Frequency of CFTR gene mutations in idiopathic pancreatitis

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SUMMARY
The prevalence of mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene in idiopathic chronic pancreatitis has been shown to be increased. The aim of this study was to prospectively analyze the frequency of such mutations in a homogeneous group of patients with idiopathic pancreatitis studied in a French Gastroenterology department.

Patients and methods — Between April 1999 and December 2001, all patients with unexplained chronic or recurrent acute pancreatitis were studied. Other causes of pancreatitis were excluded and no patient had personal signs or family history compatible with cystic fibrosis. Following informed written consent, genetic analysis for CFTR was performed using an oligonucleotid ligation assay, on the 31 most frequently known mutations of the CFTR gene. A complementary analysis for variants in exons 9, 10 and 17a, thought to be implicated in atypical cystic fibrosis, was also performed using denaturing gradient gel electrophoresis.

Results — Idiopathic pancreatitis occurred in 64 patients (chronic, n = 30; recurrent acute, n = 34) with a median age of 36 years. Eighteen CFTR mutations or variants were detected in 16 patients (25%): ΔF508 (n = 7), L997F (n = 2), E528E (n = 4), 5T (n = 5). Two patients were compound heterozygous. The frequency of ΔF508 mutations was greater than that of the general population (10.9 vs 2.4%; P < 0.001). Pancreatitis was diagnosed at a median age of 32 years in mutation-positive patients compared to 39 in mutation-negative patients. The prevalence of CFTR mutations was 35.5% in patients < 35 years against 15.1% in those > 35 years (P = 0.06). The clinical course of pancreatitis (severity and complication rates) was not altered by the presence of a mutation.

Conclusion — One-quarter of all patients and one-third of those ≤ 35 years with idiopathic pancreatitis have at least one mutation of the CFTR gene. The presence of a CFTR mutation appears to predict the development of pancreatitis at an earlier age.

RÉSUMÉ
Etude prospective de la fréquence des mutations du gène CFTR chez des malades ayant une pancréatite idiopathique

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La fréquence des mutations du gène Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) serait augmentée au cours des pancréatites chroniques idiopathiques. Le but de cette étude prospective monocentrique française était d’évaluer la fréquence des mutations du gène CFTR dans une série homogène de malades ayant une pancréatite idiopathique.

Malades et méthodes — Entre avril 1999 et décembre 2001, tous les malades ayant une pancréatite aiguë récidivante ou chronique d’origine indéterminée étaient étudiés. Une recherche exhaustive d’une cause de pancréatite était faite. Seuls étaient inclus les malades sans cause identifiée et sans signe clinique ou histoire familiale évocateurs de mucoviscidose. Après consentement éclairé, la recherche des 31 mutations du gène CFTR considérées comme les plus fréquentes a travers le monde était faite à l’aide du kit Cystic fibrosis assay. Une analyse complémentaire des exons 9, 10 et 17a par électrophorèse en gradient de gel dénaturant était réalisée dans le but de chercher des variants préalablement identifiés dans des formes frontières de mucoviscidose. Les caractéristiques morphologiques de la pancréatite, l’existence d’un diabète ou d’une insuffisance pancréatique exocrine étaient évaluées en fonction de la présence ou non d’une mutation du gène CFTR.

Résultats — Soixante-quatre malades (41 hommes, 23 femmes), d’âge médian 36 ans (14-68) ayant une pancréatite idiopathique (aiguë récidivante, n = 34 ; chronique, n = 30) étaient inclus. Dix-huit mutations ou variants du gène CFTR étaient détectés chez 16 malades (25 %) : ΔF508 (n = 7), L997F (n = 2), E528E (n = 4), 5T (n = 5). Deux malades étaient hétérozygotes composées. La fréquence observée de la mutation ΔF508 était supérieure à celle attendue dans la population générale (10.9 vs 2.4 % ; P < 0.001). L’âge médian lors du diagnostic de la pancréatite était de 32 ans chez les malades ayant au moins une mutation vs 39 ans chez les malades sans mutation identifiée. La fréquence des mutations du gène CFTR était de 35,5 % chez les malades ≤ 35 ans et de 15,1 % chez ceux > 35 ans (P = 0.06). L’histoire naturelle de la pancréatite n’était pas modifiée par l’existence d’une mutation.

Conclusion — Un quart de tous les malades et un tiers de ceux de moins de 35 ans ayant une pancréatite idiopathique ont au moins une mutation du gène CFTR. L’existence d’une mutation semble être un facteur de prédisposition à la survenue de la pancréatite à un âge précoce.
Cystic fibrosis is a severe disease transmitted by autosomal recessive inheritance affecting 1/3,500 newborns in France. The causal Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene is located on the long arm of chromosome 7 at 7q31 [1]. CFTC encodes for a 1,480 amino acid protein implicated in chloride ion transport in epithelial cells. Over 1,000 mutations and polymorphisms of the CFTR gene have been reported since its identification in 1989 [1]. The most frequent mutation in the ? F508 mutation, located on exon 10 and is identified in nearly 70% of patients with cystic fibrosis. The CFTR genotype (severe or moderate mutations) depends on the expression of the functional CFTR protein and controls phenotype severity (lung disease, pancreatic insufficiency, agenesis of the vas deferens) [2].

In two American and English studies reported in 1998 [3, 4], the prevalence of CFTR gene in patients with chronic pancreatitis (13.4% and 37%, respectively) was significantly higher than in the general population (5.3% and 2.7%, respectively). Since these reports, other groups searching for CFTR mutations in patients with pancreatitis have found varying frequency (0-62%) [5-14].

The purpose of our prospective study was to determine the prevalence of ? F508 mutation in a large homogeneous population with idiopathic pancreatitis attending a French Gastroenterology department.

Patients and methods
Inclusion criteria

Between April 1999 and December 2001, all patients with chronic pancreatitis or two or more episodes of acute pancreatitis of undetermined cause who consulted Beaujon Hospital were studied. Chronic pancreatitis was defined as the presence of at least one of the three following criteria: a) pancreatic calcifications, b) moderate or severe ductal anomalies at pancreatography (Cambridge criteria) [15], c) histological evidence of chronic pancreatitis. Acute pancreatitis was defined as the time between the first manifestation (pancreatic pain, acute pancreatitis, steatorrhea) and the current hospitalization.

Exclusion criteria

Patients with another potential cause of pancreatitis were excluded: a) careful history taking searching for excessive alcohol intake, personal and family history of cystic fibrosis, autoimmune disease, familial pancreatic disease, or medication potentially pancreatoxic; b) endoscopic ultrasound to search for pancreatic anomalies and/or biliary lithiasis, c) biopsy, endoscopic ultrasound-guided pancreatic biopsy of a pseudotumor; d) laboratory tests to search for autoimmune or metabolic disease (eosinophil polymorphonuclear counts, autoantibodies, gammaglobulins, calcium, triglycerides); e) search for cationic trypsinogen and pancreatic secretory trypsin inhibitor (PSTI) gene mutations; f) other explorations to search for etiology (salivary gland biopsy, endoscopic ultrasound-guided pancreatic biopsy of a pseudo-tumoral formation etc).

Results

Frequency of CFTR gene mutations in idiopathic pancreatitis

The following clinical and morphological data were recorded: age, gender, severity of acute episodes of pancreatitis defined by the presence of at least one necrotic area (grade D or E on Balthazar’s CT scale [20]), presence of pseudocyst, pancreatic calcifications, exocrine or endocrine pancreatic insufficiency (overt steatorrhea clearly improved by pancreatic extracts was considered diagnostic of exocrine insufficiency in these patients), diabetes mellitus (fasting glycemia = 6.7 mmol/l on at least two tests or glycemia = 10 mmol/l two hours after ingestion of 75 g glucose), mean duration of pancreatitis defined as the time between the first manifestation (pancreatic pain, acute pancreatitis, steatorrhea) and the current hospitalization.

Statistical analysis

The chi-square test and the Wilcoxon test were used as appropriate to search for factors associated with the presence of a CFTR mutation. Fisher’s exact test was used for sample sizes below five. A median test was used to compare patient age by presence of mutation.

The presence of CFTR mutations in the study population was compared with the known prevalence in the general French population using a one-sided binomial test. The prevalence of cystic fibrosis in the French population is 1:3,500. The cystic fibrosis assay kit detects approximately 85% of the mutations. The expected prevalence of the 31 most frequently observed mutations of the CFTR gene was 0.85 x 31 = 25.8%. For ? F508 (approximately 70% of the mutations), the expected prevalence was 2.4%. This is close to the 2.5% reported in other European countries and the United States [3, 4, 6, 7, 11, 12]. For the 5T variant, the prevalence is about 10% irrespective of the subject’s ethnic background [11]. The prevalence of the L997F mutation and the E528E variant is approximately 10% irrespective of the subject’s ethnic background [11]. Reprints: F. MAIRE, Fédération Médecino-Chirurgicale d’Hépato-Gastroentérologie, Hôpital Beaujon, 100 boulevard du Général Leclerc, 92110 Clichy. E-mail: frederique.maire@bjn.ap-hop-paris.fr

Abbreviations:

CFTR: Cystic fibrosis transmembrane conductance regulator
ERCP: Endoscopic retrograde cholangio-pancreatography
MRI: Magnetic resonance imaging
PSTI: Pancreatic secretory trypsin inhibitor

Study population

Between April 1999 and December 2001, 104 patients (68 men and 36 women), median age 37 years (range: 14-73) were referred to our institution for exploration of pancreatitis of undetermined cause. Exhaustive search for etiology led to the exclusion of 40 patients recognized as having autoimmune disease (n = 3), eosinophil pancreatitis (n = 2), inflammatory bowel disease (n = 2), mutation of the cationic trypsinogen (n = 3) or PSTI (n = 1) genes, papilloma, or mucinous intraductal tumor (n = 7), ampulloma (n = 1), HIV seropositivity (n = 1), recent medication with a pancreatotoxic drug (n = 2), elevated serum
Identification of CFTR gene mutations and comparison with the French population

Eighteen mutations or variants of the CFTR gene were identified in 16 of the 64 patients included in this study (25%): ΔF508 (n = 7), L997F (n = 2), E528E (n = 4), 5T (n = 5). Two patients were compound heterozygous: ΔF508/L997F and 5F508/ST. One patient was homozygous or compound heterozygous for two “severe” mutations.

The prevalence of the ΔF508 mutation was 10.9% in the study population, i.e. 4.5 times the expected prevalence in the general French population (2.4%) (P < 0.001). The prevalence of the 5T variant was 7.8% which was not statistically different from the 10% observed in the general population. The L997F mutation on exon 17a and the E528E variant on exon 10 were identified in 3.1 and 6.2% of the patients, respectively. The significance in comparison with the general population (prevalence < 1%) could not be determined due to the small number of patients.

Patient characteristics by presence of CFTR gene mutation

The sex ratio and natural history of the pancreatic disease (acute recurrent pancreatitis or chronic pancreatitis, disease duration, exocrine and endocrine insufficiency, severity of the episodes according to the Balthazar score, presence of pseudocysts or calcifications) were not related to the presence or absence of CFTR gene mutation (table I). The difference in the median age of patients with and without mutations (32 and 39 years, respectively) showed a trend towards significance (P = 0.08) as was the difference in the prevalence of mutations in patients aged = 35 years (35.5%) compared with patients aged > 35 years (15.1%) (P = 0.06). The age distribution of the patients with a mutation is presented in figure 1.

Among the 16 patients with at least one CFTR mutation, one patient had a history of childhood asthma-like bronchitis. There were no patients with a known history of ENT disease or infertility.

Discussion

This prospective study of patients with idiopathic pancreatitis referred to a French Gastroenterology department demonstrated that 25% had a mutation of the CFTR gene. This percentage lies between the 13.4% and 37% reported by Sharer et al. [3] and Cohn et al. [4] in 1998. Later studies have reported a wide variation in prevalence, ranging from 0% to 62% [5-8, 10-14, 24-26]. This wide variability could be explained by several factors, including: a) sample size — certain studies included a small number of patients; b) patient selection — most of the studies including alcoholic, metabolic, and hereditary pancreatitis — the prevalence of CFTR mutation appears to be higher in idiopathic pancreatitis than in alcoholic pancreatitis, the latter of which does not appear different to that of the general population [3, 6, 10, 11, 13, 27]; c) differences in techniques used to search for mutations and the number of mutations recognized — the highest prevalence reported (62%) was found by Bishop et al. who sequenced the entire region coding for the CFTR gene in 16 patients [8]; d) presentation of the results — certain studies reported allelic prevalence or mutation carriers while others reported gene mutations, variants and/or polymorphisms. Our work avoids some of these problems since a large homogeneous group of patients were included after an exhaustive search for etiology and application of strict exclusion criteria. In addition, we used simple targeted laboratory techniques which are easily reproducible [17, 18]. Among the 16 patients in our study with CFTR mutation, 14 were heterozygous and 2 were compound heterozygous.

The pathogenesis of cystic fibrosis can now be formally attributed to heterozygous mutation of the CFTR gene [28]. The recent report by Castellani et al. [29] that the prevalence of diverse diseases commonly related to cystic fibrosis, including pancreatitis, was not different in patients with a heterozygous CFTR mutation from that in control subjects should be interpreted with prudence because of the case-control nature of the study. On
the basis of epidemiology and causality evidence [28], it is currently accepted that CFTR mutations are factors of genetic predisposition for the development of pancreatitis. Like most authors, we found that the prevalence of CFTR mutation was higher in patients with pancreatitis than in the general population, with a relative risk ranging from 2.5 to 11 [3, 4]. The 10.9% prevalence of F508 mutation in our series was higher than the 2.4% expected in the general population (P<0.001). The ST variant appears to play a less pronounced role, as has been suggested by earlier studies, since its prevalence was close to that observed in the general population (7.8% versus 10%) [4, 11]. The significance of the L997F mutation and the ES2BE variant is not clear. The L997F mutation is generally considered as a deleterious mutation and has exhibited an abnormally high prevalence in patients with chronic pancreatitis [9]. The ES2BE variant, which could alter the splicing process of exon 10, has been demonstrated in borderline cystic fibrosis [19]. The frequency of the L997F mutation (n = 2) and the ES2BE variant (n = 4) in our study gives a prevalence higher than usually reported in the general population.

There is pathophysiological evidence supporting the implication of CFTR mutations in pancreatitis. A mixed pathogenic process is involved. Initially, lesions can be observed in the ducts (destruction by protein plugs) or the acini (pH-dependent ion exchange), two mechanisms occurring in cystic fibrosis [3]. We did not measure nasal potential or electrolytes in sweat, but earlier studies have demonstrated that these tests are normal or non-significantly altered in patients with a CFTR mutation and pancreatitis without overt cystic fibrosis [3, 4, 12]. Finally, all other causes of pancreatitis were ruled out after a rigorous and exhaustive work-up.

The presence of a CFTR gene mutation does not appear to modify the natural history of pancreatitis [7, 13]. The clinical and morphological features observed in our patients were independent of the presence of a CFTR mutation. Age alone appeared to be a predictive factor with a trend towards statistical significance. More than one-third of patients aged less than 35 years had at least one CFTR gene mutation. There has only been one previous report of this correlation [3]. Clinical expression of pancreatic disease in a young adult could be related to association with a favoring factor such as alcohol consumption, even in small quantities (regular consumption of alcohol was an exclusion criterion for this study).

In conclusion, our work confirms that in the French population, CFTR mutations are genetic susceptibility factors implicated in the development of certain types of pancreatitis. CFTR mutations can be identified with relatively simple tests in approximately one quarter of patients aged less than 35 years. It would be reasonable to propose genetic tests for all patients aged less than 35 years with chronic pancreatitis or with recurrent acute pancreatitis of undefined cause. In other situations genetic tests could be proposed on a case-by-case basis. At the present time, discovery of a mutation does not modify patient management. A cause and effect relationship has not been demonstrated and another cause cannot be ruled out. Search for other causes of pancreatitis (tumor) with a potentially specific treatment must be performed with care even in patients with a CFTR mutation.

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REFERENCES


