Effect of genotype on selected clinical features of Polish cystic fibrosis adults

Lucyna MAJKA¹, Andrzej POGORZELSKI², Witold MŁYNARCZYK¹, Jerzy ŻEBRAK², Ewa RUTKIEWICZ³, Agata NOWICKA¹, Michał WITT³

¹ Department of Pulmonary Diseases, University of Medical Sciences, Poznań, Poland
² Department of Bronchology and Cystic Fibrosis, Pediatric Division, Institute of Tuberculosis and Lung Disease, Rabka, Poland
³ Institute of Human Genetics, Polish Academy of Sciences, Poznań, Poland

Abstract. Cystic fibrosis (CF), the most common autosomal recessive disorder of Caucasians, is caused by the mutations in the gene encoding CFTR (Cystic Fibrosis Transmembrane Conductance Regulator) protein. Until now, approximately 1000 mutations of the CFTR gene have been described. The genotype-phenotype relationships in CF are still not completely understood. This study was undertaken in an attempt to characterise the distribution of CFTR mutations and their effect on selected clinical parameters in a group of Polish CF adults. A total number of 38 adult CF patients (mean age 21.6 ± 6.8); 18 females & 20 males were enrolled in the study. The CFTR gene identification was conducted with the use of PCR & InnoLipa-CF set. The assessed clinical parameters included: age at diagnosis, age, lung function test, X-ray scored in Brasfield score, weight & height. We found that: (1) the genotypes of the studied population were unevenly distributed (65.8% – genotype deltaF508/M), (2) a high percentage of 3849+10kbC→T was noted, (3) patients homozygous for the deltaF508 mutation were diagnosed significantly earlier and had a lower body mass index, (4) no differences were observed in the patients’ length of life or the progression of lung disease. Conclusions: 1. In comparison to other populations, Polish adult CF patients display a relatively higher frequency of mild mutations. 2. Late diagnosis of CF in the studied group may be partially caused by a high percentage of CFTR mutations connected with the mild course of the disease that are difficult to identify. 3. Cystic fibrosis should be more commonly taken into consideration in the differential diagnosis in adult patients with milder symptoms.

Key words: adults, CFTR, cystic fibrosis, genotype-phenotype correlation, late diagnosis.
Cystic fibrosis (CF) is the most common life limiting, autosomal recessive disorder of Caucasians. It is a multiorgan disease, which manifests itself mainly in the respiratory and digestive tracts, and the male reproductive system. The characteristic symptoms of CF are recurrent respiratory infections, steatorhea and a failure to thrive. Due to the advancements in therapy (an intensive antibiotic therapy of lung disease, physiotherapy, a high calorie diet and the pancreatic enzyme supplementation), and better diagnosis of CF in the last 40 years, the mean survival of patients increased from less than 5 years in the 1950's to 30 years currently (DODGE 1997). With the increasing percentage of adult patients in the general CF population, cystic fibrosis is no longer considered an exclusively pediatric clinical entity.

Cystic fibrosis is caused by mutations of the gene in the long arm of chromosome 7, encoding CFTR protein (Cystic Fibrosis Transmembrane Conductance Regulator). The incidence of cystic fibrosis in Caucasians varies from 1:2,000 to 1:4,000 live births. Approximately 5% of the general population carries the pathological allele. About 1,000 of the CFTR gene mutations have been described (CF GENETIC ANALYSIS CONSORTIUM, 2001) since its cloning in 1989 (RIORDAN et al. 1989, ROMMENS et al. 1989). DeltaF508 is the most common mutation in the European population (60-70% of CF chromosomes; CFGAC 1994) and also in the Polish population (56.2%; WITT et al. 1999). The CFTR protein functions as a chloride channel in the apical membrane of epithelial cells in the respiratory, gastro-intestinal and reproductive tracts, biliary and pancreatic ducts, and sweat glands (ANDERSON et al. 1991). A change in the CFTR protein structure or its absence results in a decrease of transepithelial transport of chloride ions and water. Changes in ion transport result in the change of secretion composition and in disturbances of organ functioning. The precise mechanism of this pathology is still not completely understood (QUINTON 1983, SMITH et al. 1996, WILLUMSEN, BOUCHER 1991, WINE 1997).

It was proposed that CFTR gene mutations can be classified into 5 main classes, based on the mechanism by which CFTR gene mutations disrupt the CFTR function (WELSH, SMITH 1993). Even though this mechanism seems to be known, the relationship between the genotype and organ disease is more complex and ambiguous. Genotype-phenotype correlation is best documented for pancreatic insufficiency (KRISTIDIS et al. 1992). The mutations were even divided into severe and mild, based on their influence on pancreatic sufficiency (KEREM et al. 1996). Mutations linked with pancreatic sufficiency usually coincide with a mild course of pulmonary disease (e.g., diagnosed in adulthood; DORK et al.1995) and low levels of sweat chloride (CFGAC 1994, WISCHANSKI et al. 1995). On the other hand, the occurrence of meconium ileus, CF liver disease, CF related diabetes mellitus is practically rather limited to patients with severe mutations coexisting with pancreatic insufficiency (KEREM et al. 1996).
The correlation between the genotype and lung disease is less clear. Great phenotypic variability was observed in individuals with identical genotypes (BURKE et al. 1992, CF GENOTYPE-PHENOTYPE CONSORTIUM 1993). It is now a common concept that this results from an interplay between CFTR gene mutations and the modifying genes (e.g. encoding alternative chloride channels) and various environmental factors (ZIELENSKI et al. 1996), malnutrition (NIR et al. 1996), age at diagnosis (NIXON et al. 1992, SCHECHTER, MARGOLIS 1998), or viral infections (KEREM et al. 1989).

Median survival of CF patients in the majority of North-American (COREY et al. 1997, FITZSIMMONDS 1993) and European centres (FREDERIKSEN et al. 1996) is 30 years, and the expected survival of children born in the UK in the 90’s is 40 years (ELBORN et al. 1991). The population of CF adults is still poorly studied. Progressive lung disease remains the main cause of both morbidity (75% hospitalisations) and mortality (90-97%) of CF adults (FITZSIMMONDS 1993, HODSON, 1995). Routine assessment of the lung disease progression involves lung function tests or – in advanced cases (FVC < 40%) – the blood gases analysis, sputum culture, chest X-ray picture (HILL 1998). The progression of the airway disease in CF is mainly assessed by the rate of FEV1 deterioration, which may vary from 1.79% to 9.16% per year (COREY et al. 1997). It was shown as well that the clinical presentation of patients and their lung function test values correspond well with the assessment of lung disease based on the X-ray picture (CONWAY et al. 1994, O’LAOIDE et al. 1991).

This study was undertaken in an attempt to characterise the distribution of CFTR mutations and to analyse their effect on selected clinical parameters in a population of Polish CF adults.

Material and methods

Clinical material

A total number of 38 adult CF patients, aged 18-28 (mean 21.6 y.a. ± 2.8); 18 (47%) females and 20 males (53%) were enrolled in this study. Out of that number, 14 individuals were examined at the Department of Pulmonary Diseases, University School of Medical Sciences in Poznań, whereas 24 – at the Paediatric Division of the Institute of Tuberculosis and Lung Diseases in Rabka. All the patients agreed to take part in this study and the diagnosis of CF was made in all cases according to the criteria of the Polish Working Group for Cystic Fibrosis (PGRM, 1999).

Genetical material

Molecular analysis of DNA was conducted at the Institute of Human Genetics, Polish Academy of Sciences in Poznań. The CFTR gene identification was con-
ducted with the use of PCR, InnoLipa-CF set, and restriction enzyme digestion as described previously (WITT et al. 1999).

Methods

The patients were divided into three groups according to their genotype as follows: 1) patients with genotype deltaF508/deltaF508, 2) patients with genotype (deltaF508/M (M — other mutations, both known and unknown), and 3) patients with genotype M/M.

The differences between the three groups were assessed in relation to the patients’ sex, age at diagnosis, age at the time of the study, markers of lung disease progression: lung function tests (FEV1, FVC – as the percentage of value predicted), chest X-ray picture (Brasfield score), and body mass index (BMI).

Lung function tests and chest X-ray pictures were taken on the same day/month, during the period of clinical stability. The values of FEV1 (forced expiratory volume in one second) and FVC (forced vital capacity) are the best assessments of three attempts, given as the percentage of value predicted.

Chest roentgenograms were independently assessed by two physicians according to Brasfield (BRASFIELD et al. 1979). The mean from their reading scores was obtained to get the final score.

The agreement of the local ethical committee for this study to be conducted was obtained in 1999.

All the results were statistically analysed with the use of STATISTICA 5.0 PL software.

Results

General clinical characteristics of the study population are presented in Table 1. We observed that the mean age of the study group was low (21.6 y.a. ± 2.8) and the majority of patients in the study group were diagnosed late (mean age at diagnosis – 8.3 y.a, median age at diagnosis – 6.5 y.a).

| Table 1. General clinical characteristics of the study group of Polish CF adults |
|-------------------------------------------------|-------------------|-------------------|
| Age [years]                                      | 21.6 ± 2.8        | 21.0              |
| Age of diagnosis [years]                         | 8.3 ± 6.9         | 6.5               |
| Body mass Index (BMI) [kg/m²]                    | 17.6 ± 2.4        | 17.6              |
| X-ray assessment in Brasfield score             | 16.9 ± 3.4        | 17.0              |
| FEV1 [% of predicted]                           | 52.4 ± 25.4       | 49.4              |
| FVC [% of predicted]                            | 71.1 ± 21.8       | 72.0              |

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<tr>
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<th>Min – Max</th>
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<tbody>
<tr>
<td>Age [years]</td>
<td>18 – 28</td>
<td></td>
</tr>
<tr>
<td>Age of diagnosis [years]</td>
<td>0 – 25</td>
<td></td>
</tr>
<tr>
<td>Body mass Index (BMI) [kg/m²]</td>
<td>12.5 – 23.7</td>
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<tr>
<td>X-ray assessment in Brasfield score</td>
<td>10 – 23</td>
<td></td>
</tr>
<tr>
<td>FEV1 [% of predicted]</td>
<td>18.5 – 119.0</td>
<td></td>
</tr>
<tr>
<td>FVC [% of predicted]</td>
<td>27.7 – 126.6</td>
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</table>
Table 2. The relationship between chosen clinical, spirometric and radiological parameters and genotype

<table>
<thead>
<tr>
<th>Age at study time</th>
<th>Age at diagnosis</th>
<th>FEV1</th>
<th>FVC</th>
<th>Chest X-ray assessment in Brasfield score</th>
<th>Body mass index (BMI)</th>
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<tr>
<td>%</td>
<td>[years]</td>
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<td>[%]</td>
<td>[kg/m²]</td>
</tr>
<tr>
<td>ΔF508/ΔF508 (n=6)</td>
<td>15,8</td>
<td>19,66+1,96</td>
<td>1,54±1,29</td>
<td>50,03±36,61</td>
<td>63,25±32,49</td>
</tr>
<tr>
<td>ΔF508/M (n=25)</td>
<td>65,8</td>
<td>22,08±2,95</td>
<td>10,78±7,13</td>
<td>49,86±19,51</td>
<td>69,23±15,91</td>
</tr>
<tr>
<td>M/M (n=7)</td>
<td>18,4</td>
<td>21,42±2,07</td>
<td>5,78±3,16</td>
<td>63,4±34,39</td>
<td>84,72±27,09</td>
</tr>
<tr>
<td>P value</td>
<td>Ns</td>
<td>0,004</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
</tr>
</tbody>
</table>

Ns – non significant
n – number of patients with particular genotype
M – CFTR gene mutations other than DF508

The genotypes of the studied population were unevenly distributed (Table 2). The genotype deltaF508/M was found to be the most common (25/38 patients ~65.8 %), and only 6 patients (15.8 %) were homozygous for the deltaF508 CFTR mutation. A wide variety of other CFTR mutations (described as M) – both known

Figure 1. The influence of genotype on age at CF diagnosis
and unknown, were identified in the chromosomes studied (Table 3). The high percentage of 3849 + 10kbC→T mutations was noted (7.8%).

Table 3. The frequency of the CFTR gene mutations in the study group

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>deltaF508</td>
<td>48,7</td>
</tr>
<tr>
<td>3849+10kbC→T</td>
<td>7,9</td>
</tr>
<tr>
<td>deleCFTR21 kb</td>
<td>2,6</td>
</tr>
<tr>
<td>R347P</td>
<td>2,6</td>
</tr>
<tr>
<td>621-1G→T</td>
<td>2,6</td>
</tr>
<tr>
<td>R334W</td>
<td>1,3</td>
</tr>
<tr>
<td>2789+5G→A</td>
<td>1,3</td>
</tr>
<tr>
<td>W1282X</td>
<td>1,3</td>
</tr>
<tr>
<td>G542X</td>
<td>1,3</td>
</tr>
<tr>
<td>1717-1G→A</td>
<td>1,3</td>
</tr>
<tr>
<td>G551D</td>
<td>1,3</td>
</tr>
<tr>
<td>M</td>
<td>27,6</td>
</tr>
</tbody>
</table>

M - mutations not identified

The comparison of the three groups with different genotypes showed statistically significant differences in the age at diagnosis and body mass index (Figures 1, 2, Table 2). The diagnosis of cystic fibrosis was given significantly earlier in the individuals homozygous for deltaF508 mutation (mean age of diagnosis - 1.5 years) than in the other two groups (p < 0.0002).

The same group of patients (with genotype deltaF508/deltaF508) was found to have the body mass index significantly lower than the group of patients with genotype (M/M) (p<0.03).

No difference between the studied groups in the duration of life (age at the time of study), the progression of lung diseases as assessed by the results of lung function tests and chest X-ray scored in Brasfield scale were found to reach the level of statistical significance (Table 2).
Genotype - phenotype in CF adults

The differences between patients’ weight and height in the three subgroups were not statistically significant, although there was a general pattern observed for the patients with deltaF508/deltaF508 genotype to be shorter (mean height for males – 164.6 cm ± 1.1) and lighter (mean weight for males – 47.8 kg ± 12.5) than the patients in the other groups (mean height and weight for males in group with M/M genotype – 177.2 cm ± 11.8 and 63.7 kg ± 9.1), respectively.

Discussion

In this study we analysed CFTR mutations in a group of Polish CF adults and their influence on selected phenotypic parameters. The relative frequency of the most common CFTR mutations in the study population was similar, although not equal, to that previously found in the general Polish adult CF population (Table 3, WITT et al. 1999). At the same time, the frequency of CF chromosomes with deltaF508 mutation was lower (48.7%) than in the general CF population in Poland (56%; WITT et al. 1999) and also significantly lower than in most of the other European populations (CFGAC, 1994). On the other hand, the percentage of patients with deltaF508/M genotype (65.8%) in the studied population was found to be significantly higher than in some other populations (CFGAC, 1994). A total number of 20% of patients with deltaF508/M genotype had the 3849+10kbC→T mutation on the other CF chromosome. This mutation causes a mild course of the disease and its late presentation (DORK, STUHRMANN 1995). The relative frequency of this mutation in the study group (7.89%) was slightly lower than in the general population of Polish adult CF patients (8.96%; WITT et al. 1999). Such a high frequency of this mutation is comparable only with its incidence in the Ashkenazi Jews and US Latinos (CF GEN. ANALYSIS CONSORTIUM 1994).

A comparison of the study group genotypes and also the genotypes of other Polish CF patients (WITT et al. 1999) with the CF populations with higher percentages of adults can lead to the conclusion that in fact Polish CF patients have genotypes that would predispose them to a milder course of the disease and longevity. There are quite a significant number of patients in the group studied with at least one mild allele (connected with pancreatic sufficiency). Also the percentage of patients – including children (WITT et al. 1999) – with two severe mutations (connected to pancreatic insufficiency) in the Polish CF population is considerably lower than in other populations (e.g. Danish) (CFGAC, 1994). Such a genetic heterogeneity of Polish CF patients with a high proportion of mild mutations influences the degree of identifiableness of the mutations and causes problems in genetic diagnosis and counselling.

A correlation between genotype and pancreatic status (sufficiency) in cystic fibrosis was confirmed in many studies (CF GENOTYPE-PHENOTYPE CONSORTIUM 1993, KEREM et al. 1990, KEREM, KEREM 1996). It seems that the significant variability in age at diagnosis and the level of nutrition (as assessed
by the body mass index) within the study group are a logical consequence of the above mentioned genetic heterogeneity. In our study we did not prove the correlation between genotype and weight of patients (p = 0.052, Table 2), but it can be speculated that such a correlation could have been shown if the study population was bigger.

Significant differences in the age at diagnosis between patients with deltaF508/deltaF508 and those with deltaF508/M genotype can be explained by the influence of mild mutations (M) on the late presentation of the disease. Similar differences were observed in adult CF patients in Germany, where mean age at CF diagnosis in patients with deltaF508/3849+10kbC→T genotype was 14.5 years of age, as opposed to deltaF508/deltaF508 genotype with 1.8 years of age (CFGAC, 1994, DORK, STUHRMANN 1995). Contrary to this hypothesis, there is also data showing the lack of relationship between the age at diagnosis and genotype (KRAEMER et al. 1998). In general, the diagnosis of cystic fibrosis is usually made in the majority of patients in the first months or years of life (54-70% in the first year of life; FITZSIMMONDS 1993), rarely before delivery (MONAGHAN, FELDMAN 1999), occasionally in the adulthood (POGORZELSKI 1998).

The results presented do not show any correlation between the lung function tests, radiological changes and patients genotypes. This is consistent with the findings of some previous studies (BURKE et al. 1992, HUBERT et al. 1996, KEREM et al. 1990). There are studies, however, in which significant changes among patients with different genotypes were found in some spirometric values (GAN et al. 1995, HUBERT et al. 1996, KRAEMER et al. 1998) and the age of Pseudomonas aeruginosa colonisation, that, in turn, can lead to a more significant decrease of pulmonary function (HUBERT et al. 1996). Patients with the deltaF508/deltaF508 genotype were found to be more prone to infection with mucoid forms of Pseudomonas aeruginosa (PARAD et al. 1999). In the same study it was suggested that the natural immunity against Pseudomonas aeruginosa infection is dependent on genotype. Nevertheless, it is a general consensus nowadays that pulmonary changes in CF are modulated by other modifying genes (ROMEY et al. 1999) and environmental factors (e.g. colonisation with mucoid form of P. aeruginosa) in greater extent than the pancreatic phenotype.

Conclusions

In comparison to other populations Polish adult CF patients display relatively higher frequency of mild mutations. Late diagnosis of CF in the studied group may be partially caused by high percentage of CFTR mutations connected with mild course of the disease, and also difficult to identify. Cystic fibrosis should be
more often taken into differential diagnosis in adult patients with milder symptoms.

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