4 (2.0) years. Eight patients were current smokers, seven ex-smokers, and three non-smokers. Five of the subjects did not have COPD, four were at risk stage I, II, six had moderate COPD (stage II), and three had severe COPD (stage III) according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification of the severity of COPD.

Epithelial lining fluid was collected using a microsampling probe under bronchoscopy at the main or intermediate bronchus on the tumour absent side. The albumin concentration in the extracted ELF was measured and normalised by the values in the serum. The normalised airway albumin values showed a strong correlation with the forced expiratory volume in 1 second % predicted (%FEV₁) values (r = -0.727, p = 0.0006; fig 1).

There was no significant difference in the airway albumin values according to smoking status (non-smokers: mean (SE) 1.21 (0.29)%), ex-smokers: 1.23 (0.28)%, current smokers: 1.14 (0.28%) or age. These data suggest that an increase in airway microvascular permeability may be involved in the inflammatory and subsequent obstructive process of COPD.

The precise mechanism of the microvascular hyperpermeability observed in COPD has not been well characterised. We have recently reported that oxidative and nitrosative stress is exaggerated in COPD airways. Reactive oxygen/nitrogen species such as superoxide anion and peroxynitrite may participate in the microvascular hyperpermeability of COPD airways.

At present some airway/pulmonary cells (including epithelial cells, neutrophils, and macrophages) are considered therapeutic targets for future COPD treatment. In addition to these cells, the microvascular structure may also be a target in the treatment of COPD. Furthermore, airway albumin values may be a good marker for the efficacy of COPD treatment.

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Figure 1 Relationship between normalised airway albumin and forced expiratory volume in 1 second % predicted (%FEV₁). Normalised airway albumin values were calculated as values of epithelial lining fluid/values of serum.
be typed for unlinked markers to identify any stratification is lacking concrete scientific evidence. In fact, the reference you cited—in addition to several other studies—have indicated that there are few actual examples to support this assumption and that there is growing recognition that population stratification might not be as important a problem as was originally believed, and has probably been a minor or even irrelevant factor for most non-replicated association studies.

On the other hand, an important criterion in the evaluation of genetic association studies was absent in your guidance—namely, Hardy-Weinberg equilibrium. Hardy-Weinberg disequilibrium in control subjects could result from genotyping errors, inbreeding, genetic drift, mutation, or population stratification.

In conclusion, we agree on the importance of proper selection of patients and controls, accurate definition of disease phenotype, consideration of linkage disequilibrium and haplotypes, correction for multiple comparisons, and the need for a power calculation and validation of polymorphisms, especially if they showed association with the disease. Nevertheless, we disagree with the disqualification of a study because of its limited population size if it is still able to show acceptable power, its inability to check Hardy-Weinberg equilibrium in control populations, or because of its failure to genotype the whole population for unlinked markers to exclude population stratification, especially if the genotype frequencies of the polymorphisms under study are in Hardy-Weinberg equilibrium.

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Authors’ reply
We thank Hegab and colleagues for their interest in our guidance on genetic association studies and are pleased to see we are in concordance on many issues. We will address the areas of disagreement in order.

Previous studies in Thorax
Each piece of research can only be judged fit for publication by the standards in operation at the time of submission. Genotyping has never been cheaper or more accessible, and progress in methods of analysis continues apace. If Thorax is to maintain and raise its current standing, we should aim to publish the highest quality of papers. As benchmarking practice will necessarily be more rigorous year after year, we are conscious that guidance that appears stringent today should be so to maintain relevance over the coming years. Secondly, the guidance issued was intended to be a dogma. We are aware of the difficulties in executing association studies under certain circumstances (for example, in recruiting patients for studies into rarer conditions such as cystic fibrosis).4

Required sample sizes
Hegab et al state that the required sample sizes in table 1 are excessive and advocate the use of 80% power in sample size calculations. We chose 90% for three reasons: (1) to increase confidence in our findings; finding no association—for example, if Thorax publishes five such studies in the coming year, the probability of them all being true negatives is less than a third if all have 80% power; (2) adopting lower power results in a lower positive predictive value, increasing the (already substantial) chance of false positive findings and reducing confidence in a “significant” result; and (3) authors are often overly optimistic regarding the size of effect they will find and accordingly use smaller populations: in our recent meta-analysis of association studies of ADAM33 and asthma we found an OR of 1.5 for the major risk allele, and this is a typical effect size being reported in complex disease. Even using 80% power, for this effect size and a minor allele frequency of 0.4 one would still need around 600 cases. The sample sizes given are correct for the model chosen, despite the apparently counterintuitive need for increased case numbers as the minor allele frequency increases from 0.2 to 0.4.

Functionality of polymorphisms
We emphasise that we are not “restricting the genetic association studies to functional polymorphisms”, and refer back to the initial article’s paragraph headed “SNPs, haplotypes or functionally relevant polymorphisms?”

Population stratification
As Hegab et al point out, population stratification can lead to spurious results and there has been a long running debate as to the relative contribution of this bias.5 In our guidance we refer to the frequency of sub-studies to Thorax with inappropriate study populations and that this may lead to spurious results. We state that genotyping for unlinked markers is “reasurimg”, and we stand by this.

To describe population stratification as “irrelevant” is to selectively review evidence in a field where no clear consensus exists. The paper by Wang et al cited found only a small degree of bias with stratification (in contrast to other contemporary simulation studies6), and Wang et al have since published methodology for bias correction using a null marker.7 The reference quoted provides an accessible introduction to this complex topic for the general readership Thorax attracts.8 Published examples of population substructure do exist—for example, in self-reported racial groups.9

Hardy-Weinberg equilibrium
We entirely agree that Hardy-Weinberg equilibrium should continue to be explicitly tested for in control groups, and we thank the correspondents for emphasising this.

Summary
Genetic association studies submitted to Thorax are too frequently ill planned or poorly executed by current standards. In our guidance we endeavour to highlight this problem and aim to raise the standards of submissions (and hence publications) in the coming years. We hope that authors will find these guidelines useful and challenging. As originally stated, we would always be interested in research which provides novel insight into genetic mechanisms in respiratory disease, and the guidelines are intended to be flexible in application where a strong case for an alternative approach can be made.

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