Cystic fibrosis transmembrane regulator (CFTR) ΔF508 mutation and 5T allele in patients with chronic pancreatitis and exocrine pancreatic cancer

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Abstract

Background—An increased risk of chronic pancreatitis has been described among carriers of the cystic fibrosis transmembrane regulator (CFTR) mutation. In addition, patients with cystic fibrosis may have a higher risk of exocrine pancreatic cancer.

Aims—To determine the prevalence of the ΔF508 mutation and 5T allele, the most common CFTR disease related variants, and to assess their association with lifestyle factors in an unselected series of patients with chronic pancreatitis or pancreatic cancer.

Subjects—Patients recruited to the multicentre PANKRAS II study with a diagnosis of chronic pancreatitis and pancreatic cancer from whom normal DNA was available.

Methods—The ΔF508 mutation and 5T allele were analysed using polymerase chain reaction amplified normal DNA. Information on clinical and lifestyle factors was obtained through personal interviews.

Results—Among patients with pancreatitis, no ΔF508 alleles were found and the prevalence of the 5T allele was 10.5%, similar to that described in the general population. Among patients with pancreatic cancer, the prevalence of the ΔF508 mutation and the 5T allele was 2.4% and 5.5%, respectively. 5T allele carriers with cancer consumed significantly less alcohol than non-carriers (p=0.038).

Conclusions—Our findings do not support the view that the ΔF508 mutation and 5T allele confer a higher risk of chronic pancreatitis or pancreatic cancer. Nevertheless, our data suggest that interactions between CFTR polymorphism and environmental factors may play a role in the pathogenesis of these diseases. Our study emphasises the need for a multinational study to conclusively establish the role of CFTR variants as genetic susceptibility factors for chronic pancreatitis and pancreatic cancer.

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The cystic fibrosis transmembrane regulator (CFTR) gene is expressed in a wide variety of epithelia, including the pancreatic ducts, where CFTR promotes the dilution and alkalisation of pancreatic juice. Lack of CFTR function results in inspissated pancreatic secretions. Cystic fibrosis (CF) invariably affects the pancreas and histological lesions can be identified as early as the first year of life. The spectrum of disease due to CFTR mutations is much wider than initially suspected. Abnormal CFTR genotypes are not only associated with CF but also with congenital absence of the vas deferens and possibly with various types of respiratory pathology. AF508 is the most common CFTR mutation worldwide; in Spain it accounts for approximately 53% of all CF alleles. A common variant of the poly(T) sequence in intron 8, designated 5T allele, has been associated with low levels of CFTR transcripts in some organs and with susceptibility to non-classical CF disease patterns.

We hypothesised that mutations in one allele of CFTR, possibly in association with environmental factors such as alcohol or tobacco, may be associated with the major types of chronic pancreatic pathology (that is, pancreatitis and cancer). Regarding the former, the histological aspect of CF associated lesions is very similar to that of “classical” chronic pancreatitis and is characterised by atrophy of acinar tissue, fibrosis, and inflammation. In addition, two recent reports have described an increased risk of developing chronic pancreatitis among carriers of CFTR mutations, including the ΔF508 allele. For pancreatic cancer, a large retrospective cohort study of CF patients showed an increased risk of this neoplasm (odds ratio 32, 95% confidence interval (CI) 4.8–205). Furthermore, and despite some controversy, several studies have concluded that chronic pancreatitis is a risk factor for exocrine pancreatic cancer. Therefore, it is conceivable that specific CFTR mutations confer an increased risk of pancreatic cancer through an intermediate step, histologically similar to chronic pancreatitis.

The aims of this study were: (1) to assess the prevalence of the ΔF508 and 5T CF alleles in

Abbreviations used in this paper: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane regulator; OR, odds ratio; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.
a series of unselected cases with chronic pancreatitis and exocrine pancreatic cancer; and (2) to analyse the association of these genetic traits with lifestyle factors potentially involved in these two chronic pancreatic diseases. Because a high proportion of pancreatic cancers (approximately 75%) harbour *K-ras* mutations, their association with the *CFTR* alleles was also examined.

**Methods**

**PATIENTS AND INFORMATION**

The PANKRAS II study included 602 subjects in whom a diagnosis of exocrine pancreatic cancer, biliary tract cancer, or chronic pancreatitis was suspected in five hospitals from the east coast of Spain between February 1992 and February 1995. All patients gave informed consent to participate in the study. After evaluation by a panel of experts, 118 and 185 cases had a diagnosis of chronic pancreatitis and pancreatic cancer, respectively. Chronic pancreatitis was diagnosed when: (1) pancreatic calcifications were detected by ultrasound or computed tomography; (2) fibrosis, in the absence of neoplasm, was observed in biopsies; and (3) a new episode of pancreatitis appeared in a patient with at least one previous episode of pancreatitis. Pancreatic cysts and pseudocysts were categorised as complications of chronic pancreatitis. They were subsequently classified as alcoholic on the basis of a reported consumption of >40 g of ethanol per day for men and >24 g/day for women at any time, according to recent expert recommendations. A diagnosis of idiopathic chronic pancreatitis was established in the absence of any other disease associated risk factors. Clinical information was systematically abstracted from medical records. Detailed information on lifestyle factors, diet, occupational history, and family history of cancer was obtained by direct patient interview. Patients were classified as smokers if they smoked ≥10 cigarettes or 4 cigars or 4 pipes per week for one year or more; they were classified as coffee drinkers if they reported drinking ≥2 cups per week for one year or more. Independent of the criteria used for aetiological classification, alcohol consumption was classified as heavy or light when patients drank more or less than 80 g/day for men and 40 g/day for women, respectively. From this series, all patients from whom normal DNA was available were studied, corresponding to 88 cases with chronic pancreatitis and 128 with exocrine pancreatic cancers. *K-ras* mutations in tumour tissue were analysed using the RFLP method and DNA isolated from paraffin embedded material.

**CFTR MUTATION ANALYSIS**

DNA was extracted from peripheral blood leucocytes isolated by Ficoll density gradient centrifugation using TriReagent (Molecular Research, Center Inc., Cincinnati, Ohio, USA) following the manufacturer’s instructions. When leucocytes were not available, DNA was purified from sections of normal tissue embedded in paraffin. For analysis of the ΔF508 mutation, exon 10 of *CFTR* was amplified using primers C16B and C16D as previously described by Rommens and colleagues. When DNA was obtained from tissue, a nested PCR was designed to improve the results. Firstly, a fragment was amplified using primers K10D (5'-AAT GAC CTA ATA ATG GGT TT-3') and 10i5' (5'-CAT TCA CAG TAG CTT ACC CA -3') under the following conditions: after an initial step at 95°C for three minutes, 25 cycles followed (95°C for 30 seconds, 55°C for 40 seconds, and 74°C for 50 seconds); the last cycle was followed by a seven minute step at 74°C. The second PCR was performed under the same conditions except that a total of 30 cycles were carried out with the above mentioned primers (C16B, C16D). A solution (3 µl) containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol was added to 12 µl of each sample and loaded onto a 6% non-denaturing polyacrylamide gel (20x20 cm) and electrophoresed at 200 V for two hours. After staining with ethidium bromide, fragments of either 98 bp (normal) or 93 bp (ΔF508) were visualised.

**STATISTICAL ANALYSIS**

ΔF508 and 5T allele proportions with their exact 95% confidence intervals (CI) were computed and compared with those in the general population. Fisher’s exact test was applied to compare distributions of two categorical variables and the Student’s *t* test was used to analyse the relationship between 5T allele and age. Prevalence odds ratios (OR) and their 95% CI were used to estimate the association between lifestyle factors and 5T allele and were computed by unconditional logistic regression.

**Results**

**CHRONIC PANCREATITIS**

Of the 88 patients included, 76 (86.4%) were diagnosed as having alcoholic chronic pancreatitis; in one case the disease was attributed to hypertriglyceridaemia. Eleven (12.8%) cases were classified as idiopathic chronic pancreatitis. Seventy six patients (86.4%) were men. Age ranged from 17 to 87 years (mean 46.8 years (SD 13.8)). Fifteen (17%) patients had a history of gall stones. Seventy six (91%) were smokers. Two thirds of patients were heavy drinkers and 94% drank at least two cups of coffee per week.

The ΔF508 allele was not detected in any of the 86 (95% CI 0–4.2%) cases in whom the analysis could be performed. The 5T allele was present in 9/86 cases (10.5%, 95% CI 5.2–19.4%) (table 1), a frequency similar to that found in the general population and in other studies of unselected patients with chronic pancreatitis. One case was homo-
Table 1  Sociodemographic and lifestyle factors among patients with chronic pancreatitis according to the presence of the 5T allele.

<table>
<thead>
<tr>
<th></th>
<th>Total (n (%))</th>
<th>ST (n (%))</th>
<th>Non-ST (n (%))</th>
<th>p Value</th>
</tr>
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<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>74 (86.0)</td>
<td>6 (7.8)</td>
<td>68 (81.2)</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>12 (14.0)</td>
<td>2 (25.0)</td>
<td>10 (12.5)</td>
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<tr>
<td>Age (SD)</td>
<td>48.8 (13.8)</td>
<td>47.3 (8.7)</td>
<td>49.0 (14.3)</td>
<td>0.735</td>
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<td>Consumption of tobacco1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>74 (90.2)</td>
<td>8 (10.0)</td>
<td>66 (89.2)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8 (9.8)</td>
<td>0</td>
<td>8 (10.8)</td>
<td></td>
</tr>
<tr>
<td>Consumption of alcohol2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>70 (86.4)</td>
<td>6 (7.5)</td>
<td>64 (87.7)</td>
<td></td>
</tr>
<tr>
<td>No</td>
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<td>2 (25.0)</td>
<td>9 (12.3)</td>
<td>0.297</td>
</tr>
<tr>
<td>Consumption of coffee3</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>76 (93.8)</td>
<td>7 (8.7)</td>
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<tr>
<td>No</td>
<td>5 (6.2)</td>
<td>1 (12.5)</td>
<td>4 (5.5)</td>
<td>0.418</td>
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</table>

*With respect to the prevalence in the general population.1
1Information was not available for four cases.
2Information was not available for nine cases.
3Information was not available for five cases; data from both light and heavy drinkers are pooled.

Table 2  Sociodemographic and lifestyle factors among patients with exocrine pancreatic cancer according to the presence of the 5T allele.

<table>
<thead>
<tr>
<th></th>
<th>Total (n (%))</th>
<th>ST (n (%))</th>
<th>Non-ST (n (%))</th>
<th>p Value</th>
</tr>
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<tbody>
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<td>Sex</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>62 (56.4)</td>
<td>4 (6.7)</td>
<td>58 (55.8)</td>
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</tr>
<tr>
<td>Women</td>
<td>48 (43.6)</td>
<td>2 (3.3)</td>
<td>46 (44.2)</td>
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<tr>
<td>Age (SD)</td>
<td>67.1 (12.6)</td>
<td>62.5 (10.8)</td>
<td>67.4 (12.6)</td>
<td>0.356</td>
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<td>Consumption of tobacco1</td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>54 (53.5)</td>
<td>4 (6.7)</td>
<td>50 (47.6)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>47 (46.5)</td>
<td>2 (3.3)</td>
<td>45 (42.4)</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>74 (70.0)</td>
<td>2 (3.3)</td>
<td>72 (76.5)</td>
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</tr>
<tr>
<td>No</td>
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<td>4 (6.7)</td>
<td>22 (33.5)</td>
<td>0.038</td>
</tr>
<tr>
<td>Consumption of coffee3</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>87 (89.0)</td>
<td>6 (10.0)</td>
<td>81 (86.2)</td>
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<td>No</td>
<td>8 (8.2)</td>
<td>0</td>
<td>8 (10.8)</td>
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</table>

*In comparison with the prevalence in the general population.1
1Information was not available for nine cases.
2Information was not available for 10 cases; data from both light and heavy drinkers are pooled.

Discussion

In this study we analysed the prevalence of the most common CFTR mutation and the 5T allele among patients with chronic pancreatitis or exocrine pancreatic cancer from the eastern Spanish coast. The two series of patients were not previously selected on the basis of known risk factors, either environmental or genetic, and hence represent the spectrum of patients seen at general and university hospitals in Spain. The analysis was restricted to AF508 carriers and 5T alleles because they are the most common disease associated variants of CFTR in the general population. In addition, CF alleles in the Mediterranean area display a much greater heterogeneity than in the UK or USA: more than 200 mutations account for approximately 85% of CF alleles,10 hampering complete mutation analysis.

The frequency of the AF508 mutation observed in our series of patients with chronic pancreatitis (0/86) was significantly lower than that reported by Sharer and colleagues10 (p=0.004) and Cohn and colleagues11 (p=0.001) and was not significantly different from that expected in the general population of Spain.8

Sharer and colleagues10 examined 22 mutations, accounting for 95% of all mutations in patients with CF in the UK, in an unselected series of patients with chronic pancreatitis. In this group, 18/134 patients (13.4%, 95% CI 8.2–20.4%) had a CFTR mutation in one chromosome, a prevalence higher than that in the general population, estimated as 5.3%. The authors concluded that the presence of a CFTR
mutation is associated with an increased risk of developing chronic pancreatitis. The rate of CFTR mutation carriers was slightly higher among non-smokers (p=0.081) and among those who were not heavy drinkers (p=0.074). Twelve of the 18 cases carried the ΔF508 mutation, with a prevalence of 9% (12/134 patients, 95% CI 4.71–15.12%), a significantly higher proportion than that reported in the general population in the UK (estimated as 3%). ΔF508 mutations were more prevalent among non-smokers (p=0.014) and among those who were not heavy drinkers (p=0.043).

Cohn and colleagues studied CFTR alleles, accounting for approximately 75% of all CF causing mutations in the USA, in a highly selected group of 27 patients with idiopathic chronic pancreatitis. Ten were carriers of at least one abnormal allele and, of these, five were ΔF508 mutation carriers. The prevalence of the ΔF508 mutation in these patients was significantly higher than expected (prevalence ratio 11, 95% CI 1.1–537.1).

Our study and that of Sharer and colleagues are similar regarding inclusion of an unselected group of patients with chronic pancreatitis and the prevalence of heavy alcohol drinkers (64% and 53%; p=0.117). Nevertheless, in our series we could not confirm a higher risk of developing chronic pancreatitis among ΔF508 carriers. This discrepancy cannot be explained by differences in known lifestyle factors associated with the disease.

The different conclusion drawn by Cohn and colleagues on the role of ΔF508 as a genetic susceptibility factor may result from the different case selection criteria applied in both studies. The small number of cases with idiopathic chronic pancreatitis in our series hampered a conclusive result.

Regarding the 5T allele, our data are in agreement with the findings of Sharer and colleagues and Cohn and colleagues and indicate that this allele does not confer a significant risk of chronic pancreatitis. The agreement of the three studies is important from a public health standpoint because the 5T allele is the most common disease associated polymorphism in the CFTR gene described to date in the general population.

The role of CFTR in chronic pancreatitis is as yet unknown. Studies performed so far have not explored the whole gene and have focused on mutations involved in CF. Nevertheless, it is possible that milder alterations in CFTR are present in the whole population of patients with chronic pancreatitis or in selected subgroups. New studies should include a large control group drawn from the general population, focus on patients well defined from a phenotypic point of view, and analyse amino acid variants that apparently are not involved in classical CF. The importance of sample size in independent studies, it points to a potential gene-environment interaction not previously identified in patients with this neoplasm.

Together with previous reports, our findings emphasise the need for a multinational study to conclusively establish the role of CFTR mutation/polymorphisms as genetic susceptibility factors for chronic pancreatitis and pancreatic cancer.

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Appendix

CENTRES AND MEMBERS OF THE PANKRAS II STUDY GROUP


