Clinical phenotype of cystic fibrosis patients with the G551D mutation

D.M. COMER1, M. ENNIS2, C. MCDOWELL3, D. BEATTIE4, J. RENDALL1, V. HALL1 and J.S. ELBORN1,2

From the 1Regional Adult Cystic Fibrosis Centre, Belfast City Hospital, 2Respiratory Medicine Research Group, Centre for Infection and Immunity, The Queen’s University of Belfast, 3Biostatistician, The Northern Ireland Clinical Research Support Centre, The Royal Group of Hospitals and 4Regional Genetics Laboratories, Belfast City Hospital, Belfast, N Ireland, UK

Received 18 May 2009 and in revised form 4 August 2009

Summary

Background: Data on whether the phenotype of cystic fibrosis (CF) patients with compound heterozygosity for G551D (Gly551Asp) differs from patients with F508del (Phe508del) homozygous mutations is divergent.

Aim: We hypothesized that CF patients with the G551D mutation would have less severe disease than F508del homozygotes.

Design: We compared the clinical phenotype of adult patients with a G551D mutation with adult patients homozygous for F508del and those with the missense mutation R117H (Arg117His). Compound heterozygotes for the G551D and R117H were analysed separately.

Methods: Data were collected for 101 adult CF patients. Group 1–4 represents in order F508del homozygote patients (n=61), those with the G551D mutation and a more severe mutation (n=13), those with R117H mutation and a more severe mutation (n=23) and also those compound for both the R117H and G551D mutations (n=4).

Results: Our findings have shown that adult patients with the G551D mutation and a second severe mutation have a milder clinical phenotype than F508del homozygous adult patients. Higher FEV1 and body mass index and less impaired glucose tolerance was demonstrated in the patients with G551D and R117H compared to F508del homozygotes. There was a reduced yearly rate of decline of FEV1 ($P < 0.05$), infection with Pseudomonas aeruginosa along with reduced burden of care. Compound heterozygosity for G551D and R117H mutations was associated with normal spirometry, body mass index, no chronic infection and no symptoms.

Conclusions: Mutations on different chromosomes are not independent of each other for the overall impact on the amount of functional CFTR. This study suggests that patients with the G551D mutation and a second severe mutation have a milder clinical phenotype than F508del homozygous patients, but the phenotype is not as mild as patients with the R117H mutation.

Introduction

Cystic fibrosis (CF), the most common recessively inherited disease in North West European populations, has a diverse clinical phenotype attributable to the numerous mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (CFTR). The clinical impact of any given mutation on CFTR function varies depending on the underlying cellular mechanisms involved. These mutations can be divided into six classes (defective protein synthesis, abnormal processing and trafficking, defective regulation, decreased conductance, reduced synthesis/trafficking and decreased

Address correspondence to J.S. Elborn, Regional Adult Cystic Fibrosis Centre, Belfast City Hospital, Belfast, N Ireland, Antrim BT9 7AB, UK.

© The Author 2009. Published by Oxford University Press on behalf of the Association of Physicians. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org
stability). Individual phenotypes (such as exocrine pancreatic status, degree of sweat gland dysfunction and age of diagnosis) are dependent, to varying degrees, on the class of genetic mutation. The correlation between the ‘severity’ of the mutation and clinical outcomes is strongest for pancreatic function, which is not easily modified by environmental factors. In contrast, access to specialized centres, advances in therapeutic strategies for CF and lifestyle factors can influence lung function in addition to genotype alone. Furthermore, the high degree of clinical variability for any given mutation (as illustrated by CF siblings) supports the concept that factors in addition to CFTR function alone are important.

The G551D (Glycine to Aspartate change in nucleotide 1784 in exon 11) mutation is a Gly to Asp substitution at amino acid position 551. There is considerable current interest in treating patients with the G551D mutation with drugs that potentiate its function. These pharmacological agents have shown promise in phase 2 clinical trials leading to changes in nasal potential difference and improved lung function. The prevalence of this particular mutation is high in Northern Ireland.

Data on whether the phenotype of patients with compound heterozygosity for G551D differs from patients with F508del (Deletion phenylalanine in codon 508 in chromosome 7) homozygous mutations are divergent. No difference was shown between the G551D mutation and the F508del mutation for age at diagnosis, sweat chloride, measures of nutritional status, lung function, Pseudomonas aeruginosa infection and exocrine pancreatic function. At an early age, G551D patients had pancreatic insufficiency (PI). In contrast, a case study of two CF patients, who were heterozygous with the G551D mutation and a second severe mutation, showed a mild pulmonary and intestinal phenotype. More recently in a large study of the 11 most common CF mutations using US CF Registry data, the severity of the G551D mutation was similar to F508del for lung function, exocrine pancreatic status and P. aeruginosa infection but significant differences for age at diagnosis, sweat chloride, height and weight were demonstrated. There was a nonsignificant trend towards better survival for the G551D/F508del patients than homozygotes for F508del.

In this study, we hypothesized that CF patients with the G551D mutation would have less severe disease than those F508del homozygotes. To address this, we compared the clinical phenotype of patients with a G551D mutation, a Class 3 mutation, with patients homozygous for F508del and those with the missense mutation R117H (Arginine to histidine mutation of residue 117), a Class 2 and 4 mutation, respectively. Including the R117H mutation, a missense mutation in exon 4 and the most common mutation in its class, allowed a comparison across three groups to be established. Compound heterozygotes for the G551D and R117H were studied as a separate group.

**Patients and Methods**

One hundred and eighty-four patients who attend the regional adult CF service at the Belfast City Hospital were studied. Using the UK CF Registry, data were collected for the following parameters: birth date; age of diagnosis; gender; genotype; forced expiratory volume in 1 s (FEV1) expressed as a percentage predicted of normal values; yearly decline of FEV1 [(100−% predicted FEV1)/age]; whether the patient took azithromycin; body mass index [BMI, weight (kilograms)/height (m²)]; number of days over the previous 12 months when the patient was treated with intravenous antibiotics (IV Ab); use of rhDNase; use of maintenance inhaled antibiotics; infection with P. aeruginosa (defined as two positive sputum cultures within any 6 month period); a previous culture of Burkholderia cepacia complex; presence of impaired glucose tolerance (IGT) or overt CF-related diabetes mellitus (CFRD) established by oral glucose tolerance testing; and exocrine pancreatic status (defined as insufficient if clinical assessments deemed a requirement for pancreatic-enzyme supplementation and sufficient if not the case).

Four groups of patients were analysed from the total patient cohort according to their genotype. Group 1 included F508del homozygote patients, Group 2 with the G551D on either chromosome (and without the R117H mutation), Group 3 with the R117H mutation on either chromosome (and without the G551D mutation) and Group 4 compound heterozygotes for the R117H and G551D mutation.

Statistical analysis was performed using version 17.0 of the Statistical Package for the Social Sciences (SPSS). In the case of categorical variables, binary logistic regression analysis was used to compare Group 1 with each of the other three groups and univariate analysis of variance with age as a covariate for the continuous variables. For each variable, the trend across Groups 1–4 was established and if significant, Groups 2–4 were compared with Group 1 for statistical significance. Fisher’s Exact test was used to establish any association between genotype and gender.
Results

Group 1 (F508del homozygote patients) included 61 patients, Group 2 (patients heterozygous for G551D) 13 patients, Group 3 (patients heterozygous for R117H) 23 patients and Group 4 (compound R117H/G551D patients) 4 patients. The patients in Group 4 are not related. In Group 3, 96% of the patients, and 75% of those in Group 4, had the 5-thymidine (5T) tract variant in intron 8. Two patients in Group 2 (G551D/621+1G→T, G551D/3659delC) and 1 in Group 3 (R117H/E60X) were heterozygous with a CFTR mutation other than F508del. These combinations of mutations have been reported to have a clinical phenotype dominated by the milder mutation under investigation and so were included.13–15 Full details of the genotypes within each group are shown in Table 1.

There was no significant association between genotype and gender, or any difference between the mean age of the patients between the four groups. The mean age of diagnosis increased across Groups 1–4 [3.9 (8.1), 3.9 (5.7), 16.4 (19.1) and 13.8 (16.7) years] reaching statistical significance between Groups 1 and 3 only (P < 0.001). P. aeruginosa infection was lower across the groups (P < 0.001, OR 0.1 for Group 3). Only F508del homozygote patients were chronically infected with B. cepacia complex. BMI increased across the groups [21.4 (2.8), 23.7 (3.0), 24.7 (3.5) and 26.6 (1.1) kg/m²; P < 0.05 for all three]. FEV1 increased across the groups [56.3% (22), 77.5% (18.8), 83.4% (21.2) and 100% (2.4); P < 0.05 for all three]. There was also a significant difference for the yearly rate of decline of FEV1 [1.6 (0.9), 0.9 (0.7), 0.5 (0.7) and 0 (0.1)%/year; P < 0.05 for all]. Percentage use of azithromycin was greater in Group 1 than each of the Groups 3 and 4. The decreased use of mucolytic from Groups 1 to 4 was statistically significant for Group 3 only (P < 0.05, OR 0.2). Likewise, there was decreasing requirement for inhaled antibiotics across the groups. The reduced percentage of PI was significant for all the three groups in comparison to Group 1 (P < 0.05, OR 0.07; P < 0.001 OR 0.03 and P < 0.05, OR 0.2). The percentage of IGT/CFRD decreased across the groups. Phenotypic variables for Groups 1–4 are shown in Table 2.

Discussion

Comparing F508del homozygous CF patients to those heterozygous for G551D and R117H, we have shown that the latter two groups individually have better lung function, slower rate of decline in FEV1, older age at diagnosis, better nutritional status, reducing infection with P. aeruginosa and B. cepacia complex, reduced burden of care and improved pancreatic status and glucose tolerance.

McKone et al.’s study, in contrast to our data, showed no significant difference in pancreatic status and FEV1 for those heterozygous for the G551D mutation compared with the F508del homozygous patients. The trend for reduced infection with P. aeruginosa and the statistically better nutritional status that we have shown for G551D patients compared to F508del homozygous is in keeping with McKone et al.’s findings. The average age of patients in this cohort was 10.9 years, significantly lower than the patients in our study. Subtle differences in FEV1 between genotypes are likely to be amplified with the passage of time and progression of disease, and so this difference would be more apparent in our group of patients.11

Since the isolation of the gene responsible for CF was identified in 1989, over 1600 disease associated mutations have been identified.3 As the vast majority are individually rare, accurate genotype–phenotype studies of many of these mutations are challenging. For many monogenic diseases, accurate phenotype predictions allow appropriate prognostic counselling. Recent interest in drugs that potentiate CFTR expression in G551D patients and the inconsistency in previous reports as to the overall severity of this particular phenotype make further study important.

The R117H mutation is commonly associated with the genetic polymorphism of the polypyrimidine tract in intron 8 giving rise to variable amounts of functional CFTR. This polymorphism exists on an intron-8 polythymidine sequence (IVS8) as a 5-, 7- or 9-thymidine (T) variant. These alleles cause intron

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F508del/F508del</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>G551D/F508del</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>G551D/621+1G→T</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>G551D/3659delC</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>R117H/F508del</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>R117H/E60X</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>G551D/R117H</td>
<td>4</td>
</tr>
</tbody>
</table>
Phenotypic variables for Groups 1–4 (gender ratio, mean value± SD and range for continuous variables, percentage with OR for categorical variables)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1: F508del homozygote</th>
<th>Group 2: G551D+ severe mutation</th>
<th>Group 3: R117H+ severe mutation</th>
<th>Group 4: R117H/G551D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>41/20</td>
<td>7/6</td>
<td>14/9</td>
<td>2/2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27.6 ± 6.9 (17.7 to 48.2)</td>
<td>26.3 ± 4.5 (20.1 to 35.5)</td>
<td>31.7 ± 13.3 (19.8 to 76.6)</td>
<td>35.3 ± 3.2 (31.3 to 39)</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>3.9 ± 8.1 (3 to 26)</td>
<td>3.9 ± 5.7 (1 to 19)</td>
<td>16.4 ± 19.1 (0 to 73.4)**</td>
<td>13.8 ± 16.7 (0 to 29.3)</td>
</tr>
<tr>
<td>Percentage predicted FEV₁ (%)</td>
<td>56.3% ± 22 (19 to 110)</td>
<td>77.5% ± 18.8 (50 to 110)*</td>
<td>83.4% ± 21.2 (27 to 116)**</td>
<td>100% ± 2.4 (97 to 102)</td>
</tr>
<tr>
<td>Yearly rate of decline of FEV₁ (%/year)</td>
<td>1.6 ± 0.9 (–0.5 to 4.0)</td>
<td>0.9 ± 0.7 (–0.3 to 2.4)*</td>
<td>0.5 ± 0.7 (–0.7 to 1.8)*</td>
<td>0 ± 0.1 (0 to 0.1)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.4 ± 2.8 (15.1 to 29.9)</td>
<td>23.7 ± 3.0 (17.5 to 28.4)*</td>
<td>24.7 ± 3.5 (18.3 to 30.6)**</td>
<td>26.6 ± 1.1 (25.2 to 28)</td>
</tr>
<tr>
<td>Infection with <em>P. aeruginosa</em></td>
<td>75%</td>
<td>62% (OR: 0.5)</td>
<td>26% (OR: 0.1)**</td>
<td>0%</td>
</tr>
<tr>
<td>Culture of <em>B. cepacia</em> complex</td>
<td>16%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Use of azithromycin</td>
<td>56%</td>
<td>54% (OR: 0.9)</td>
<td>35% (OR: 0.4)</td>
<td>0%</td>
</tr>
<tr>
<td>Use of rhDNase</td>
<td>61%</td>
<td>39% (OR: 0.4)</td>
<td>22% (OR: 0.2)*</td>
<td>0%</td>
</tr>
<tr>
<td>Number of days of IV Ab</td>
<td>21.8 ± 24.3 (0 to 98)</td>
<td>8.7 ± 17.4 (0 to 60)</td>
<td>4.7 ± 12.3 (0 to 42)*</td>
<td>0 (0)</td>
</tr>
<tr>
<td>IGT/CFRD</td>
<td>23%</td>
<td>8%</td>
<td>4%</td>
<td>0%</td>
</tr>
<tr>
<td>Use of inhaled antibiotic</td>
<td>74%</td>
<td>31% (OR: 0.5)</td>
<td>26% (OR: 0.4)</td>
<td>0%</td>
</tr>
<tr>
<td>Pancreatic insufficient</td>
<td>97%</td>
<td>69% (OR: 0.07)*</td>
<td>48% (OR: 0.03)**</td>
<td>25% (OR: 0.02)*</td>
</tr>
</tbody>
</table>

*Compared to Group 1 (P<0.05); **Compared to Group 1 (P<0.001); OR: Odds ratio relative to Group 1.

The understanding that phenotype is dominated by the milder of the two mutations may be an oversimplification. In our study, the lack of any significant CF phenotype in patients compound for R117H/G551D compared with R117H/F508del mutations is intriguing. Compound heterozygosity for G551D/R117H mutations was associated with normal spirometry, BMI, no chronic infection and no symptoms. In this context, it is possible that mutations on different chromosomes are not independent of each other for the overall impact on the amount of functional CFTR. Interpreting the combination of mutations as opposed to simply accepting the milder mutation as dominant may well be more appropriate. Previous published data support this concept as homozygotes for G551D have an older age at diagnosis, reduced amount of exocrine PI and intestinal complications than those compound for G551D/F508del mutations. Therefore, the correlation between genotype and phenotype is strongest for exocrine pancreatic function; however, this relationship is not consistent. Only 52% of patients who are compound heterozygotes for R117H, where the second mutation was a severe mutation, were pancreatic sufficient. In the Cystic Fibrosis Genotype–Phenotype Consortium, 87% of patients with the R117H/F508del mutation had pancreatic sufficiency; however, the older age in our cohort (31.7 ± 13.3 years) vs. 23.5 ± 9.6 years) may account for this difference. The particularly high incidence of R117H on an IVS8-5T background in our centre, with its associated 5T background in our centre, with its associated
more severe phenotype, could be contributory to these divergent findings.

Many of the clinical outcome variables studied cannot be interpreted independently. With progression of disease and declining lung function, the increased work of breathing increases the metabolic rate and can predispose to undernutrition. Acquisition of P. aeruginosa can lead to a more rapid decline in FEV₁ and more frequent exacerbations and hence greater burden of care. The presence of PI leads to malabsorption of nutrients and fall in BMI. Patients in Group 1 had a lower incidence of CFRD than those in Groups 2 and 3, consistent with previous investigators.

Our study has limitations, primarily, the relatively small numbers studied. If more patients were available, it would be more appropriate to compare patients individually, matched for age and gender, in order to allow a direct comparison in genotype. Also, previous investigators have determined pancreatic function by a variety of different methods (such as 72-h faecal-fat study, pancreatic-stimulation test, serum trypsinogen assay or subjective assessment of the need for pancreatic enzyme supplementation) making direct comparison of pancreatic status tenuous. Many of the patients in this study will have started taking pancreatic-enzyme supplementation immediately after diagnosis and so our data will be an overrepresentation of the overall amount of PI, particularly in G551D and R117H patients.

Our data suggest that additional genetic or environmental factors, in addition to CFTR function alone, contribute to the maintenance of the pancreatic sufficient state. There is a high variability in phenotype within identical CFTR genotypes. However, as modifier genes remain to be identified, clinicians need to be mindful of consensus statements advising that genotype alone should not be used to predict clinical outcomes at the time of diagnosis. In conclusion, this study reports that patients with the G551D mutation and a second severe mutation have a milder clinical phenotype than F508del homozygous patients.

Acknowledgement
All the authors have approved the final version of the manuscript and all those who have contributed to it have been acknowledged.

Conflict of interest: None declared.

References


