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%): ∆F508 (n = 7), L997F (n = 2), E528E (n = 4), 5T (n = 5). Two patients were compound heterozygous. The frequency of ∆F508 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

Étude 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 à travers le monde était faite à l’aide du kit Cystic fibrosis assay. Une analyse supplémentaire 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’âgemedian 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 %) : ∆F508 (n = 7), L997F (n = 2), E528E (n = 4), 5T (n = 5). Deux malades étaient hétérozygotes composites. La fréquence observée de la mutation ∆F508 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 précoc.
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 T7q31 [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 very variable prevalence (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 in accordance with the French Consensus Conference as the presence of pancreatic pain and elevated serum lipase (or amylase) (= 3N) or in accordance with the French Consensus Conference as the presence of pseudocysts, pancreatic calci

Detection of CFTR gene mutation

A 10 ml blood sample was drawn after the patients gave their written informed consent for the study. Genomic DNA was extracted from nucleated cells using the standard guanidium chloride technique. Search for the 31 most frequently observed mutations of the CFTR gene was performed with the cystic fibrosis assay kit (aligconucleotide ligation assay (OLA), Perkin Clmer Applied Biosystmes, CA, USA). This kit detects approximately 82% of the mutations observed in populations with cystic fibrosis. A complementary analysis for variants in CFTR gene exons 9, 10 and 17a (mutation L997F, variants ES28E and ST), thought to be implicated in atypical cystic fibrosis (idiopathic bronchectasis, bilateral agenesis of the vas deferens), was also performed using denaturing gradient gel electrophoresis [8, 18, 19].

Clinical and morphological features

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 pseudocysts, pancreatic calcifications, exocrine or endocrine pancreatic insufficiency (ovet steatorrhoea clearly improved by pancreatic extracts was considered diagnostic of exocrine insufficiency in these patients), diabetes mellitus (fasting glycemia = 6.7 mmol/l on at 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, steatorrhoea) 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 frequent mutations was thus 2.8 % (1/3,500 × 0.85 ≤ 2%). 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, 6, 7, 11, 12]. For the ST variant, the prevalence is about 10% irrespective of the subject’s ethnic background [11]. The prevalence of the L997F mutation and the ES28E variant is < 1% [21-23].

Statistical significance was set at 5% for all tests.

Results

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), eosi

Exclusion criteria

Patients with another potential cause of pancreatitis were excluded: daily alcohol intake > 40 g for 2 years, biliary lithiasis, metabolic disorders (elevated serum triglycerides, calcium), autoimmune disease (confirmed by significantly elevated autoantibody level, histological evidence), inflammatory bowel disease (Crohn’s disease, ulcerative colitis), medication with known pancreatic toxicity, seropositivity for human immunodeficiency virus, mutation of cationic trypsinogen or PSTI genes, pancreas divisum, or pancreas tumor (in particular papillary or mucinous intraductal tumors).

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

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ABBREVIATIONS :
Exon 17a and the E528E variant on exon 10 were identified in 10% of the study population. The L997F mutation on exon 10 was 7.8% which was not statistically different from the 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.

Tableau I – Clinical characteristics according to the presence of CFTR gene mutations in the 64 patients with idiopathic pancreatitis.

<table>
<thead>
<tr>
<th>CFTR Mutation present (n = 16)</th>
<th>CFTR Mutation absent (n = 48)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>39</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>8/8</td>
<td>33/15</td>
</tr>
<tr>
<td>Recurrent acute pancreatitis/chronic pancreatitis [n]</td>
<td>8/8</td>
<td>26/22</td>
</tr>
<tr>
<td>Mean duration of pancreatitis years</td>
<td>3.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Severity of acute pancreatitis (Balthazar score D or E)</td>
<td>3 (21%)*</td>
<td>10 (27%)*</td>
</tr>
<tr>
<td>Pseudocysts</td>
<td>0</td>
<td>5 (10.4%)</td>
</tr>
<tr>
<td>Calciﬁcations</td>
<td>5 (26%)*</td>
<td>11 (50%)</td>
</tr>
<tr>
<td>Exocrine insufﬁciency</td>
<td>1 (12%)*</td>
<td>9 (41%)*</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>4 (8.3%)</td>
</tr>
</tbody>
</table>

NS: P > 0.05; * among the 51 patients with at least one acute episode (14 had a mutation and 37 did not); ** among the 30 patients with chronic pancreatitis

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 studied 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
References


