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Original article

Genetic analysis of the bicarbonate secreting anion exchanger SLC26A6 in chronic pancreatitis

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ABSTRACT

Background: Pancreatic ductal HCO_3^- secretion is critically dependent on the cystic fibrosis transmembrane conductance regulator chloride channel (CFTR) and the solute-linked carrier 26 member 6 anion transporter (SLC26A6). Deterioration of HCO_3^- secretion is observed in chronic pancreatitis (CP), and *CFTR* mutations increase CP risk. Therefore, *SLC26A6* is a reasonable candidate for a CP susceptibility gene, which has not been investigated in CP patients so far.
Methods: As a first screening cohort, 106 subjects with CP and 99 control subjects with no pancreatic disease were recruited from the Hungarian National Pancreas Registry. In 60 non-alcoholic CP cases the entire *SLC26A6* coding region was sequenced. In the Hungarian cohort variants c.616G > A (p.V206M) and c.1191C > A (p.P397=) were further genotyped by restriction fragment length polymorphism analysis. In a German replication cohort all exons were sequenced in 40 non-alcoholic CP cases and variant c.616G > A (p.V206M) was further analyzed by sequencing in 321 CP cases and 171 controls.

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Results: Sequencing of the entire coding region revealed four common variants: intronic variants c.23 + 78_110del, c.183-4C > A, c.1134 + 32C > A, and missense variant c.616G > A (p.V206M) which were found in linkage disequilibrium indicating a conserved haplotype. The distribution of the haplotype did not show a significant difference between patients and controls in the two cohorts. A synonymous variant c.1191C > A (p.P397=) and two intronic variants c.1248 + 9_20del and c.-10C > T were detected in single cases.

Conclusion: Our data show that *SLC26A6* variants do not alter the risk for the development of CP.

Introduction

Chronic pancreatitis (CP) is an intractable inflammatory disease of the pancreas, leading to progressive and irreversible destruction of the parenchyma. In the majority of patients the etiology is complex, the disease results from the interaction of multiple risk factors. CP is most commonly associated with excessive alcohol consumption but other factors such as smoking, metabolic disturbances, anatomic abnormalities, autoimmunity and genetic variations have also been implicated. Discoveries of association of CP with *PRSS1* [1], *SPINK1* [2] and *CTRC* [3] gene mutations suggest a central role for trypsin in the development of CP, while association with *CFTR* [4] variants highlight the importance of ductal changes contributing to disease development.

Fluid and HCO_3^- secretion is a vital function of pancreatic ductal epithelium and is fundamental for the integrity of the tissue. The human pancreas secretes 1–2 L of alkaline, isotonic juice per day, which contains concentrations of bicarbonate that may exceed 140 mM [5]. This bicarbonate rich fluid flushes out digestive enzymes from the ductal tree, facilitates solubilization of macromolecules, neutralizes the protons secreted by acinar cells, prevents premature activation of trypsinogen and neutralizes gastric acid in the duodenum providing an optimal pH environment for digestive enzymes.

One of the functional consequences of CP is the reduction in secretin-stimulated bicarbonate content in pancreatic juice [6]. On the other hand, in cystic fibrosis (CF), pancreatic HCO_3^- secretion is impaired. Impaired HCO_3^- secretion disrupts the physiological interaction between acinar and duct cells, resulting in decreased intraluminal pH, premature trypsinogen activation, impaired acinar secretion, obstruction of the lumen with protein plugs and finally destruction of the parenchyma [7]. Several pancreatitis associated toxic factors, such as ethanol [8], bile acids [9], trypsin [10] and cigarette smoke extract [11] displayed inhibitory effects on bicarbonate secretion and influenced the activity of the cystic fibrosis transmembrane conductance regulator chloride channel (CFTR). Not only functional inhibition, but also genetic defects of *CFTR* can increase the risk for pancreatitis. Association of *CFTR* mutations and the development of CP [4,12], and recurrent acute pancreatitis [14] has been reported. CF-causing severe and mild *CFTR* variants increase the risk 2.9 and 4.5-fold respectively [15]. These observations indicate that insufficient electrolyte transport is pathogenic for CP (Fig. 1).

Pancreatic bicarbonate secretion is not only dependent on *CFTR* but also on the solute-linked carrier 26 (SLC26) anion transporters, localized in the apical membrane of the ductal cells. SLC26 isoforms constitute a conserved family of anion transporters with 10 distinct members. All SLC26 isoforms - except for SLC26A5 (prestin) - are versatile anion exchangers mediating apical chloride/base exchange in epithelial tissues. Several diseases have been linked to mutations in members of the family, including diastrophic dysplasias (SLC26A2), congenital chloride diarrhea (SLC26A3), Pendred's syndrome (SLC26A4), hearing loss (SLC26A5) and asthma (SLC26A9) [16,17].

The SLC26A6 anion exchanger is expressed in the apical membrane of pancreatic ducts, intestinal epithelium and kidney proximal tubule [17]. It mediates multiple anion exchange modes, including, Cl^-/HCO_3^- exchange, Cl^-/formate exchange and Cl^-/oxalate exchange. Earlier studies revealed a direct molecular interaction between CFTR and two SLC26 exchangers, namely A3 and A6, which results in mutual upregulation of their transport activity [18]. This process is mediated by binding of the regulatory (R) domain of CFTR to the highly conserved STAS (sulphate transporter and anti-sigma antagonist) domain of SLC26 and this interaction is required for activation of both SLC26 transporters and CFTR. Notably, CF causing *CFTR* mutations that retain normal or substantial Cl^- conductance exhibited a severe defect in CFTR dependent Cl^-/HCO_3^- exchange activity. This indicates that impairement of the coupled bicarbonate transport mechanism is sufficient to damage pancreatic function even in the presence of CFTR Cl^- channel activity [19]. On the basis of its localization in the apical membrane of the pancreatic duct and its function as a Cl^-/HCO_3^- exchanger, SLC26A6 has been proposed to be a major contributor to the apical HCO_3^- secretion in the pancreatic duct [20]. However, the role of genetic variations in *SLC26A6* has remained unexplored in CP.

In this study, we sequenced the entire coding region of *SLC26A6* in 100 non-alcoholic CP cases. The identified variants were further investigated in Hungarian and German cohorts of non-alcoholic and alcoholic CP.

Methods

Subjects and study design

The study protocol was approved by the national ethical review committee ETT-TUKEB (22254-1/2012). All patients gave written informed consent for genetic analysis. The study included CP patients originating from Hungary (n = 106) and Germany (n = 361). Clinico-pathological information on individual patients including symptoms, diagnostic criteria and etiology were collected from medical records and questioners completed by the patients. Diagnosis of CP was based on at least two of the following criteria: constant or recurrent abdominal pain, calcifications on sonography or CT, ductal irregularities on ERCP or MRCP examination, EUS based diagnosis of CP and histologically confirmed CP. According to etiology, patients were divided into alcoholic CP and non-alcoholic CP groups. Alcoholic CP was defined by consumption of more than 80 g/d (man) ethanol or more than 60 g/d (women) for at least two years. 99 Hungarian and 171 German control subjects were recruited from adult volunteers who considered themselves generally healthy, from inpatients who had no history of pancreatic disease and from blood donors (Table 1).

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