Cystic fibrosis transmembrane regulator (*CFTR*) Δ F508 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 Δ F508 mutation and 5T allele, the most common *CFTR* disease related variants, and to assess their association with life-style 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 Δ F508 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 Δ F508 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 Δ F508 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 Δ F508 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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Keywords: cystic fibrosis transmembrane regulator gene; Δ F508 mutation; 5T allele; genetic susceptibility; chronic pancreatitis; pancreatic cancer

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.¹ Lack of *CFTR* function results in inspissated pancreatic secretions.² Cystic fibrosis (CF) invariably affects the pancreas and histological lesions can be identified as early as the first year of life.³ The spectrum of disease due to *CFTR* mutations is much wider than initially suspected.⁴ Abnormal *CFTR* genotypes are not only associated with CF but also with congenital absence of the vas deferens⁵ and possibly with various types of respiratory pathology.^{6,7} Δ F508 is the most common *CFTR* mutation worldwide; in Spain it accounts for approximately 53% of all CF alleles.⁸ 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 organs⁹ and with susceptibility to non-classical CF disease patterns.⁵

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.³ In addition, two recent reports have described an increased risk of developing chronic pancreatitis among carriers of *CFTR* mutations, including the Δ F508 allele.^{10,11} 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).¹² Furthermore, and despite some controversy, several studies have concluded that chronic pancreatitis is a risk factor for exocrine pancreatic cancer.^{13,14} 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 Δ F508 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 1995.¹⁵⁻¹⁸ 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.¹⁵⁻¹⁷ 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.¹⁶⁻¹⁷ 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.¹⁹ 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.²⁰ Detailed information on lifestyle factors, diet, occupational history, and family history of cancer was obtained by direct patient interview.²¹ 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.¹⁸ 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.¹⁵⁻¹⁸⁻²²

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 $\Delta F508$

mutation, exon 10 of *CFTR* was amplified using primers C16B and C16D as previously described by Rommens and colleagues.²³ 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 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 μ l) containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol was added to 12 μ l of each sample and loaded onto a 6% non-denaturing polyacrylamide gel (20 $\times$ 20 cm) and electrophoresed at 200 V for two hours. After staining with ethidium bromide, fragments of either 98 bp (normal) or 95 bp ($\Delta F508$) were visualised. Analysis of the 5T polymorphism was performed as previously described.⁵

Because paraffin embedded material posed some problems for PCR amplification, results for $\Delta F508$ were obtained in 86 (98%) patients with pancreatitis and 127 (99%) with pancreatic cancer, and for the 5T polymorphism in 86 (98%) and 110 (86%) patients, respectively.

STATISTICAL ANALYSIS

$\Delta F508$ 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.²⁴

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 $\Delta F508$ 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 population⁵⁻²⁵ and in other studies of unselected patients with chronic pancreatitis.¹⁰ One case was homo-

Table 1 Sociodemographic and lifestyle factors among patients with chronic pancreatitis according to the presence of the 5T allele

	Total (n (%))	5T (n (%))	Non-5T (n (%))	p Value
n	86	9 (10.5)	77 (89.5)	1.000*
Sex				
Men	74 (86.0)	8 (88.9)	66 (85.7)	1.000
Women	12 (14.0)	1 (11.1)	11 (14.3)	
Age (SD)	48.8 (13.8)	47.3 (8.7)	49.0 (14.3)	0.735
Consumption of tobacco ¹				
Yes	74 (90.2)	8 (100)	66 (89.2)	1.000
No	8 (9.8)	0	8 (10.8)	
Consumption of alcohol ²				
Yes	70 (86.4)	6 (75.0)	64 (87.7)	0.297
No	11 (13.6)	2 (25.0)	9 (12.3)	
Consumption of coffee ²				
Yes	76 (93.8)	7 (87.5)	69 (94.5)	0.418
No	5 (6.2)	1 (12.5)	4 (5.5)	

*With respect to the prevalence in the general population.⁵

¹Information was not available for four cases.

²Information 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

	Total (n (%))	5T (n (%))	Non-5T (n (%))	p Value
n	110	6 (5.5)	104 (94.5)	0.303*
Sex				
Men	62 (56.4)	4 (66.7)	58 (55.8)	0.695
Women	48 (43.6)	2 (33.3)	46 (44.2)	0.356
Age (SD)	67.1 (12.6)	62.5 (10.8)	67.4 (12.6)	
Consumption of tobacco ¹				
Yes	54 (53.5)	4 (66.7)	50 (52.6)	0.683
No	47 (46.5)	2 (33.3)	45 (47.4)	
Consumption of alcohol ²				
Yes	74 (74.0)	2 (33.3)	72 (76.5)	0.038
No	26 (26.0)	4 (66.7)	22 (33.5)	
Consumption of coffee ²				
Yes	87 (87.0)	6 (100)	81 (86.2)	1.000
No	13 (13.0)	0	13 (13.8)	

*In comparison with the prevalence in the general population.⁵

¹Information was not available for nine cases.

²Information was not available for 10 cases; data from both light and heavy drinkers are pooled.

zygous for the 5T allele: a 42 year old man with a history of heavy alcohol consumption and an episode of acute pancreatitis nine years prior to the diagnosis of chronic pancreatitis.

The 5T allele was similarly distributed among cases with alcoholic and idiopathic chronic pancreatitis ($p=0.329$). Age, sex, and tobacco, coffee, and heavy alcohol consumption were similar among cases with and without the 5T allele (table 1).

EXOCRINE PANCREATIC CANCER

Seventy six (59.4%) of 128 cases included in this series were men. Age ranged from 36 to 94 years (mean 66.5 years (SD 12.7)). In 63.3% of patients the tumours were located in the head of the pancreas and 46.9% were disseminated at the time of diagnosis. In 27 subjects the diagnosis was made on clinical grounds; the remaining 101 cases were confirmed cytohistologically. Eighty five (84.2%) tumours were ductal adenocarcinomas. Other histologies were mucinous adenocarcinomas (three), papillary adenocarcinoma (one), cystadenocarcinoma (one), adenosquamous carcinoma (one), squamous cell carcinoma (two), and undifferentiated carcinoma (eight). Tobacco consumption was known for 119 patients and 57% of them were smokers. Data on alcohol consumption were available for 118 cases; 89 (75.4%) were daily drinkers and 22% were classified as heavy drinkers. Eighty six percent of 118 cases drank two or more cups of coffee per week. Mutational analysis of *K-ras* was conducted in

tumour tissue from 86 patients; 80% had a codon 12 mutation.

The $\Delta F508$ mutation was detected in 3/127 cases (2.4%, 95% CI 0.5–6.8%). This prevalence is not significantly different from that estimated in the general population in Spain (2%).⁸ The age of $\Delta F508$ carriers with pancreatic cancer was similar to that of the rest of cases (mean 70 years *v* 67 years, respectively) and two were women. No particular characteristics were noted in the three mutated cases in terms of medical history, tumour characteristics, or family history of cancer.

The 5T allele was found in 6/110 cases (5.5%, 95% CI 2.2–12.0%), a prevalence approximately half that expected in the general population⁵ ($p=0.303$) and was lower than that found among our patients with chronic pancreatitis ($p=0.118$). None of the three $\Delta F508$ carriers had the 5T allele.

Age, sex, tumour site and stage at diagnosis, histology, and tobacco and coffee consumption were similar among 5T allele carriers and non-carriers (table 2). However, a 6.6-fold increase (95% CI 0.94–55.83) in the probability of being an alcohol drinker was found among non-5T carriers compared with carriers ($p=0.038$) (table 2). None of the 5T allele carriers was a heavy drinker compared with 22.3% of non-carriers ($p=0.338$). There was no association between the presence of *K-ras* mutations and CFTR genotype (data not shown).

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 $\Delta F508$ mutations 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,²⁶ hampering complete mutation analysis.

The frequency of the $\Delta F508$ 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 $\Delta F508$ 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%). $\Delta F508$ mutations were more prevalent among non-smokers ($p=0.014$) and among those who were not heavy drinkers ($p=0.043$).

Cohn and colleagues¹¹ 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 $\Delta F508$ mutation carriers. The prevalence of the $\Delta F508$ 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 colleagues¹⁰ 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 $\Delta F508$ carriers. This discrepancy cannot be explained by differences in known lifestyle factors associated with the disease.

The different conclusion drawn by Cohn and colleagues¹¹ on the role of $\Delta F508$ 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 colleagues¹⁰ and Cohn and colleagues¹¹ and indicate that this allele does not confer a significant risk of chronic pancreatitis.²⁷ 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 these studies is illustrated by a recent report aimed at assessing the association between *CFTR* variants and asthma.²⁸ It is likely that only prospective multicentre studies will yield conclusive results on the role of *CFTR* mutations in distinct subgroups of chronic pancreatitis patients.

This is the first study on the prevalence of *CFTR* mutations among patients with exocrine pancreatic cancer and our findings suggest that carriers of the $\Delta F508$ or 5T alleles are not at significant risk of developing this tumour. Nevertheless, we cannot exclude the possibility that *CFTR* mutations play a role in a subset of patients with pancreatic cancer. Identification of low penetrance genes or alleles involved in the pathogenesis of common diseases is increasingly important.²⁹ It has been postulated that such genes (or alleles) may modify the effects of environmental agents on disease development.²⁹ One example is the association between *CFTR* mutations and non-smoker status observed in the study of Sharer and colleagues (1998).¹⁰ We found that alcohol consumption in the six patients with pancreatic cancer who carried the 5T allele was different from that observed in non-carriers. While this observation should be confirmed in large and 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

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