Cystic fibrosis genotype and bacterial infection: a possible connection

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ABSTRACT

Patients with cystic fibrosis (CF) have repeated bacterial infection of the airways, which can lead to chronic infection. There is evidence that disease severity is determined by the genetic mutations present. This study aims to establish if CF genotype is related to the frequency and types of airway bacterial infection. Adult patients attending the regional CF unit are followed for two years and assigned to one of three groups depending on whether they are chronically infected with *Burkholderia cepacia* complex (BCC) organisms, *Pseudomonas aeruginosa*, or neither of these organisms. Genotype analysis is performed on all patients to determine which of the cystic fibrosis transmembrane regulator (*CFTR*) gene mutations are present. The numbers and types of organism with the CFTR mutations isolated from sputum are identified. Data are available on 59 patients: 15 colonised with BCC, 24 colonised with *P. aeruginosa*, and 20 not colonised with either organism. Twenty patients were homozygous for ΔF508, 25 were heterozygous, and the ΔF508 mutation was not present in the remaining 14 patients. Patients homozygous or heterozygous for the ΔF508 mutation had an increased likelihood of colonisation with BCC or *P. aeruginosa*, an increased number of positive sputum cultures and a higher frequency of multiple infecting organisms. Cystic fibrosis mutational analysis identified seven patients who had the R117H mutation. These patients were less likely to be colonised with BCC or *P. aeruginosa*. In conclusion, patients homozygous or heterozygous for the ΔF508 deletion are more likely to suffer airway colonisation with BCC or *P. aeruginosa*, with increased numbers of positive sputum cultures and infecting organisms. Those with the R117H mutation are less likely to be colonised by Gram-negative organisms.

KEY WORDS: Bacterial infection. Burkholderia cepacia complex. Cystic fibrosis. Genotype. Pseudomonas aeruginosa.

Introduction

Patients with cystic fibrosis (CF) have repeated bacterial infection of the airways, which can lead to a chronic condition. Over 1000 mutations have been identified in the cystic fibrosis transmembrane regulator (*CFTR*) gene.[1] There is inconclusive evidence that pulmonary disease severity is determined by the genetic mutations present, but stronger associations have been determined for pancreatic status.[2,3]

It has been shown, however, that patients homozygous for 'severe mutations' present earlier with more severe respiratory disease and have a higher *Pseudomonas aeruginosa* colonisation rate.[4] This group of patients also has a more rapid decline in their forced expiratory volume in one second (FEV_1) measurement and requires more courses of antibiotic therapy.[5]

Studies also suggest that the presence of the R117H mutation confers a better prognosis in terms of pancreatic sufficiency and mortality rate.[6,7] Exacerbations associated with the identification of an infecting organism are associated with a more rapid decline in lung function, admission to hospital and earlier acquisition of *P. aeruginosa*.[8–10] It is also known that those patients who are infected with *Burkholderia cepacia* complex (BCC) have a worse prognosis, a more rapid decline in lung function, and increased mortality.[11]

It is not clear, however, whether or not a patient's genotype determines the types of organism with which they become infected during exacerbations, and therefore this study aims to discover if a patient's genotype affects the frequency and type of bacterial infection / co-infection and colonisation.

Materials and methods

Patients and bacteriology

Data were collected on all patients (>16 years old) attending the regional adult CF unit at the Belfast City Hospital over two consecutive years. The majority of patients were diagnosed with CF by the neonatal immunoreactive trypsinogen (IRT) test screening programme, with subsequent confirmation by sweat test (sweat chloride >60 mmol/L) and/or mutation analysis.

Sputum samples were obtained for each patient at regular clinic attendances (three monthly intervals) as well as twice during hospital admissions. Culture for BCC was performed using selective agar comprising Columbia agar base (CM331, Oxoid, Hampshire, UK) with 5% defibrinated horse blood (E & O Laboratories, Bonnybridge, Scotland). All isolates

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Table 1. Median (interquartile range) of number of sputum specimens, organisms and co-infecting organisms (per patient) in each group. Mean (±SD) number of individual co-infecting organisms isolated per patient in each group, patient age, FEV_1 and change in weight.

	ΔF508 homozygous	ΔF508 heterozygous	ΔF508 not present
Number of patients	20	25	14
Male/female	15/5	18/7	7/7
Mean age (years)	23.7±6.2	22.0±12.9	22.7±4.3
FEV_1 (litres)	3.3±1.1	2.3±1.2	2.1±0.9
Δ Weight (kg)	(+) 0.37±3.14	(+) 0.86±3.26	(+) 1.65±3.52
BCC infected	7**	7**	1
P. aeruginosa infected	12**	11**	1
Not colonised	1	7	12
Specimens per patient#	17.0** (8.0 – 26.5)	18.0** (4.0 – 36.0)	6.3 (0 – 9.8)
Organisms per patient†	26.0 (12.5–35.0)	21.0 (7.0–50.0)	0 (0–11.0)
Co-infecting organisms per patient‡	7.0* (0.3–10.8)	7.0* (1.0–23.5)	0 (0–4.3)
P. aeruginosa co-infection	10.6±17.1	6.9±14.5	0.9±3.0
Staphylococcus aureus co-infection	1.6±2.3	4.9±7.6	1.4±4.8
Haemophilus influenzae co-infection	0.8±1.9	0.8±1.5	1.4±4.0
Streptococcus pneumoniae co-infection	0.5±1.1	0.1±0.3	0.3±1.1
Stenotrophomonas maltophilia co-infection	0.7±1.5	4.4±9.1	0.4±0.9
Other organism co-infection	2.2±5.2	2.7±4.9	1.1±2.0

#Mean total number of positive sputum cultures per patient over the complete study.

†Total number of different bacterial organisms isolated in that group of patients during the study period.

‡Number different bacterial organisms isolated, excluding BCC or *P. aeruginosa* in those patients colonised with these bacteria.

*$P<0.05$, **$P<0.01$

were grouped into cultural phenotypes displaying similar visual characteristics and one colony from each was identified by API (bioMerieux).

Sputum culture results were retrieved from the bacteriology database for all samples submitted over the study period. Patients were then divided into three groups depending on whether they were chronically infected with BCC organisms, had *P. aeruginosa* identified on two or more occasions over a 12-month period, or were not chronically infected with either of these organisms. A note was also made about whether the positive culture consisted of one organism or if additional organisms were identified. In the BCC and *P. aeruginosa* groups, when more than one organism was identified from a sputum culture the additional organism(s) where recorded as co-infecting, as where all organisms in cultures of two or more infecting organisms in the group that showed neither BCC nor *P. aeruginosa* infection.

Co-infecting organisms were divided into groups: *P. aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Stenotrophomonas maltophilia* and all other organisms, including fungi. Spirometry (Vitalograph α, Buckingham, UK) and oxygen saturation measurements were noted at the beginning and end of the study, and the best measurement for each year was recorded. Weight was recorded at the beginning and end of the two-year period.

Genotyping

Extraction of DNA from whole blood was performed using either a guanidine hydrochloride method[12] or the GenFast DNA extraction system (Whatman, London, UK) and stored at –80°C. Initial mutation screening was performed using the Elucigene kit (Orchid Biosciences), the most recent version of which (CF29) can detect 29 of the most common *CFTR* gene mutations.

Approximately 99% of Northern Irish patients with classical CF symptoms show at least one *CFTR* mutation.[13] Patients heterozygous for a mutation or who were negative on screening but had a confirmed diagnosis were screened for additional mutations. Mutation analysis was performed by a combination of denaturing gradient gel electrophoresis (DDGE), later superseded by temporal temperature gradient gel electrophoresis (TTGE), and direct sequencing. Primers and melting profile data for TTGE were supplied by Ingeny International and sequencing primers were those used by Zielinski *et al*.[14]

The majority of *CFTR* exons were screened initially using DDGE/TTGE analysis, and DNA was amplified using the standard polymerase chain reaction (PCR) conditions from Ingeny International, followed by heteroduplex generating conditions. Products were run on the BioRad D-Code system and the acrylamide gels were visualised using ethidium bromide staining and ultraviolet (UV) transillumination. Band shifts were characterised by direct fluorescence dye terminator sequencing on an ABI 310 or ABI3100 instrument. Patients negative on TTGE screening were screened by direct sequencing for all *CFTR* exons.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version II package. Characteristics of the subjects are presented as mean (± SD).

Table 2. Median (interquartile range) of number of sputum specimens, total organisms and of co-infecting organisms (per patient) in each group. Mean (± SD) number of individual co-infecting organisms isolated per patient in each group, patient age, FEV_1 and change in weight.

	R117H present	R117H not present
Number of patients	7	52
Male/female	4/3	36/16
Mean age (years)	25.1±5.9	22.4±9.6
FEV_1 (litres)	2.7±1.1	2.4±1.2
Δ Weight (kg)	(+) 1.1±1.6	(+) 0.9±3.5
BCC infected	0	15**
PA infected	1	23**
Not colonised	6	14
Total specimens per patient#	3.0 (0–27.0)	15.0 (3.3–31.5)
Total organisms per patient†	6.0 (0–32.0)	19.0 (4.0–41.0)
Co-infecting organisms per patient‡	2.0 (0–10.0)	4.5 (0–14.5)
P. aeruginosa co-infection	0.2 ± 0.4	6.7 ± 13.6
Staphylococcus aureus co-infection	6.1±8.1	2.5±5.4
Haemophilus influenzae co-infection	1.4±1.6	0.9±2.5
Streptococcus pneumoniae co-infection	0±0	0.3±0.9
Stenotrophomonas maltophilia co-infection	3.6±8.2	2.0±6.0
Other organism co-infection	1.6±2.6	2.2±4.7

#Mean total number of positive sputum cultures per patient over the complete study.
†Total number of different bacterial organisms isolated in that group of patients during the study period.
‡Number different bacterial organisms isolated, excluding BCC or *P. aeruginosa* in those patients colonised with these bacteria.
**$P<0.01$

Numbers of sputum specimens and the organisms isolated are presented as median and interquartile ranges. Data between the groups were compared using analysis of variance and the χ^2 test for linear-by-linear association. A probability (P) value of less than 0.05 was considered statistically significant.

Results

Data were available on 59 patients attending the regional adult CF centre at the time of the study. Mean (SD) age was 26 (±8.5) years and there were 15 patients in the BCC group, 24 in the *P. aeruginosa* group and 20 in the group that exhibited neither infection.

Analysis by DF508 mutation

Twenty patients were homozygous for ΔF508, 25 were heterozygous and ΔF508 was not detected in the remaining 14 patients. A linear-by-linear association using the ΔF508 mutation showed that those patients homozygous ($P=0.002$; odds ratio [OR]: 5.0, 1.4, 18.6) or heterozygous ($P < 0.001$; OR: 114, 7.3, 5108) for this deletion were more likely to be colonised with BCC or *P. aeruginosa*. Further comparison showed that those homozygous or heterozygous for the ΔF508 mutation were more likely to be colonised with an infecting bacterium ($P<0.001$; relative risk 5.8 [1.6, 20.9]).

This group also showed a trend towards increased total numbers of different infecting organisms ($P=0.07$), as patients with these genotypes isolated organisms from their sputum more frequently over the duration of the study. In addition, higher numbers of positive sputum cultures from those patients homozygous or heterozygous for the ΔF508 mutation ($P<0.001$) were noted, as well as an increased incidence of co-infection with multiple organisms ($P<0.05$). Full details are shown in Table 1.

Analysis by R117H mutation

Cystic fibrosis mutational analysis identified seven patients who had the R117H mutation. These patients showed a linear-by-linear association, with reducing likelihood of chronic infection in the BCC, *P. aeruginosa* and non-infected groups, respectively ($P=0.005$). In other words, patients with the R117H mutation were less likely to be colonised with BCC or *P. aeruginosa* (OR: 0.06 [0.00–0.60]).

Comparison of patients with and without the R117H mutation showed no significant difference in total numbers of positive culture specimens seen. Patients were less likely to require treatment with prophylactic antibiotic therapy if they had the R117H mutation. Three patients were known to have the R117H polyT5 mutation, one of whom was colonised with *P. aeruginosa*. This was the only patient to be colonised in this group.

Comparison of genotype mutations with changes in FEV_1 during the study showed no trends. Further subgroup analysis of the infecting organisms did not identify any link between mutation group and type of co-infecting bacterium; in other words the patient's genotype did not appear to be linked with the type of co-infecting organism isolated.

Discussion

The main findings of this study are that patients homozygous or heterozygous for the ΔF508 mutation are more likely to be colonised with BCC or *P. aeruginosa*, more likely to have a co-infection with additional organisms, and also have more infections with multiple additional infecting organisms. In contrast, patients with the R117H mutation are less likely to be colonised with BCC or *P. aeruginosa*. Patient genotype is not associated with the type of co-infecting organism isolated.

These findings are supported by previous investigators who reported higher *P. aeruginosa* colonisation rates in patients heterozygous for ΔF508.[4] However, higher colonisation with BCC in patients with this genotype has not been demonstrated previously, nor has lower colonisation with BCC or *P. aeruginosa* in patients without the ΔF508 mutation. No difference was seen between mutation group and decline in FEV_1; however, this may be a reflection of the smaller group sizes or duration of the study.

Those patients who did not have the ΔF508 mutation did not demonstrate a different profile of co-infecting organisms. In particular, these patients (non-infected group) did not have infections with 'atypical' or unusual bacteria (other organism co-infection) any more frequently than those patients with the ΔF508 mutation (homozygous or heterozygous).

These findings have important implications for counselling CF patients. Using genotyping, it is now possible to determine which patients are at increased risk of colonisation with BCC, *P. aeruginosa* or from bacterial pulmonary infection. Thus, as patients with the ΔF508 mutation are more likely to have chronic BCC and/or *P. aeruginosa* infection, it is important to further emphasise compliance with good infection control procedures in such patients.

The findings of this study are based on observations from the Northern Ireland CF adult population and, although obtained from a relatively small group with a high frequency of the R117H mutation, these findings are important. Thus, further investigation is warranted to confirm these results in a larger population and to determine how genotype mutations affect the CF airway and the host's defence response to bacterial infection and colonising flora.

Patients homozygous or heterozygous for the ΔF508 deletion are more likely to suffer from airway colonisation with BCC or *P. aeruginosa*, with increased frequency of positive sputum cultures and greater numbers of infecting organisms. Those with the R117H mutation are less likely to be colonised.

Dr. Chris Patterson (Department of Epidemiology, Queen's University, Belfast) performed the statistical analysis of all data.

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