

# PostScript

## LETTERS TO THE EDITOR

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### TIMP-3 promoter gene polymorphisms in BFL

Bird fanciers' lung (BFL) is a form of hypersensitivity pneumonitis induced by inhalation of antigens from birds.<sup>1</sup> Only a small percentage of bird fanciers will develop BFL, so it is likely that these patients have a certain genetic predisposition to the disease.<sup>1</sup>

Matrix metalloproteinases (MMP) are zinc enzymes responsible for the degradation of the extracellular matrix. The proteolytic activities of MMP are counter-regulated by tissue inhibitors of MMP (TIMP). Hill found a decreased carriership of the rare TIMP-3 -1296C and -915G promoter alleles in Mexican patients with pigeon induced BFL, suggesting a protective effect of these alleles against the development of this disease.<sup>2</sup>

Only two previously published genetic association studies to date have focused on the susceptibility to BFL and both were performed in Mexican pigeon breeders.<sup>2,3</sup> We have undertaken a study to validate the association between BFL susceptibility in Mexicans and TIMP-3 promoter polymorphisms in a group of Dutch white patients with BFL.

Forty one patients with BFL (35 keeping pigeons, 10 keeping budgerigars, 3 keeping parrots and 1 keeping canaries; 19 women and 22 men) and 335 controls were genotyped using sequence specific primers and polymerase chain reaction. The diagnosis of BFL was established in concordance with the criteria used in the Mexican study.<sup>2</sup> The control group comprised healthy employees from our hospital. We did not include a group of bird fanciers without BFL since Hill did not

find differences in TIMP-3 allele distributions between Mexican controls with or without exposure to birds.<sup>2</sup>

The Dutch population was in Hardy-Weinberg equilibrium. In contrast to the previous TIMP-3 study in Mexicans, we found 100% linkage between the -1296T and -915A alleles and between the -1296C and -915G alleles in subjects homozygous for the respective alleles. We were therefore able to deduce two haplotypes (TA and CG). The TIMP-3 CG haplotype frequency in BFL patients was significantly lower than in controls ( $p = 0.0434$ ; OR 0.513 (95% CI 0.277 to 0.950;  $p = 0.0312$ ); table 1).

Hill described a similar association in Mexican patients with BFL. We found a reduction of the rarer TIMP-3 alleles in Dutch patients with BFL (-1296C and -915G, -11%), comparable to the reduction found in Mexican BFL patients (-1296C, -12.6%; -915G, -10.8%; table 1). However, there were differences between the findings of the two studies. The TIMP-3 -1296C and -915G allele frequencies in Dutch controls were significantly lower than in the Mexican controls (-1296C,  $p = 0.0008$ ; -915G,  $p = 0.0183$ ; table 1).<sup>2</sup> A search on the National Center for Biotechnology Information website showed similar TIMP-3 -1296C frequencies in Dutch and American controls (30 mother-father-child trios from Utah with northern and/or western European ancestry; [http://www.ncbi.nlm.nih.gov/SNP/snp\\_viewTable.cgi?pop=1409](http://www.ncbi.nlm.nih.gov/SNP/snp_viewTable.cgi?pop=1409); [http://www.ncbi.nlm.nih.gov/SNP/snp\\_ref.cgi?rs=9619311](http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=9619311)). BFL in Mexicans has a similarly poor prognosis to idiopathic pulmonary fibrosis, which contrasts with the more benign clinical course in Europeans.<sup>4,5</sup> Although most patients in our study had severe symptoms at presentation with profound pulmonary function abnormalities, symptoms and pulmonary function improved in the majority of cases during follow up. Furthermore, we included an approximately equal number of male and female patients and bird fanciers who kept birds other than pigeons, while all the Mexican patients were female and kept pigeons only.<sup>2</sup> Despite these genotypical and phenotypical differences, the rarer TIMP-3 promoter alleles were protective in both ethnic populations which makes an underlying functional cause of the CG haplotype likely.<sup>2</sup>

In conclusion, we found a decreased carriership of the TIMP-3 CG haplotype in Dutch patients with BFL, indicating a protective effect against the development of this disease. Studying the influence of polymorphisms on disease susceptibility in multiple ethnically and

geographically distinct disease and control populations is important. Our study is the first to confirm an association between polymorphisms and susceptibility to BFL, which adds importance to the relationship between TIMP-3 promoter polymorphisms and BFL. However, the mechanism by which the TIMP-3 variants may cause such a protective effect has yet to be determined.

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### References

- Bourke SJ, Dalphin JC, Boyd G, et al.** Hypersensitivity pneumonitis: current concepts. *Eur Respir J* 2001;**32**:81-92s.
- Hill MR, Briggs L, Montano MM, et al.** Promoter variants in tissue inhibitor of metalloproteinase-3 (TIMP-3) protect against susceptibility in pigeon breeders' disease. *Thorax* 2004;**59**:586-90.
- Camarena A, Juarez A, Mejia M, et al.** Major histocompatibility complex and tumor necrosis factor-alpha polymorphisms in pigeon breeder's disease. *Am J Respir Crit Care Med* 2001;**163**:1528-33.
- Sansores R, Salas J, Chapela R, et al.** Clubbing in hypersensitivity pneumonitis. *Arch Intern Med* 1990;**150**:1849-51.
- Bourke SJ, Banham SW, Carter R, et al.** Longitudinal course of extrinsic allergic alveolitis in pigeon breeders. *Thorax* 1989;**44**:415-8.

### Late CF caused by homozygous IVS8-5T CFTR polymorphism

The distribution of cystic fibrosis (CF) transmembrane conductance regulator (CFTR) genotypes is not well characterised in patients with CF diagnosed after childhood, the majority of whom are compound heterozygotes for  $\Delta F508$ .<sup>1</sup> We describe such a patient with a rare genotype more commonly associated with inherited infertility in males.

A 54 year old man who had never smoked was referred with bilateral bronchiectasis and chronic sinusitis. He had no known allergy, no history of pancreatitis, and no family history of CF or consanguinity. Obstructive infertility with azoospermia had been established by spermography. The patient reported recurrent lower respiratory tract infections since childhood and pneumonia at the age of 45. He had undergone sinus surgery for nasal polyposis.

CF was suspected. A first sweat test was positive with a chloride concentration of 65 mmol/l (normal <40 mmol/l). The patient had chronic cough productive of purulent sputum, mild dyspnoea, chronic nasal

**Table 1** TIMP3 -1296T>C and -915A>G allele frequencies in Mexican and Dutch controls and BFL patients

	TIMP3 -1296T>C		TIMP3 -915A>G	
	Mexican <sup>2</sup>	Dutch	Mexican <sup>2</sup>	Dutch
Controls	(n=323)	(n=335)	Controls	(n=323)
T	416 (64.4)	490 (73.1)	A	433 (67.0)
C	230 (35.6)	180 (26.9)	G	213 (33.0)
BFL	(n=115)	(n=41)	BFL	(n=115)
T	177 (77.0)	69 (84.1)	A	179 (77.8)
C	53 (23.0)	13 (15.9)	G	51 (22.2)

Data are given as absolute numbers with percentages in parentheses.

obstruction with nasal polyps and anosmia. His weight was 70 kg and his height 1.75 m. He had no digestive symptoms. Lung and heart auscultation was normal. A chest CT scan showed diffuse bronchiectasis predominating in the right upper and left lower lobes (fig 1). Lung function was near normal with forced expiratory volume in 1 second (FEV<sub>1</sub>) of 3.1 l (89% predicted), FEV<sub>1</sub>/forced vital capacity 0.73, total lung capacity 7 litres (100% predicted), and forced expiratory flow<sub>25-75%</sub> 61% of predicted. Arterial oxygen tension was normal. Both sputum and bronchoalveolar lavage cultures were positive for mucinous *Pseudomonas aeruginosa* but no mycobacteria or fungi were found. Serological examination for *Aspergillus fumigatus* was negative. Exocrine pancreatic sufficiency was confirmed by normal elastase levels in the stools. A second sweat test was normal (25 mmol/l).

A screening test for the 22 most frequent mutations of the CFTR gene encountered in France was negative. However, mutations of the CFTR gene were confirmed by the presence of homozygosity for the 5T allele in the polythymidine tract of intron 8 (IVS8-5T) with 11 TG repeats. The M470V polymorphism was absent. Sequencing of the full CFTR coding sequence including all 27 exons and the flanking splice sites showed no other mutation.

This patient had clinical features typical of CF involving several organs (bilateral

bronchiectasis, chronic sinus disease, male infertility) together with two pathogenic CFTR gene mutations, so a diagnosis of non-classic CF can be made.<sup>2</sup> The sweat test was positive on only one of two occasions, suggesting partial dysfunction of the CFTR protein.<sup>3</sup>

The IVS8-5T allele is associated with poorly effective usage of the intron 8 splice acceptor site compared with the two other existing alleles (7T and 9T) and results in frequent skipping of exon 9. Patients homozygous for the IVS8-5T allele have lower than normal levels of full length CFTR messenger RNA<sup>3</sup> and protein. Heterozygous IVS8-5T polymorphism is considered equivalent to a "mild" CFTR mutation. When *in trans* with a known CFTR mutation, the IVS8-5T allele may be responsible for congenital bilateral absence of the vas deferens or recurrent pancreatitis.<sup>4</sup> It may modulate the variable expression of "mild" CFTR mutations such as when present *in cis* of the R117H mutation, thus causing a CF phenotype.

Compound heterozygotes with IVS8-5T and ΔF508 may present with classic or late onset CF.<sup>1</sup> Whether IVS8-5T homozygosity may be sufficient by itself to cause disease has not hitherto been established. Non-classic CF was reported in a 48 year old woman homozygous for IVS8-5T, but the M470V polymorphism and TG12 repeat sequence known to modulate the disease penetrance of IVS8-5T were also present.<sup>5</sup>

This observation shows that individuals homozygous for the IVS8-5T allele as the sole variation of the whole CFTR coding sequence may present as non-classic CF with sinopulmonary disease and male infertility. However, given the high prevalence of the IVS8-5T allele (5–10% in the general population), the expected frequency of individuals homozygous for IVS8-5T may be higher than the prevalence of CF, suggesting that other factors may contribute to the disease. The IVS8-5T allele should be included in the systematic screening for CFTR mutations in patients with suspected or confirmed CF.

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References

- 1 Rodman DM, Polis JM, Heltshe SL, et al. Late diagnosis defines a unique population of long-term survivors of cystic fibrosis. *Am J Respir Crit Care Med* 2005;171:621–6.
- 2 Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. *J Pediatr* 1998;132:589–95.
- 3 Chu CS, Trapnell BC, Curristin S, et al. Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nat Genet* 1993;3:151–6.
- 4 Boyle MP. Nonclassic cystic fibrosis and CFTR-related diseases. *Curr Opin Pulm Med* 2003;9:498–503.
- 5 Noone PG, Pue CA, Zhou Z, et al. Lung disease associated with the IVS8 5T allele of the CFTR gene. *Am J Respir Crit Care Med* 2000;162:1919–24.

CORRECTION

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In the paper entitled "Relationship between reduced forced expiratory volume in one second and the risk of lung cancer: a systematic review and meta-analysis" by S Wasswa-Kintu et al which appeared on pages 570–575 of the July 2005 issue of *Thorax*, the correct figure for the worldwide mortality from lung cancer in 2000 (mentioned in the second line of the first paragraph) is 0.85 million, not 328 million as stated in the article.

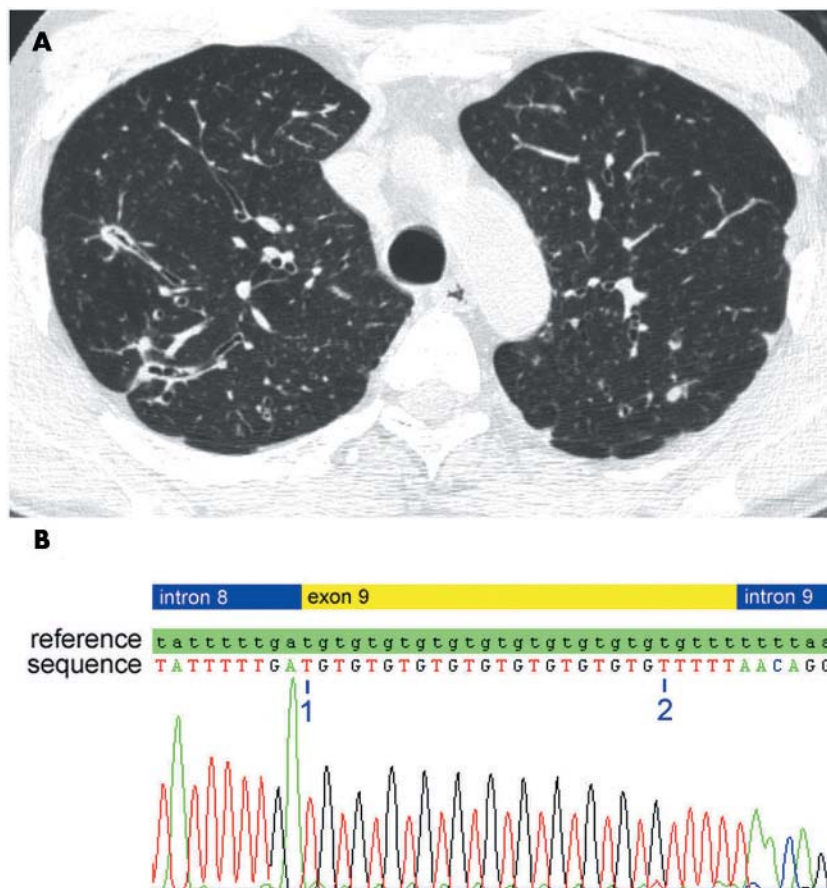


Figure 1 (A) Chest CT scan showing bronchiectasis and bronchial wall thickening predominating in the right upper lobes. (B) Sequencing of the IVS8 locus. 1 indicates the first T of the 11 TG repeats and 2 shows the first T of the 5T motif of the IVS8 locus. The reference DNA sequence is indicated as "reference" and the DNA sequence of the patient is indicated as "sequence".