Cytolysin-positive *Enterococcus faecalis* is elevated in patients with chronic alcoholic pancreatitis

Clara Perrin[1], Vinciane Rebours[2,3], Nicolas Trainel[1], Cosmin Sebastian Voican[1,4], Gabriel Perlemuter[1,4], Anne-Marie Cassard[1*], and Dragos Ciocan[1,4*]

[*]Corresponding authors:

Dragos Ciocan: Hôpital Antoine-Béclère, Hepato-Gastroenterology and Nutrition, 157 rue de la porte de Trivaux, 92140 Clamart, France.Tel: +33 1 45 37 43 71, Fax: +33 1 45 37 40 44, dragosmarius.ciocan@aphp.fr

Anne-Marie Cassard: INSERM U996, 32 rue des carnets 92190 Clamart, France. Tel: +33 1 41 28 80 37, Fax: +33 1 46 32 79 93, cassard.doulcier@u-psud.fr

[1]Univ. Paris-Saclay, Inserm U996, Inflammation, Microbiome and Immunosurveillance, 92140, Clamart, France

[2]INSERM UMR 1149, DHU UNITY, CRI, University Paris 7, Paris, France

[3]AP-HP, Pancreatology Department, Beaujon Hospital, Clichy, France

[4]AP-HP, Hepato-Gastroenterology and Nutrition Department, Hôpital Antoine-Béclère, Clamart, France

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ABSTRACT

Introduction: Patients with alcoholic hepatitis have an increase in cytolysin-producing *Enterococcus faecalis* that correlates with disease severity and mortality.

Aim: To determine whether patients with chronic alcoholic pancreatitis have an elevated abundance of cytolysin-producing *E. faecalis*.

Methods: Quantification by qPCR of cytolysin-producing *E. faecalis* in controls and patients with alcoholic hepatitis or pancreatitis.

Results: Patients with alcoholic pancreatitis had a higher proportion of intestinal cytolysin-positive *E. faecalis* than healthy controls and patients with alcoholic hepatitis.

Conclusion: Cytolytic *E. faecalis* may also be involved in this other alcohol-related complication and benefit from targeted microbiota editing strategies.

INTRODUCTION

Chronic, excessive alcohol consumption leads to several diseases, including alcoholic hepatitis (AH) and chronic alcoholic pancreatitis (CAP). These diseases are life-threatening complications of chronic alcohol consumption, with a high rate of mortality, and they have no specific treatment, except alcohol withdrawal. Several studies have shown that the intestinal microbiota plays a role in the pathogenesis of these two diseases and that patients with CAP or AH display altered and specific gut dysbiosis (1,2). Among the differences observed in the intestinal microbiota, we previously reported a higher relative abundance of *Enterococcus* in patients with CAP (2).

A recent study by Duan et al. found that patients with AH have an elevated relative abundance of a particular type of fecal *Enterococcus faecalis* that produces a bacteriocin, cytolysin, which causes hepatocyte death. This bacteria is associated with more severe clinical outcomes and increase mortality in these patients (3). However, it was not clear in this study whether the alcoholic patients with alcoholic hepatitis that were included had any other alcohol-related complications. This could be of major interest, as the microbiome-editing strategy proposed by the authors could also be extended to other alcohol-induced complications if the relative abundance of this strain of *Enterococcus faecalis* is also elevated in such complications. We thus aimed to determine whether patients with chronic alcoholic pancreatitis also exhibit an elevated relative abundance of cytolysin-producing *Enterococcus faecalis* and compare their profile to that of patients with alcoholic hepatitis.

METHODS

Three groups of patients were included in the study: healthy controls (HC) (n = 28), patients with AH and without CAP or acute pancreatitis (n = 27) and patients with CAP and without AH (n = 24). Fecal samples were frozen at -80°C and bacterial DNA was extracted as previously described (4). We amplified three bacterial genes: total bacterial 16S, *E. faecalis* 16S, and *E. faecalis cytolysin L (cylL$_L$)* (see Supplementary Material for details).

RESULTS

The patient characteristics are summarized in Table 1. We included patients with chronic alcohol consumption and two types of alcohol-related complications, AH without CAP and CAP without AH. Patients with CAP had a significantly higher abundance of fecal *E. faecalis* than healthy controls (HC) ($p < 0.0001$) and AH patients ($p = 0.0004$) (Figure 1A).

There was no difference in the relative abundance of *E. faecalis* between AH patients and HC (Figure 1A). Genomic DNA of *E. faecalis* was detected in 96% (23/24) of CAP patients, 89% (25/28) of HC ($p > 0.05$), and 85% (23/27) of AH patients ($p > 0.05$) (Figure 1B). CAP patients were more frequently cytolysin-positive (23/24, 96%) than HC (11/28, 39%, $p < 0.001$) or AH patients (13/27, 48%, $p < 0.001$, Figure 1C). In addition, the relative abundance of cytolysin-positive *E. faecalis* was elevated in CAP and AH patients ($p = 0.0006$ and $p= 0.0067$) but not HC (Figure 1D). The relative abundance of cytolysin-positive *E. faecalis* did not correlate with pancreatic disease severity, neither in terms of biological (inflammation and albumin levels) nor radiological (necrosis and inflammation) severity criteria.

DISCUSSION

Overall, our data show that the relative abundance of cytolysin-positive *E. faecalis* is elevated in two different alcohol-related complications i.e. AH and CAP. Therefore, an increase in the relative abundance of cytolysin-positive *E. faecalis* is due to alcohol-consumption itself rather than to a specific complication. Toxicity of cytolytic *E. faecalis* against a human intestinal epithelial cell line has been shown (5) suggesting that it acts directly against the intestinal barrier. Moreover, studies in mice have shown that gut dysbiosis is involved in the pathogenesis of pancreatitis by inducing alterations in intestinal barrier function (6). Cytolysin levels increased significantly in the livers of mice given cytolytic *E. faecalis*, suggesting a direct toxic effect of cytolysin in the liver (3). Therefore, as alcohol itself increases gut permeability, the alcohol-related increase in cytolysin-positive *E. faecalis* in CAP may also be involved in the inflammatory process that leads to CAP. Accordingly, *Enterococcus* is the most common bacteria found in the bile of patients with CAP and antibodies against *E. faecalis* capsular polysaccharide are elevated in the blood of these patients (7). We therefore suggest that targeting cytolysin-positive *E. faecalis* using the bacteriophages described by Duan et al. could also be of great interest for CAP patients.

In summary, we confirm, in our independent French cohort, that patients with AH and no pancreatic complications have an elevated prevalence of cytolysin-positive *E. faecalis* and show that the prevalence of cytolysin-positive *E. faecalis* is also elevated in another alcohol-related complication, CAP without alcoholic AH. This could be of great interest in battling this life-threatening complication, which has no specific treatment and which could benefit from the microbiota-editing strategy using targeted bacteriophages described by Duan et al (3). Further studies are needed to confirm the role of cytolysin-positive *E. faecalis* in CAP and consider it as a therapeutic target in patients.

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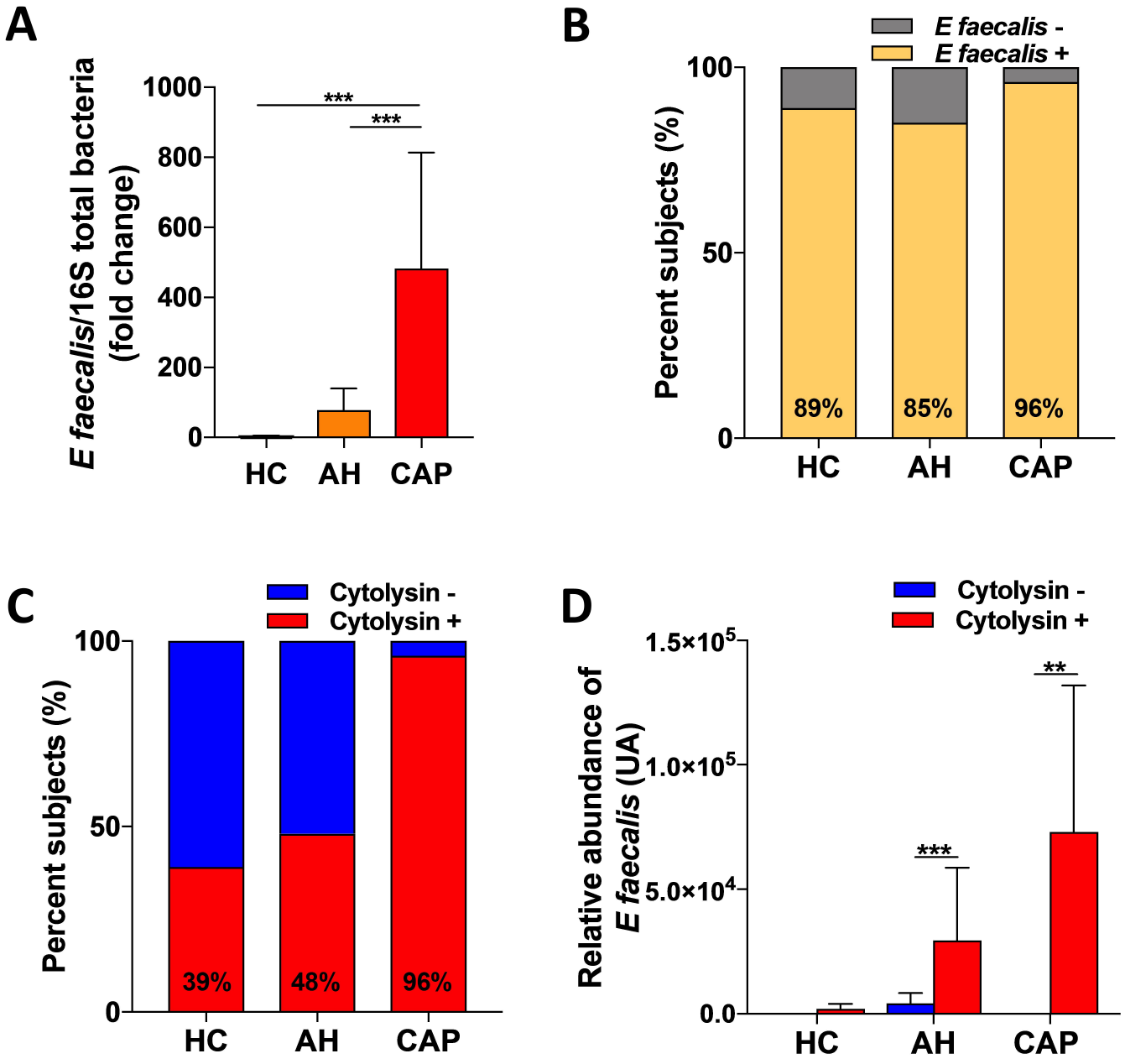
Table 1. Clinical characteristics of the three groups of patients.

	Chronic alcoholic pancreatitis (CAP, n = 24)	Severe alcoholic hepatitis (AH, n = 27)	Healthy controls (HC, n = 28)
Age (years)* **	51.4 ± 9.8	52.5 ± 11.5	34.9 ± 11.6
Sex (male/female)* **	21/3	23/4	12/16
BMI (kg/m^2)*	22.3 ± 3.3	26 ± 5.2	23 ± 3.6
Alcohol intake (g/day) *	143.8 ± 90	106 ± 65.5	
Duration of intake (years)**	13 ± 3.9	21 ± 10.8	
Smoking (%)	22 (92)	17 (65)	
Type 2 diabetes (%) **	10 (42)	2 (8)	
PPI use (%)	10 (45)	8 (30)	
CRP (mg/L)*	37.4 ± 72..1	31.88 ± 25..3	
AST (U/L)* **	38 ± 34..3	242 ± 495..7	
ALT (U/L)	43.9 ± 43..9	66.4 ± 88..4	
Total bilirubin (µmol/L)* **	25 ± 68	210.5 ± 172	
GGT (U/L)* **	182..9 ± 287..9	392.8 ± 314..5	
Glycaemia (mmol/L)* **	7.3 ± 1.9	5.4 ± 1	
Serum albumin (mg/dL)	31 ± 7.8	26.9 ± 4,8	
Platelets (x10^9/L)* **	325 ± 129	109 ± 88	
Prothrombin time (%)* **	94 ± 13.9	39 ± 13.5	
Cirrhosis (%)* **	0	27 (100)	
Liver biopsy (%)* **	0	27 (100)	
Maddrey score		55.9 ± 21.3	
MELD score		21.5 ± 6.5	
Exocrine pancreatic insufficiency (%)**	6 (25)	0	

Data is expressed as the mean ± SD for continuous variables and n (%) for discrete variables**.** PPI: proton pump inhibitors, CRP: C-reactive protein, AST: aspartate transaminase, ALT: alanine transaminase, GGT: gamma-glutamyl transpeptidase, MELD: Model for End-Stage Liver Disease. *p<0.05, **p<0.01, *** p<0.001

FIGURE LEGENDS

Figure 1. Fecal samples from CAP patients contain more *E. faecalis* and cytolysin than those of AH patients and HC. CAP: chronic alcoholic pancreatitis, AH: severe alcoholic hepatitis, HC: healthy controls. **(a)** Fold change of *E. faecalis* relative abundance in fecal samples from HC (n = 28) and AH (n = 27), and CAP patients (n = 24), assessed by qPCR. **(b)** Percentage of fecal samples positive for *E. faecalis* in HC (n = 28) and AH (n = 27) and CAP patients (n = 24), assessed by qPCR. **(c)** Percentage of cytolysin positive fecal samples in HC (n = 28) and AH (n = 27) and CAP patients (n=24), assessed by qPCR. **(d)** Relative abundance of *E. faecalis* in fecal samples from AH and CAP patients and HC whose fecal samples were cytolysin-positive or cytolysin-negative, assessed by qPCR. Data are shown as the mean ± SEM. Significant results for *p < 0.05, **p < 0.01, and ***p < 0.001 were determined by Mann-Whitney tests, unless stated otherwise.


A
E faecalis/16S total bacteria (fold change)
1000
800
600
400
200
0
***
***
HC
AH
CAP
B
E faecalis -
E faecalis +
Percent subjects (%)
100
50
0
89%
85%
96%
HC
AH
CAP
C
Cytolysin -
Cytolysin +
Percent subjects (%)
100
50
0
39%
48%
96%
HC
AH
CAP
D
Cytolysin -
Cytolysin +
Relative abundance of E faecalis (UA)
1.5×10^5
1.0×10^5
5.0×10^4
0.0
***
**
HC
AH
CAP