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Letter to the Editor

Alpha-1 antitrypsin restores colonic epithelial permeability in irritable bowel syndrome with diarrhea

Sir,

Irritable bowel syndrome (IBS) is an intestinal functional disorder affecting 1 in 10 adults. Serine protease (SP)-mediated increase in gut epithelial permeability (EP) has been proposed as a mechanism causing IBS with diarrhea (IBS-D) [1,2] and abdominal pain in IBS-D is correlated to increased SP activity [1]. Sources of SP in the colon include exocrine pancreas, immune cells, and intestinal microbiota [3]. We hypothesized that a lack of serine protease inhibitors (serpins) is responsible for increased SP activity in IBS-D. Alpha-1 antitrypsine (A1AT) is a ubiquitous and natural protease inhibitor, the main known serpin in the human colon [4]. We set out to examine whether A1AT levels in the colon of patients with IBS-D are comparable to healthy subjects (HS), if A1AT levels are correlated with gut protease activity and whether treatment with A1AT could restore normal colonic permeability in IBS-D subjects.

Twenty colonic biopsies were taken during standard colonoscopy from HS (N = 7), IBS-D (N = 8), and Crohns disease (CD) (N = 5) patients. A1AT and total SP activity (PA) were measured in colonic supernatants (CS) by commercial assays. Effect of A1AT on EP was assessed by measuring transfer of fluorescein isothiocyanate-conjugated salt across T84 gut epithelial cells cultured in Transwell® inserts. Patients with CD acted as positive controls.

Our results have demonstrated that IBS-D (16 [0-123]) and CD patients (22 [0-87]) had ~10-fold lower A1AT in their CS than CS of HS (226 [170-267] μg/ml, $P < 0.01$) [Figure 1a]. A1AT levels were inversely associated with protease activity [Figure 1b]; the later which was strongly correlated with severity of the disease. CS from IBS-D and CD subjects significantly increased T84 epithelial cell permeability compared to CS from HS ($P < 0.01$) [Figure 1c]. Both of these increases were completely abolished following addition of exogenous A1AT ($P < 0.01$), and responses were similar to HS.

Here we report, for the first time, reduced level of serpin (A1AT) in the colon of IBS-D and CD subjects compared to healthy controls. This reduction is associated with elevated protease activity in both groups and later is positively correlated with disease severity. Exogenous addition of A1AT restores normal permeability and corrects colonic barrier dysfunction in IBS-D patients. These results support further studies in humans to

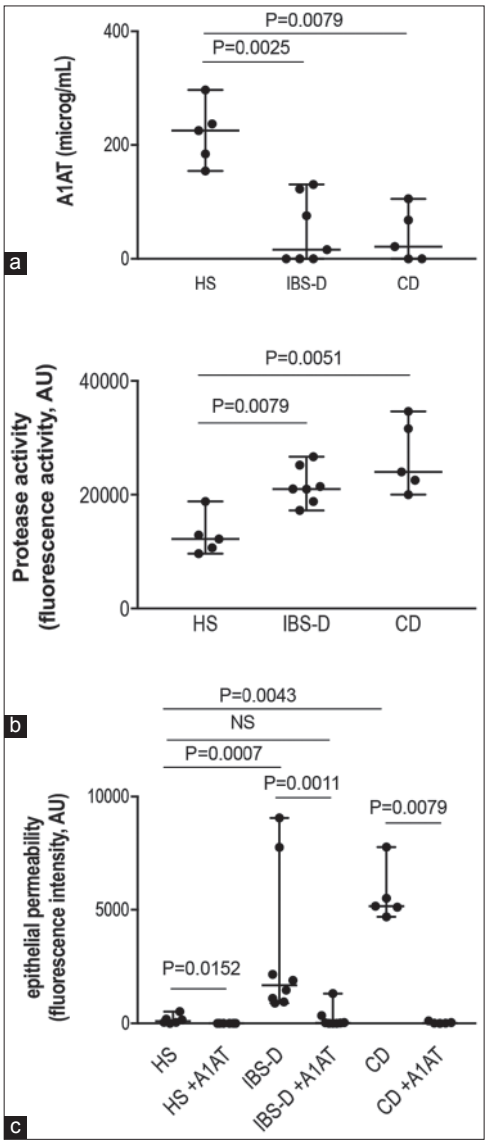

Figure 1: Measurement of alpha-1 antitrypsine (A1AT) (a) and protease activity (b) in the colonic supernatant (CS) from healthy subject (HS) (N = 7), irritable bowel syndrome with diarrhea patients (N = 8) or patients with Crohn's disease (N = 5). *In vitro* permeability of T84 cells in the presence of CS before and after exogenous addition of alpha-1 antitrypsine (c). Epithelial permeability is measured as transfer of fluorescein isothiocyanate-conjugated salt across gut epithelial cells. The results are shown as median and interquartile range. Significance is set at $P < 0.05$

examine the potential use of A1AT in clinic to treat IBS-D; perhaps delivered as a probiotic vector as it has previously been done in patients with CD [5].

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