

Angiotensin-converting-enzyme gene polymorphisms, smoking and chronic obstructive pulmonary disease

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Abstract: While tobacco smoking is the main risk factor for chronic obstructive pulmonary disease (COPD) only a fraction of smokers go on to develop the disease. We investigated the relationship between the insertion (I) – deletion (D) polymorphisms in the Angiotensin converting enzyme (ACE) gene and the risk of developing COPD in smokers by determining the distribution of the ACE genotypes (DD, ID and II) in 151 life-long male smokers. 74 of the smokers had developed COPD (62 ± 2 years; FEV₁ 44 ± 6 % reference) whereas the rest retained normal lung function (56 ± 2 yrs; FEV₁ 95 ± 3 % reference). In addition, we genotyped 159 males recruited randomly from the general population. The prevalence of the DD genotype was highest ($p = 0.01$) in the smokers that developed COPD and its presence was associated with a 2-fold increase in the risk for COPD (OR 2.2; IC95% 1.1 to 5.5). Surprisingly, the 151 individuals in the smoking population did not demonstrate Hardy-Weinberg equilibrium unlike the 159 recruited from the general population. Our results suggest that ACE polymorphisms are associated with both the smoking history of an individual and their risk of developing COPD.

Keywords: chronic bronchitis, COPD, dopamine, drug addiction, emphysema

Introduction

Chronic obstructive pulmonary disease (COPD) is now the sixth largest cause of death in the world and its prevalence is expected to increase in the future (Murray and Lopez 1997). Tobacco smoking is the main risk factor for COPD but despite this only a fraction of smokers go on to develop the disease (Barnes 2000). While it has been suggested that susceptibility to tobacco smoke may reflect a genetic component of the disease (Barnes 1999) the only genetic factor identified so far in predisposing individuals to COPD is the α_1 -antitrypsin deficiency (Silverman and Speizer 1996).

The Angiotensin-converting enzyme (ACE) is a zinc metallo-peptidase that is highly expressed in lungs, where it degrades bradykinin and catalyses the formation of the Angiotensin II (AII); a powerful vasoconstrictor, inflammatory modulator and cellular growth factor (Woods et al 2000). ACE activity is increased in COPD (Brice et al 1995; Ucar et al 1997) but it remains to be established whether this is a cause or consequence of the disease process.

The ACE gene is located in chromosome 17q23 and shows a functional polymorphism consisting of an insertion (I) or deletion (D) of a 287bp fragment that determines three genotypes (DD, ID and II) (Rigat et al 1992) where the presence of the D allele is associated with higher ACE activity (Rigat et al 1990; Busjahn et al 1998; Chagani et al 1999; Montgomery et al 1999). Given that previous studies show increased ACE activity in COPD patients (Brice et al 1995; Ucar et al 1997) the aim of the present study was to test the hypothesis that the DD genotype is more prevalent in smokers that develop COPD.

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Methods

Population and ethics

We genotyped 151 male life-long smokers, 74 of whom had developed moderate-severe COPD (FEV_1 44 ± 6 % reference) and the rest retaining normal lung function (FEV_1 95 ± 3 % reference). All COPD patients were in a stable phase of the disease (not having had an exacerbation for, at least, three months preceding their inclusion to the study). We exclude subjects with other chronic lung diseases (asthma, bronchiectasis, lung cancer and interstitial lung diseases) and/or chronic extrapulmonary disorders such as, diabetes, arterial hypertension, cancer, immune diseases and cardiac, hepatic or renal failure. In addition, we genotyped 159 healthy males recruited randomly from the general population. All individuals were from the Balearic Islands and belonged to the same Caucasian Mediterranean ethnic background. Participants were not relatives and all gave informed consent to the study that was approved by the ethical review board of the Hospital Universitari Son Dureta.

Lung function

Forced spirometry (GS, Warren E Collins, Braintree, MA, USA) was determined according to international guidelines (Sanchis et al 1989) and reference values were those for a Mediterranean population (Roca et al 1986).

ACE genotyping

Genomic DNA was extracted from venous blood using standard methods (Promega, Madison, WI, USA) and stored at -80°C for subsequent analysis. The ACE genotype was determined using the polymerase chain reaction (PCR). Since the D allele can be more effectively amplified than the longer I allele, increasing the potential for ID individuals to be genotyped as DD (Ueda et al 1996), we used two different genotyping methods. Initially, the genotype was determined using the method of Rigat et al (1992) where the PCR products were a 190 bp (allele D) and a 490 bp (allele I) (Figure 1, Panel a). Subsequently, the genotype was confirmed with the method described by Evans et al (1994) where the amplified products were a 84 bp (allele D) and a 65 bp (allele I) (Figure 1, Panel b).

Statistical analysis

Results are shown for continuous variables as mean and its standard error, and for discrete variables as percentages. Quantitative variables were compared between groups using

ANOVA. Genotype distributions were compared between smokers with and without COPD using Pearson's Chi-Square test, from which the odds ratio (OR) and the 95% confidence interval (95% CI) were calculated. Genotype frequencies were compared with values predicted by Hardy-Weinberg equilibrium equation ($p^2 + 2pq + q^2 = 1$) by the Chi-Square test, where p is frequency of dominant allele D and q is frequency of recessive allele d. A p value of less than 0.05 was considered significant.

Results

Age, smoking exposure and body mass index were similar in smokers with and without COPD (Table 1) and, by definition, the smokers with COPD showed characteristics of airflow obstruction (Table 1).

The distribution of the ACE genotypes in smokers that developed COPD was significantly different ($p = 0.023$) from those with normal lung function (Table 2). As hypothesized, the DD genotype was found to be more prevalent in patients with COPD (36.5% vs. 16.9%, $p = 0.01$) and the presence of the DD genotype in a smoker was calculated to give a 2-fold increase in the risk of developing COPD (OR 2.2; IC95% 1.1 to 5.5).

Surprisingly, we found Hardy-Weinberg disequilibrium in the smoking population ($n = 151$, $p = 0.001$). Deviations from this equilibrium can be caused by either genotyping errors and/or stratifications within the population being studied (Xu et al 2002) and we excluded the first possibility by using two different genotyping techniques. To address the second possibility, we further genotyped 159 randomly recruited individuals from the same population as our smokers and found their distribution of ACE genotypes to be in Hardy Weinberg equilibrium (Table 3). This observation further supports the validity of our genotyping techniques but more importantly, suggests that smoking may be associated with the ACE genotype. In fact, an excess of the heterozygote

Table 1 Clinical and functional data of the two life-long smoker groups

	Smokers with COPD (n = 74)	Smokers with normal lung function (n = 77)
Age (yr.)	62 ± 2	56 ± 2
Smoking history (pack-yr.)	49 ± 5	42 ± 5
Body mass index (Kg /m ²)	25 ± 3	27 ± 1
FEV ₁ (% reference)	44 ± 6	95 ± 3 *
FEV ₁ /FVC (%)	44 ± 3	78 ± 2*

* $p < 0.001$

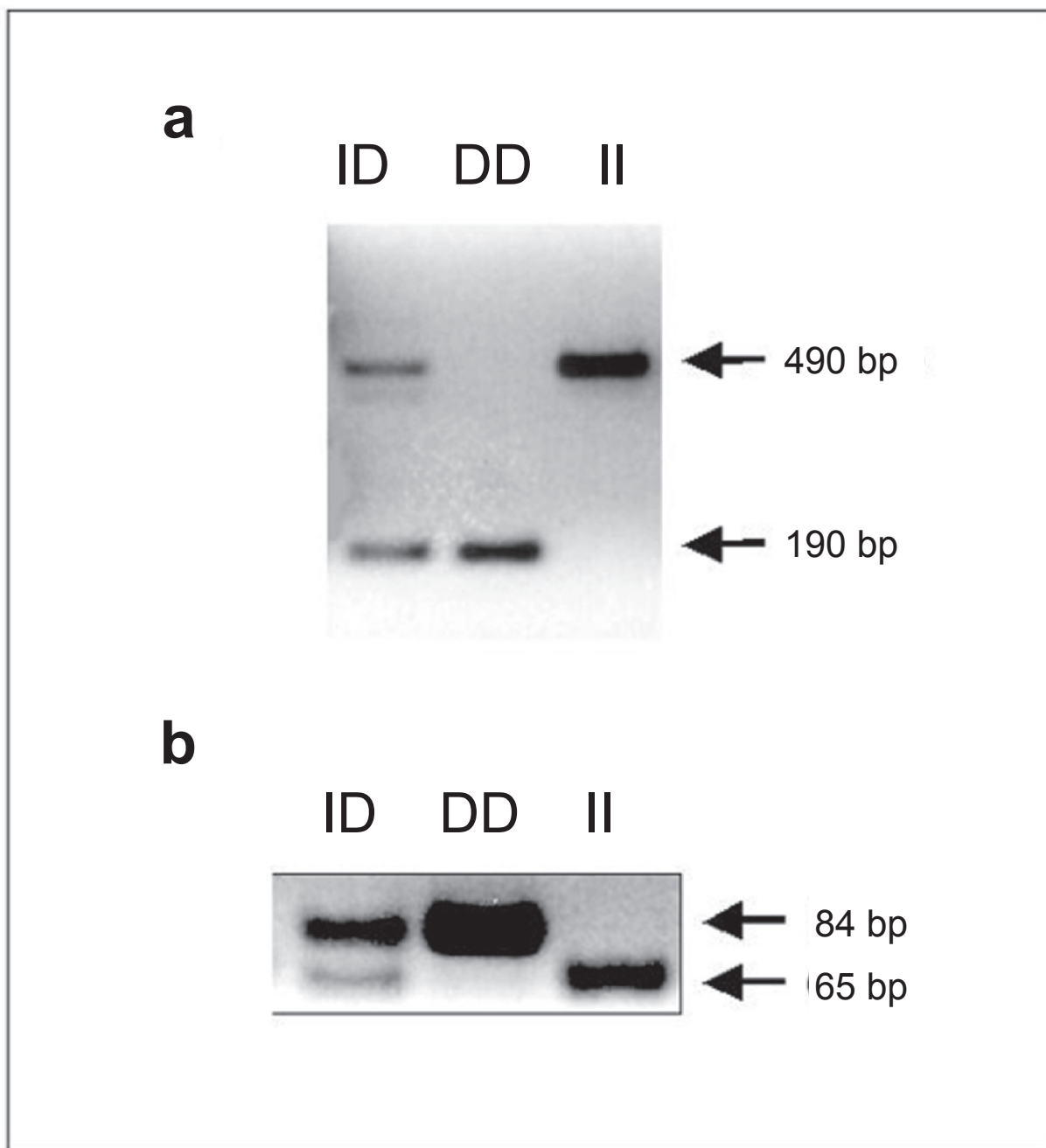


Figure 1 Determination of DD, ID, and II genotypes by PCR. **Panel a** shows a representative experiment using the method described by Rigat et al (1992). PCR products are a 190 bp fragment (D genotype) and a 490 bp fragment (I genotype). Heterozygotes (ID) have both the 190 bp and 490 bp fragments. **Panel b** shows a representative experiment using the method described by Evans et al (1994). PCR products are a 84 bp (D genotype) and a 65 bp (I genotype). Heterozygotes (ID) have both the 84 bp and 65 bp fragments.

(ID) was found in smokers ($p = 0.05$) irrespective of whether they had developed COPD (Table 3).

Discussion

This study shows that the DD ACE genotype is associated with an increased risk of a smoker to developing COPD and, unexpectedly, that the ACE heterozygote is more prevalent in individuals that smoke.

ACE and COPD

Previous studies have shown an association between ACE genotype and several phenotypic characteristics of COPD, for example, the development of pulmonary vascular remodelling, right ventricular failure and skeletal muscle strength (van Suylen et al 1999; Kanazawa et al 2000; Hopkinson et al 2004). To our knowledge, however, this is the first study to show that the presence of the DD genotype

Table 2 Distribution of the ACE genotypes in the different groups studied

		DD	ID	II	Total
Life-long smokers					
	<i>COPD</i>				
	n	27	40	7	74
	%	36.5	54.1	9.5	100
	<i>normal lung function</i>				
	n	13	53	11	77
	%	16.9	68.8	14.3	100
	<i>Total</i>				
	n	40	93	18	151
	%	26.5	61.6	11.9	100
General population					
	n	53	79	27	159
	%	33.3	49.7	17.0	100

increases the risk of a smoker developing chronic airflow obstruction. Plasma ACE activity is increased in COPD (Brice et al 1995; Ucar et al 1997) and while we did not measure it here the D allele is associated with higher plasma ACE activity (Rigat et al 1990; Busjahn et al 1998; Chagani et al 1999; Montgomery et al 1999). The high prevalence of the DD genotype observed in the smokers that developed COPD is therefore consistent with increased plasma ACE activity in these patients (Brice et al 1995; Ucar et al 1997). More importantly, our results suggest that the increased ACE activity reported in COPD (Brice et al 1995; Ucar et al 1997) can be related to the genotype and is not a consequence of the disease (leading to translational or post-translational modification of the protein).

Our study was not designed to investigate the potential mechanisms linking the ACE genotype to the pathogenesis of COPD. However, several possibilities can be conceived to explain a higher risk of COPD among smokers who carry the DD genotype. As discussed earlier, the presence of the D allele will increase ACE activity (Rigat et al 1990; Busjahn et al 1998; Chagani et al 1999; Montgomery et al 1999) and, in turn, cause higher Angiotensin II (AII) levels. Increased

AII levels will contribute to the inflammatory response that characterizes COPD (Barnes 1999, 2000) by (1) up-regulating expression of the endothelial adhesion molecules ICAM-1 and VCAM-1 (Guba et al 2000) and facilitating neutrophil retention within the lungs (Selby et al 1991; Keatings and Barnes 1997), (2) activating the neutrophil respiratory burst and contributing to oxidative stress (Zafari et al 1998), (3) enhancing airway hyper-responsiveness (Myou et al 2000), (4) regulating vascular smooth-muscle cell growth and migration (Hatakeyama et al 1994), that may contribute to pulmonary hypertension in COPD and (5) modulating tissue repair and fibrosis (Woods et al 2000).

ACE and smoking

We found that our smoking population was in Hardy-Weinberg disequilibrium and are confident that this did not result from genotyping errors or population stratification due to the reasons given previously.

There are a number of assumptions required to evaluate whether the genotype distribution within a population fulfils the requirements for Hardy – Weinberg equilibrium, namely that the gene is located in a somatic chromosome,

Table 3 Allele/Genotype frequencies and test of Hardy-Weinberg (HW) equilibrium

		General population				Smokers			
f(D)		0.581				0.573			
f(I)		0.419				0.427			
	O	E	X ²	P	O	E	X ²	P	
DD	53 (33.3%)	53.85 (33.9%)	0.01	>0.9	40 (26.5%)	49.5 (32.8%)	1.82	>0.1	
ID	79 (49.7%)	77.41 (48.7%)	0.02	>0.5	93 (61.6%)	73.8 (49%)	4.99	0.026	
II	27 (17%)	27.91 (17.4%)	0.009	>0.9	18 (11.9%)	27.5 (18.2%)	3.28	>0.05	
HW equilibrium			0.039	>0.5			10.09	0.0027	

f = observed frequency of each allele (D or I); O = observed genotype numbers; E = expected genotype numbers under a Hardy-Weinberg (HW) equilibrium assumption; X² = Chi-square values; P = probability of difference

the organism has discrete generations, there is random mating within a single population and an infinite population size (or sufficiently large so as to minimize the effect of genetic drift). Finally, there should be experiences of no selection, no mutation, and no migration (gene flow). We believe all apply in the populations studied here; however, replication by different investigators in different populations would be welcome to identify any causal link between the ACE gene and COPD. Departure from Hardy-Weinberg equilibrium also occurs when the selection criteria are based on disease-susceptibility genotypes rather than on independently selected alleles. In fact, marker-disease association can be detected by testing for Hardy-Weinberg disequilibrium at a marker locus (Nielsen et al 1998). Therefore, to explain the excess ID genotype observed among smokers (Table 3), we propose the existence of linkage disequilibrium between the trait of being a life-long smoker and the ACE locus. This interpretation is supported by a recent study showing that the ACE ID polymorphism influences the amount of cigarettes smoked per week (Hubacek et al 2001).

Molecular heterosis occurs when heterozygous subjects for a given polymorphism show a significantly greater or lesser effect for a trait than in homozygous subjects for either allele. We found molecular heterosis in smokers, where a significant proportion of individuals carried the ID genotype (Table 3). It is worth noting here that molecular heterosis (and Hardy-Weinberg disequilibrium) has also been found in studies of the dopamine D₂ receptor gene (DRD₂) in smoking males (Lee et al 2002; Lee 2003).

The biological basis of nicotine dependence is not fully understood, but it is well established that nicotine stimulates the central dopamine reward pathway (Pontieri et al 1996; Pontieri et al 1998; Alcantara et al 2003). Like nicotine (Zhou et al 2001), Angiotensin II also modulates dopamine release in the striatum (Jenkins et al 1997; Zhou et al 2001) and it is likely that the ACE gene is involved in the regulation of the dopamine reward pathway. This provides a mechanistic basis for the interpretation of our observation of Hardy-Weinberg disequilibrium and molecular heterosis in smokers.

Conclusion

Our study shows, first, that the presence of the DD ACE genotype increases the risk of smokers going on to develop COPD and, second, that smoking is linked to a Hardy-Weinberg disequilibrium in the allelic distribution of the ACE gene consistent with it playing a role in smoking addiction.

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Note

XB and NGM equally contributed to this work.

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