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Mutation analysis of PRSS1, SPINK1 and CFTR gene in patients with alcoholic and idiopathic chronic pancreatitis: A single center study

PANCREAS

Gürhan Şişman[1], Murat Tuğcu[2], Kerimova Ayla[3], Özdemir Sebati[1], Hakan Şentürk[4]

[1]Department of Gastroenterology, İstanbul University Cerrahpasa Medical Faculty, İstanbul, Turkey
[2]Department of Internal Medicine, İstanbul University Cerrahpasa Medical Faculty, İstanbul, Turkey
[3]Department of Medical Genetics, İstanbul University Cerrahpasa Medical Faculty, İstanbul, Turkey
[4]Department of Gastroenterology, Bezmialem University Faculty of Medicine, İstanbul, Turkey

Original Article

ABSTRACT

Background/Aims: A relation between some genetic mutations and chronic pancreatitis (CP) has been reported. However, the relation of genetic mutation to alcoholic CP (ACP) and idiopathic CP (ICP) still remains controversial. In this study, we investigated the prevalence of protease serine 1 (PRSS1), serine protease inhibitor, Kazal type 1 (SPINK1) SPINK1 and cystic fibrosis transmembrane conductance regulator (CFTR) mutations in ACP and ICP patients in Turkey.

Materials and Methods: Forty-one patients with ACP and 38 patients with ICP were enrolled, and 35 healthy individuals served as controls. The PRSS1 and SPINK1 mutations were investigated by the polymerase chain reaction (PCR)-restriction fragment-length polymorphism (RFLP) technique. The CFTR mutation was examined with PCR direct sequencing.

Results: The mean ages of the ACP, ICP and healthy control groups were 53.2, 40.4 and 46.3 years, respectively. A CFTR F508 mutation was detected as a heterozygote in one (2.4%) patient with ACP. In the ICP and control populations, PRSS1, SPINK1 and CFTR mutations were not detected.

Conclusion: This study shows that PRSS1, SPINK1 and CFTR mutations do not play a role in ACP and ICP patients.

Keywords: Chronic pancreatitis, SPINK1, PRSS1, CFTR

INTRODUCTION

Chronic pancreatitis (CP) is a chronic destructive disease that is characterised by irreversible morphological changes, function loss and, in the last stages of the disease, endocrine and/or exocrine disorders due to continuous inflammation (1). In the Western countries, the most common etiological factor is alcohol abuse (2). Other causes include metabolic, anatomical, obstructive, and autoimmune etiological factors (3,4). Recently, several genetic risk factors for CP, mostly idiopathic or hereditary in form, have been recognised, such as: the cationic trypsinogen gene, also known as protease serine 1 (PRSS1); the serine protease inhibitor, Kazal type 1 (SPINK1); and the cystic fibrosis transmembrane conductance regulator (CFTR) gene. In addition, around one-third of all patients have no etiological factor identified, and these cases are classified as idiopathic chronic pancreatitis (ICP) (5-7).

The aim of this study was to determine the frequency of PRSS1, SPINK1 and CFTR mutations in patients with alcoholic CP (ACP) and ICP compared with the healthy control volunteers.

MATERIALS AND METHODS

Patients

A total of 79 unrelated patients with CP (41 with ACP and 38 with ICP) and 35 healthy controls were enrolled in the present investigation at the Istanbul University Cerrahpasa Medical Faculty Department of Gastroenterology. The diagnosis of CP was made by confirmation of the presence of a typical history of recurrent attacks of pancreatitis and radiological findings of either pancreatic calcification, revealed by computed tomography, or pancreatic ductal changes in endoscopic retrograde pancreatography or endosonography.

Address for Correspondence: Gürhan Şişman, Department of Gastroenterology, İstanbul University Cerrahpasa Medical Faculty, İstanbul, Turkey
E-mail: sisman1981@hotmail.com
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Clinical characteristics of patients

There were 33 males and 8 females in the ACP group and 32 males and 6 females in the ICP group. Thirty-five healthy controls, comprising 22 males and 13 females, were also included. The mean ages of the ACP, ICP and healthy control groups were 53.2, 40.4 and 46.3 years, respectively.

Alcoholic CP was diagnosed in male patients who consumed more than 80 grams (g)/day of alcohol and in female patients who consumed at least 40 g/day of alcohol for 5 or more years prior to the first symptoms of the disease (4).

Idiopathic CP was diagnosed when possible etiological factors, such as alcohol abuse and positive family history, were absent (4). Other possible risk factors for the development of CP were evaluated through laboratory tests (hypercalcemia and hypertriglyceridemia) and by radiology (magnetic resonance cholangiopancreatography or endosonography) of both the biliary and pancreatic ducts.

For the control group, healthy unrelated volunteers from our hospital staff were enrolled. Blood samples were withdrawn using EDTA tubes after obtaining written informed consent.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes using the spin-column method (QiAmp DNA blood mini kit from Qiagen) according to the manufacturer's recommendations.

PRSS1 mutation detection

The R122H mutation was detected by the method polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) using specific primer sequences: 5′ GGT CCT GGG TCT CAT ACC TT 3′ and 5′ GTA ATG GGC ACT CGA AAT GT 3′. PCR was performed using 3 µL of genomic DNA template, 1.5 U of Taq polymerase (TaKaRa, BIOKOM), a 0.2 µM concentration of each primer, 2.5 µL of buffer, and a 50-µM concentration of each dNTP in a 25-µL reaction volume. Thirty cycles of PCR were performed (30 s at 94°C, 30 s at 60°C and 30 s at 72°C) and final extension performed for 5 min at 72°C in an automated thermal cycler GeneAmp PCR 2400 (made by Perkin-Elmer of Norwalk, USA). The PCR products were then digested with restriction endonuclease BbrPI for 2 h at 37°C. The digested amplification products were electrophoresed on 7% polyacrylamide gel (PAGE) and stained with ethidium bromide, generating two fragments of 327 bp and 228 bp. After digestion, the obtained product had an additional length of 208 bp.

The N29I mutation of exon 2 was detected by the PCR amplification using specific primer sequences: 5′ CCATCTTACCCAACCTCAGTAG 3′ and 5′TGATGACAGATCGTTGGGGGCTAGA 3′. The reaction program was as follows: an initial denaturation for 5 min at 94°C and an additional denaturation of 30 cycles of 30 s at 94°C; 30 s of hybridization for 30 s at 56°C; 45 s of primer extension at 72°C; and a final extension for 10 min at 72°C in a GeneAmp PCR 2400 automated thermal cycler. The products were then digested with restriction endonuclease Sau3A. PCR-amplified products were electrophoretically separated on 7% PAGE and stained with ethidium bromide. In the presence of N29I mutation, two bands of 207 and 23 bp were seen, whereas only one band of 1,018 bp was seen on the gel.

SPINK1 mutation detection

Genomic DNA from the patients and the controls was analysed for a common mutation of SPINK1. The N34S mutation of exon 3 was detected by PCR-RFLP using specific primer sequences: 5′ TTC TGT TTA ATT CCA TTTTT AGGCCA AAT GCT GCA 3′ and 5′ GGC TTTT ATC ATACAA GTG ACT TCT 3′. The primers were designed to introduce a PstI endonuclease restriction site in sequences containing the N34S mutation and a BsrDI endonuclease restriction site in wild-type sequences. PCR was performed using 3 µL of genomic DNA template, 1.5 U of Taq polymerase (TaKaRa, BIOKOM), a 0.2 µM concentration of each primer, 3 µL of 10 × PCR buffer, a 75-nM concentration of each dNTP, and a 30-µL reaction volume. Twenty cycles of PCR were performed (30 s at 94°C, 30 s at 60°C and 60 s at 72°C) in a GeneAmp PCR 2400 automated thermal cycler. The PCR products were then digested with restriction endonuclease PstI and BsrDI. Undigested amplification products were 320 bp long. After digestion with PstI, a product of 286 bp was obtained from mutant sequences, and an identical result was achieved from wild-type sequences after digestion with BsrDI. Heterozygote samples produced products of both 320 and 286 bp after digestion with either endonuclease.

CFTR mutation detection

DNA extraction from non-coagulated blood was performed by a column-based extraction kit: QiAamp DNA mini kit or NucleuoSpin Blood (MACHEREY-NAGEL cod NLMAA1001 of Germany). DNA concentrations were measured spectrophotometrically using the NanoDrop (Thermo Scientific). DNA samples were multiplied by multiplex PCR with a CF 22Mut and CF 14Mut+Tn strip assay kit which has 36 common mutations of the CFTR gene (DF508, DI507, F508C, I502T, 1706del17, 1677del TA, G542X, 1717-1G>A, R553X, Q552X, G551D, S549R(A>C), N1303K, 4016insT, R1162X, R1158X, W1282X, G1244E, 2789+5G>A, 2183AA>G, 711+5G>A, 711+1G>T, G85E, 3849+10kbC>T, 621+1G>T, R117H, D1152H, L1065P, R1066H, L1077P, 4382delA, 1259insA, 852del22, R347P, T338I, S912X and Allele5T-7T-9T). The PCR forward and reverse primers used were 5´ AAT GTC AAC TGC TTG AGT GT 3´ and 5´ GAA GCA GGC ATA ATG ATT CT 3´, respectively.

Polymerase chain reaction was performed using 3 µL of genomic DNA template, 1.5 U of Taq polymerase (Perkin-Elmer), a 0.4-µM concentration of each primer, 4 µL of 10 × PCR buffer, a 74-nM concentration of each dNTP and 1.5 mM of MgCl2 in a 32-µL reaction volume.

Thirty cycles of PCR were performed (30 sec at 94°C, 120 sec at 53°C and 120 sec at 72°C). Cycle sequencing was performed on the products directly using a 96-capillary ABI3730xl automatic sequencer (Applied Biosystems). If the mutation of F508 was found, reverse sequencing with primer 5´GGA TCC AAA TGA GCA CTG GGT TC 3´ was performed to confirm the result.

Ethics committee

The study was undertaken under strict ethical guidelines and we obtained the written consent of every participant. The study was approved by the local ethics committee of the Istanbul University Cerrahpasa Medical Faculty.

Statistical analyses

All statistical analyses were carried out using the software SPSS 13.0 for Windows. For analysis of the difference in the allele and genotype frequencies of mutants, we used Fisher's exact probability test. Significance was set at a p value of 0.05.

RESULTS

R122H and N29I mutation analysis on the PRSS1 gene

R122H and N29I mutation analysis on the PRSS1 gene was performed on the ACP and ICP patient groups and the control group. Mutations were not present in any of the three groups.

N34S mutation analysis on the SPINK1 gene

N34S mutation analysis on the SPINK1 gene was performed on the ACP and ICP patient groups and the control group. Mutations were not present in any of the three groups.

Mutation analysis on the CFTR Gene

Mutation analysis on the CFTR gene was performed on the ACP and ICP patient groups and the control group. Only 1 patient (2.4%), from the ACP group, had the heterozygote F508 mutation. None of the groups had the 5T allele.

DISCUSSION

Many studies have been done regarding the role of SPINK1, PRSS1 and CFTR mutations in the etiology of CP. Our study is the first of its kind to be done in the adult Turkish population regarding these gene mutations and their relationships with ACP and ICP.

SPINK1, PRSS1 and CFTR mutations in ACP patients

The pathogenesis of ACP still remains controversial. There are many theories, such as: channel obstruction due to protein plugs; direct toxicity of ethanol; oxidative stress; and the effects of ethyl fatty acids produced during the non-oxidative metabolisation of ethanol (3,8). However, none of these theories can give a clear explanation of the pathogenesis. The role of genetic factors in ACP has been shown in previous studies. PRSS1, SPINK1 and CFTR gene mutations are the main focus of these research projects (9-11).

Mutations of the PRSS1 gene cause autosomal dominant hereditary pancreatitis at a high prevalence of about 80% (12). This mutation's analysis has always been negative in ACP (13-16), confirming that PRSS1 mutations play no role in pancreatic damage in patients who drink alcohol. A recent study also showed that PRSS1 mutations were not detected in Indian patients with hereditary and nonhereditary CP, including ACP (17). One study from Brazil managed to detect an E79K mutation in one patient (1.5%) with ACP in an 82-patient CP group (18). We studied the most common mutations on the PRSS1 gene: R122H and N29I. In correlation with previous studies, we did not detect any R122H and N29I mutations on the PRSS1 gene in patients with ACP.

The role of the SPINK1 mutation in ACP is still controversial. In previous studies, SPINK1 N34S mutations are commonly found in 1% to 4% of the general population, but are more frequent (6%) in patients with ACP (12,19,20). These results suggest that SPINK1 mutations do not play an important role in the majority of patients (15,21). On the other hand, a study done in India showed a much higher prevalence of SPINK1 mutation: 26.8% in ACP patients with a study population of 41 patients (17). In contrast to Western or Indian patients, PRSS1 mutations were not detected in our patients with ACP. In our study, mutational screening of the SPINK1 gene was performed by techniques similar to other studies, so there are no methodological problems in our series. The differences in results can be explained by genetic differences between ethnic populations.

Currently, more than 1,000 mutations have been described in the CFTR gene. Several initial studies looking at limited mutations, such as F508del and the 5T allele, failed to identify an association with ACP (22-25). CTFR gene mutations in ACP patients were also studied by different research groups. Sharer et al. looked for 22 different mutations; in patients with ACP, the CFTR mutation rate was 8.5%; significantly higher than the control group (26). However, subsequent studies failed to establish any significant difference between ACP patients, the healthy population and the control groups (13,14,25,27). Two studies from Brazil showed that different methods could affect the results of the study. The first, done by Bernardio et al., found a 4.7% CFTR mutation rate; the second study, done by Costa et al., used a different method and found a higher mutation rate of 11.8% (18,28). In our study, we found heterozygous F508 mutation in 1 patient (2.4%) within a 41-patient ACP group. We used CFTR17 and CFTR19 kits on the CFTR gene to study the most common 36 mutations. If we would have studied the whole gene or used a different method, we might have found a similar rate to other studies. This suggests that there might be CFTR mutations that we could not detect with our method.

SPINK1, PRSS1 and CFTR in ICP patients

Mutations of the PRSS1 gene have shown a variable low incidence (0%-19%) in ICP, which suggests a possible role for them (12,29,30). A recent study from Poland showed a higher incidence (21.4%) of PRSS1 mutations in ICP patients (8). In our study, we did not find any R122H or N29I mutations in ICP pa-

tients. In contrast to previous studies, PRSS1 mutations were not detected in our patients with ICP.

The frequency of SPINK1 mutation in patients with ICP is reported in a wide range in different studies. Pfutzer et al. reported 12% homozygous and 25% heterozygous N34S mutations in a 57-patient ICP series (31). Other studies from Germany and the USA established an association between juvenile ICP and SPINK1 mutations (32,33). The mean age was 19.5 in a group of 12 patients with early onset ICP; SPINK-1/N34S mutation was not found in subgroup analysis of our study. This result is likely to be related to genetic diversity among ethnic groups and the small number of patients in our study.

Cystic fibrosis transmembrane conductance regulator mutations in ICP patients have been researched in many different studies. Sharer et al. reported a 13.4% mutation rate in a group of 134 ICP patients; however, Cohn et al. reported a higher rate of 37% in a group of 27 CP patients (27,34). As with ACP patients, this wide range of prevalence in ICP patients can be a result of the number of patients studied, the selection of patients, method differences, investigated mutation numbers or statistical calculation and presentation differences of the results. In our study, we looked for the 36 most common mutations of CFTR in the group of 38 ICP patients and did not detect any mutations in our population.

Limitations of our study included absence of epidemiologic data about patients with CP in Turkey and the analysis of the most common genetic mutations. Thus, our study was not able to exactly present genetic mutations in Turkey; comprehensive mutation analysis in a greater number of patients is needed.

In conclusion, these results show that PRSS1, SPINK1 and CFTR mutations do not have a role in ACP and ICP within our study group. Further exhaustive studies are needed to show other unknown possible genes that might be related to ACP and ICP.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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