

*Rapid communication*

## Human ACE gene polymorphism and distilled water induced cough

Alyn H Morice, Andrew J Turley, Kate Linton

**Abstract**

**Background** – Inhibitors of angiotensin converting enzyme (ACE) cause a non-productive cough. The insertion/deletion polymorphism of ACE was used as a genetic marker to investigate the relationship between ACE genotype and cough sensitivity.

**Methods** – A double blind cough challenge was performed in 66 normotensive subjects (34 men) of mean age 34.8 years (range 18–80) using aerosols of distilled water. The number of coughs during the one minute exposure to water was recorded. DNA samples from venous blood were amplified by the polymerase chain reaction and resolved on a 1% agarose gel. They were analysed for the presence of a polymorphism in intron 16 of the ACE gene consisting of an insertion (I) or deletion (D) of an Alu repetitive sequence 287 base pairs long.

**Results** – The distribution of genotypes was 20 II, 26 ID, and 20 DD. The cough response was significantly ( $p < 0.01$ ) related to the ACE genotype, the mean number of coughs being 15.8, 11.3, and 9.6, respectively, in subjects with the II, ID, and DD genotypes.

**Conclusions** – The observation that cough challenge is dependent on ACE genotype in normal subjects is evidence of a link between ACE activity and the cough reflex.

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to an Alu repetitive sequence 287 base pairs (bp) long has been described.<sup>2</sup> The ACE insertion/deletion polymorphism was found to be associated with serum levels of ACE and, in subjects who are homozygous for the D allele, the mean serum level of ACE is nearly twice as high as that of subjects homozygous for the I allele.<sup>3</sup>

Since the introduction of ACE inhibitors for the treatment of hypertension and heart failure in the late 1970s their use has grown steadily. However, it was not until 1985 that the association of ACE inhibitors with a dry cough was noted.<sup>4</sup> This side effect of ACE inhibitors appears to be related to the class of the drug rather than to individual compounds as it has been reported with all ACE inhibitors in current use.<sup>5</sup>

ACE inhibitors have been shown to cause a leftward shift in the concentration-response curve for the tussive agent capsaicin, and it has been postulated that inhibition of ACE leads to accumulation of protussive peptides such as bradykinin and substance P which cause the heightened cough response.<sup>6</sup> Despite much research, the biochemical mechanism of ACE inhibitor cough remains to be elucidated.

Following the discovery of a genetic polymorphism within the ACE gene, we suggested a relationship between the polymorphism and the cough reflex.<sup>7</sup> However, a simple relationship between ACE genotype and ACE inhibitor cough does not exist. In this study we have postulated that the response to cough evoked by distilled water may be related to the ACE gene polymorphism with subjects of genotype II showing an excess of cough.

**Methods**

Sixty six healthy volunteers (34 men) of mean age 34.8 (range 18–80) years were studied. None gave a history of recent respiratory tract infection and none had asthma or were current smokers. All gave informed consent and the study was approved by the hospital ethics committee.

**OBJECTIVE MEASURE OF COUGH**

Cough challenges were performed as previously described<sup>8</sup> using aerosols of distilled water generated by an ultrasonic nebuliser (DeVilbiss)

The Section of Respiratory Medicine, University Department of Medicine and Pharmacology, Royal Hallamshire Hospital, Sheffield S10 2JF, UK  
A H Morice  
A J Turley  
K Linton

Correspondence to:  
Dr A H Morice.

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Angiotensin converting enzyme (ACE) or kininase II plays a key part in the renin-angiotensin system. ACE is a non-specific zinc metallopeptidase which not only cleaves angiotensin I to produce the potent vasoconstrictor angiotensin II, but also catalyses the metabolism of other small peptides such as bradykinin, substance P, and enkephalins. Human endothelial ACE consists of 1306 amino acids. The gene is located on chromosome 17q23, spans approximately 21 kb, and contains 26 exons.<sup>1</sup> A polymorphism in intron 16 consisting of the insertion (I) or deletion (D) of a DNA fragment corresponding

Table 1 Comparison of ACE genotypes

Genotype	Men	Women	Total
<b>II</b>			
n	12	8	20
Mean age (years)	34.9	30.5	33.2
Age range (years)	19–71	19–56	19–71
Mean no. of coughs	16	15.5	15.8
<b>ID</b>			
n	13	13	26
Mean age (years)	35.3	40.9	38.1
Age range (years)	18–67	21–80	18–80
Mean no. of coughs	8.5	13.7	11.3
<b>DD</b>			
n	9	11	20
Mean age (years)	33.5	31.1	32.2
Age range (years)	20–76	21–57	20–76
Mean no. of coughs	9.9	9.1	9.6

run at maximum output. Subjects were asked to breath with tidal respiration. The number of coughs during the minute of challenge was recorded as tussive blasts by an observer blinded to the genotypes of the subjects.

#### IDENTIFICATION OF ACE GENOTYPE

Five ml of venous blood was obtained and stored at  $-40^{\circ}\text{C}$  until the study was complete. Samples were amplified by the polymerase chain reaction (PCR) using primers flanking the corresponding region in intron 16 of the ACE gene in accordance with a method modified from that of Rigat *et al.*<sup>2</sup> All samples were analysed with a water blank and a known ID genotype as a control marker. The PCR products were resolved on a 1% agarose gel and visualised with ethidium bromide staining. The 190 bp and 490 bp alleles were observed corresponding to the ACE diallelic polymorphism.

#### STATISTICAL ANALYSIS

The data were analysed using the Mann Whitney test and p values of  $<0.05$  were considered statistically significant.

#### Results

The distribution of genotypes in the study population is given in table 1. The mean numbers of coughs for subjects of II and DD

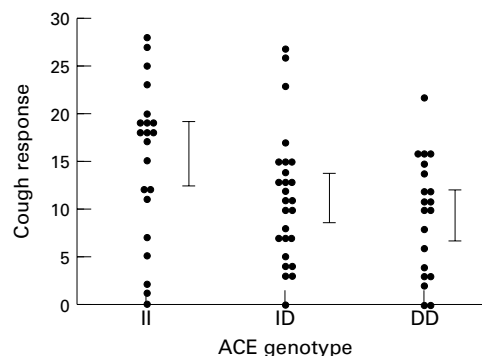


Figure 1 Relationship between ACE genotype and cough frequency as assessed by a one minute inhalation of ultrasonically nebulised distilled water. II = homozygote insertion polymorphism, DD = homozygote deletion polymorphism, ID = heterozygote.

genotypes were 15.8 (range 1–28) and 9.6 (range 0–22) respectively;  $p < 0.01$ . The mean number of coughs for subjects of the ID genotype was 11.3 (range 0–27). Individual data with 95% confidence intervals are shown in fig 1.

#### Discussion

The involvement of ACE in the human cough reflex was revealed by the development of a dry cough in up to 15% of patients receiving treatment with ACE inhibitors.<sup>9</sup> This side effect occurs with compounds of differing chemical structure and is therefore likely to be due to the inhibition of ACE activity rather than to any idiosyncratic reaction. In vivo, ACE is capable of metabolising several small peptides including substance P and bradykinin. We have previously shown that ACE inhibition causes a change in the concentration-response relationship to tussive challenge with capsaicin and have hypothesised that an increase in the perineuronal concentration of substance P underlies the increased cough sensitivity which occurs in ACE inhibitor cough.<sup>6</sup> Indeed, increased levels of substance P are found in the sputum of patients with ACE inhibitor cough.<sup>10</sup>

The cough produced by hypotonic solutions has been extensively studied and is characterised by rapid tachyphylaxis.<sup>8</sup> The number of coughs produced per minute is proportional to the concentration of chloride in the nebulised solution, an observation which has been ascribed to the lack of a “permeant anion” since replacement of chloride by acetate or bicarbonate still leads to coughing.<sup>11</sup> Hypotonic solutions presumably alter the ionic composition of the airway surface liquid and extracellular fluid in adjacent paracellular spaces causing discharge of the putative cough receptor. Airways surface liquid is the inner serous fluid lining the trachea and bronchi and consists of a layer 15–30  $\mu\text{m}$  thick with a chloride content of approximately 80 mmol/l.<sup>12</sup> The hypotonic composition of this layer is governed by active transport of ions – particularly the inward transport of sodium – by epithelial cells. Inhalation of distilled water may be expected to reduce the chloride content of airways surface liquid further.

Human somatic ACE is an extensively glycosylated protein of 170 kilodaltons which is uniquely reliant on monovalent anions, particularly chloride, for activity.<sup>13</sup> We hypothesised that cough produced by distilled water was caused by a local inhibition of ACE activity resulting from a reduction of chloride concentration in the airway surface liquid, and that subjects with genetically determined low ACE levels would therefore be more susceptible to cough produced by distilled water. Our results show that those subjects known to exhibit lower ACE activity (II genotype) have a significantly greater cough response than those of the DD genotype.

Surprisingly, there is no obvious relationship between ACE inhibitor cough and the ACE gene polymorphism in Caucasian subjects.<sup>14</sup> Treatment with ACE inhibitors leads to a

slowly developing but marked reduction of ACE activity which may be compensated by an increase in activity of other peptide degrading pathways. One such pathway is as neutral endopeptidase (NEP) which has been shown to regulate both substance P metabolism and cough in animal models.<sup>15</sup> Patients with ACE inhibitor cough may thus be deficient in this alternative pathway of degradation of pro-tussive peptides rather than in basal ACE activity.

In summary, we have shown an increased sensitivity of the cough reflex to stimulation with distilled water in subjects with the II genotype of ACE polymorphism. The link between ACE activity and cough sensitivity supports a physiological role for ACE in the cough reflex in man.

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