EDITORIAL

Tumour necrosis factor and lymphotoxin A polymorphisms: a relationship with COPD and its progression?

H.M. Boezen* and D.S. Postma[#]

In this issue of the *European Respiratory Journal*, TANAKA *et al.* [1] present the results of their study on polymorphisms in tumour necrosis factor (TNF) and lymphotoxin A (LTA) in relation to lung function in smokers. They designed two nested case-control studies in the Lung Health Study, originally enrolling almost 6,000 smokers with mild-to-moderate chronic obstructive pulmonary disease (COPD). They conclude that there exist no significant associations between polymorphisms in TNF or LTA and lung function in COPD patients of Caucasian origin, contrary to previously reported studies in Asians. It is important to note that this study assesses the genetics of progression of COPD and that the study cannot make any inference from this population as to whether the genes under study are COPD susceptibility genes. To meet this aim, one needs a general population study in which smokers with and without accelerated lung function loss are compared.

These findings are of importance, since they confirm and extend previously reported negative findings in Caucasians; among them is one of the authors' own studies that included only two polymorphisms in the genes [2]. In the present study, the authors analyse a sample that is sufficiently large to exclude potential negative findings due to small study populations, and thus lack of power. Indeed, one of the strengths of the study by TANAKA *et al.* [1] is the *a priori* power calculation, to ensure a sufficiently large sample to detect differences in distributions of polymorphisms between their COPD subgroups.

Unfortunately, too many studies on genetics of COPD are still being performed in COPD patients derived from population samples investigators happen to have at hand, including patients with inappropriately defined COPD phenotypes. This increases the likelihood of spurious results, since genotyping in excessively small sample sizes will lead to positive results in a pre-defined number of studies (e.g. 5%, if $\alpha=0.05$ is accepted). Thus, it is not surprising that a fair amount of positive results from genetic studies of COPD have not been replicated in similar or even larger populations. There is another reason

why it is important to publish (negative) studies like that of TANAKA *et al.* [1]. There may be an unjustified bias towards lack of publication of negative findings, and moreover it is necessary to discover that previously reported findings are unjustly called positive. Presumably, reviewers and journals are more likely to accept first-time positive findings than first-time negative findings. As for researchers, in order to prove that previous positive findings are in fact negative, large efforts and costs are required, since one needs a study sample that is easily three or four times as large as the one from the original study [3].

Another potential drawback of current genetic research practice also surfaces in the study of TANAKA *et al.* [1], namely the apparent difficulties of transposing results from one population to another. Findings in Asians are often not replicated in Caucasian subjects. True associations found in Asians may be missed in Caucasian populations as a result of different, usually lower, prevalences of the polymorphisms under study in Caucasian subjects. However, this explanation might be too simple. If the decision to study a particular gene is based on biological plausibility or homology with other diseases, one would expect that the majority of positive associations reported in Asians could be replicated in Caucasian subjects, assuming that the function of the polymorphism under study is the same in different races. Clearly, this presumption automatically stresses the importance of thorough research on the functionality of polymorphisms that have been found to be positively associated with COPD. In our opinion, this type of research will be crucially important for further understanding the pathophysiological processes that evolve in the pathway from genetics to phenotypic expression of COPD.

Apart from insufficiently large population samples, differences in the definition of the exact phenotypic outcome under study may also lead to difficulties in replicating results. A strength of the study of TANAKA *et al.* [1] is that the authors clearly define COPD in two ways, and study these phenotypic outcomes in two different nested case-control samples. First, they compare (a sample of) subjects with the fastest and the slowest lung function decline. Secondly, they compare subjects with the highest and lowest post-bronchodilator % predicted forced expiratory volume in one second (FEV1). Fortunately, both nested case-control samples are mutually exclusive, since one might expect a number of subjects who have the fastest decline

*Depts of Epidemiology and [#]Pulmonology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

CORRESPONDENCE: D.S. Postma, University Medical Center Groningen, Dept Pulmonary Diseases, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Fax: 31 503619320. E-mail: d.s.postma@int.umcg.nl

TABLE 1 Studies investigating links between tumour necrosis factor (TNF), lymphotoxin A (LTA) and chronic obstructive pulmonary disease (COPD)

First author [Ref.]	Gene	SNPs	Population	Results
COPD progression				
SANDFORD [2]	TNF	G-308A	591 smokers mild-to-moderate COPD; C	Negative Level of FEV ₁ decline
TANAKA [1]	TNF	G-308A, G-238A, rs3091257, rs769178	538 COPD (fast and slow decline)	Negative Fast/slow decliners
	LTA	A252G, G368A, T495C, C723A, rs2009658, rs3093543	1065 COPD (highest and lowest FEV ₁); C	Level of FEV ₁
SAKAO [7]	TNF	G-308A	84 COPD; A	Positive/negative Extent of emphysema
KEATINGS [8]	TNF	G-308A	106 COPD; C	Positive Mortality Level of FEV ₁
HIGHAM [9]	TNF	G-308A	84 COPD; C	Negative Level of FEV ₁ Diffusion capacity
COPD susceptibility				
RUSE [10]	TNF	G-308A	220 COPD; 141 controls; C	Negative COPD
PATUZZO [11]	TNF	G-308A	66 COPD, 23 bronchiectasis 45 lung disease/no obstruction 98 controls; C	Negative All four groups
	LTA			
KEATINGS [8]	TNF	G-308A	106 COPD; 99 Controls	Negative
BRÖGGER [12]	TNF	G-308A, G-376A, G-238A	244 COPD; 248 controls; C	Negative COPD
FERRAROTTI [13]	TNF	G-308A	63 COPD	Negative COPD
	LTA	SNP in intron 1	86 healthy smokers; C	
	TNF-r-1	Exon 1		
	TNF-r-2	Exon 6		
KUCUKAYCAN [14]	TNF	G-238A, G-308A, G-376A, G+489A	169 COPD; 358 controls; C	Positive G+489A COPD
MATHESON [15]	TNF	G-308A	1232 general population; C	Positive Reduced FEF ₂₅₋₇₅
JIANG [16]	TNF	G-308A	111 COPD; 97 controls; A	Negative COPD
HERSH [17]	TNF	G-308A	n=949, in 127 extended pedigrees of early onset COPD; C 304 COPD (NETT study, 441 controls (NAS study); C	Positive COPD Negative COPD
CHIERAKUL [18]	TNF	G-308A	57 smoking COPD 67 smoking controls; A	Negative COPD
SEIFART [19]	TNF	G-308A	113 COPD	Negative
	LTA	Intron 1 -252	113 CHD; 243 controls; C	All groups
HIGHAM [9]	TNF	G-308A	84 COPD; 63 (ex-) smokers; 199 controls; C	Negative COPD Airflow obstruction
ISHII [20]	TNF	G-308A	53 COPD; 65 controls; A	Negative COPD
HUANG [21]	TNF	G-308A	42 COPD; 42 (ex-) smokers; 99 population; A	Positive COPD

SNPs: single nucleotide polymorphisms; C: Caucasians; FEV₁: forced expiratory volume in one second; A: Asians; FEF₂₅₋₇₅: forced expiratory flow between 25 and 75% of forced vital capacity.

to have the lowest post-bronchodilator % pred FEV₁. Indeed, the authors indicate there was an overlap of ~25% between the two groups. However, the mutual exclusion of the two nested case-control samples does not withhold the fact that both COPD phenotypes might be highly correlated, and therefore the two different outcomes are not as “different” as might be suspected at first glance. Thus, the study would have gained in relevance if the cross-sectional (nested) case-control analyses had been extended to longitudinal analyses. This would have given insight into the potential (lack of) association of single nucleotide polymorphisms (SNPs) in the continuous range of lung function. Clearly, information about the potential (lack of) association between SNPs and lung function should preferably not be reduced to lack of associations in the extremes of the lung function continuum, but cover the whole range of lung

functions in-between, representing a different phenotype to that of the extreme lung function decliners. Our own studies in a very large general population cohort showed that it is important to study both high excess decline and no decline, as TANAKA *et al.* [1] did, as well as decline in lung function over the years, since both phenotypes may be different expressions of disease, and important associations may be identified studying the whole continuum of lung function [4, 5].

It is important to note that TANAKA *et al.* [1] studied the association between TNF and LTA and COPD progression in a cohort of smokers and ex-smokers. Phenotypic expression of COPD in smokers may be different from phenotypic expression of COPD in nonsmokers, although the latter group is hardly ever studied. Although it is generally accepted that the

majority of COPD patients are smokers or ex-smokers, pulmonologists observe subjects who claim they have never smoked in their life but who have developed COPD. It is possible that within this type of subject, genes other than those that are important for the development of COPD in smokers play a role. Therefore, we advocate additional studies on the genetics of COPD in these subjects, probably in relation to environmental exposures, since a majority of these never-smokers who have developed COPD might be passive smokers.

The Lung Health Study [6] has collected unique longitudinal data on lung function of (ex-) smokers, including detailed information on whether individuals quit or sustained smoking over many years. This allows study of the effect of quitting *versus* sustained smoking on the course of lung function. Across the 5-yr follow-up period, among sustained quitters, females gained more in % pred FEV₁ than males [6]. Thus, smoking cessation has an even clearer advantage for females than it does for males. Therefore, analyses stratified according to sex, investigating the effects of TNF or LTA polymorphisms in quitters and sustained smokers, might yield important information about pathophysiological mechanisms underlying the sex differences in susceptibility to cigarette smoke.

In conclusion, TANAKA *et al.* [1] report, contrary to previously reported positive studies in Asians on chronic obstructive pulmonary disease susceptibility, a lack of association between tumour necrosis factor or lymphotoxin A and lung function progression in patients with mild-to-moderate chronic obstructive pulmonary disease in Caucasian subjects. The sample size and methodology of this study suggest that we can finally conclude that there is no association within white subjects. Reading various other papers on the subject with conflicting results (clearly not all quoted by TANAKA *et al.* [1]; table 1), tumour necrosis factor and lymphotoxin A could still be given the benefit of doubt as candidate genes for the development of chronic obstructive pulmonary disease in other populations.

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