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## CORRESPONDENCE

# CTLA4 polymorphisms and COPD

*To the Editors:*

We read with interest the article by ZHU *et al.* [1] describing the association of two single nucleotide polymorphisms (SNPs; rs231775 and rs3087243) within the CTLA4 gene and chronic bronchitis. Both of these polymorphisms may be risk factors for autoimmunity [2], and are often referred to as A49G and CT60, respectively. As alluded to briefly by the authors in their discussion, there has been recent interest in a possible role for autoimmunity in chronic obstructive pulmonary disease (COPD), hence shared genetic susceptibility with other autoimmune diseases would support this hypothesis. Although the authors report their association in two independent cohorts, general acceptance of the importance of this locus would require further validation in other COPD populations. We would like to add support to their results, by reporting association of rs231775 in an $\alpha_1$-antitrypsin deficient ($\alpha_1$-ATD) cohort, which also tended to associate with the same feature in the 1958 birth cohort.

428 unrelated Caucasian PiZZ subjects were fully characterised regarding COPD phenotypes, as described previously [3], and genotyped for rs231775 and rs3087243 using TaqMan® (Applied Biosystems, Foster City, CA, USA) genotyping technologies. Association with chronic bronchitis and forced expiratory volume in 1 s ($FEV_1$) were sought using logistic and linear regression, respectively, adjusting for age, sex and pack-yrs smoked. The characteristics of the group are shown in table 1. The genotyping success rate exceeded 90%, and both SNPs were in the Hardy–Weinberg equilibrium. Data pertaining to $FEV_1$ associations with both SNPs and the surrounding region were obtained from published online data of the 1958 birth cohort [4].

The G allele of rs231775 associated with lower $FEV_1$ (B= -4.22; p=0.04), but not with chronic bronchitis. No associations were seen with rs3087243. In data deposited by J. Todd (University of Cambridge, Cambridge, UK) and published online by the 1958 birth cohort [4], rs231775 tended to be associated with $FEV_1$, such that those with the G allele had lower $FEV_1$ compared with AA homozygotes (B= -0.053, 95% CI -0.101- -0.005; p=0.09). An SNP just 4 kb towards the 5′ end of the gene from rs231775 was significantly associated (B=0.098, 95% CI 0.032 -0.163; p=0.01).

**TABLE 1** Characteristics of the $\alpha_1$-antitrypsin deficiency cohort

| | |
|---|---|
| **Age yrs** | 50.34±0.54 |
| **Male** | 252 (58.9) |
| **Pack-yrs smoked** | 15.91±0.71 |
| **$FEV_1$ % pred** | 53.80±1.59 |
| ***K*CO % pred** | 69.70±1.01 |
| **Chronic bronchitis** | 146 (34.1) |

Data are expressed as frequency (%) or mean±SEM. $FEV_1$: forced expiratory volume in 1 s; % pred: % predicted; *K*CO: transfer coefficient of the lung for carbon monoxide.

It is recognised that subjects with $\alpha_1$-ATD exhibit similar genetic associations for COPD phenotypes to those seen in usual COPD [5], such that the effect of any individual polymorphism is additive to $\alpha_1$-ATD. In this cohort, association with chronic bronchitis was not seen, perhaps because the study was underpowered. Although there was some linkage disequilibrium (LD) between rs231775 and rs3087243 ($r^2$=0.59), slightly higher than that observed by ZHU *et al.* [1] in their cohorts, no associations with the latter SNP were seen. The degree of LD between the SNPs may account for their shared susceptibility to chronic bronchitis in the original publication by ZHU *et al.* [1], which was not detectable due to lower power in our dataset. In autoimmune disease the peaks of association within *CTLA4* are not exactly centred on rs231775 and rs3087243 [2], which might account for the lack of association with the latter SNP here, and the association upstream of rs231775 in the 1958 birth cohort. This suggests that more detailed mapping of *CTLA4* polymorphisms in COPD may be able to detect the true associated variant.

Our work also supports a role for *CTLA4* in COPD, albeit with a different related phenotype. The suggestive data from the 1958 birth cohort goes some way to corroborating this. Further study of autoimmunity associated loci in COPD may help to establish whether this is an important aspect of pathogenesis.

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# Equipment for multiple breath washout

*To the Editors:*

We read with interest the article ''Paediatrics in Berlin'' [1] in a recent issue of the *European Respiratory Journal*, which summarised the paediatric topics of the European Respiratory Society's Annual Congress in Berlin.

In this article, inert gas multiple breath washout (MBW) technique was highlighted as a promising tool for assessing parameters of ventilation inhomogeneity, such as the lung clearance index (LCI) for detecting early peripheral airway disease, *e.g.* in cystic fibrosis. However, equipment using mass spectrometry, the current ''gold standard'' for MBW, has no prospect to become commercially available. Alternatively, an ultrasonic flow sensor or an infrared analyser has been introduced for indirectly measuring gas concentrations during a washout procedure. Our recent study reporting within-test repeatability and between-test reproducibility of the LCI in healthy children and adolescents using a side-stream ultrasonic flow sensor [2] was cited in this article [1].

As there has already been a lot of confusion regarding equipment for MBW, we feel it is important to emphasise that we did not use equipment sold by Eco Medics (Dürnten, Switzerland) for any of our studies in preschool children through to adults.

Rather, we have developed equipment based on a similar flow sensor but with the sensor in a side-stream position, utilising a sophisticated valve system controlled by the appropriate software. This equipment is not yet commercially available but was developed as EasyOne Pro, MBW module, in collaboration with ndd Medical Technologies (Zurich, Switzerland). This prototype equipment has been validated in a stepwise approach including comparison with the gold standard mass spectrometry [3], proof of hygienic safety [4], demonstration of feasibility in patients with cystic fibrosis and healthy controls [5], data on short-term and long-term variability in healthy subjects [6] and will now be used in a multicentre trial.

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**Statement of Interest:** A statement of interest for M. Gappa can be found at www.erj.ersjournals.com/misc/statements.dtl

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*From the authors:*

We thank S.I. Fuchs and M. Gappa for their interest in our review [1]. The points they make are of general importance. As with all lung function measurements, lung clearance index (LCI) must be measured with equipment that has adequate frequency responses, precision and stability, and has been suitably calibrated. Their work with an ultrasonic flow meter is welcome, because it has been compared with a gold standard, and may make measurement of LCI more accessible. The use of equipment which has not been so carefully validated must be firmly discouraged.

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