

PostScript

LETTERS TO THE EDITOR

Inhaled corticosteroids and long term outcome in adults with asthma

The concept that treatment with inhaled corticosteroids (ICS) may improve long term lung function by reversing or minimising the effects of airway remodelling remains an attractive one but, until now, unproven. It is therefore with much interest that we read the publications of two observational studies by Lange *et al*¹ and by Dijkstra *et al*.² Both groups of authors hypothesised that asthmatic individuals continuously treated with ICS would have a less pronounced decline in forced expiratory volume in 1 second (FEV₁) than those not treated with ICS, and their studies indeed suggest that long term treatment with ICS in asthmatic adults is associated with a more favourable decline in FEV₁ with age compared with the natural course of the disease.

Obviously, the most interesting next question is to what extent further improvement in FEV₁ is possible. Unfortunately neither study addressed the issue of possible additional anti-inflammatory treatment. The assessment of FEV₁ after maximal bronchodilation and a course of systemic corticosteroids in these groups of patients would have been most interesting because it might show the maximal attainable functional outcome in adults who have had asthma for many years.

Similarly, one could study growth of lung function in childhood and adolescence to assess whether this is affected by asthma and can be reversed by anti-inflammatory treatment. Lange *et al*,¹ Dijkstra *et al*,² and the accompanying editorial by Ernst³ refer to the CAMP study⁴ as one which showed that ICS treatment may not even improve FEV₁ in children with mild asthma compared with placebo. The CAMP study did indeed assess lung growth from postbronchodilator FEV₁ as the primary outcome variable, but it lacked the proper design to address this issue fully because bronchodilation was not maximal and ICS treatment was started in a daily dose of 400 µg and tapered off or stopped based on symptoms. Because treatment based on symptoms does not take into account of—and is likely to underestimate—the degree of inflammation,⁵ such a study design is probably biased towards underestimation of the maximal attainable level of function.

In our own study we observed that FEV₁ after maximal bronchodilation was normalised in children with moderately severe asthma who continued to inhale 600 µg budesonide daily, and also when they had become completely asymptomatic.⁶ This suggests that ICS treatment can completely normalise FEV₁. Whether similar favourable effects of ICS on postbronchodilator FEV₁ can also be demonstrated in adult patients with long term asthma is unknown.

We therefore think that future long term outcome studies in adults with asthma should try to estimate the maximal attainable lung function.

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Susceptibility to high altitude pulmonary oedema: role of ACE and ET-1 polymorphisms

High altitude pulmonary oedema (HAPE) is a severe form of altitude illness that develops in travellers on rapid ascent to or physical exertion at altitudes of >2500 m.¹ The disease is characterised by pulmonary hypertension, uneven vasoconstriction, and overperfusion which is thought to cause stress failure of pulmonary capillaries leading to alveolar flooding.¹ Since uneven pulmonary vasoconstriction appears to play an important part in the development of HAPE, the genes involved in maintaining pulmonary vascular tone—for example, angiotensin converting enzyme (ACE) and endothelin-1 (ET-1)—could be possible candidates for HAPE.

Earlier studies showed that the selective pressure of hypobaric hypoxia acted in favour of those alleles of ACE and ET-1 which were

beneficial in maintaining a healthy state at high altitude.^{2,3} On the other hand, unfavourable alleles are likely to contribute to the susceptibility to HAPE. This hypothesis lends support to an earlier report on the allelic variants of endothelial nitric oxide synthase gene.⁴

We therefore investigated ACE insertion/deletion (I/D) (GenBank accession no X62855) and ET-1 5'-untranslated region (UTR) microsatellite (CT)_n-(CA)_n (GenBank accession no J05008), -3A/-4A (rs10478694), G2288T (rs2070699), and Lys198Asn (rs5370) polymorphisms in 64 patients with HAPE (HAPE-p) and 53 HAPE resistant controls (HAPE-r). The HAPE-r were healthy individuals who had climbed 2–3 times to altitudes greater than 3500 m and carried out routine strenuous physical activities without suffering from HAPE; in contrast, the other group suffered from HAPE on their very first visit. The study groups consisted of age matched (30–40 years) individuals of the same ethnicity. An institutional review committee approved the investigation and all subjects gave informed consent.

HAPE was diagnosed on the basis of the criteria described earlier.⁴ After recovery the HAPE-p were examined to exclude the possibility of any previous cardiopulmonary diseases. The subjects were genotyped for the five polymorphisms of the two genes using primers and conditions shown in table S1 (available online at <http://www.thoraxjnl.com/supplemental>). The plasma ACE levels were measured by a kinetic method using N-(3-[2-furyl] acryloyl)-Phe-Gly-Gly as substrate. The plasma ET-1 levels were determined by ELISA (Assay Designs, Ann Arbor, USA). SPSS statistical software for Windows (release 10), EPIINFO 6, and SNP Alyze program (Version 3.1, Dynacom, Mobara-shi, Japan) were used to perform the statistical analysis.

The mean (SD) ACE activity and ET-1 levels were significantly higher in HAPE-p than in HAPE-r (84.6 (26.2) v 40.7 (12.1) U/l and 8.0 (2.5) v 3.5 (0.7) pg/ml, respectively; both p<0.0001). Furthermore, a direct relationship was observed between ACE activity and ET-1 levels in HAPE-p and HAPE-r ($r = 0.31$, $p = 0.03$ and $r = 0.32$, $p = 0.02$, respectively), which reflects their interaction. ACE generates angiotensin II which induces ET-1 transcription and secretion in vitro in a variety of cell types including endothelial and vascular smooth muscle cells.⁵ ET-1 is also involved in the regulation of ACE activity in vivo independently of ACE expression.⁶

The polymorphisms were in Hardy-Weinberg equilibrium in both groups and are shown in table 1. The ID+DD and GT+TT genotypes of ACE I/D and ET-1 G2288T polymorphisms were over-represented in HAPE-p ($p = 0.03$ and $p = 0.002$, respectively), with D and T alleles being more frequent in HAPE-p than HAPE-r. The (CT)_n-(CA)_n repeats were segregated and recognised as shorter (13–30) and longer (31–45) based on our earlier observation.³ However, unlike in our previous report, the shorter and longer repeats did not correlate with ET-1 levels. Analysis of the possible genotype combinations between the five polymorphisms showed that there were significantly fewer genotype combinations II/longer repeats and II/GG between the two genes in HAPE-p than in HAPE-r ($p = 0.02$ and $p = 0.002$, respectively). The longer repeats/

Table 1 Distribution of *ACE*I/D and *ET-1* -3A/-4A, G2288T and Lys198Asn polymorphisms and their combinations in the HAPE-p and HAPE-r

Genotypes/ genotype combinations*	HAPE-p (n = 64)	HAPE-r (n = 53)	χ^2	p value	OR (95% CI)
<i>ACE</i>					
II	18 (28)	23 (43)			
ID	34 (53)	21 (40)			
DD	12 (19)	9 (17)	5.10	0.07	
ID+DD	46 (72)	30 (57)	4.91	0.03	1.94 (1.08 to 3.50)
<i>ET-1</i>					
Longer repeats†	17 (27)	19 (35)			
Shorter repeats†	47 (73)	34 (65)	1.15	0.28	1.46 (0.80 to 2.66)
-3A/-3A	44 (69)	35 (66)			
-3A/-4A	20 (31)	18 (34)	0.21	0.65	0.87 (0.48 to 1.58)
GG	15 (23)	23 (43)			
GT	37 (58)	22 (42)			
TT	12 (19)	8 (15)	9.09	0.01	
GT+TT	49 (77)	30 (57)	9.05	0.002	2.53 (1.37 to 4.65)
Lys198Lys	22 (34)	17 (31)			
Lys198Asn	32 (50)	27 (52)			
Asn198Asn					
Lys198Asn+	10 (16)	9 (17)	0.20	0.90	
Asn198Asn	42 (66)	36 (69)	0.65	0.76	0.87 (0.48 to 1.58)
<i>ACE+ET-1</i>					
II/Longer repeats	1 (1)	4 (8)			
Remaining combinations	63 (99)	49 (92)	5.70	0.02	0.12 (0.01 to 0.95)
II/-3A/-3A	13 (21)	15 (28)			
Remaining combinations	51 (79)	38 (72)	1.32	0.25	0.68 (0.36 to 1.31)
II/GG	5 (7)	12 (23)			
Remaining combinations	59 (93)	41 (77)	10.04	0.002	0.25 (0.10 to 0.62)
II/Lys198Lys	9 (14)	8 (16)			
Remaining combinations	55 (86)	45 (84)	0.15	0.69	0.85 (0.39 to 1.86)

HAPE-p, individuals with high altitude pulmonary oedema; HAPE-r, individuals resistant to high altitude pulmonary oedema.

The genotypes and genotype combinations are presented as number (%).

*Genotype combinations were grouped into wild-type genotype combinations and remaining combinations.

†(CT)_n-(CA)_n repeats were segregated and recognised as shorter (13–30) and longer (31–45).

Lys198Lys genotype combination within *ET-1* was significantly less in HAPE-p than in HAPE-r (1% v 9%, odds ratio 0.10 (95% CI 0.01 to 0.82), $p = 0.01$). Our findings support the hypothesis that *ACE* and *ET-1* polymorphisms have a role in the susceptibility to HAPE. The findings of the present study and earlier reports on adaptation to high altitude^{2,3} together indicate that *ACE* and *ET-1* genes are significant in high altitude physiology.

In conclusion, this study showed that *ACE* and *ET-1* variants have independent and interactive roles in the susceptibility to HAPE. Higher *ACE* activity and *ET-1* levels correlated with HAPE. The *ACE* I/D and *ET-1* G2288T polymorphisms emerged as noteworthy variants showing association with HAPE. Owing to the small sample size, the difference in representation of the polymorphisms and their correlation with biochemical parameters did not reach greater statistical significance; in particular, we consider our data on the genotype combinations between the polymorphisms of the two genes to be preliminary. Nevertheless, since HAPE samples are difficult to obtain, the findings of this study are important and warrant confirmation in a larger sample. The results of this study may find application in identifying individuals with a predisposition to HAPE.

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Details of the primers and conditions for genotyping the five polymorphisms are shown in table S1 available on the *Thorax* website at <http://www.thoraxjnl.com/> supplemental.

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NSIP in a curry sauce factory worker

Curry powder and ground pepper are commonly used spices in many countries of the world. Although a case of bronchiolitis obliterans organising pneumonia has been reported in a worker who inhaled spice dust in a potato chip factory,¹ we report the first case of non-specific interstitial pneumonia (NSIP)² with bronchiolar lesions associated with curry powder and ground pepper.

A 50 year old male smoker (20 pack-years) developed a cough with sputum and shortness of breath on both working days and non-working days and was admitted to our hospital 1 month after developing the symptoms. He had worked in a factory that produced curry sauce for 13 years. His job was to carry sacks filled with curry powder (containing a mix of ground spices) and ground pepper on his shoulders and to empty them into a large curry sauce cooker without any equipment to protect against dust inhalation.

Physical examination on admission revealed inspiratory crackles in the bilateral lower lungs without digital clubbing. Serum markers for interstitial pneumonia, surfactant protein D (SP-D), and KL-6 were raised to 2410 ng/ml and 5570 U/ml, respectively. A high resolution computed tomographic (HRCT) scan of the chest revealed multiple irregular consolidations along with bronchovascular bundles and, in the subpleural lesions, trivial pleural effusion and cystic air spaces in the bilateral apex portions (fig 1A). Bronchoalveolar lavage was performed: the total cell count was 4.1×10^6 /ml with 23.8% macrophages, 70.4% lymphocytes, 0.2% neutrophils, 2.6% eosinophils, and 3.0% basophils. The CD4+/CD8+ lymphocyte ratio was 0.94 and pathogenic organisms were not detected.

Specimens obtained by video assisted thoracoscopic surgery from the right lung (S³ and S⁸) 2 months after the onset of disease revealed a cellular and fibrosing NSIP pattern with polypoid granulation tissues in a few respiratory bronchioles and alveolar ducts. The bronchioles showed reparative proliferation of the bronchiolar epithelium and infiltration of eosinophils, lymphocytes, and multinucleated giant cells, together with stenosis with intraepithelial infiltration of lymphocytes. These findings suggest an association between the patient's disease and the particles he inhaled while working.³

Four months after the onset of disease his serum SP-D and KL-6 levels had fallen and the consolidations seen on the HRCT scan had spontaneously resolved. Lymphocyte stimulation tests (LST) using the patient's peripheral blood lymphocytes were positive for the curry powder, ground black pepper, and ground