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LETTER

CFTR and asthma in the French EGEA study

It has been suggested that ΔF508 heterozygosity[1] in the *CFTR* gene and other variants[2] could be risk factors for asthma. Results regarding ΔF508 come from a Danish study conducted in about 9000 subjects from the general population.[1] However, the hypothesis that ΔF508 heterozygosity in the *CFTR* gene could protect against asthma, was proposed earlier after a study conducted in obligate heterozygotes.[3] Recently, a case control study based on 144 asthmatics recruited in emergency rooms in Barcelona and a first control group of 41 spouses of CF carriers showed an excess of heterozygotes for aminoacid variants in the asthmatics, R75Q, G576A, R668C and L997F being the most frequent. Furthermore, in the asthmatic group only, a positive association of MM or MV in M470V variant in the previous aminoacid variants was observed, whereas the 5T allele in the IVS8-(T)n was never associated with these variants. In a second control group of 184 blood donors, however, the proportion of the four previous aminoacid variants was similar to the distribution in the asthmatics.[2]

The potential association of ΔF508 and other CFTR aminoacids and IVS8-(T)n variants to asthma has been studied in the French epidemiological study on the genetics and environment of asthma, bronchial hyper-responsiveness and atopy (EGEA). In that study, cases were recruited in chest clinics in five cities in France with a standardised definition of asthma.[4] They were examined together with population-based controls and first degree relatives of the cases by a standardised protocol. Association of ΔF508 heterozygosity to positive skin prick test of *Aspergillus* in a subsample of relatives and controls has been previously reported.[5] In the present analysis, 247 cases and 233 population-based controls (204 without asthma) were compared. They were carefully matched for place of birth (Table 1), taking five regions based on previous knowledge of genetic markers within France.[6] Asthmatics were well characterised[7] and had more severe symptoms than those in the general population, with around one third of asthmatic cases having been hospitalised for asthma, and more than two thirds on inhaled steroids.

All subjects were typed blindly to case control status for ΔF508, IVS8-(T)n and M470V in Poitiers. Due to the work load, only the four most common variants previously observed in asthmatics (R75Q, G576A, R668C and L997F), which accounted for 50% of mutations in asthmatics in the Barcelona study, were typed in the EGEA study as previously in the second control group in Barcelona, using the same techniques.[2] The most common mutation in cystic fibrosis, ΔF508 was analysed by acrylamide gel electrophoresis, and the missense variant M470V[8] was analysed by DGGE. The detection of the IVS8-5T, -7T and -9T variants[9] was performed by non-denaturing polyacrylamide gel electrophoresis after PCR amplification. Three missense variants R75Q,[10] G576A,[11] L997F[11] were analyzed by restriction analysis. Variant R668C[11] was analyzed by SSC *A*.

Prevalences in the whole sample of 480 subjects were 2.9%, 3.8%, 4.0%, 4.2% and 0.6% for heterozygosity for ΔF508, R75Q, G576A, R668C and L997F, respectively. Regarding M470V, 18.3%, 47.9% and 33.8% were MM, MV and VV and 5T/- occurred in 8.8%.

Comparisons between cases and various control groups were performed in order to increase the contrast: cases *vs* all 233 controls (representing the general population), cases *vs* the 204 non-asthmatic controls, and cases *vs* the 174 non-asthmatic controls with both parents without asthma. As conclusions were similar, figures (Table 1) are given for the comparison with the greatest phenotypic contrast only. Controls were older than cases, which also increases the contrast for a disease with a variable age of onset. For ΔF508, the odds ratios were greater than 1, but not statistically significant. No particular pattern was observed considering FEV_1, FVC or bronchial responsiveness. Any variant (R75Q, R668C, G576A, L997F), carriers of M allele (M470V), or 5T/-, shows odds ratios lower than 1, which were not statistically significant, except for 5T/-. That unexpected negative association with 5T/- needs confirmation in other studies. None of the variants studied was significantly related to the region of birth in France. Analyses taking into account the region of birth led to the same conclusions. Relations between variants suggesting linkage disequilibrium were evidenced. ΔF508, 576A, 668C were significantly ($P = 0.001$) related to the M allele in M470V, whereas the opposite non-significant trend was observed for 75Q. IVS8-(T)n was also related ($P = 0.001$) to M470V as 97% of VV *vs* 39% of MM were 7T7T. The association of M470V with the other variants was similar in cases and controls.

The EGEA study differs in its population and its design from previous studies on ΔF508. It is based on a case-control approach with careful matching of cases and controls in respect of geographic origin. A first possible method of assessing the relationship of ΔF508 to asthma consists of comparing the prevalence of asthma in obligate heterozygotes and in non-carriers.[3,12] In the first study, Schroeder *et al* first estimated the expected percentage of ΔF508 carriers in relatives of various degrees of relationship to cystic fibrosis patients. They then compared it with the observed number of carriers in those relatives who were asthmatics and concluded that heterozygotes had three times less chance of developing asthma than wild homozygotes. The limitations of this study were the diagnosis of asthma based on non-

Table 1 Comparison of cases and controls from the EGEA study

	Asthma cases	*Controls: no asthma* *Both parents: no asthma*	P *value* *OR [95% CI]*
Number	247	174	
Demographic and clinical characteristics			
Geographical origin within France			
Paris, %	24.9	25.8	0.98
North West, %	11.0	9.8	
South West, %	5.3	4.6	
North East, %	6.9	8.1	
South East, %	51.8	51.7	
Age, m±SD	30.2±17.9	34.7±16.1	0.01
[range]	[7.0–68.8]	[7.4–64.7]	
Sex, % males	57.1	49.4	0.12
Atopy (weal ≥ 3mm, any of 11 allergens), %	76.8	34.8	0.001 6.4 [4.2–9.7]
IgE, IU/ml, GM	246	36	0.001
Hay fever or childhood eczema, %	61.4	30.5	0.001 3.6 [2.4–5.5]
FEV_1 % predicted, m±SD	0.88±0.19	1.04±0.15	0.001
FVC % predicted, m±SD	0.99±0.16	1.04±0.15	0.001
Methacholine challenge, number[a]	113	127	
PD_{20} 4mg, %	92.9	22.8	0.001 44.4 [22.4–87.8]
CFTR data			
ΔF508, %	3.2	2.9	0.83 1.13 [0.36–3.52]
R75Q, %	2.4	5.2	0.14 0.46 [0.16–1.28]
G576A, %	3.6	4.0	0.84 0.90 [0.33–2.47]
R668C, %	3.6	4.6	0.62 0.79 [0.30–2.07]
L997F, %	0.4	0.6	1.0[b]
R75Q or G576A or R668C or L997F, %	6.9	9.8	0.28 0.68 [0.34–1.37]
M470V			
MM, %	18.2	17.2	
MV, %	46.2	50.6	0.66
VV, %	35.6	32.2	
IVS8-(T) n, 5T/–, %	6.9	12.6	0.05 0.51 [0.27–0.99]

[a]FEV_1 > 80% predicted and no contraindications. [b]Fisher exact test.

standardised records, potential non-paternal transmission in these families and limitations inherent in the study of obligate heterozygotes.

A second design, in which the prevalence of asthma in carriers and non-carriers drawn from the general population are compared, was used in the Danish study. In the 250 heterozygotes detected in more than 9000 subjects, 9% reported a history of asthma versus 6% in the other subjects, corresponding to an odds ratio of 1.60 [1.04–2.47] which after adjustment for sex, age, and other factors remained statistically significant with an odds ratio of 2.0 [1.2–3.5]. Results from the EGEA study, although not significant, are compatible with the data of that large population survey. The advantage of the Danish study is the representativeness of the population, the objective measures of lung function and the large sample size. The weakness is the little information available regarding asthma.

Although differences in population could explain the differences between asthmatics in Barcelona and in France, the most likely hypothesis is that the four variants which were unusually frequent in the asthmatics in the first study do not relate to asthma, since a similar distribution to that of

the cases was observed in a sample of blood donors in Barcelona, unconnected, and if anything, an opposite trend was observed in the French case control study, with a careful match according to place of birth. Other studies on a sufficiently large sample size of appropriately matched cases and controls with complete screening of the *CFTR* gene would be necessary to exclude any association between the *CFTR* gene and asthma. In conclusion, results do not support the hypothesis of any important role of ΔF508 or the other aminoacid variants currently studied in asthma.

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