Application of molecular techniques in population genetic studies of cystic fibrosis in the Netherlands

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Relative frequencies of CFTR mutations in The Netherlands: Regional variation is present even in a small country

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(submitted)

Abstract

Cystic fibrosis (CF) is one of the most common autosomal recessive disorders in white populations. Significant regional differences of CF mutations among affected individuals have been reported.

We studied the geographic distribution of the relative frequencies of the three most common Dutch CF mutations, ΔF508, A455E, and G542X, by analyzing data on residences of CF patients.

Significantly higher relative frequencies of the A455E mutation and the G542X mutation were observed in the South-west and the South-east, respectively. For the ΔF508 mutation a rather uniform distribution of relative frequencies was found. The results of our study show that even in a small country like The Netherlands certain CF mutations may be more common in one region than in another.

Introduction

Cystic fibrosis (CF) is one of the most common autosomal recessive disorders in white populations. The classic clinical picture is characterised by chronic pulmonary
disease, pancreatic insufficiency and elevated concentrations of sweat electrolytes [1]. In The Netherlands the birth prevalence of CF has been estimated to be 1 in 3600, corresponding to a carrier frequency of 1 in 30 [2].

Since the identification of the CF gene and its major mutation ΔF508 in 1989 [3,4,5], over 500 mutations have been described. In a recent study we found a ΔF508 carrier frequency of one in 42 in the Dutch population. This corresponds with an estimated total CF carrier prevalence of 1 in 31, based on an estimated relative ΔF508 frequency of 73.3% [6]. The second and third most common mutations, A455E and G542X, represent approximately 3 and 2%, respectively [7]. All other identified mutations were detected in less than two percent of all CF chromosomes each.

As significant regional differences in the relative frequencies of CF mutations have been reported -with France as an example [8,9]- and migration from one to another part of the country used to be uncommon in The Netherlands until only a few decades ago, we studied regional differences in relative frequencies of Dutch cystic fibrosis mutations. In view of the small numbers of other mutations only ΔF508, A455E and G542X were treated individually.

Patients and methods

In The Netherlands DNA testing for CF mutations is performed in two centres for Clinical Genetics, one located in the west (Erasmus University Rotterdam) and one in the North (University of Groningen). Both laboratories receive blood samples from patients throughout the country. In the summer of 1994 samples from 634 independent Dutch CF patients (i.e. 1268 alleles) had been analyzed for diagnostic purposes. Of the patients studied we collected data on the results of CF mutation detection, on the residential area (residence and province), and on the age of the patient at receipt of the blood specimen. Patients were excluded if no information on their residence was available.

DNA testing

The detection of the ΔF508, A455E, and G542X mutations in Rotterdam was
performed as described by Kerem et al. [10]. In Groningen the ΔF508 mutation detection was carried out according to Scheffer et al. [11] and the analysis for the G542X mutation as described by Ferrie et al. [12]. The A455E mutation was detected with a specific amplification refractory mutation system (ARMS)(HS, unpublished).

Data analysis

The Netherlands consists of 12 provinces. First, the distribution of the ΔF508 mutation over the 12 provinces was compared with the distribution of the non-ΔF508 mutations. Departure from a random distribution was tested for by means of the chi-square test (with 11 degrees of freedom). Next, in view of the small expected numbers of the A455E and G542X mutations in most provinces, provinces were combined in three groups. Group 1 consisted of the Northern and Eastern provinces (1-6 in figure 1), group 2 comprised the provinces called Noord-Holland, Zuid-Holland and Utrecht (7-9 in figure 1) which contain the major cities of the country, and group 3 included the remaining Southern provinces (10-12 in figure 1). This grouping may seem rather arbitrary but takes account of some natural barriers: the former Zuider Zee between group 1 and group 2, and the major rivers of Rhine and Meuse between group 3 and the rest of the country. The distribution of the ΔF508, A455E, G542X, other known mutations and unknown mutations over the three groups of provinces was then analyzed (chi-square test with 8 degrees of freedom). To study the distribution of the ΔF508, A455E and G542X in more detail, we calculated standard deviation (SD) scores for the proportion of the particular mutation in each of the 12 Dutch provinces. The expression used for the SD score was as follows:

\[ D_x = \left( \frac{A_x - A_t \times n_x}{n_t} \right) / \left( A_t \times n_x / n_t \right)^{1/2} \]

\[ D_3 = \text{standard deviation score per province } x \]
\[ A_x = \text{number of CF chromosomes with a particular mutation found in province } x \]
\[ A_t = \text{number of CF chromosomes with a particular mutation found in The Netherlands} \]
\[ n_x = \text{number of CF chromosomes studied in province } x \]
n_t = number of CF chromosomes studied in The Netherlands

In provinces with standard deviation scores of <-2 or >2, the mutation under study forms a significantly lesser or greater proportion of CF chromosomes in that area when compared to the national average.

The major CF clinics, which have integrated DNA diagnostics for CF more routinely in patient care (unpublished results, Dutch CF registry), are not randomly distributed throughout The Netherlands. We, therefore, did not calculate frequencies relative to the size of the population of the region.

Results

Of the 634 CF patients we were able to retrieve information on places of residence in all but 36 (5.7%) cases. This resulted in a total of 1196 CF chromosomes that were included in the analysis. In this study population the ΔF508 mutation was present in 75% of the CF chromosomes. The relative frequencies of the A455E and G542X mutations were 3.7% and 2.2% respectively. Other identified mutations accounted for 7.4% of CF chromosomes. In 11.5% of the CF chromosomes no known mutation was found.

The relative frequencies of ΔF508, A455E, G542X, other identified CF mutations, and unknown CF mutations by region are shown in table 1. When comparing the distribution of ΔF508 over the 12 provinces to the distribution of non-ΔF508 mutations (all together), no significant differences were seen indicating that its relative frequency is rather uniformly distributed. When comparing the distributions of the ΔF508, A455E, G542X, other known mutations, and unknown mutations over the three groups of provinces a significant deviation from a random distribution was observed (\( \chi^2 = 22.1, p<0.01 \)). This high score is caused mainly by an abnormal distribution of the A455E and G542X mutations, while the ΔF508 and the other mutations contributed much less to the chi-square. SD scores of the relative frequencies of ΔF508, A455E and G542X were calculated by province, and are shown in figure 1. The ΔF508 mutation was uniformly distributed without SD values more or less than 2. However, significantly more CF chromosomes in the South-West of The Netherlands carried the A455E mutation: in the provinces Zuid-
Holland and Zeeland this mutation was identified in 6.2% (SD= 2.45) and 11.1% (SD=2.84) of the chromosomes, respectively. The only other province in which the A455E mutation was relatively frequent was Utrecht (SD=1.38), a central region that flanks Zuid-Holland.

For the G542X mutation a significantly larger proportion when compared with the national average was observed in Limburg (8.6%, SD=3.33), in the Southeast of the country. In the adjacent province Noord-Brabant this mutation was also relatively frequent (SD=1.27).

When looking at the ages of CF patients at the time of DNA analysis a remarkable dissimilarity was observed between patients with different mutations. The mean age for patients with a double ΔF508 mutation or a ΔF508 mutation and a G542X mutation (only three homozygotes for G542X were found) was 11.5 years and 8.9 years respectively. The mean age for patients with a ΔF508 and an A455E mutation (no homozygotes for A455E were found) was 23.3 years. No patients were identified who were compound heterozygotes of an A455E and a G542X mutation.

<table>
<thead>
<tr>
<th>Province¹</th>
<th>ΔF508</th>
<th>A455E</th>
<th>G542X</th>
<th>Other known mutations</th>
<th>Unknown mutations</th>
<th>Total number of CF chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groningen (1)</td>
<td>28</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>Friesland (2)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Drenthe (3)</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>Overijssel (4)</td>
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<td>1</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>62</td>
</tr>
<tr>
<td>Flevoland (5)</td>
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<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Gelderland (6)</td>
<td>68</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>11</td>
<td>88</td>
</tr>
<tr>
<td>Utrecht (7)</td>
<td>63</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>74</td>
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<tr>
<td>Noord-Holland (8)</td>
<td>142</td>
<td>3</td>
<td>6</td>
<td>16</td>
<td>29</td>
<td>196</td>
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<tr>
<td>Zuid-Holland (9)</td>
<td>251</td>
<td>22</td>
<td>7</td>
<td>24</td>
<td>52</td>
<td>356</td>
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<td>Zeeland (10)</td>
<td>36</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>54</td>
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<td>Noord-Brabant (11)</td>
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<td>7</td>
<td>15</td>
<td>19</td>
<td>200</td>
</tr>
<tr>
<td>Limburg (12)</td>
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<td>5</td>
<td>4</td>
<td>3</td>
<td>58</td>
</tr>
<tr>
<td>The Netherlands</td>
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<td>44</td>
<td>26</td>
<td>43</td>
<td>138</td>
<td>1196</td>
</tr>
</tbody>
</table>

Table 1. Numbers of ΔF508, A455E, G542X, and other mutations by province in Dutch CF patients
Figure 1. The standard deviation (SD) scores of the relative frequencies of the ΔF508 (a), A455E (b), and G542x (c) mutations per province in The Netherlands. The numbers refer to the provinces mentioned in table 1.
Discussion

The results of our study show that in The Netherlands certain CF mutations among CF patients are more common in one area than in another. We observed this for the A455E and the G542X mutation, but not for the ΔF508 mutation.

With respect to the ΔF508 mutation we earlier performed a study on the frequency of ΔF508 carriers among blood donors which showed a lower prevalence of carriers in the North of the country [6]. As in the present study no differences were observed in relative frequencies for the ΔF508 mutation between the Dutch provinces, this suggests a lower prevalence of CF and CF carriernesship (all mutations together) in the North. In fact this phenomenon has been suggested before in other studies on the prevalence of CF in the Netherlands [2,13].

The A455E mutation has mainly been described in The Netherlands and in Canada [7]. In the latter it has been introduced during the period 1650-1900 [14,15]. Some of the Canadian and Dutch patients share a common haplotype over distances up to 25 cM surrounding the A455E mutation [16], which suggests that this mutation has been derived from a not too distant common predecessor. Furthermore, it is associated with a less severe CF phenotype [17], which results in a higher prevalence among adult CF patients. In this study this is reflected by the higher age of patients with a A455E mutation when compared to those with a ΔF508 mutation. As one of the major clinics for adult CF patients is located in the province Zuid-Holland, in the west of the country, this confronted us with a possible confounding factor. In order to distinguish between a truly high prevalence of the A455E mutation in the South-West and a confounder effect, we repeated the data analysis by calculating standard deviation scores for CF patients under 16 years of age (706 CF chromosomes, A455E in 1.4%). This resulted again in significantly higher frequencies and deviation scores for Zuid-Holland (3.5%, SD=2.28) and Zeeland (9.4%, SD= 3.78), while the relative frequencies of the A455E mutation in other provinces were not different from the results in the total group, thus confirming our first observation. Similarly analysis of the distribution of the ΔF508 and the G542X mutations in patients under 16 years of age confirmed the results in the total group.

The G542X mutation has been detected in various populations [7] with a tendency to be more frequent in South European countries. While the A455E mutation gives rise to mild disease, the G542X mutation is associated with more
severe symptoms and pancreatic insufficiency [18]. In this study we found a higher frequency of this mutation in the South, predominantly in the province of Limburg.

In conclusion, the results of this study show that even in a relatively small country like The Netherlands regional differences of CF mutations among affected individuals can be observed.

Acknowledgements

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