CLINICAL RESEARCH ON ALCOHOLIC LIVER AND PANCREATIC DISEASES

Individual susceptibility to alcoholic pancreatitis
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Abstract

The observation that only a minority of heavy drinkers develop pancreatitis has prompted an intensive search for a trigger factor/cofactor/susceptibility factor that may precipitate a clinical attack. Putative susceptibility factors examined so far include diet, smoking, amount and type of alcohol consumed, the pattern of drinking and lipid intolerance. In addition, a range of inherited factors have been assessed including blood group antigens, human leukocyte antigen serotypes, alpha-1-antitrypsin phenotypes and several genotypes. The latter group comprises mutations/polymorphisms in genes related to alcohol-metabolizing enzymes, detoxifying enzymes, pancreatic digestive enzymes, pancreatic enzyme inhibitors, cystic fibrosis and cytokines. Disappointingly, despite this concerted research effort, no clear association has been established between the above factors and alcoholic pancreatitis. Experimentally, the secretagogue cholecystokinin (CCK) has been investigated as a candidate ‘trigger’ for alcoholic pancreatitis. However, the clinical relevance of CCK as a trigger factor has to be questioned, as it is difficult to envisage a situation in humans where abnormally high levels of CCK would be released into the circulation to trigger pancreatitis in alcoholics. In contrast, bacterial endotoxemia is a candidate cofactor that does have relevance to the clinical situation. Plasma lipopolysaccharide (LPS, an endotoxin) levels are significantly higher in drinkers (either after chronic alcohol intake or a single binge) compared to non-drinkers. We have recently shown that alcohol-fed animals challenged with otherwise innocuous doses of LPS exhibit significant pancreatic injury. Moreover, repeated LPS exposure in alcohol-fed rats leads to progressive injury to the gland characterized by significant pancreatic fibrosis. These studies support the concept that endotoxin may be an important factor in the initiation and progression of alcoholic pancreatitis. Scope remains for further studies examining proteins related to cellular anti-oxidant defenses, minor cystic fibrosis (CF) mutations and trans-heterozygosity involving a combination of mutations of different genes (such as CFTR alterations combined with SPINK1 or PRSS1 variants), as potential triggers of alcoholic pancreatitis.

Key words
alcoholic pancreatitis, susceptibility, trigger factor.

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Introduction

The association between alcohol abuse and pancreatitis has long been known and accepted. As early as in 1878, Friedreich wrote ‘I am inclined to believe that a general chronic interstitial pancreatitis may result from excessive alcoholism (drunkard’s pancreas)’.1 Although this initial observation by Friedreich concerned alcohol-associated chronic pancreatic injury, there is now sufficient evidence to indicate that pancreatic injury due to alcohol abuse often manifests clinically as an acute episode of pancreatic necroinflammation (acute pancreatitis). Repeated acute episodes can result in chronic pancreatitis characterized by acinar atrophy and fibrosis (the ‘necrosis-fibrosis’ sequence).

The incidence of alcoholic pancreatitis is known to be proportional to the level of alcohol consumption, suggesting that alcohol exerts dose-related effects on the pancreas.2,3 However, it is also clear that only a minority of heavy drinkers develop alcoholic pancreatitis, indicating that some individuals have an increased susceptibility to the disease.4,5 Over the past 20 years, a concerted effort has been made to identify potential ‘susceptibility/trigger’ factors that may be responsible for initiating alcoholic pancreatitis. Ideally, for such ‘susceptibility’ studies, the key comparison should be between alcoholics with pancreatitis and alcoholics without pancreatitis. The inclusion of alcoholics (with equivalent lifetime consumption of alcohol) as controls ensures that any differences observed between the two groups can be attributed to the presence or absence of pancreatitis and not simply to alcoholism per se. Unfortunately, numerous studies in the literature have only used the general population as controls, making the interpretation of results difficult.
 SUSCEPTIBILITY TO ALCOHOLIC PANCREATITIS

To date, numerous factors have been assessed for their putative role in conferring increased susceptibility to alcoholic pancreatitis (Table 1). These include diet, smoking, type of alcohol consumed, the pattern of drinking, lipid intolerance and a range of inherited factors (discussed in detail later). With regard to diet, both poor nutrition and hypernutrition have been implicated in alcoholic pancreatitis. Experimentally, malnutrition or protein deficiency has been reported to cause acinar atrophy and ductal damage in the pancreas. In contrast, increased nutrient intake has been reported to result in increased digestive enzyme synthesis and/or secretion which is postulated to predispose the pancreas to autodigestive injury. However, clinical studies examining diet in alcoholics with and without pancreatitis have failed to find significant differences in protein, carbohydrate or fat intake in the two groups. These findings indicate that macronutrient intake does not play a major role in the pathogenesis of alcoholic pancreatitis. More work is needed to assess the role of micronutrients in alcoholic pancreatitis.

The possible association between smoking and alcoholic pancreatitis has been examined by several investigators, but the issue remains controversial. In 1987, Lowenfels and colleagues studied a native American Indian population and reported that smoking was a risk factor for alcoholic pancreatitis. However, this was a retrospective study relying on hospital admission records. Therefore, the accuracy of assessment of tobacco intake in the study subjects was uncertain. More recently, Maisonneuve et al. reported that alcoholic pancreatitis developed earlier in smokers than in non-smokers. These findings were similar to those reported earlier by Bourliere and colleagues. However, both these studies were confounded by the fact that, compared to non-smokers, a higher proportion of smokers were also heavy drinkers. Thus, the groups being compared differed with respect to two variables, alcohol intake and tobacco consumption. Notably, when the amount of alcohol consumed by the two groups was similar, no association could be detected between smoking and alcoholic pancreatitis. Thus, it appears unlikely that smoking is an initiating factor for alcoholic pancreatitis. However, it is possible that it plays a role in disease progression.

The influence of beverage type and drinking pattern on the development of alcoholic pancreatitis has always intrigued researchers and several series of cases of pancreatitis have variously reported a predominance of beer, wine or spirits in the index population (see Haber et al. for review). Most recently, Nakamura et al. have reported that patients consuming spirits (gin, brandy, whiskey, shochu [a rice-based spirit]) have a higher risk of developing alcoholic chronic pancreatitis than patients drinking beverages with lower alcohol content. However, they also report that the amount of alcohol consumed per day was greater in spirits drinkers indicating that it is the high alcohol consumption (rather than the beverage type) that is associated with pancreatitis. Other controlled studies have also demonstrated that the type of alcoholic beverage does not influence susceptibility to pancreatitis (see Haber et al. for review).

Although binge drinking has been reported to precede attacks of pancreatitis, it is evident that most patients have consumed alcohol steadily over many years. An early study has reported an increased proportion of binge drinkers among alcoholics with pancreatitis compared to those without pancreatitis. However, a subsequent

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**Table 1** Susceptibility factors in alcoholic pancreatitis

<table>
<thead>
<tr>
<th>Susceptibility factor</th>
<th>Susceptibility factor (Yes/no)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet</td>
<td>No</td>
<td>Wilson et al. 1985^9</td>
</tr>
<tr>
<td>Beverage type</td>
<td>No</td>
<td>Wilson et al. 1985^9</td>
</tr>
<tr>
<td>Drinking pattern</td>
<td>Yes</td>
<td>Nakamura et al. 2004^15</td>
</tr>
<tr>
<td>Smoking</td>
<td>No</td>
<td>Wilson et al. 1985^9</td>
</tr>
<tr>
<td>Inherited factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human leukocyte antigen</td>
<td>No</td>
<td>Wilson et al. 1984^25</td>
</tr>
<tr>
<td>Alpha-1-antitrypsin deficiency</td>
<td>No</td>
<td>Haber et al. 1991^21</td>
</tr>
<tr>
<td>Cystic fibrosis genotype</td>
<td>No</td>
<td>Norton et al. 1998,23 Haber et al. 1999^19</td>
</tr>
<tr>
<td>Alcohol-metabolizing enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>No</td>
<td>Frenzer et al. 2002,26 Verlaan et al. 2004^28</td>
</tr>
<tr>
<td>CYP4502E1</td>
<td>No</td>
<td>Frenzer et al. 2002^28</td>
</tr>
<tr>
<td>Cholesteryl ester lipase</td>
<td>Yes</td>
<td>Miyasaka et al. 2005^37</td>
</tr>
<tr>
<td>Trypsinogen gene mutations</td>
<td>No</td>
<td>Perri et al. 2003^23</td>
</tr>
<tr>
<td>PSTI/SPINK1 mutations</td>
<td>No</td>
<td>Schneider et al. 2003^27</td>
</tr>
<tr>
<td>Cytokines</td>
<td>No</td>
<td>Witt et al. 2001^32</td>
</tr>
<tr>
<td>Detoxifying enzymes</td>
<td></td>
<td>Schneider et al. 2004^25</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>No</td>
<td>Frenzer et al. 2002^28</td>
</tr>
<tr>
<td>UDP-glucuronosyl transferase</td>
<td>Yes</td>
<td>Ockenga et al. 2003^27</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>te Morsche et al. 2007^29</td>
</tr>
</tbody>
</table>

^Studies that did not include alcoholics without pancreatitis as controls.
study by Wilson et al. has reported that drinking patterns were similar in alcoholics with and without pancreatitis. Thus, the association of binge drinking with the development of alcoholic pancreatitis remains uncertain.

Hyperlipidemia/hypertriglyceridemia is another factor that has been assessed for its potential role in triggering alcoholic pancreatitis. Prolonged and heavy drinking per se is associated with hypertriglyceridemia (fasting and postprandial) attributable in large part to increased hepatic synthesis of very low-density lipoproteins and, in the presence of liver disease, reduced hepatic removal of triglycerides. In view of this and the long-established association of hypertriglyceridemia with pancreatitis, it would be reasonable to hypothesize that elevated serum lipid levels play a role in alcoholic pancreatitis. In a carefully controlled study, Haber et al. gave an oral lipid load to alcoholics with and without pancreatitis and found that the plasma triglyceride levels were similar in both groups. Even after the addition of ethanol to the oral lipid load, there was no difference observed between plasma triglyceride levels in alcoholics with pancreatitis and alcoholics without pancreatitis. These findings argue against a major role for hypertriglyceridemia in the development of alcoholic pancreatitis.

Several inherited factors have been examined for their possible role in the pathogenesis of alcoholic pancreatitis. These include blood group antigens, human leukocyte antigen (HLA) serotypes, alpha-1-antitrypsin phenotypes and several genotypes such as those associated with cystic fibrosis, cytokines (transforming growth factor beta [TGF-β], tumor necrosis factor α [TNFα], interleukin-10 and γ-interferon), alcohol-metabolizing enzymes (alcohol dehydrogenase [ADH], aldehyde dehydrogenase [ALDH] and cytochrome P450 2E1 [CYP2E1]) and detoxifying enzymes such as UDP glucuronosyltransferase (UGT1A7) and glutathione S-transferase. Mutations of genes related to pancreatic proteins that may play an important role in autodigestive injury to the gland have also been examined. These include trypsin itself and other housekeeping proteins such as pancreatic secretory trypsin inhibitor (PSTI), also known as serine protease inhibitor Kazal type 1 (SPINK-1), which inactivates inappropriately activated trypsin within the cell. Unfortunately, none of the above studies has been able to demonstrate an unequivocal association between a candidate factor and susceptibility to alcoholic pancreatitis.

Space constraints preclude a detailed discussion of each of the above inherited factors. However, it may be pertinent to describe recent studies related to specific factors such as alcohol-metabolizing enzymes, the cystic fibrosis transmembrane regulator (CFTR) and the trypsin inhibitor SPINK-1.

Polymorphisms of alcohol-metabolizing enzymes have attracted interest as potential susceptibility factors, as there is ample evidence from experimental studies demonstrating that the metabolites of alcohol have deleterious effects on the pancreas. Polymorphisms have been identified in genes coding for the enzymes responsible for the oxidation of alcohol to acetaldehyde (i.e. alcohol dehydrogenase [ADH]) and cytochrome P450 2E1 (CYP2E1) and for the enzyme that catalyzes the conversion of acetaldehyde to acetate, namely acetaldehyde dehydrogenase (ALDH). Frenzer and colleagues have reported a case–control study comparing the genotype frequency of ADH2, ADH3, ALDH2 and CYP2E1 in a Caucasian cohort comprising patients with alcoholic cirrhosis (57 patients) and alcoholic pancreatitis (71 patients), alcoholics without evidence of organ damage (57 subjects) and healthy subjects (200). The authors reported an increased frequency of specific genotypes (ADH3*2/*2 and possibly ADH2*1/*1 which both encode less active enzymes) in patients with alcoholic cirrhosis compared to patients with alcoholic pancreatitis and healthy controls. However, this frequency was not significantly different when compared to alcoholic controls. Notably, the authors found no association between polymorphisms for ADH2, ADH3, ALDH2 and CYP2E1 and alcoholic pancreatitis. These findings are similar to an earlier case–control study analyzing the above genes in Chinese patients with acute alcoholic pancreatitis, in alcoholic patients without organ damage, in patients with pancreatic disease of non-alcoholic origin and in patients with alcoholic liver disease.

More recently, Verlaan et al. have reported a study assessing ADH3 and CYP2E1 polymorphisms in patients with alcoholic pancreatitis, hereditary pancreatitis, and idiopathic pancreatitis, as well as in alcoholics without pancreatitis and healthy controls. The researchers reported a trend to a higher frequency of the CYP2E1 intron 6D allele in patients with alcoholic pancreatitis compared to healthy or alcoholic controls. The functional significance of this finding is unclear, although the authors hypothesize that the intron 6D allele may be associated with less efficient microsomal CYP2E1 enzyme activity resulting in a shift from microsomal to cytosolic ethanol oxidation and damage to vital intracytoplasmic structures within the cell. No significant difference was observed in ADH3 genotype frequency between patients with pancreatitis of various etiologies and alcoholic controls.

The studies described above are relevant to the oxidative pathway of alcohol metabolism. A second, increasingly well-recognized pathway of alcohol metabolism in the pancreas is the non-oxidative pathway involving the conversion of alcohol to fatty acid ethyl ester (FAEE), a reaction that is catalyzed by a group of enzymes collectively called FAEE synthases. Interestingly, a promising association has been recently reported between a polymorphism of the gene for carboxylester lipase (CEL, one of the candidate FAEE synthase enzymes) and the risk of developing alcoholic pancreatitis. The study included a population of 100 alcoholics with chronic pancreatitis, 52 alcoholics, 50 non-alcoholic pancreatitis patients, 96 hyperlipidemia patients and 435 control subjects. The authors report an increased frequency of a variable number of tandem repeat (VNTR) polymorphisms in the coding region of the CEL gene in patients with alcoholic pancreatitis compared to the other groups, including alcoholics without organ damage. The functional significance of this polymorphism is not yet clearly defined but it has been suggested that it might influence CEL protein stability and/or secretion.

Cystic fibrosis is an autosomal recessive disease characterized by pulmonary dysfunction and pancreatic insufficiency. It results from mutations in the gene coding for CFTR (cystic fibrosis transmembrane regulator), an apical membrane chloride channel that regulates fluid and electrolyte secretion in digestive and respiratory tracts. Cystic fibrosis is a known association of pancreatitis, but the role of CFTR mutations in alcoholic pancreatitis is unclear. In 1998, Sharer et al. reported that the frequency of CFTR mutations in patients with alcoholic chronic pancreatitis was twice that expected in the general population. However, using appropriate controls (i.e. alcoholics without pancreatitis), two other studies found no differences in the frequency of mutations (15 common
mutations assessed in one study\textsuperscript{23} and polymorphisms of the variable length polythymidine tract assessed in the other\textsuperscript{19} between alcoholics with and without pancreatitis. Large studies analyzing the entire coding sequence of the \textit{CFTR} gene in alcoholics with and without pancreatitis are needed to comprehensively address the possible role of \textit{CFTR} mutations in alcoholic pancreatitis.

Premature intracellular activation of trypsinogen to trypsin leading to a cascade of activation of other proteolytic enzymes is now accepted as a key event in the pathogenesis of pancreatitis. Perhaps the most compelling evidence in support of this concept was the discovery, in patients with hereditary pancreatitis, of a mutation in the cationic trypsinogen gene (\textit{PRSS1}) which resulted in a trypsin molecule resistant to degradation.\textsuperscript{40} Several other mutations of both the cationic and anionic trypsinogen genes have since been described. A variant of the anionic trypsinogen gene (\textit{PRSS2}) resulting from a substitution of glycine by arginine at codon 191 (p.G191R) has been shown by \textit{in vitro} studies to have a protective function.\textsuperscript{41} Activation of a recombinant form of G191R by enterokinase or trypsin \textit{in vitro} resulted in a complete loss of trypsin activity due to the introduction of a novel tryptic cleavage site making the enzyme hypersensitive to autocatalysis. Thus, it is possible that the G191R \textit{PRSS2} variant reduces intrapancreatic trypsin activity and protects against cellular injury. Interestingly, G191R has been reported to be significantly less common in patients with alcoholic CP compared to healthy controls. However, the significance of these findings is uncertain, as the \textit{PRSS2} variant was not assessed in alcoholics without pancreatitis.

In recent years, researchers have turned their attention to the pancreatic secretory trypsin inhibitor PSTI, also known as serine protease inhibitor, Kazal type 1 (\textit{SPINK1}). The hypothesis is that, in addition to ‘gain of function’ mutations in \textit{PRSS1} as a cause of pancreatitis, chronic pancreatitis may also result from ‘loss of function’ mutations in pancreatic trypsin inhibitors. Since the initial report of a c.101A > G transition leading to substitution of asparagine by serine at codon 34 (p.N34S) in the \textit{SPINK1} gene,\textsuperscript{42} several additional mutations have been reported predominantly in patients with idiopathic chronic pancreatitis. An association between the N34S mutation and alcoholic CP has been reported by Witt \textit{et al.}, with the mutation being found in 5.8% patients with alcoholic CP compared to 1.0% in alcoholic controls without CP.\textsuperscript{32} A subsequent study has reported an N34S frequency of 6.3% in alcohol-related CP compared to 1.6% in the general population, but this study did not use alcoholics without pancreatitis as controls.\textsuperscript{33}

The search for a susceptibility/trigger factor for alcoholic pancreatitis has not been limited to clinical studies alone. Researchers have also used experimental models of chronic alcohol administration to investigate possible ‘triggerers’ of alcohol-related pancreatic injury. The secretagogue cholecystokinin (CCK) has been investigated as a candidate ‘trigger’ by several research groups. An early study by Quon \textit{et al.}\textsuperscript{43} reported that chronic ethanol administration (using the Lieber-DeCarli pair-feeding model) intensified pancreatic injury induced by CCK, but this finding was disputed by a later study using the same model of ethanol feeding.\textsuperscript{44} Subsequently, Pandol \textit{et al.} have reported that chronic ethanol administration via gastrostomy catheters sensitizes the rat pancreas to the development of CCK-induced pancreatitis.\textsuperscript{45} Two recent studies have also demonstrated that pancreatic injury can be induced by repeated cerulein (a CCK analogue) injections in rats\textsuperscript{46} and mice\textsuperscript{47} on an alcohol diet. While these findings are of interest and the studies may have developed useful animal models for future work, the clinical relevance of cerulein as a trigger factor has to be questioned. In humans, CCK is released in picomolar quantities after meals and it is difficult to envisage a situation where abnormally high levels of CCK would be released into the circulation to trigger pancreatitis in alcoholics.

One possible cofactor that does have relevance to the clinical situation is bacterial endotoxemia. It is well established that chronic alcohol intake is associated with increased gut permeability and translocation of Gram-negative bacteria (such as \textit{Escherichia coli}) across the mucosal barrier in both humans and experimental animals.\textsuperscript{48,49} Thus, bacterial components (endotoxins) can enter the circulation and reach the pancreas. Lipopolysaccharide (LPS), a major component of the cell wall of Gram-negative bacteria, is an endotoxin known to exert deleterious effects on organs such as the liver and lungs by impairing microcirculation, promoting cytokine release and inducing oxidant stress. Plasma LPS levels have been shown to be significantly higher in drinkers (either after chronic alcohol intake or a single binge) compared to non-drinkers and in patients with alcoholic liver disease compared to those with liver disease of other etiologies.\textsuperscript{50} The role of LPS in alcoholic pancreatitis has attracted some attention in recent years. Fortunato \textit{et al.} have examined the effects of chronic alcohol feeding and a single injection of LPS, alone and in combination on rat pancreas.\textsuperscript{51} The authors report that while alcohol or LPS alone did not cause any overt pancreatic damage, administration of LPS to alcohol-fed rats resulted in necrosis and inflammation in the pancreas, suggesting that alcohol may sensitize the gland to the toxic effects of otherwise innocuous doses of LPS. Most recently, Vonlaufen and colleagues have provided strong evidence that not only do otherwise innocuous doses of endotoxin initiate overt pancreatic injury in alcohol-fed rats, but importantly, repeated exposure to endotoxin results in progressive pancreatic damage in these animals resulting in the hallmarks of chronic pancreatitis, namely acinar atrophy and fibrosis.\textsuperscript{52} The authors further demonstrate that pancreatic stellate cells play a central role in the alcohol-related pancreatic fibrosis in this model. In view of these experimental findings, clinical studies assessing plasma endotoxin levels in alcoholics with and without pancreatitis would be an important step towards clarifying the role of endotoxin in alcoholic pancreatitis.

In summary, the cofactor/trigger factor/susceptibility factor predisposing an individual to develop alcoholic pancreatitis remains unidentified. However, there are candidate factors that have not yet been examined fully, including polymorphisms of genes that code for proteins relevant to cellular anti-oxidant defenses, minor cystic fibrosis (CF) mutations, trans-heterozygosity involving combination of mutations of different genes (such as \textit{CFTR} alterations combined with \textit{SPINK1} or \textit{PRSS1} variants) and environmental factors such as bacterial endotoxin and dietary micronutrient intake.

**Conflict of interest**

No conflict of interest has been declared by the authors.
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S67
Susceptibility to alcoholic pancreatitis


