usual care. Conversely, for a biomarker that costs $253, a 96.7% accuracy is required to remain cost-saving. Above $253, the biomarker-based approach is no longer cost-saving vs. usual care, even when the test is 100% accurate at detecting IBS-D. **Conclusion**: These results can guide investigators as they develop, validate, and price IBS-D biomarkers for use in everyday clinical practice. Based on the natural history and usual costs of care for IBS-D, any biomarker exceeding $253, regardless of diagnostic accuracy, will not be cost-saving. However, future analyses should also evaluate cost per quality-adjusted life year (QALY) gained to explore the balance between biomarker cost and QALY benefits from more accurate testing.

TABLE. Base-case probability and cost estimates used in the decision model.

Clinical probabilities, %	Base-case estimate
Has IBS-D if meets Rome IV criteria	85.3%
Has Celiac disease if meets Rome IV criteria	3.6%
Has microscopic colitis if meets Rome IV criteria	9.8%
Has inflammatory bowel disease if meets Rome IV criteria	1.3%
IBS-D patient responds to amitriptyline	54.1%
IBS-D patient responds to low-FODMAP diet	63.1%
IBS-D patient responds to rifaximin	44.0%
IBS-D patient responds to eluxadoline	24.5%
Patient with organic disease has placebo response to IBS therapy	23.9%
Cost estimates, $	**Base-case estimate**
CRP and ESR blood tests	$17
Celiac serologies	$34
Esophagogastroduodenoscopy with biopsies	$363
Colonoscopy with biopsies	$444
Initial dietician visit	$134
Follow-up dietician visit	$57
Initial gastroenterologist visit	$173
Follow-up gastroenterologist visit	$80
Amitriptyline 1 month supply	$9
Rifaximin 2-week treatment course	$1,649
Eluxadoline 1 month supply	$1,168
Budesonide 2-month treatment course	$3,612
Inflammatory bowel disease treatment for 1 month	$520

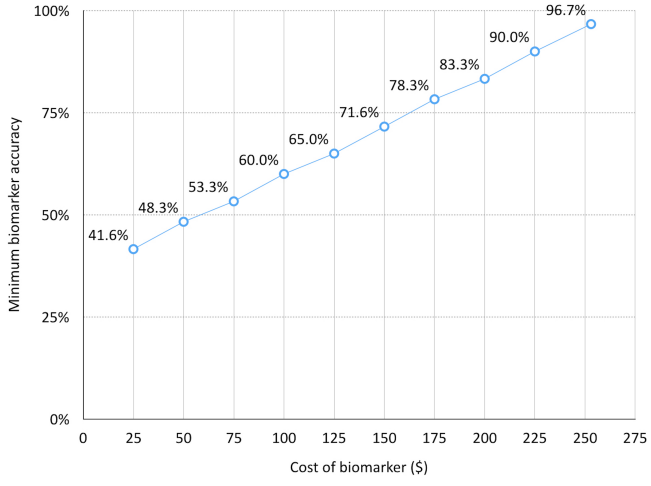

FIGURE. Biomarker accuracy required for the IBS-D biomarker-based approach to be cost-saving vs. usual care, according to biomarker cost. We calculated accuracy through the following equation: biomarker sensitivity x IBS prevalence among Rome IV positive individuals + biomarker specificity x (1 - IBS prevalence among Rome IV positive individuals).

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LONG NONCODING RNA UC-173 ENHANCES GUT BARRIER FUNCTION BY PROMOTING TRANSLATION OF TIGHT JUNCTION CLAUDIN-1

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Epithelial cells line the intestinal mucosa and form an important barrier to a wide array of noxious substances in the lumen. The functional integrity of the intestinal epithelial barrier fully depends on specialized structures composing different intercellular junctions (IJs) including tight junctions (TJs) and adherent junctions (AJs), but the exact mechanism underlying the control of cellular levels of IJs remains largely unknown. Long noncoding RNAs (lncRNAs) are a huge class of RNAs spanning >200 nucleotides and lacking protein-coding capacity that are involved in many aspects of multiple biological and pathological processes in a tissue-specific pattern. *Uc-173* belongs to a new subset of lncRNAs that are transcribed from ultraconserved regions and are absolutely conserved among human, mouse, and rat genomes. We have recently demonstrated that lncRNA *Uc-173* is highly expressed in the gut mucosa and that elevation of *Uc-173* levels stimulates intestinal mucosal regeneration. In this study, we further examined if *Uc-173* regulates the intestinal epithelial barrier function by using *in vitro* and *in vivo* models. ***Methods:*** Studies were carried out in Caco-2 cells and mice. Epithelial barrier function was evaluated by transepithelial electrical resistance (TEER) and paracellular flux of FITC-dextran. *Uc-173* gain-of-function study was performed by transfection with pCMV-driven Uc-173 expression vector, whereas locked nucleic acid-modified (LNA) anti-Uc-173 oligos (LNA-Anti-Uc173) was used for its loss-of-function study. Translational efficiency was studied by new protein synthesis and polyribosome profiling analysis. ***Results:*** *Uc-173* silencing by transfection with LNA-Anti-Uc173 for 48 h specifically decreased TJ claudin-1 levels, although it failed to alter cellular abundances of TJs ZO-1, occludin, and claudin-3 and AJs E-cadherin and α-catenin. Uc-173 silencing decreased claudin-1 at the translation level. *Uc-173* antagonism also disrupted epithelial barrier function as indicated by a decrease in the TEER values and an increase in the levels of paracellular flux of FITC-dextran in Uc-173-silence cells and by an increased level of gut permeability in mice treated with LNA-Anti-Uc173 for 4 days. In contrast, ectopically expressed Uc-*173* increased claudin-1 translation and enhanced the barrier function. Uc-173-overexpression cells exhibited increased newly claudin-1 protein synthesis and significant shifting of the *claudin-1* mRNA to the high-translating polysomal fractions without effect on its total mRNA level. *Uc-173* was found to directly bind to microRNA-29b (miR-29b) and acted as a sponge to prevent miR-29b-induced inhibition of claudin-1 translation. ***Conclusions:*** These results indicate that lncRNA *Uc-173* enhances intestinal barrier function by increasing claudin-1 translation at least partially through down-regulation of miR-29b.

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ROLE OF AUTOPHAGY RELATED PROTEIN ATG6/BECLIN 1 IN INTESTINAL TIGHT JUNCTION BARRIER

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Background: Defective tight junction (TJ) barrier has been postulated as a key pathogenic factor for Inflammatory Bowel Disease. Previously we have shown that autophagy, a cell survival mechanism, enhances intestinal epithelial TJ barrier function. Autophagy related protein ATG6/beclin 1 is a key protein in the autophagy pathway and also has been shown to play a role in endocytic pathway. The role of beclin 1 in intestinal TJ barrier is not known. **Aim:** This study examined the role of beclin 1 in intestinal epithelial TJ barrier function. **Methods:** The role of beclin 1 in TJ barrier function was studied in a cell culture model of filter grown human intestinal Caco-2 monolayers and by mouse colonic perfusion. Transepithelial resistance (TER) and flux of paracellular probe was used to measure TJ barrier function. Other laboratory techniques included co-immunoprecipitation (co-IP), confocal immunofluorescence (IF), and siRNA transfection. **Results:** In Caco-2 cells, beclin 1 was immunoprecipitated with TJ protein occludin in co-IP analysis and also found to be co-localized with occludin on the membrane in IF examination. Treatment of Caco-2 cells with beclin 1 cell permeable peptide (Tat-beclin 1, 2-20μM) and not scrambled peptide reduced TER and increased paracellular mannitol flux, in a concentration and time dependent manner. Tat-beclin 1 treatment also caused increased cytoplasmic co-localization of occludin with caveolae marker caveolin-1 and resulted in a decrease in total occludin protein level. On the contrary, beclin 1 siRNA transfection of Caco-2 cells increased the TER and reduced mannitol flux. Inhibition of ERK MAPK prevented Tat-beclin 1-induced reduction in TER, increase in paracellular mannitol flux, and caveolar internalization of occludin. Tat-beclin 1 treatment disrupted mechanistic target of rapamycin complex mTORC2, involved in actin cytoskeleton organization, as evidenced by loss of co-localization between rictor, a key mTORC2 scaffolding protein and mTOR. In further investigation of the role of autophagy in beclin 1 mediated occludin endocytosis, induction of autophagy with starvation (EBSS media) or rapamycin resulted in reduced beclin 1-occludin association in co-IP and IF. Starvation or rapamycin treatment also prevented Tat-beclin 1-induced increase in TJ permeability, cytoplasmic occludin-caveolin-1 co-localization, and reduction in occludin level. In mouse colon, beclin1 co-localized to occludin in IF examination. Perfusion of mouse colon with beclin 1 peptide caused an increase in colonic TJ permeability that was prevented by *in vivo* induction of autophagy with rapamycin. **Conclusions:** These data suggest that beclin 1 regulates TJ barrier via endocytosis of occludin in ERK and mTORC2 dependent way. Upstream autophagic stimuli of starvation or rapamycin treatment superseded and prevented beclin 1-mediated occludin endocytosis.

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LOSS OF INTESTINAL EPITHELIAL DESMOGLEIN 2 LEADS TO DESMOSOMAL REMODELLING AND INCREASED INTESTINAL PERMEABILITY AND PREDISPOSES TO DEVELOPMENT OF COLITIS AND ADENOMA

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Introduction: To protect the organism against luminal pathogens while enabling selective uptake of nutrients, intestinal epithelia contain the apical junctional complex (AJC) consisting of tight junctions, adherens junctions and desmosomes. Desmosomes constitute the least investigated AJC component and are composed of transmembrane cadherins of the desmoglein (Dsg) and desmocollin (Dsc) type that are associated through the intracellular plaque proteins plakoglobin, plakophilin and desmoplakin to the cytoplasmic keratin intermediate filament cytoskeleton. Dsg2 and Dsc2 are the major desmosomal cadherins of intestinal epithelia. Dsg2 was shown to be deregulated in Crohn's disease and was implicated in colon cancer cell proliferation. **Aims & methodology:** Given that constitutive Dsg2 knockouts are embryonal lethal, we generated intestinal epithelium-specific, conditional Villin-Cre DSG2 knockouts ($DSG2^{\Delta IEC}$). The intestinal phenotype was evaluated under basal conditions, after short-term (4 days) and long-term (7 days) 1.6% dextran sodium sulfate (DSS) or infection with $1x10^9$ *Citrobacter rodentium*. Intestinal alterations were assessed by histological/immunological staining, immunoblotting, qRT-PCR and electron microscopy. Administration

of FITC-dextran and BrdU was used to analyze paracellular permeability and cellular proliferation, respectively. Additionally, growth of small intestinal organoids was assessed. The impact of Dsg2 on intestinal tumor development was carried out by crossbreeding with APC^{min} mice. **Results:** A reduction of Dsg2 expression was observed in Crohn's disease patients as well as in murine colitis models. $DSG2^{\Delta IEC}$ mice displayed a robust knockout of intestinal Dsg2, resulting in profound alterations in the desmosomal composition and a moderate change in tight junctional proteins. The overall histology was inconspicuous, but $DSG2^{\Delta IEC}$ animals exhibit a greater intercellular space width, a somewhat increased intestinal permeability and a faster cell migration along the crypt axis. No obvious differences in the development of $DSG2^{\Delta IEC}$ mice and isolated intestinal organoids were seen. DSS challenge resulted in markedly more severe colitis with accelerated weight loss, elevated bacterial translocation to mesenteric lymph nodes and increased epithelial inflammation in $DSG2^{\Delta IEC}$ mice. *C. rodentium* infection led to significantly stronger crypt hyperplasia and a pronounced immune response. Both models showed increased epithelial cell loss. DSG2-deficient $DSG2^{\Delta IEC}$ mice displayed increased small intestinal tumor development. All three models revealed enhanced intestinal permeability and accelerated IL-22/pSTAT3/RegIIIbeta signalling. **Conclusions:** Our results identify Dsg2 as an essential component of intestinal desmosomes, whose loss is of functional relevance since it predisposes to different forms of epithelial injury.

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DELETION OF TRPV6 CHANNEL IN MICE BLOCKS ETHANOL-INDUCED DISRUPTION OF COLONIC EPITHELIAL JUNCTIONS, BARRIER DYSFUNCTION AND LIVER DAMAGE

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Background: Disruption of intestinal epithelial tight junctions (TJ), gut barrier dysfunction and endotoxemia plays an important role in the pathogenesis of alcoholic liver disease (ALD). Our previous *in vitro* studies showed that Ca^{2+} influx and transient receptor potential vanilloid-6 (TRPV6) channel play crucial role in ethanol and acetaldehyde-induced TJ disruption and barrier dysfunction in Caco-2 cell monolayers. In this study, we evaluated the effect of chronic ethanol feeding on gut and liver injury in TRPV6 knockout ($TRPV6^{-/-}$) mice. **Methods:** Wild type (WT) and $TRPV6^{-/-}$ mice were fed 1-6% ethanol (0% 2d, 1% 2d, 2% 2d, 4% 1 wk, 5% 1 wk and 6% 1 wk) in Lieber-DeCarli liquid diet; control mice were pair fed with iso-caloric maltodextrin. Gut permeability was measured by vascular-to-luminal flux of FITC-inulin *in vivo*. Endotoxemia was assessed by measuring plasma endotoxin level. TJ (occludin and ZO-1) and adherens junction (AJ) (E-cadherin and b-catenin) integrity was analyzed by immunofluorescence confocal microscopy. Protein thiol oxidation was evaluated by measuring reduced and oxidized protein thiols by fluorescence staining using BODIPY FL-*N*-(2-aminoethyl) maleimide as a probe. Liver triglyceride and plasma AST/ALT were measured to assess liver injury. Expression of antioxidants (SOD1, SOD2, Trx1, catalase, GPX1 & Nrf2), cytokine (IL-1β & TNFα) and chemokine (MCP-1, CCL5, CXCL1 & CXCL2) genes were evaluated in liver and distal colon by qRT-PCR. **Results:** Ethanol feeding significantly increased colon length in WT mice, but not in $TRPV6^{-/-}$ mice. Ethanol feeding significantly increased inulin permeability in the distal colon that was associated with redistribution of TJ and AJ proteins from the junctions. Ethanol-induced colonic epithelial permeability and TJ disruption was absent in $TRPV6^{-/-}$ mice. Ethanol increased plasma endotoxin level in WT mice, but not $TRPV6^{-/-}$ mice. Ethanol feeding increased liver triglyceride level that was associated with histological lesions and elevated plasma AST/ALT in WT mice. Ethanol-induced liver injury and plasma AST/ALT elevation were minimal in $TRPV6^{-/-}$ mice, suggesting that TRPV6 channel plays an important role in the pathogenesis of ALD. In WT mice, ethanol depleted reduced protein thiols and increased oxidized protein thiols in colonic epithelium and liver, which was associated with down regulation of antioxidant genes; these effects of ethanol were minimal in $TRPV6^{-/-}$ mice. Ethanol feeding up regulated inflammatory cytokine and chemokine gene expression in WT mice, but not in $TRPV6^{-/-}$ mice. **Conclusion:** These data demonstrate that TRPV6 plays an important role in ethanol-induced colonic epithelial TJ disruption, mucosal barrier dysfunction, endotoxemia and liver damage, and that TRPV6 channel blockers may bear therapeutic value in the treatment of ALD and other alcohol-related diseases.

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PP2A-ASSOCIATED PROTEIN Î‘4 IS ESSENTIAL FOR NORMAL INTESTINAL EPITHELIAL BARRIER FUNCTION BY MODULATING MUCOSAL MATURATION

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The mammalian intestinal epithelium is among the most rapidly self-renewing tissue in the body and forms an important barrier to a wide array of noxious substances in the lumen. Mucosal maturation is crucial for maintaining the epithelial integrity and normal functions including the epithelial barrier, but the exact mechanisms underlying these processes remain largely unknown. PP2A-associated protein α4 is a recently identified multiple functional protein that forms a stable complex with the catalytic subunits of PP2A and is implicated in a variety of distinct cellular processes and functions. Our previous studies demonstrate that α4 regulates intestinal mucosal homeostasis and that target deletion of α4 in the intestinal epithelial cells (IECs) inhibits gut mucosal regeneration and protection. In this study, we further tested the hypothesis that α4 plays a role in the regulation of gut permeability by altering mucosal maturation. ***Methods:*** Studies *in vivo* were conducted in intestinal epithelial tissue-specific α4 deletion mice (IE-$\alpha4^{-/-}$) that were generated in our group recently. Experiments *in vitro* were performed in differentiated intestinal epithelial cells induced by stable *Cdx2*-transfection (IEC-Cdx2L1 line). Epithelial barrier function was assayed by transepithelial electrical resistance (TEER) and membrane-impermeable trace molecule FITC-dextran. Gut mucosal maturation was measured by immunohistochemistry staining of lysozyme (marker for paneth cells) and cell migration. ***Results:*** Levels of α4 mRNA and protein in the small and large intestinal mucosa were undetectable in IE-$\alpha4^{-/-}$ mice, although α4 expression was normal in other tissues. This intestinal epithelial-specific α4 deletion disrupted the intestinal epithelial barrier function, since IE-$\alpha4^{-/-}$ mice exhibited a remarkable decrease in the levels of tight junction (TJ) proteins ZO-1, claudin-1, and claudin-3 (by >75%) and adherens junction E-cadherin in the small intestinal mucosa. Gut permeability to FITC-dextran in IE-$\alpha4^{-/-}$ mice increased significantly compared with that observed in control littermates. IE-$\alpha4^{-/-}$ mice also displayed a significant delay in mucosal maturation as indicated by hyperplasia in the crypts, irregular lining of lysozyme positive cells, and inhibition of IEC migration along crypt/villus axis. Consistently, α4 silencing by transient transfection with siRNA targeting α4 mRNA (siα4) in cultured IEC-Cdx2L1 cells also inhibited expression of TJs and E-cadherin and resulted in the epithelial barrier dysfunction *in vitro*. The TEER values were decreased by ~70% in cells transfected with siα4 for 48 h, whereas paracellular flux of FITC-dextran increased by >40%. ***Conclusion:*** These results indicate that α4 is necessary for maintaining normal intestinal epithelial barrier function at least partially by enhancing mucosal maturation.

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SOLUBLE PROTEIN P40 PRODUCED BY *LACTOBACILLUS RHAMNOSUS* GG ENHANCES TIGHT JUNCTION PROTEIN EXPRESSION AND IMPROVES EPITHELIAL INTEGRITY BY PREVENTING ALTERATION OF DNA METHYLATION AND HISTONE ACETYLATION IN HUMAN COLONOIDS

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Disruption of intestinal epithelial barrier function appears to play an important role in the pathogenesis of chronic inflammatory intestinal disorders. Altered gut permeability may increase bacterial load and dietary antigens in the lamina propia leading to mucosal inflammation. Clinically, probiotics such as Lactobacilli has been shown to improve barrier function in experimental animals and reduce symptoms in IBS patients. Using an enteroid model system, we recently showed that the supernatant of Lactobacillus rhamnosus GG (LGG) prevented IFN gamma-induced epithelial barrier damage and this is mediated by a secreted soluble protein P40 (Gastroenterology 2016). But, the mechanism by which P40 improves epithelial barrier function is unclear. We hypothesize that P40 acts via epigenetic regulatory pathways to increase the synthesis of junction proteins Z01 and occludin. To test this hypothesis we designed a strategy to synthesize P40. The coding sequence for P40 was PCR amplified from LGG genomic DNA and subcloned into the PET28b+ expression vector. The protein was produced using the E. coli strain BL21 (DE3)/pLysS and purified by nickel nitrilotrialetic acid agarose and fast protein liquid chromatography. Using a reductionist approach, we employed human colonoid, a 3D structure grown from human colonic biopsies. Fecal supernatant (FSN) from IBS-D patients was used to induce epithelial barrier damage resulting in a 40% and 50% reduction in gene expression of occludin and ZO-1. ($p<0.05$) These changes were accompanied by a 28% increase in DNA methyltransferase (DNMT1) protein expression and a 40% decrease in acetyl-histone H3 (ep300) protein expression. Pretreatment with P40 prevented the changes in DNMT1 and ep300 and normalized the protein expression of Z01 and occludin. In separate studies we used in-outside human colonoid structures to evaluate the permeability of the epithelium. Human colonoids were injected with the fluorescence dye FD4 and images were obtained at different time points. Under control conditions, the human colonoids retained 70% of FD4 over 10h. Treatment of the colonoids with IBS-FSN impaired permeability resulting in 10% retention of the dye at 10 h. These changes were prevented by P40. Silencing DNMT1 gene in human colonoids with specific siRNA also prevented the leakage of dye evoked by IBS-FSN. In conclusion, P40 is the first probiotic bacterial product demonstrated to promote junction protein expression and maintain mucosal epithelial barrier function by epigenetic regulation. Our findings provide a molecular basis for therapeutic application of probiotic bacterial products on inflammation-mediated intestinal disorders.

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A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND CLINICAL TRIAL OF THE JAPANESE HERBAL MEDICINE RIKKUNSHITO FOR PATIENTS WITH FUNCTIONAL DYSPEPSIA: THE DREAM STUDY

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Background/Aim: Functional dyspepsia (FD) is a heterogeneous disorder involving several pathogenic factors. Therefore, the current pharmacological therapy options for FD are still limited. Rikkunshito (RKT), a traditional Japanese herbal medicine, has various pharmacological actions such as stimulation of gastric emptying, regulation of ghrelin secretion, and improvement of stress-induced gastric hypersensitivity. This prospective, randomized, multicenter, placebo-controlled study was performed to clarify the efficacy of RKT in patients with FD. Methods: One hundred ninety-two patients who met the Rome III criteria for FD were enrolled from March 2014 to January 2016 at 56 hospitals in Japan. Patients with Helicobacter pylori-positivity, severe depression, or severe heartburn were excluded from this study. One hundred twenty-eight patients whose FD symptoms did not improve after 2 weeks of single-blind placebo treatment in a run-in period were randomly assigned to 8 weeks of RKT at 7.5 g/day (RKT group, n = 65) or placebo at 7.5 g/day (PL group, n = 63). The primary efficacy endpoint was global assessment of overall treatment efficacy (OTE). The secondary efficacy endpoints were improvements in upper gastrointestinal symptoms, psychological symptoms, and quality of life (QOL). Upper gastrointestinal symptoms were evaluated by the modified Frequency Scale for the Symptoms of Gastroesophageal Reflux Disease and the Patient Assessment of Upper Gastrointestinal Disorders-Symptom Severity Index (PAGI-SYM). Psychological symptoms were evaluated by the Hospital Anxiety and Depression Scale (HADS), and QOL was evaluated by the Global Overall Symptom scale (GOS) and 8-Item Short-Form Health Survey (SF-8). Results: The OTE after the 8-week treatment was significantly higher in the RKT than PL group ($p = 0.019$). In the assessment of upper gastrointestinal symptoms using the PAGI-SYM, although the overall scores decreased in both the RKT and PL groups after the treatment, the degrees of improvement in postprandial fullness/early satiety ($p = 0.001$), bloating ($p = 0.002$), and the overall scores ($p = 0.018$) were significantly higher in the RKT than PL group. The degrees of improvement in the overall score ($p = 0.027$) and subscale scores (anxiety, $p = 0.016$) of the HADS were