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SHORT REPORT

Increased frequency of CFTR gene mutations in sarcoidosis: a case/control association study

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A complete screening of the *CFTR* gene by DGGE and DNA sequencing was performed in patients with sarcoidosis. In 8/26 cases, missense and splicing *CFTR* gene mutations were found, a significant difference over controls (9/89) from the same population (*P* = 0.014). The odds ratio for a person with a *CFTR* gene mutation to develop the disease is 3.95 (1.18 < OR < 13.26). Seven different *CFTR* gene mutations were observed: *R75Q*, *R347P*, *621 + 3 A/G*, *1898 + 3 A/G*, *L997F*, *G1069R*, and a novel mutation which was detected in this study, *I991V*. *R75Q* mutation was present in 3/26 patients, a significant increase (*P* = 0.01) in cases over controls, indicating its preferential association with sarcoidosis. A trend towards disease progression was observed in patients with *CFTR* gene mutations compared to patients without mutations. These data suggest that *CFTR* gene mutations predispose to the development of sarcoidosis. *European Journal of Human Genetics* (2000) **8**, 717–720.

Keywords: *CFTR* gene mutations; sarcoidosis; pulmonary diseases; cystic fibrosis; genotype–phenotype correlations

Introduction

Sarcoidosis is a multisystem granulomatous disorder characterised by non-caseous granulomata and an accumulation of immunocompetent cells at sites of disease activity.[1] The aetiology of the disease is not known, but there is a growing body of evidence strongly suggesting that in sarcoidosis, as in other complex traits, environmental factors may contribute to the onset of the disorder in genetically predisposed individuals.[2] A genetic component in sarcoidosis is indicated by the varying incidence among different ethnic groups, and by the occurrence of familial clustering of cases.[3,4] Furthermore, genetic factors are believed to play an important role in determining the pattern of the disease, its severity, and prognosis: in other words, genetic variation may underlie the different phenotypes of the disease.[5] No linkage to a chromosomal region where candidate genes for two granulomatous disorders with clinical similarities to sarcoidosis are located has been observed in African-American sib pairs with sarcoidosis.[6] During a *CFTR* gene mutation screening in pulmonary diseases, we previously analysed a group of patients with a variety of obstructive and non-obstructive pulmonary diseases.[7] In that paper, we reported that 5/8 (62%) of sarcoidosis patients had a mutation in the *CFTR* gene. This high incidence in a limited number of patients prompted us to analyse a second enlarged series of patients to confirm the data. We now report that in 26 new patients with sarcoidosis there is an increased frequency of mutations in the *CFTR* gene. These data confirm the initial finding, and indicate that the *CFTR* gene may increase the genetic susceptibility to the development of the disease.

Preliminary findings were communicated at two international meetings.[8,9]

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Materials and methods

Patients

Twenty-six adult unrelated consecutive patients with sarcoidosis were collected. All patients were Italians of white ancestry, and living in a northern region of Italy (Lombardy). Diagnosis and assessment of sarcoidosis was performed according to the recently published guidelines.[10] Chest

radiographic staging was assessed according to De Remee.[11] Twenty-three out of 26 patients had sarcoidosis confirmed by one or more biopsies. Two patients refused biopsy. Both were in chest radiologic stage II and they showed clinical features consistent with clinical diagnosis of sarcoidosis. In one patient, presenting with classical Löfgren's syndrome, confirmatory biopsy was not needed.[10] For these reasons, the three patients with clinical diagnosis of sarcoidosis were included in our series (see Table 1).

The control group was formed by 89 consecutive patients presenting to the same department for other pulmonary diseases (27 with chronic bronchitis, 25 with emphysema, 26 with lung cancer, four with active tuberculosis, five with bacterial pneumonia, two with spontaneous pneumothorax.[7] All subjects were of the same ethnic and geographic origin as the sarcoidosis patients. In the control group, assessment of the underlying disease excluded the coexistence of sarcoidosis.

Mutation analysis

Genomic DNA was extracted from peripheral whole blood samples by standard methods.[12] A complete analysis of all the 27 exons of the *CFTR* gene and their intronic flanking regions was performed by denaturing gradient gel electrophoresis (DGGE), as previously reported.[7] By this method, individual genotypes were correctly determined for 73/74 *CFTR* gene mutations not originally detected by DGGE, and located at different sites all along the gene, with a sensitivity of 98.6%.

Mutations detected by DGGE analysis were identified by automatic DNA sequencing with the ABI Prism 377 sequence analyser (PE Applied Biosystems, Foster City, CA, USA). Moreover, individuals were genotyped for three intronic mutations: *IVS8-6* T_nN,[13] *IVS8* $(TG)_m$-T_n,[14] 3849 + 10Kb C→T.[15]

Analysis of the effect of mutations on splicing efficiency was performed with the use of the software BCM Gene Finder/HSPL, available on the Baylor College of Medicine Search Launcher website (http://dot.imgen.bcm.tmc.edu:9331).

Statistical analysis

The frequency of mutations was determined by patient counts. Differences between patients and controls were compared by Fisher's exact test, using the EPI Info software (version 5.01). A P value of less than 0.05 was considered to indicate statistical significance. From the previous finding of 5/8 sarcoidosis patients with *CFTR* gene mutations compared with 9/89 individuals with other pulmonary diseases from the same population,[7] it was calculated that a new sample of 28 patients would have the power of 80% to detect a *CFTR* gene effect with an odds ratio of 5.

Results

We performed the complete DGGE analysis of the *CFTR* gene in the 26 patients with sarcoidosis. We found eight patients with mutations (30.8%) in the *CFTR* gene, as reported in Table 2, column 2. This frequency represents a significant

Table 1 Characteristics of the sarcoidosis patients (n = 26)

Sex	Females 10 Males 16
Age of onset (yrs, mean ± SD)	38.4 ± 9.2
Stage	I 6 (23%) II 12 (47%) III 8 (30%)
Pulmonary function tests (mean ± pred.) Obstructive syndrome	FEV1 93% – FVC 92% 8/26 (30%): FEV1 71% – FVC 79%
Biopsy	23/26 (88%) Scalene lymph. 10 Lung 9 (TBB 4; Surgical 5) Other sites 4
Extrapulmonary localisations	6/26 (23%) Eye 3 Skin 3
Duration of the disease (months mean ± sd)	67.6 ± 47.1
Follow up available (> 1 yr)	24/26 (92%) Remission 5/24 (20%) Persistence 19/24 (80%)
Patients with relapses	10/24 (42%) 4 pts with 1 relapse 4 pts with 2 relapses 1 pts with 3 relapses 1 pts with 4 relapses

Table 2 CFTR genotypes of sarcoidosis patients (n = 26)

ID	*Missense and splicing mutations*	*Same sense and intronic mutations*	*TGm-Tn*	*M470V*
11	G1069R		11–7/12–7	V/V
13	R347P	4404 C/T	10–7/11–7	M/V
15	R75Q, 1898+3A/G	186-13 C/G	11–7/11–7	V/V
20	621+3 A/G		10–7/10–7	M/M
21	R75Q		11–7/11–7	V/V
27	R75Q	1716 G/A	10–7/11–7	M/V
31	I991V		11–7/10–9	M/V
32	L997F	4002 A/G, 3041-71 G/C	10–9/11–9	M/V
10		4404 C/T	11–7/11–7	V/V
18		4002 A/G	11–7/12–7	M/V
24		3417 A/T	11–7/11–7	V/V
28		4002 A/G	11–7/11–7	M/V
34		4002 A/G	11–7/11–7	M/V
26			12–5/11–7	V/V
16			12–5/11–7	V/V
9			11–7/11–7	V/V
12			12–7/10–9	M/M
14			11–7/10–9	M/V
17			11–7/11–7	V/V
19			11–7/10–9	M/V
22			10–7/11–7	M/V
23			10–7/11–7	M/V
25			10–7/11–7	M/V
29			11–7/11–9	M/V
30			11–7/11–7	V/V
33			11–7/12–7	V/V

The phase of the mutations is not known, as no segregation analysis was possible.

increase over controls (9/89; 10.1%, Fisher's exact test, P = 0.014; OR = 3.95, 1.18–13.26). Seven different missense or splicing mutations were found in the eight patients: *R75Q, R347P, 1898 + 3 A/G, 621 + 3 A/G, L997F, G1069R, I991V*. *R75Q* was present in three patients (nos. 15, 21, 27). This is a significant increase over controls (3/26 *vs* 0/89, respectively, P = 0.01). *R75Q* has been described in CF and related diseases, as disseminated bronchiectasis,[7,16] and allergic broncho-pulmonary aspergillosis.[17] One patient (no. 15) had two *CFTR* mutations (*R75Q*, 1898 + 3 A/G), but it was not possible to determine the phase. *R347P* is known to cause CF.[18] *1898 + 3 A/G* has been found in 1/225 genes from an Italian CF birth cohort we have previously described.[19] The mutation *1898 + 3 A/G* abolishes the donor splice site (program cited in methods), with the possible consequence of exon 12 skipping from mature mRNA. *621 + 3 A/G, L997F,* and *G1069R* have been described in rare CF cases (Cystic Fibrosis Genetic Analysis Consortium website: http://www.genet.sickkids.on.ca). Mutation *621 + 3 A/G* abolishes the donor splice site (program cited in Methods), with the possible consequence of exon 4 skipping from mature mRNA. *L997F* was found also in disseminated bronchiectasis.[7,16] *I991V* is a novel mutation here described for the first time: it changes isoleucine to valine (both hydrophobic residues) in the second transmembrane domain. Isoleucine is a conserved residue in 4/5 species, and valine is present in the fifth species.[20]

The frequency of three common mutations which modify gene expression by alternative splicing (*IVS8* TG_m–T_n), or by the efficiency of protein maturation (*M470V*) was not different from controls (see Table 2, columns 4 and 5, respectively). In particular the IVS8-5T allele, which determines the production of increased amounts of transcripts lacking exon 9,[21] was found in 2/26 patients, not statistically different from controls (12/89).

Several common polymorphisms not thought to cause CF, and including same sense mutations (*1716 G/A, 2694 T/G, 3417 A/T, 4002 A/G, 4404 C/T,* and *4521 G/A*), and deep intronic mutations (*186-13 C/G, 875 + 40 G/A, IVS6* $(GATT)_n$, and *3041-71 G/C*) were found. No significant difference was detected between cases and controls for any of these common polymorphisms.

Discussion

These data indicate a significant excess of *CFTR* gene mutations in sarcoidosis patients. The data confirm, in a second series of patients from the same department, the excess of *CFTR* gene mutations found in our first report, in which 5/8 patients with sarcoidosis were found to carry *CFTR* gene mutations.[7] The total of this study plus the previous one is 13/34 sarcoidosis patients with at least one *CFTR* mutation (P = 0.0006; OR = 5.5, 1.88 < OR < 16.41). With the exception of two novel mutations, *E826K*[7] and *I991V* (this study), all the mutations present in the 34 patients with sarcoidosis (*R75Q, 621 + 3 A/G, R347P, DF508, 1898 + 3 A/G, V754M, L997F, G1069R, 4382 del A*) have also been observed in CF and CF-related diseases. Two recurrent mutations were observed in sarcoidosis: *R75Q* found in 3/34 patients and in 0/89 controls (P = 0.02), and *L997F* found in 2/34 patients and in 0/89 controls (NS). *R75Q* may therefore be a *CFTR* gene mutation characteristic of sarcoidosis.

When the 34 sarcoidosis patients are subdivided into those with (*CFTR+*) and those without (*CFTR-*) *CFTR* gene mutations, and several clinical parameters are analysed, no significant differences were observed for mean age and symptoms at onset of the disorder, duration of the disease, outcome, and development of airflow obstruction. Interesting, but not significant, differences were the increase in *CFTR+* patients compared with *CFTR-* patients of:

1) chest radiologic stage I at entry (39% *vs* 24%);
2) relapses (58% *vs* 44%);
3) extrapulmonary localisations (31% *vs* 19%); and
4) chest radiologic stage progression (30% *vs* 11%), respectively.

This is an indication of a possible increased severity of the disease in the presence of *CFTR* gene mutations.

The mechanism of *CFTR* gene involvement in sarcoidosis aetiopathogenesis is unknown. Sarcoidosis is a complex disease in which genetic and environmental factors may play

a role. Based on the fact that the granulomatous inflammation in sarcoidosis may be determined by a bacterium or a virus, and that the *CFTR* gene acts as a receptor for *Pseudomonas aeruginosa*[22] and *Salmonella typhi*,[23] an hypothesis is that the presence of *CFTR* gene mutations modify the effect of infection on disease onset and development. Further studies are needed to investigate the possible role of CFTR in determining a genetic predisposition to the disease.

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