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Novel Non-Invasive Biomarkers for the Diagnosis and Management of Celiac Disease

Lead Guest Editor: Anil K. Verma
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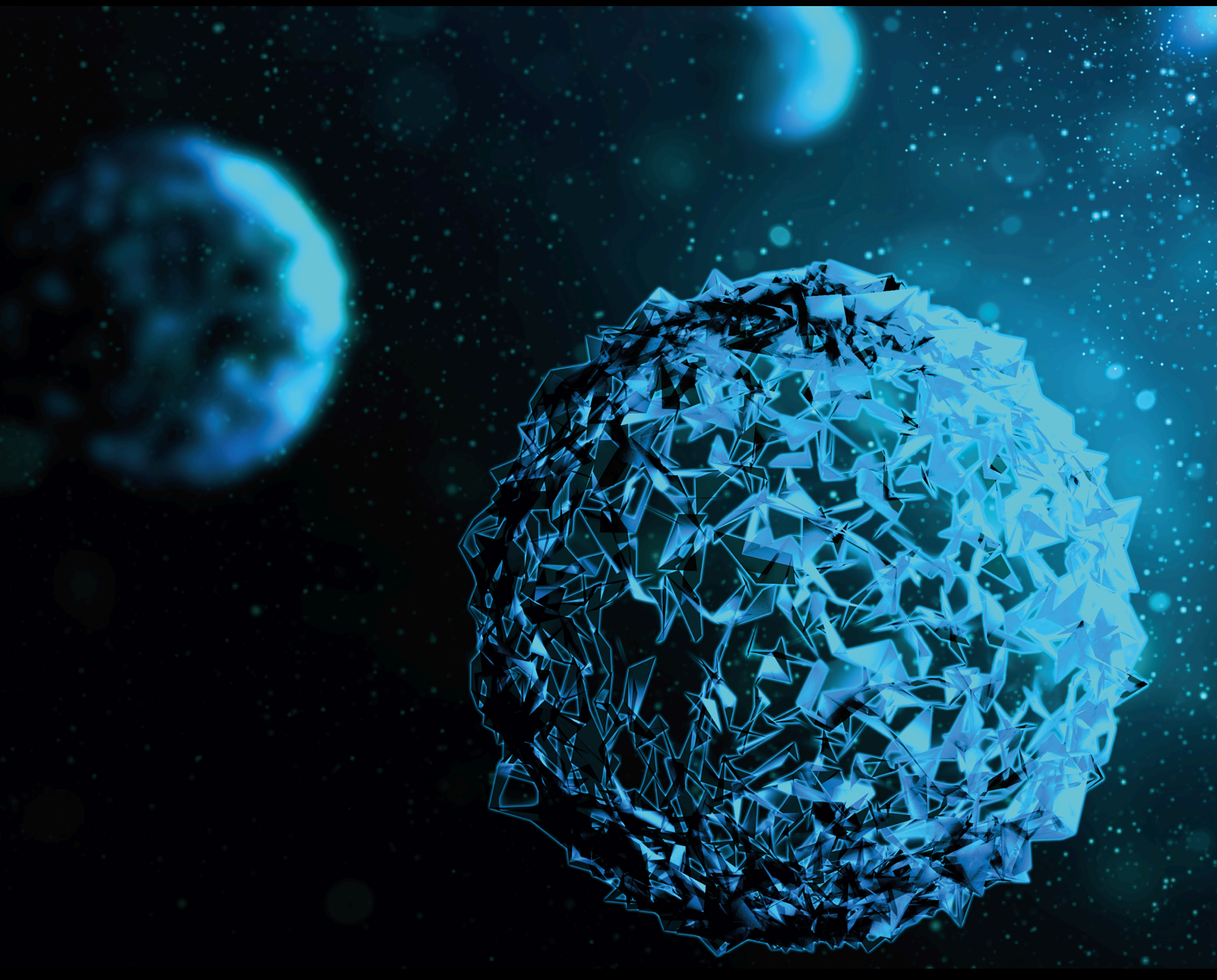

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Contents

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Review Article

Emerging Biomarkers for Screening and Management of Celiac Disease

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Celiac disease (CeD) is a chronic, immune-mediated enteropathy that is precipitated by dietary gluten in genetically predisposed individuals expressing HLA-DQ2 and/or HLA-DQ8. In the current clinical practice, there are many serologic studies to aid in the diagnosis of CeD which include autoantibodies like IgA antitissue transglutaminase, antiendomysium, and antideamidated forms of gliadin peptide antibodies. Small intestinal biopsy has long been considered an essential step for the diagnosis of CeD. However, in the recent era, researchers have explored the possibility of CeD screening and diagnosis without endoscopy or biopsy. The newer emerging biomarkers of CeD appear promising in diagnostic evaluation and subsequent monitoring of disease. In this review of literature, we have explored the emerging biomarker-based diagnostic evaluation and monitoring of CeD.

1. Introduction

Celiac disease (CeD) is a chronic, immune-mediated multisystem disorder that is precipitated by gluten in diet of individuals with HLA DQ2 and/or DQ8 positivity [1–4]. The estimated global prevalence of CeD is very high (1–1.5%) [5]. CeD is characterized by villus atrophy of the duodenal mucosa associated with malabsorption of the nutrients and the subsequent clinical disease. The clinical spectrum of CeD includes intestinal as well as extraintestinal symptoms, such as anemia, fatigue, and dermatitis herpetiformis [6, 7]. In the past, typical CeD (now called classical CeD) denoted a clinical presentation with signs and symptoms of malabsorption, such as diarrhoea, steatorrhea, weight loss, and nutritional deficiencies. The term is of limited use as most of the patients do not present with so-called classical manifestations and a half may present with nondiarrhoeal form [8, 9]. Presentations previously described as atypical CeD and now termed nonclassical CeD (e.g., osteoporosis, anemia,, abdominal bloating, fatigue, and infertility) are now more common [7]. Asymptomatic CeD (also called silent CeD) is usually identified by using CeD-specific serology and is characterized by duodenal villous atrophy in individuals who lack symptoms or signs of CeD. Potential celiac disease denotes patients with normal small intestinal histology and positive CeD-specific serology [10, 11].

In the current clinical practice, there are many serologic studies to aid in the diagnosis of CeD which include autoantibodies like IgA antitissue transglutaminase (tTG), antiendomysial antibody (EMA), and antideamidated forms of gliadin peptide antibodies (DGP) [12]. Small intestinal histology showing villous atrophy has long been considered as an essential prerequisite for the diagnosis of CeD. The only proven treatment is adoption of a lifelong gluten-free diet

(GFD), and clinical and histologic relapse occurs invariably when gluten is reintroduced. The genetically predisposing factors most extensively studied in CeD patients are HLA-DQ2 and/or HLA-DQ8, which are identified in almost 90-95% of patients [7].

2. A Case for Novel Biomarkers: Historical Prospective

Antigliadin antibodies (AGA) were the first serological marker for CeD that came into the picture in 1960s but soon went out of picture because of poor sensitivity and specificity. Both IgA and IgG antibodies were utilized in the diagnosis initially; however, presently, IgG AGA are in use for the diagnosis of nonceliac gluten sensitivity (NCGS). The remarkable step in celiac diagnosis came in the 1990s with the discovery of EMA that had a very high sensitivity and specificity of ~95%. But, EMA needed immunofluorescence which was cumbersome and required expertise that got solved with the invention of IgA anti-tTG with almost similar diagnostic accuracy. Now, we have IgA anti-tTG, antideamidated gliadin peptide (anti-DGP), and EMA that are most commonly used in the present scenario for diagnosis. In the last two decades, we have progressed from serology-based tests to point-of-care testing (POCT), genetic testing, testing of antibodies in other fluids (saliva, faeces, and stools), and tests to ascertain villous atrophy (I-FABP and citrulline).

3. Novel Biomarkers

3.1. Point-of-Care Testing. Point-of-care testing (POCT) has revolutionized the diagnosis of various diseases. Card-based POCTs have made diagnosis of CeD rapid and easy. A study by Lau et al. explored the role of Sintex POCT, based on detection of DGP either IgA/IgG via immunochromatographic principle. It is a card-based test with three lines in the card: one detecting the presence of IgA/IgG against DGP, another detecting serum IgA, and a control line. This test requires 25 mcg/L of blood by finger prick and showed a sensitivity and specificity comparable to IgA tTG and EMA in symptomatic patients. Moreover, patient preference was markedly in favour of POCT vis a vis serological tests (90% vs. 2.8%) [13]. In another study from India involving the pediatric population, Biocard, a lateral flow immunochromatographic strip system, showed a sensitivity and specificity of 83% and 93%, respectively, against a gold standard of combination of duodenal biopsy and EMA [14]. In a meta-analysis by the same group, the sensitivity and specificity of tTG/DGP/tTG+AGA-based tests was 90% and 95%, respectively [15]. Hence, it could be concluded that in resource constrained settings, POCTs are reliable methods to diagnose CeD.

3.2. Detection of CeD Autoantibodies in Saliva and Faeces. Saliva and stool samples are an excellent specimen for screening of CeD, as these samples are easily obtained by noninvasive methods and do not need a venepuncture. A test available to measure IgA anti-tTG in saliva consists of a fluid-phase radioimmunoassay method. It has been shown to have a good sensitivity and specificity in a study involving 5000 children, where 31 out of 32 serology-positive children had positive salivary assay [16]. Despite a good diagnostic accuracy, the test has inherent problems associated with the use of radioisotopes as well as radioactive waste disposal. Adornetto et al. described an enzyme-linked immunomagnetic electrochemical assay for measuring IgA anti-tTG in saliva, based on magnetic beads to support the immunological chain reaction and differential pulse voltammetry as the detection technique. This method has high specificity and sensitivity, bypassing the problems intrinsic to the radioimmunoassay method [17]. Although these results are encouraging, these tests need more data to recommend their use as a method of screening.

Studies have explored stools as a possible sample for detecting CeD antibodies. Di Tola et al. showed that the area under the curve (AUC) for IgA anti-tTG (AUC = 0.862, $p < 0.0001$), IgA anti-DGP (AUC = 0.822, $p < 0.0001$), and IgA/IgG tTG/DGP (AUC = 0.783, $p = 0.0003$) in faecal samples are very significant [18]. However, the sensitivity of 76% for faecal IgA antibodies against tTG makes it unsuitable as a screening for CeD.

3.3. Intestinal-Fatty Acid Binding Protein (I-FABP). I-FABP is a small cytosolic protein (15 kDa) and serves as a marker for enterocyte damage. It is present in mature enterocytes and on enterocyte damage gets rapidly released into the circulation. I-FABP is most commonly found in the small intestine, jejunum in particular and that too at the distal villi that are the site of early damage in CeD. Therefore, circulating I-FABP is a surrogate marker for the extent of intestinal epithelial cell injury. It is a valuable marker in the evaluation of intestinal epithelial damage in various disease states such as mesenteric infarction, intestinal ischemia, and necrotising enterocolitis [19, 20]. Studies have shown that patients with untreated CeD have elevated levels of I-FABP, and these levels normalize after initiation of a GFD [21–23]. In a study involving patients with up to 10-fold tTG rise and villous atrophy vis a vis patients without villous atrophy and only tTG rise, mean I-FABP levels were significantly higher in patients with villous atrophy (784.7 pg/ml vs. 172.7 pg/mL, $p < 0.001$) and I-FABP levels declined on GFD [21]. Moreover, I-FABP levels recovered rapidly on GFD, implying that plasma I-FABP may also be used for monitoring disease activity in CeD patients on a GFD. The positive predictive value for CeD of an increased I-FABP level in children with elevated CeD autoantibody titres and HLA-DQ2 and/or -DQ8 positivity was 100%. The negative predictive value of I-FABP in this group was 50% with a sensitivity and specificity of 84.7% and 100%, respectively, for the detection of CeD in these patients [23]. In another study involving 68 children (CeD = 49 and controls = 19) with raised IgA tTG, I-FABP concentration was significantly higher in cases than controls (458 pg/ml vs. 20 pg/ml), only 2 out of 19 controls had raised I-FABP, and out of them one later turned out to be celiac and I-FABP correlated with the degree of villous atrophy [22]. In a study from India, diagnostic accuracy of I-FABP >1100 pg/ml was 78% in a cohort of celiac patients, and this

value decreased on GFD [24]. Hence, I-FABP has been proposed as a marker for no biopsy approach in patients not qualifying for 10-fold tTG rise [22].

3.4. Plasma Citrulline. Citrulline, a nonessential amino acid, is synthesized specifically in small intestinal enterocytes; hence, its levels are representative of the synthetic function of enterocytes. The first evidence of the role of citrulline in assessing enterocyte mass was shown by Crenn et al. in patients of short bowel syndrome who had significantly raised levels in comparison to controls (20 ± 13 vs. 40 ± 10 μmol L, $p < 0.001$) and levels of citrulline also correlated with the length of the resected intestine [25]. Evaluating the role of citrulline in disorders other than short bowel, Crenn et al. reported its value in correlation to villous atrophy. Values of <10 μmol/L, 10-20 μmol/L, and 20-30 μmol/L correlated with total villous atrophy, proximal only villous atrophy, and partial villous atrophy, respectively [25]. In a meta-analysis by Fragkos and Forbes, plasma citrulline level of 20 μmol/L had a sensitivity and specificity of ~80%. But, in inflammatory states, citrulline may be decreased without any intestinal malabsorption as nitric oxide and arginine gets depleted in inflammatory states leading to reduction in citrulline [26]. In a recent study from India, plasma citrulline level < 30 μmol/L had a diagnostic accuracy of 89% with a sensitivity and specificity of 95% and 90%, respectively, for predicting villous atrophy of Marsh grade > 2 [24]. Hence, citrulline may be used as a marker to predict villous atrophy in patients unwilling for biopsy and for follow-up of patients on GFD.

3.5. HLA Typing (Genetic Screening). The cohort of patients with CeD who do not have classical symptoms of CeD gave birth to an idea called "celiac iceberg." Experts have proposed expansion of the "iceberg" to include patients who are genetically susceptible to CeD, i.e., HLA DQ2- and/or HLA DQ8-positive patients [27, 28]. ESPGHAN-2012 had proposed "triple test" strategy for no-biopsy approach of diagnosing CeD, i.e., very high fold IgA-tTG serology, EMA-IgA positivity, and HLA DQ2/DQ8 positive [3]. The recent ESPGHAN-2020 guidelines have removed the HLA typing from the no-biopsy approach of diagnosing CeD [29]. The basis of this stems from multiple European studies that have shown no additional benefit of doing HLA typing over and above high tTG and EMA [30, 31].

Genetic screening provides a novel methodology that could be used to obtain accurate estimates of the at-risk individuals of CeD. The screening also detects false-positive IgA tTG serology in adults at average risk of CeD as CeD is almost always found in patients possessing genes encoding either HLA-DQ2.5, DQ2, or DQ8. HLA typing has a high negative predictive value for CeD albeit a very poor positive predictivity as 30%-40% of the general population harbours these genes [32]. Up to 90%-95% of patients with CeD in Western cohorts have HLA-DQ2 heterodimer (HLA-DQ2.5), encoded by DQ A1∗0501 and DQB1∗0201 alleles. HLA DQ8 accounts for the remaining 5%-10% [33]. In a Western cohort, only 0.5% of CeD patients were HLA DQ2/DQ8 negative, emphasizing that populations without DQ2/DQ8 genes have a very less chance of developing CeD [34]. Hence, genetic testing is an important tool in excluding CeD in cases of diagnostic dilemma.

3.6. Neoepitopes of Tissue Transglutaminase and Deamidated Gliadin Peptide. A recent study has shown a complex of tTG- and DGP-synthesized peptides to be a method of high diagnostic accuracy for CeD, with a sensitivity and specificity found to be 99% and 100%. These neoepitopes showed more reactivity in patients on GFD with healed mucosa in comparison to patients with unhealed mucosa. The overall diagnostic accuracy of these epitopes in diagnosing villous atrophy in patients on GFD was 90% which was better than other serological tests [35]. Though promising, this biomarker needs further data and validation in different racial cohorts for its use for screening/monitoring response in preference to antibodies that are presently in use.

3.7. HLA-DQ-Gluten Tetramers. A lot of people now are or on self-prescribed GFD, and diagnosing CeD in this subset is a problem as serology and histology has less accuracy in these patients. Guidelines recommend a gluten challenge with 3-6 grams of gluten in these patients prior to any serology testing or duodenal biopsy. Detection of HLA-DQ-gluten tetramers in blood detects CD4-positive T-cell reactivity in patients of CeD and has been found to have good diagnostic accuracy in patients already on GFD. These tetramers have a high sensitivity and specificity for patients on GFD, i.e., 100% and 90%, respectively. Hence, for patients on a gluten-containing diet, this test offers comparable sensitivity and specificity via antibody tests [36].

3.8. Peptide-Functionalized Gold Nanoparticles. Gold nanoparticles (AuNPs) are small gold particles with a diameter of 1 to 100 nm. There are sensing platforms based on the optical characteristics of AuNPs for the molecular detection and recognition of disease biomarker. Colorimetric sensors based on AuNPs have been applied for identifying targets, DNA, protein conformations, and enzyme activity, where they have demonstrated high sensitivity and effectiveness. There has been advent of newer designs of these nanoparticles that have more enhanced and controlled surface chemistry to be used for sensing applications. Peptide-functionalized nanoparticles (PFNs) are one of the prototype of such sensing platform [37].

Recent studies have demonstrated the potential of PFNs as a colorimetric sensor, using AuNPs coated with a peptide sequence derived from the gliadin protein, for screening CeD. A deamidated peptide sequence is derived from α-gliadin amino acids that detects the immunogenic peptide sequence acting as a trigger for CeD [37]. The AuNP peptide assay seems promising for development as a POCT as it is based on the formation of a precipitate, and there occurs a reduction in color of a positive sample in the presence of a CeD-specific autoantibody, with an overall accuracy of 86.6% [38].

3.9. Regenerating Gene Ia. The regenerating gene (REG) is a multigene family in humans and encodes small multifunctional secretory proteins that might be involved in cell

proliferation, differentiation, and regeneration. The REG Ia protein is expressed in the liver, pancreas, and the gastrointestinal tract [39]. Recent data has shown that REG Ia levels are significantly higher in sera of CeD patients, while its levels are not increased in other autoimmune diseases like pernicious anemia and T1DM, and serum REG Ia levels decrease on GFD. Microarray analysis demonstrates that GFD in CeD patients reverses the altered transcription of genes in small-bowel biopsy samples, suggesting that the detected alterations in CeD are caused by the reaction to gluten and not by a primary defect [40]. The decrease in REG Ia levels in sera after GFD coincides with the fall of autoantibodies to transglutaminase. The decrease of REG Ia levels in sera correlates well with the decrease of IgA anti-tTG levels as both start to decline soon after removal of gluten [41]. REG Ia appears to be a promising biomarker for CeD that can help in both diagnosis and to ascertain compliance with the GFD.

3.10. CD3 Immunohistochemical Stain. The use of CD3 IHC expression represents a sensitive and specific tool to distinguish IELs from epithelial cells especially in Marsh 1 cases because the occurrence of IELs by itself is not specific for CeD and can be observed in other forms of intestinal inflammations. Current studies show that ≥30 IEL/100 epithelial cells are detected in 68 to 100% of CD3 positive cases, i.e., CD3+ [42].

There is a significant relationship between the count of CD3+ T-lymphocytes per 100 epithelial cells and the histopathological changes in the duodenal biopsy according to Marsh classification. Moreover, the immunohistochemical staining of CD3 in intraepithelial T-lymphocytes could help in definite assessment in 43.3% of the patients with Marsh grade 1 histological lesion. In addition to that, the IHC expression of CD3+ marker provides a hint about its distribution of within the lymphocyte whether global surface or clonal surface and intracytoplasmic to diagnose refractory CeD [42].

3.11. Mucosal IgA-tTG and EMA Deposits. tTG antibodies are produced primarily at an intestinal level by specific B lymphocytes, and once produced, tTG antibodies are deposited in the small intestinal mucosa, even before they can be detected in the bloodstream [12]. Therefore, these autoantibody deposits in biopsies can ascertain the diagnosis in borderline cases, primarily in patients with seronegative CeD [43]. In this setting, also the EMA assay in cultured intestinal mucosa biopsies before and after an in vitro gliadin challenge may be an additional tool to either confirm or exclude the presence of a gluten-related enteropathy [44]. It is important to note that IgA-tTG is produced in small intestine, and hence, the deposits preclude the development of a positive IgA-tTG serology [12]. These deposits are helpful in predicting progression of potential CeD to CeD and may help in a case where diagnosis cannot be made on biopsy and serology. Flow cytometry of intestinal epithelial lymphocytes showed increased IELs in active CeD and a 97% specificity for CeD diagnosis [45]. We had shown the utility of these deposits in establishing these deposits in the esophagus, stomach, and colon and found significantly more deposits at these sites in comparison to the controls [46]. We have also shown the utility of these deposits in patients with celiac-related liver disease.

4. Conclusion

Prior to the advent of serology, diagnosis of CeD was cumbersome and required biopsy in all cases. The need to avoid biopsy was a great impetus for the scientific community to look for novel biomarkers. Nowadays, the increased diagnostic accuracy of the newly emerged plasma biomarkers and those in the pipeline suggests a paradigm change in adult CeD diagnosis. There is, however, the need for more data to predict villous atrophy and obviate the need of biopsy. We also need biomarkers for diagnosing CeD with good accuracy in special subgroups of patients such as those with seronegative CeD, patients already on GFD, and borderline patients for the diagnosis of CeD. We also need more biomarkers for predicting villous atrophy that obviates the need for biopsy as well as for monitoring of the disease.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Screening of Altered Metabolites and Metabolic Pathways in Celiac Disease Using NMR Spectroscopy

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Background. Celiac disease (CeD) is an autoimmune intestinal disorder caused by gluten protein consumption in genetically predisposed individuals. As biopsy sampling is an invasive procedure, finding novel noninvasive serological markers for screening of at-risk CeD population is a priority. Metabolomics is helpful in monitoring metabolite changes in body fluids and tissues. In the present study, we evaluated serum metabolite levels of CeD patients relative to healthy controls with the aim of introducing new biomarkers for population screening. *Method*. We compared the serum metabolic profile of CeD patients ($n = 42$) and healthy controls ($n = 22$) using NMR spectroscopy and multivariate analysis. *Result*. 25 metabolites were identified by serum metabolic profiling. Levels of 3-hydroxyisobutyric acid and isobutyrate showed significant differences in CeD patients' samples compared with healthy controls ($p < 0.05$). According to pathway analysis, our data demonstrated that changes in nine metabolic pathways were significantly disrupted/affected in patients with CeD. These enriched pathways are involved in aminoacyl-tRNA biosynthesis; primary bile acid biosynthesis; nitrogen metabolism; glutamine and glutamate metabolism; valine, leucine, and isoleucine biosynthesis and degradation; taurine and hypotaurine metabolism; glyoxylate and dicarboxylate metabolism; glycine, serine, and threonine metabolism; and arginine biosynthesis. *Conclusion*. In summary, our results demonstrated that changes in the serum level of 25 metabolites may be useful in distinguishing CeD patients from healthy controls, which have the potential to be considered candidate biomarkers of CeD.

1. Introduction

Celiac disease (CeD), an autoimmune intestinal disorder that affects up to 1% of the world population, is caused by the ingestion of gluten (found in wheat, barley, and rye) in genetically predisposed individuals carrying HLA-DQ2 or HLA-DQ8 [1–3]. Gluten peptides trigger an immune reaction, which damages the small intestinal villi and causes nutrient malabsorption. Iron deficiency, osteoporosis, and bone disease, followed by mineral deficiencies, are known as celiac disease-associated disorders [4–7].

CeD diagnosis is mainly based on a combination of specific serological and histological evaluations. The small intestinal biopsy is considered the gold standard for adult CeD diagnosis. A certain level of expertise and skill is needed for the assessment of intestinal biopsies, and variability in sample quality and subjective interpretation can affect the diagnostic accuracy [8, 9]. Moreover, biopsy sampling is an

invasive procedure and efforts are being made to find alternatives to this method [10]. In some cases, discrepancies between the clinical, histology, and serology findings make CeD diagnosis difficult. In particular, minor small bowel mucosal changes in latent CeD subjects usually lead to a misinterpretation [11]. The discovery of new diagnostic biomarkers can be a basis for the development of a point-of-care type of assays for monitoring celiac disease directly by the affected individual or by the healthcare professionals. Several approaches can be applied in identifying such novel celiac biomarkers, including microarray-based techniques, proteomics, and metabonomics. Metabolomics, genomics, transcriptomics, and proteomics can analyze living organisms and provide a better understanding of cellular biology. Each organism, organ, tissue, or cell has a characteristic metabolic profile that can be altered in response to pathophysiological stimuli or genetic modification [12]. Metabolomics can describe the biological changes by monitoring metabolites in body fluids and tissues and improve the understanding of the main mechanisms behind diseases. It can also help in the discovery of potential prognostic and diagnostic biomarkers of disease by describing biological changes between the target and control groups and give a cell physiology snapshot by metabolic profiling determination. In addition, metabolomics can provide a picture of cell function under specific conditions and physiological states [12–15]. Sometimes, tissue damage in diseases changes metabolic profiles in body fluids such as blood [12]. In 2015, Sharma et al. demonstrated that villus damage in the epithelial layer of the small intestine from CeD patients affects blood metabolic profile and proposed that measuring the altered levels of blood metabolites may provide a metabolic signature for intestinal damage [16].

Nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry are useful analytical techniques to describe metabolic changes in response to disease, drugs, diet, toxins, and nutrient intake. The advantages of NMR spectroscopy include the need for a very small sample volume, simple preparation, high reproducibility, lower analysis time, and lower maintenance cost, providing complete information about a large number of metabolites [17]. Metabolomics based on NMR combined with multivariate data analysis has proven to be a very powerful method to determine changes in metabolite concentration in data consisting of a large number of samples.

As the current serologic tests for celiac disease can be accompanied by false-negative results (due to patients' IgA deficiency) or false-positive results (due to other autoimmune diseases and intercurrent infections) and since celiac disease is known as a pathology with a direct impact on metabolism [18–20], an NMR profile of serum metabolites may significantly improve the diagnosis process of the disease [20]. In particular, NMR-based metabonomic analysis of biological fluids can trace small changes of target metabolites, which doubled its importance [20]. Today, NMR is widely used to clarify the pathophysiology of different disorders (such as neurological disorders, cancer, gastrointestinal diseases, and cardiovascular disease) and identify diagnostic biomarkers for them [21–25]. Previous studies reported characteristic metabolic alterations of inflammatory bowel disease, fatty liver disease, *Helicobacter pylori* infection, etc. [20, 26, 27].

Bertini et al. introduced some metabolomics biomarkers in the serum and urine of CeD patients which were significantly different between healthy controls and patients. In addition, in this study, the serum and urine of CeD patients were examined after 12 months of GFD and no significant variations in levels of other resonances were found between patients and healthy subjects [20].

In this study, we compared serum metabolites of untreated CeD patients and healthy controls to introduce novel reliable diagnostic biomarkers for CeD screening.

2. Materials and Methods

2.1. Study Population and Sample Collection. Forty-two active adult CeD patients, who did not start a gluten-free diet (22 females and 20 males with a mean age of 33 ± 10 years (mean ± SD)), were recruited from the Research Center for Gastroenterology and Liver Diseases at the Taleghani Hospital from August 2019 to February 2020. The CeD diagnosis was based on positive serology (anti-endomysial (EMA) and anti-transglutaminase-2 (TG2) antibodies) confirmed by villous abnormalities subclassified into modified Marsh grade ≥ 2 [28]. Patients with positive tTGA and EMA serology tests but with Marsh 0/1 lesions were excluded from the analysis. Pregnant and lactating women, patients with any other autoimmune/gastrointestinal diseases, and patients who had a history of nonsteroidal anti-inflammatory drug intake were excluded too. Twenty-two gender- and age-matched healthy controls (10 females and 12 males with a mean age of 35 ± 12 years (mean ± SD)) were also recruited as the control group. Both CeD subjects and healthy controls had no significant past medical history such as hypertension or diabetes mellitus (Table 1).

This study was approved by the ethical committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RETECH.REC.1399.1146). Participants were informed about the content of the study, and written consent was signed by all of them.

Blood samples (5 mL) were collected in the morning after overnight fasting into plastic serum tubes. The tubes were placed vertically at room temperature (22°C) for 20 minutes and centrifuged at 10,000 rpm using an Eppendorf centrifuge at room temperature for 10 minutes. The serum supernatant was removed into a fresh polypropylene tube, immediately frozen, and stored at -80°C until NMR analysis.

2.2. ^{1}H NMR Spectroscopy. Acquiring NMR is similar to our previous work with a new analysis [29–31]. Hydrogen-1 NMR or ^{1}H NMR spectroscopy analysis of serum samples was performed using a Bruker Avance 400 spectrometer, operating at 400 MHz ^{1}H resonance frequency. A 5 mm high-quality NMR tube (Sigma-Aldrich, RSA) was used.

After inserting 10% D_2O (deuterium oxide, 99.9%D, Aldrich Chemicals Company) into the serum sample of each individual, the ^{1}H NMR spectra were acquired immediately and referenced to the chemical shift of lactate at $\delta = 1.33$.

Table 1: Baseline demographics of the study participants.

Demographic factors	Study groups	
	Celiac disease patients ($n = 42$) Mean ± SE	Healthy controls ($n = 22$) Mean ± SE
Age (years)	33 ± 10	35 ± 12
Males	20 (47.6%)	12 (54.5%)
Females	22 (52.4%)	10 (45.5%)
Baseline height (cm)	159 ± 33	162 ± 22
Baseline weight (kg)	56 ± 43	61 ± 21
Marsh classification		
Marcsh 2	9 (21.5%)	0
Marsh 3	33 (78.5%)	0

The D_2O provided a field-frequency lock solvent for the NMR spectrometer. Typically, ^1H NMR spectra were measured with the following parameters: spectral width: 8389.26 Hz; time-domain points: 32 K; number of scans: 154; acquisition time: 2 s; spectrum size: 32 K; and line broadening: 0.3 Hz. In order to enhance visualization of the low-molecular weight metabolites and to assuage protein and lipoprotein's broad signals, the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence was applied for serum sample analysis [32–34].

2.3. Data Preprocessing. Phase and baseline distortions were manually corrected for all ^1H NMR spectra within XWINNMR (version 3.5, Bruker Spectrospin Ltd.). ^1H NMR spectral processing (baseline correction, normalization, and alignment) was performed using ProMetab software (version prometab_v3_3) [35] in MATLAB (version 6.5.1, MathWorks, Cambridge, UK). In order to remove the effects of the residual water peak in the region, δ_{1H} = 4.5-5.5 ppm was set to zero in all NMR spectra. This software integrates the bins across the spectral regions of 0.02 ppm width within the range of 0.2 and 10.0 ppm. Then, baseline correction and alignment were done by ProMetab software in MATLAB. To decrease any significant concentration differences between samples, data were mean-centered and Pareto-scaled after importing data into SIMCA.

3. Statistical Analysis

SIMCA software version 14.0 (Umetrics, Umeå, Sweden) and SPSS 16.0 (SPSS, Inc., Chicago, IL) were used for analyzing metabolomics results. SIMCA is used widely as a commercial tool in metabolomics data analysis. Principal component analysis (PCA) was applied as an unsupervised statistical method to find outliers, patterns, and trends within the dataset and visualize intrinsic clusters [36]. Also, Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) was performed as a supervised statistical method on NMR data using SIMCA to identify metabolite fingerprint differences and construct predictive models.

R^2X, R^2Y, and Q^2, three goodness parameters for the OPLS-DA model, were calculated using the default leave-one-out (LOO) procedure to describe the quality of the OPLS-DA model [37]. To evaluate the OPLS-DA prediction performance, the receiver operating characteristic (ROC) curve was used and the area under the ROC (AUC) value was calculated using SPSS 16.0 (SPSS, Inc., Chicago, IL). Specificity and sensitivity were determined according to the prediction of the sample class using the 7-fold cross-validation [38].

4. Metabolite Identification

Identification of metabolites was done manually based on signal multiplicity and assignments, which were published in the literature [39, 40], and online databases such as the Biological Magnetic Resonance Data Bank (BMRB) (http://www.bmrb.wisc.edu/metabolomics/) [41] and the Human Metabolome Database (HMDB) (http://hmdb.ca/) [42].

5. Metabolic Pathway Analysis

By MetaboAnalyst 4.0 (accessible at http://www.metaboanalyst.ca/), a simple and freely available tool that combines pathway enrichment analysis and topology analysis, metabolic pathway analysis was performed. The online software MetaboAnalyst with 6292 metabolite sets, 15 model organisms, and three types of biofluids (cerebrospinal fluid, blood, and urine) was used widely in metabolomics studies [43, 44].

The metabolic pathways that are used by MetaboAnalyst are the basis of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Identified metabolites by NMR, which showed significant differences between celiac patients' serum samples and healthy controls, were entered into MetaboAnalyst. The Homosapiens library, hypergeometric test default, and relative betweenness centrality algorithms were chosen as the options for the enrichment analysis and pathway topology analysis. In each pathway, the numbers of involved metabolites (hits) were reported. The most important pathways, with $p$values and false discovery rates (FDR) less than 0.05, were considered significant [45].

6. Results

6.1. Comparison of Altered Serum Metabolic Profiles between CeD Patients and Healthy Controls. After NMR spectral preprocessing, the resulting binned data including 64 samples and 408 variables were analyzed by unsupervised PCA to find patterns, trends, and outliers. Two samples from CeD patients were located far away from the 99% Hotelling's T^2 confidence limit and were considered outliers. After excluding two outliers, PCA was carried out again. PCA score plots showed that the CeD group is not separated clearly from the healthy control group (R^2X: 0.817; Q^2: 0.58) (Figure 1(a)). Then, OPLS-DA was performed to detect alterations between two groups and identify the different metabolic patterns and the potential biomarkers. The OPLS-DA score plots (R^2X: 0.603; R^2Y: 0.967; Q^2: 0.93; and p value: 7.51E

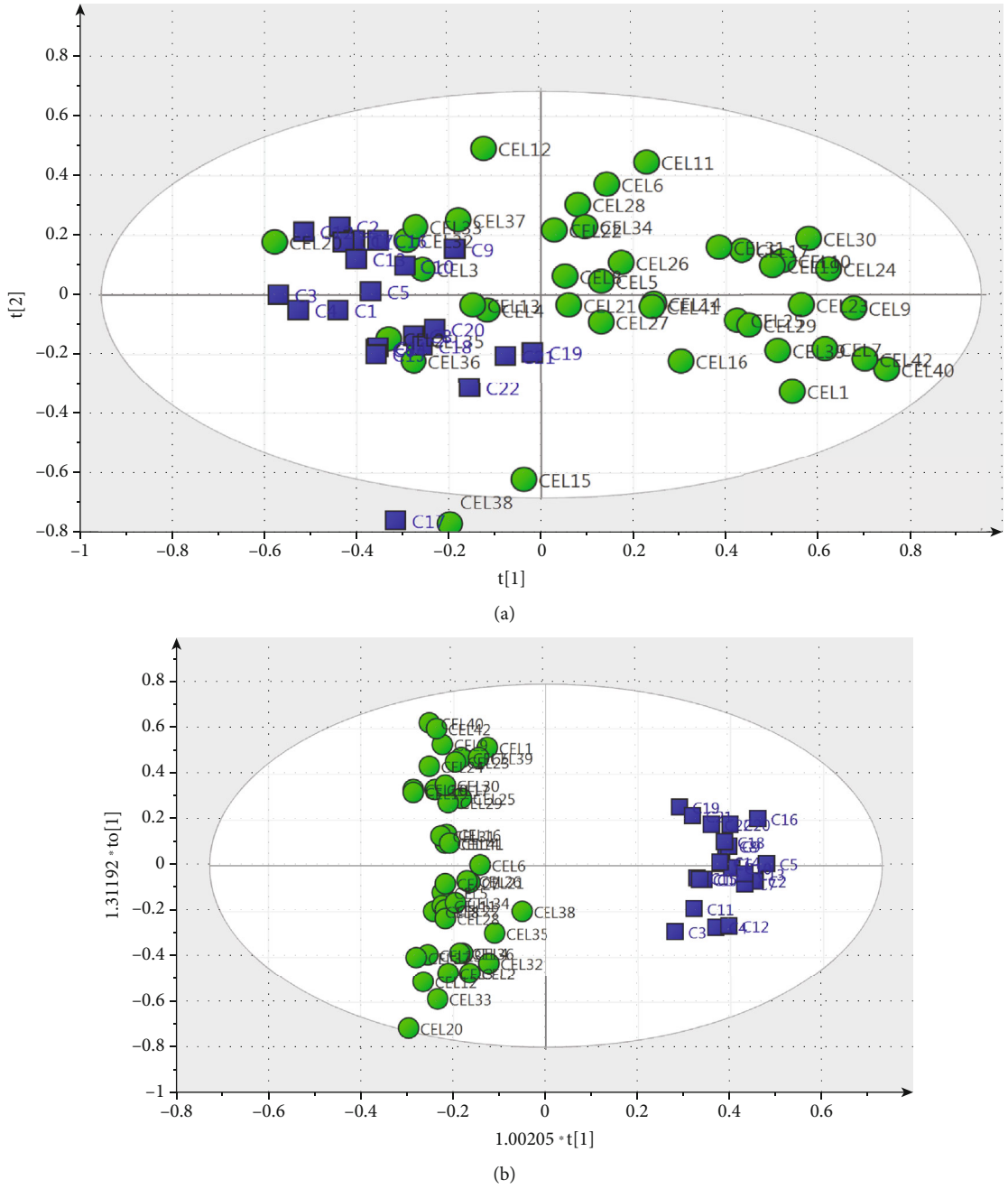

Figure 1: Multivariate statistical analysis from NMR-based metabolic profiling. (a) PCA score plot with all variable unit variance scaled. (b) OPLS-DA score plot of the CeD group versus the healthy control group. Circle, CeD; square, healthy controls.

-28) showed that the CeD group was distinct from healthy controls.

To further validate the diagnostic performance, the ROC curve was used and the calculated AUC value was 1 in this model (Supplementary figure (available here). These results indicated that the OPLS-DA model had a high predictive power between the CeD group and the healthy control group, showing that NMR-based fingerprinting could be used to differentiate CeD subjects from healthy controls.

Metabolites responsible for separating CeD samples from healthy controls in the OPLS-DA model are shown in Table 2. Data analysis based on their chemical shifts and signal multiplicity according to online databases (http://hmdb.ca) and the literature showed changes in 25 different metabolites which were related to the following: amino acids (glutamine (Gln), isoleucine (Ilu), lysine (Lys), valine (Val), proline (Pro), serine (Ser), and glutamic acid (Glu)); bile acids (chenodeoxycholic acid (CDCA), taurocholic acid (TCA), cholic acid (CA), glycocholic acid (GCA), and lithocholic acid (LC)); fatty acids (elaidic acid (EA), linoleic acid (LIN), stearic acid (SA), and propionic acid (PA)); triglycerides (tg); glucose (Glc); cholesterol; 3-hydroxyisobutyric acid (3-HIB); isobutyrate; betaine (Bet); taurine; choline (Cho); and acetylcholine (Ach) between CeD and healthy controls.

Table 2: Differential serum metabolites between CeD samples and healthy controls using NMR.

No.	Metabolite	δ_{1H} (ppm)[a]	Fold change[b]	Direction of variation[c]	Biochemistry pathway
1	Glycocholic acid	3.47	1.49	↑	Primary bile acid biosynthesis
2	Chenodeoxycholic acid	1.99, 1.97, 2.01	1.59	↑	Primary bile acid biosynthesis
3	Glucose	3.35, 5.23, 3.83, 3.39	1.61	↑	Glycolysis
4	Betaine	3.89	1.67	↑	Choline oxidation Glycine, serine, and threonine metabolism
5	Taurine	3.41	1.70	↑	Primary bile acid biosynthesis
6	Taurocholic acid	0.87, 0.81, 0.83, 0.85	1.71	↑	Primary bile acid biosynthesis
7	Choline	3.19	1.74	↑	Lipid metabolism
8	Cholic acid	1.27	1.89	↑	Primary bile acid biosynthesis
9	Acetylcholine	3.21	2.15	↑	Acetylcholine biosynthesis
10	Lithocholic acid	1.25	2.33	↑	Primary bile acid biosynthesis
11	3-Hydroxyisobutyric acid	1.11	3.56	↑	BCAA catabolism Gut microbiota
12	Isobutyrate	1.13	9.1	↑	Gut microbiota
13	Glutamine	2.47	1.55	↓	Amino acid metabolism
14	Elaidic acid	1.59	1.50	↓	Fatty acid metabolism
15	Linoleic acid	1.35	1.65	↓	Fatty acid metabolism
16	Isoleucine	0.93	2.28	↓	Amino acid metabolism
17	Triglycerides	4.15	1.67	↓	Lipid metabolism
18	Lysine	3.73	1.48	↓	Amino acid metabolism
19	Stearic acid	1.37	2.12	↓	Fatty acid metabolism
20	Cholesterol	3.51, 0.91	1.42	↓	Steroid biosynthesis
21	Valine	1.01	1.57	↓	Amino acid metabolism
22	Proline	2.09	1.41	↓	Amino acid metabolism
23	Propionic acid	1.07	2.05	↓	Fatty acid metabolism
24	Serine	3.93	1.51	↓	Amino acid metabolism
25	Glutamic acid	2.07	1.94	↓	Amino acid metabolism

Abbreviations: BCAA: branched-chain amino acid; NMR: nuclear magnetic resonance. [a]Chemical shift scale of the NMR signal used for the quantification of metabolites. [b]Fold change for each chemical shift was calculated based on the median values. [c]Increased or decreased metabolites in the CeD group compared with the healthy control group.

NMR spectroscopy of serum samples showed higher levels of glucose, bile acids, betaine (Bet), taurine, 3-HIB, isobutyrate, and Ach and reduced levels of Gln, Ilu, Lys, Val, Pro, Ser, Glu, fatty acids (EA, LIN, SA, and PA), cholesterol, and triglycerides in specimens of CeD patients than healthy controls.

6.2. Metabolic Pathway Analysis of Altered Profiles. Based on the identified metabolites in serum, metabolic pathways were investigated by applying the MetaboAnalyst 3.0 server. Nine metabolic pathways including aminoacyl-tRNA biosynthesis; primary bile acid biosynthesis; nitrogen metabolism; glutamine and glutamate metabolism; valine, leucine, and isoleucine biosynthesis and degradation; taurine and hypotaurine metabolism; glyoxylate and dicarboxylate metabolism; glycine, serine, and threonine metabolism; and arginine biosynthesis were altered in CeD serum samples (Figures 2 and 3).

Statistics related to pathways with major changes based on p value and FDR indicated that only two pathways (aminoacyl-tRNA biosynthesis and primary bile acid biosynthesis) showed p value < 0.05 and FDR < 0.05, whereas seven pathways showed only p value < 0.05 (Table 3).

7. Discussion

Blood biochemical composition is known as the main basis of clinical biochemistry to describe pathological conditions. Potential biomarkers associated with different diseases can be described by metabolite analysis of biological samples. Moreover, using the obtained profile may be helpful in achieving a better understanding of the disease pathogenesis and mechanism from a holistic point of view.

As the metabolome shows alteration of both the genome and the proteome, an NMR-based metabolic profile, when paired with a transient statistical analysis, provides a comprehensive metabolic picture of such a multifactorial pathology. CeD metabolomics may identify new molecular mechanisms, which can clarify CeD-related symptoms as currently there is no explanation for them. In this study, we analyzed CeD patients' and healthy controls' serum metabolite levels and found that there is a distinct pattern

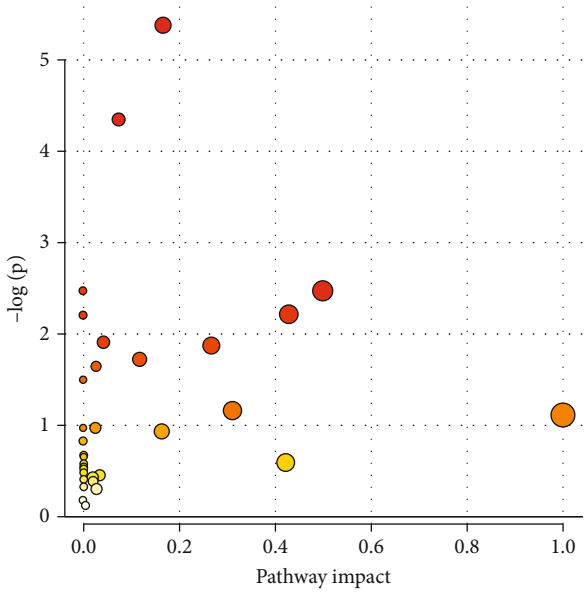

Figure 2: Pathway analysis overview showing altered metabolic pathways in serum from CeD subjects using MetaboAnalyst 4.0. (1) Aminoacyl-tRNA biosynthesis. (2) Primary bile acid biosynthesis. (3) Nitrogen metabolism. (4) Glutamine and glutamate metabolism. (5) Valine, leucine, and isoleucine biosynthesis and degradation. (6) Taurine and hypotaurine metabolism. (7) Glyoxylate and dicarboxylate metabolism. (8) Glycine, serine, and threonine metabolism. (9) Arginine biosynthesis.

between*t*values and a metabolic signature for CeD in serum samples from celiac patients.

Our results showed changes in the level of 25 metabolites, which can be useful in distinguishing CeD patients from the healthy control group (Table 2). These altered metabolites are related to lipid, carbohydrate, and amino acid metabolism.

Higher levels of glucose in celiac samples can be related to the upregulation of glucose intake at the microvillus membrane surface due to their altered lipid-to-protein ratio and impairment of one or more steps in the glycolysis process [20].

When glycolysis is disturbed and reduced, fatty acid β-oxidation, the second major metabolic pathway responsible for energy supply, is usually overstimulated to produce energy. Malabsorption and the increase of fatty acid β-oxidation explain lower fatty acid levels in CeD serum [46].

In gluten metabolism conditions, amino acids can also be used as energy sources and affect cellular metabolism and immune system signaling [47, 48]. In glycolysis impairment, the amino acid carbon backbone can convert into citric acid cycle intermediates or their precursors to provide energy. Therefore, the decrease in serum concentration of amino acids of CeD cases can be due to the decreased amino acid absorption as a result of villous atrophy and their participation in energy production [49, 50].

We demonstrated that the isobutyrate and 3-HIB levels were increased and the FA level was decreased in CeD serum samples when compared to healthy controls. 3-HIB, an intermediate of valine catabolism, is secreted from muscle cells, enhances muscle lipid accumulation, regulates endothelial fatty acid (FA) transport, and connects the regulation of FA flux to catabolism of the branched-chain amino acids (BCAAs; valine, leucine, and isoleucine) [51]. Decreased glycolysis and increased fatty acid oxidation caused higher levels of BCAA. High activity of BCAA aminotransferase increased BCAA catabolism in muscles [52]. Increased levels of 3-HIB in CeD subjects can be related to the increased catabolic flux of BCAAs or microbial activity. Increased BCAA catabolic flux causes 3-HIB secretion from muscle and imports excessive transendothelial FA into the muscle. Elevated 3-HIB levels indicate secretion of BCAA catabolic flux and regulate metabolic flexibility in muscles and the heart. It has been shown that 3-HIB can be used as a risk indicator of insulin resistance (IR) and the future development of type 2 diabetes (T2D) [51, 53].

Isobutyrate and 3-HIB are produced from glucose and amino acids (valine) in gut microbiota. The gut microbiota, formed by a large number of microorganisms, produces some compounds under the influence of environmental stimuli and affects the host metabolome and its health. According to the studies, the gut microbiota composition, which can be influenced by genetic factors including HLA molecules, may have a role in the development of several immune-based disorders. A limited number of studies have reported a link between alterations in the gut microbiota and the onset of intestinal diseases such as inflammatory bowel disease and celiac disease. For instance, Bodkhe et al. reported significant decreases in *Lactobacillus sakei* and total *Lactobacillus* populations in GFD-treated celiac patients compared to untreated and healthy subjects [54]. Serena et al. in their study observed the change in blood microbiome composition and taxonomic diversity in the samples of adult CeD subjects compared with healthy controls [55]. Moreover, Leonard and coworkers using the Celiac Disease Genomic, Environmental, Microbiome, and Metabolomic (CDGEMM) study, which is about understanding the role of the gut microbiome as an additional factor in the susceptibility to autoimmune diseases, revealed that several microbial species, functional pathways, and metabolites might be specific to CeD [56, 57]. In this regard, Olshan et al. in a recent study reported significant differences at both the strain level and the species level for bacteria and viruses and in functional pathways in breast milk composition of subjects with CeD on a gluten-free diet than healthy controls [58]. Rheumatoid arthritis and celiac disease are two diseases with similarities such as HLA mutations that show similar microbial dysbiosis, which can lead to worsening both diseases' severity [59].

Gut microbiota productions can be absorbed by the colonic epithelium; they enter the bloodstream and play an important role in regulating the metabolism of glucose, fatty acids, and cholesterol. Changes in serum metabolite levels in CeD have been identified and suggest significant changes in the gut microbiota of CeD subjects [60–65].

The reduction of cholesterol concentration in CeD patients than healthy controls can be related to intestinal malabsorption, decreased cholesterol genesis, increased bile

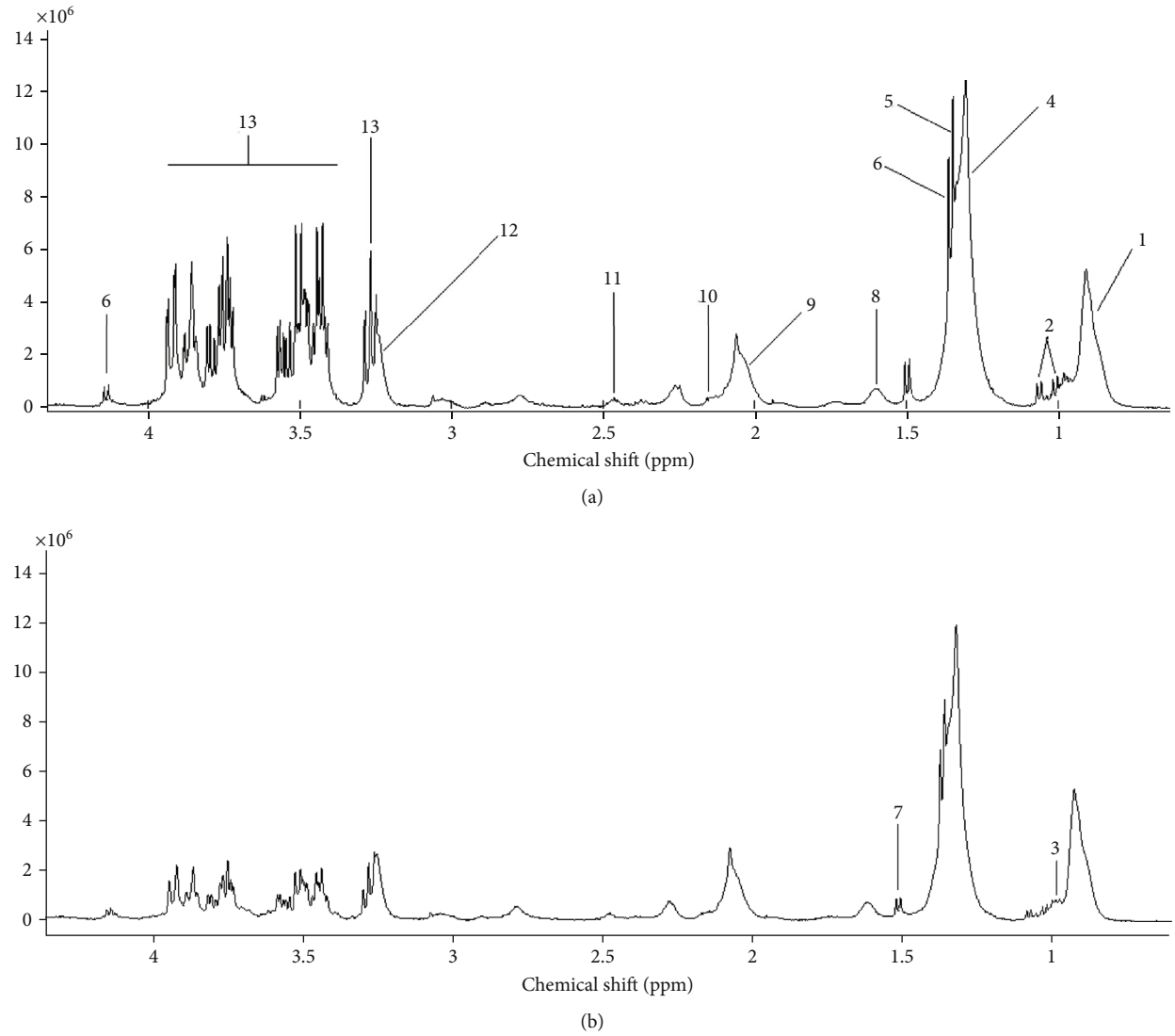

Figure 3: Representative 400 MHz one-dimensional CPMG ^{1}H NMR spectrum of celiac disease (a) and healthy control (b) subjects. (1) Lipid: LDL CH_3-$(CH_2)_n$. (2) Valine. (3) Leucine. (4) Lipid: VLDL $(CH_2)_n$-CO. (5) Threonine. (6) Lactate. (7) Alanine. (8) Lipid: VLDL CH_2-CH_2-CO. (9) Lipid: CH_2-CH=CH. (10) Glutamate+glutamine. (11) Glutamine. (12) Choline. (13) α-Glucose and β-glucose.

Table 3: Significant pathway based on p values/FDR.

	Pathway name	Total[a]	Hits[b]	p	−log (p)	FDR	Impact[c]
1	Aminoacyl-tRNA biosynthesis	48	7	4.058E-6	5.3917	3.4087E-4	0.16667
2	Primary bile acid biosynthesis	46	6	4.4108E-5	4.3555	0.0018525	0.07433
3	Nitrogen metabolism	6	2	0.0033199	2.4789	0.069718	0.0
4	D-Glutamine and D-glutamate metabolism	6	2	0.0033199	2.4789	0.069718	0.5
5	Valine, leucine, and isoleucine biosynthesis	8	2	0.0060807	2.216	0.08513	0.0
6	Taurine and hypotaurine metabolism	8	2	0.0060807	2.216	0.08513	0.42857
7	Glyoxylate and dicarboxylate metabolism	32	3	0.012055	1.9188	0.13782	0.04233
8	Glycine, serine, and threonine metabolism	33	3	0.013126	1.8819	0.13782	0.26741
9	Arginine biosynthesis	14	2	0.018672	1.7288	0.17427	0.11675

Abbreviations: FDR: false discovery rate. [a]The total number of metabolites in each pathway. [b]The number of identified metabolites in each pathway. [c]The pathway impact is based on scores from topology analysis.

acid biosynthesis, and elimination of high-cholesterol feces. Failure to increase cholesterol levels in CeD patients under treatment indicates that intestinal malabsorption is less involved in this process [66]. We indicated that bile acids such as chenodeoxycholic acid, taurocholic acid, cholic acid, glycocholic acid, and lithocholic acid have a higher concentration in CeD serum samples in comparison to healthy controls. These compounds and taurine are synthesized from cholesterol during the primary bile acid biosynthesis mechanism [66–69].

The size of the bile acid pool is affected by the microbial metabolism of bile acids in the intestines. Bile acids regulate the gut microbiome at the highest toxemic levels. The host and microbiome regulate the size of the bile acid pool. A large pool of a conjugate of hydrophilic bile acids is produced by the host. The members of the microbiome use bile acids and their compounds. Bacterial overgrowth, inflammation, antibiotic therapy, diet (such as gluten-free diet), and disease states affect the microbiome-bile acid pool balance [70].

We demonstrated that Bet and choline have a high concentration in the CeD group compared to healthy controls. Choline plays a critical role in lipid metabolism and methylation [71]. It is an essential component of the lipids present in the plasmatic membrane and structural lipoproteins and a precursor of acetylcholine. Bet is synthesized from choline oxidation and glycine during glycine, serine, and threonine metabolism. Choline and Bet are important sources of one-carbon units and are involved in the pathogenesis of various disorders such as chronic diseases and neurological developmental disorders [72]. High choline concentrations in plasma are associated with cardiovascular risk factor profiles. Moreover, altered choline metabolite levels may act as biomarkers for changes in membrane metabolism in CeD patients. Choline and Bet have opposite relationships with the major components of metabolic syndrome and have a key role in disease prevention and risk assessment. The presented results indicate their involvement in the pathogenesis of various chronic diseases [72, 73].

Higher levels of ACh in CeD may be related to increased acetylcholine biosynthesis from acetyl-CoA and inhibition or inactivation of acetylcholinesterase (AChE), the enzyme responsible for the ACh degradation in cholinergic neurons [74]. ACh, a neurotransmitter, is synthesized from choline and acetyl-CoA. High levels of acetyl-CoA are obtained from the β-oxidation cycle. Excessive accumulation of ACh at the synapses and neuromuscular junctions causes symptoms of both nicotinic toxicity and muscarinic toxicity. Fatigue and muscle weakness in CeD may be an immune-mediated neurological disorder, which is caused by an increase in Ach concentration [75, 76].

These biomarkers still need confirmation through additional techniques, such as LC/MS and 2D NMR. Moreover, in the present study, metabolic pattern differences between CeD and other gastrointestinal diseases have not been studied. These were our study limitations. Further studies with other techniques and in different societies are needed to confirm/reject the result of our study.

8. Conclusion

Analysis of CeD patients' and healthy controls' serum metabolite levels showed that changes in the serum level of 25 metabolites can be useful in distinguishing CeD patients from the healthy control group and may be considered candidate biomarkers of CeD, which needs to be confirmed by the results of subsequent studies. Our results may further enhance the understanding of impaired metabolic pathways in CeD.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Figure 1: ROC curve analysis for the predictive power of serum biomarkers for distinguishing CeD from healthy controls using the OPLS-DA model. Serum metabolite biomarkers by OPLS-DA yielded an AUC value of 1. *(Supplementary Materials)*

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Review Article

The Role of lncRNAs in Regulating the Intestinal Mucosal Mechanical Barrier

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lncRNA is a transcript that is more than 200 bp in length. Currently, evidence has shown that lncRNA is of great significance in cell activity, involved in epigenetics, gene transcription, chromatin regulation, etc. The existence of an intestinal mucosal mechanical barrier hinders the invasion of pathogenic bacteria and toxins, maintaining the stability of the intestinal environment. Serious destruction or dysfunction of the mechanical barrier often leads to intestinal diseases. This review first summarizes the ability of lncRNAs to regulate the intestinal mucosal mechanical barrier. We then discussed how lncRNAs participate in various intestinal diseases by regulating the intestinal mucosal mechanical barrier. Finally, we envision its potential as a new marker for diagnosing and treating intestinal inflammatory diseases.

1. Introduction

Previous studies have found that although there are as many as 3 billion base pairs in the human genome, few can encode proteins, yet most of them produce a class of RNA, noncoding RNA (ncRNA). It cannot encode proteins. This type of RNA is rich in species, including microRNAs (miRNAs), small nucleolar RNAs (SnoRNAs), lncRNAs, and circRNAs [1]. Among them are lncRNA transcripts longer than 200 bp but do not encode proteins. lncRNA is a by-product of RNA polymerase II (Pol II), with a methyl guanosine cap at its 5′ end and a poly tail at its 3′ segment [2]. Compared to other categories of ncRNAs, lncRNAs show a surprisingly wide range of size, shape, and functionality. These characteristics give them the functional potential that cannot be underestimated [3]. The development of third-generation sequencing technology has expanded and improved the existing lncRNA annotations rapidly, economically, and effectively so that an increasing number of new lncRNAs have been discovered and annotated [4]. lncRNAs are usually located in the nucleus or cytoplasm. They can regulate chromatin and assemble membranous nucleosomes through interactions with other genetic materials such as DNA, RNA, and proteins. Furthermore, they can also change the stability and translation of cytoplasmic mRNAs and interfere with signaling pathways [5]. The rich function of lncRNAs affects gene expression in many physiological and pathological processes. Studies have confirmed that they are involved in neuronal diseases [6], immune response [7], and cancer [8]. Recent findings have shown that lncRNAs play an essential role in intestinal diseases, such as inflammatory bowel disease (IBD), and can potentially be used as biomarkers and therapeutic targets.

More than 10 trillion bacteria, fungi, viruses, and other microorganisms are present in the intestinal mucosa [9]. The intestinal mucosal mechanical barrier prevents these uninvited guests from invading the internal environment. It maintains the dynamic balance between these intestinal florae and intestinal epithelial cells. Moreover, it is the transportation carrier between the body and nutrients, water, and waste [10]. The intestinal mucosal mechanical barrier is the most important part of the intestinal mucosal barrier, which is essentially a defensive layer composed of intestinal mucosal epithelial cells and tight junctions between cells and the bacterial membrane. Its existence can effectively prevent intestinal mucosal injury [11]. Pathophysiological changes, including

trauma, local ischemia, total parenteral nutrition (TPN), and intestinal obstruction, can cause acute or chronic damage to the intestinal mucosal barrier [12]. It has been confirmed that intestinal mucosal barrier injury can increase intestinal permeability, usually accompanied by critical diseases, including septicemia and multiple organ failure. This injury may result in high morbidity and mortality rates [13].

Therefore, the identification and characterization of early biomarkers for intestinal inflammatory diseases have become a priority. This review discusses the role of lncRNAs in regulating the mechanical barrier and how they coregulate each other and their target genes.

2. Intestinal Mucosal Mechanical Barrier

The mechanical barrier of the intestinal mucosa consists of intestinal epithelial cells and tight junctions between cells and the bacterial membrane. Intestinal epithelial cells contain absorptive cells, Paneth cells, and goblet cells (Figure 1). Intestinal epithelial cells are phagocytosed by bacteria. Paneth cells can secrete lysozyme, natural antibiotic peptides, human defensin 5, and human defensin 6. With the deepening of research, the role of Pan's cells in inhibiting bacterial translocation and preventing enterogenous infection has been gradually explored [14]. Goblet cells secrete mucus glycoproteins, preventing digestive enzymes and harmful substances from damaging epithelial cells in the digestive tract. It can wrap bacteria and compete with pathogenic microorganisms to inhibit adhesin receptors on intestinal epithelial cells. It inhibits bacterial adhesion and colonization in the intestine, thereby restraining the proliferation of intestinal bacteria and intestinal infection [15]. Intercellular junctions are composed of tight junctions, adhesive junctions, gap junctions, and desmosome junctions, of which tight junctions are the core [16]. The tight junctions between adjacent cells are tightly arranged by tight junction protein particles. There are many kinds of proteins, including claudin, occludin, junctional adhesive molecule (JAM), and zonula occludens (ZO) [17]. When there are more than two forms of intercellular junctions between the sides of the adjacent intestinal epithelial cells, we can call it the tight junction complex. The gap between the complexes is so narrow that only water molecules and small molecular water-soluble substances can selectively pass through [18]. The tight junction between cells is similar to an iron fence, which closes the gap between intestinal epithelial cells. It can prevent foreign pathogens from entering the lamina propria, so as to prevent the activation of immune cells in the lamina propria, avoiding the occurrence of abnormal mucosal immune reactions [19]. From a macropoint of view, intestinal motor function is also part of the intestinal mechanical barrier, which prevents the attachment of intestinal bacteria and promotes the self-cleaning function of the intestinal tract [20].

3. Regulatory Effects of Different lncRNAs on Intestinal Mucosal Mechanical Barrier

3.1. lncRNA H19. lncRNA H19, located in the endometric region 11P15.5 of the human chromosome, is a long noncoding RNA with a length of 2.3 kb, first discovered in 1991 [21]. Its expression reaches its highest level in the human embryo and decreases with aging [22]. Previous studies have found that lncRNAH19 plays a role in physiological and pathological processes such as inflammation, aging, and tumorigenesis [23, 24]. In recent years, an increasing number of studies have reported that it can regulate the intestinal mucosal mechanical barrier through various mechanisms and indirectly participate in the progression of intestinal diseases.

3.1.1. Regulation of Intestinal Mucosal Epithelial Cell Function and Tight Junction by lncRNA H19. Yu et al. [25] suggested that the defense function of Paneth cells and goblet cells in the intestinal mucosal mechanical barrier was enhanced if the H19 gene was specifically knocked out in mice. Autophagy of the intestinal mucosa was also improved. On the contrary, the overexpression of H19 significantly inhibited the functions of Paneth cells and goblet cells, and the autophagy of intestinal mucosa for self-renewal was also significantly weakened, possibly leading to damage to the intestinal mucosa mechanical barrier. Previous studies have found that the RNA-binding protein HUR can effectively regulate the intestinal mucosal mechanical barrier by binding directly to lncRNA H19 [26]. Zou et al. [27] suggested that H19 could indirectly destroy the tight junction of the intestinal mechanical barrier by increasing the expression of miR-675, destroying the structure of ZO-1 and E-cadherin in tight junction proteins, and inhibiting their translation. By increasing the content of HUR, which competes for the binding of H19 and whose effect on miR-675 was weakened, the upregulated expression of ZO-1 and E-cadherin could be detected, and the function of the intestinal mucosal mechanical barrier gradually recovered. The authors suggest that H19 can destroy the defense function of the intestinal mucosal mechanical barrier by increasing the expression level of downstream miR-675. These two studies illustrate that H19 can destroy the intestinal mucosal mechanical barrier. However, many studies have found that H19 has an opposite effect on the intestinal barrier through some mechanisms. Li et al. [28] found that intestinal autophagy was activated after severe burn using a mouse model; this could increase the transcription level of lncRNA H19 and suggested that lncRNA H19 might regulate the repair of EGF after intestinal mucosal injury after burn through miRNA LET-7G. Another study verified this conjecture and found that the autophagy-mediated H19 expression increased in the intestine of severely burned mice and acted as a sponge combined with let-7 g to regulate EGF, suggesting that H19 may be a therapeutic target and biomarker of intestinal mucosal injury after burn [29]. In addition, H19 can also regulate the intestinal mucosal mechanical barrier by regulating the expression of AQPs. Aquaporin (AQP) is a small (30 kDa/monomer) hydrophobic membrane integrin belonging to the special superfamily membrane integrin of MIP (main intrinsic protein). AQPs are responsible for transporting liquids and electrolytes [30]. AQP3 mainly exists in human intestinal epithelial cells. Because of its existence, the intestinal mucosa can reverse the osmotic gradient to complete the absorption and

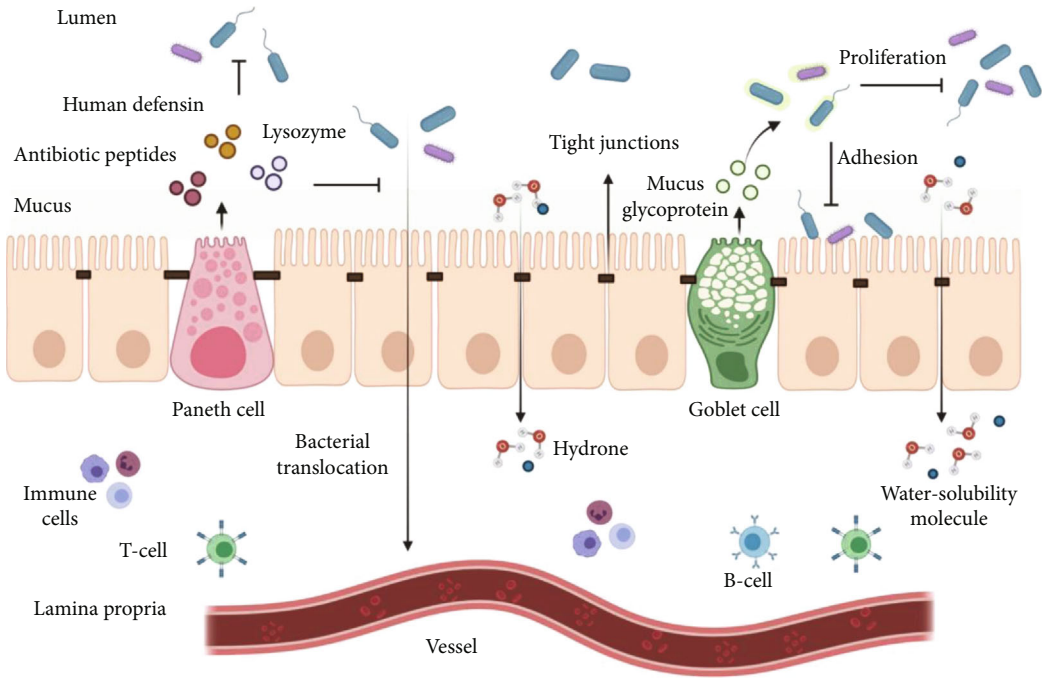

Figure 1: The tight junction between intestinal mucosal epithelial cells and adjacent cells constitute the intestinal mucosal mechanical barrier jointly, which prevents intestinal microorganisms and foreign pathogens from entering the intestinal environment and prevents the occurrence of inflammation.

elimination of water to achieve the normal function of the intestine and the balance of human body fluid. Zhang et al. [31] found that cell permeability increased significantly after the knockout of AQP3 in a Caco-2 cell line, which may be related to the inhibition of tight junction proteins in the intestinal mucosal mechanical barrier, but the specific mechanism is still unclear. In addition, Chao et al. [32] found that compared to healthy colon tissue, the expression of lncRNA H19, AQP1, and AQP3 in the colon tissue of patients with IBS-D decreased. The author further demonstrated that the expression of lncRNA H19 was positively correlated with AQP1 and AQP3. This experiment revealed that lncRNA H19 might participate in developing IBS-D by regulating the intestinal mucosal mechanical barrier. Geng et al. [33] detected the level of lncRNAH19 in intestinal tissues of IBD mice and humans and found that its expression level was significantly higher than that in normal tissues. H19 may promote the proliferation of intestinal epithelial cells and repair inflammatory mucosa by inhibiting the expression of p53 protein, microRNA34a, and let-7 and promote IEC proliferation and epithelial regeneration.

3.1.2. Inhibiting the Expression of Vitamin D Receptor (VDR). VDR is a nucleophilic protein that can mediate 1,25(OH) D and exerts its biological effects. In recent years, many studies have reported the involvement of VDR in the process of ulcerative colitis (UC) and other diseases [34]. Furthermore, several studies have shown that $1,25(OH)_2D_3$ can effectively prevent intestinal mucosal mechanical barrier damage [35]. Chen et al. [36] detected the expression levels of H19, miR-675-5p, and VDR in 12 patients with UC. The results showed that, compared to normal tissues, the expression of VDR was significantly downregulated in patients with UC, while the expression of H19 was significantly increased. In an experimental study of the Caco-2 cell line, it was found that overexpressed lncRNA H19 could inhibit the expression of VDR by upregulating miR-675-5p. Yet, this effect could be partially weakened by the miR-675-5p inhibitor. Based on this, the authors inferred that lncRNA H19 could inhibit the expression of VDR and damage the intestinal mucosal mechanical barrier. At the same time, miR-675-5p mentioned in the experiment can only partially affect the effect of lncRNA H19. Therefore, the research on VDR mentioned above still needs to be further improved.

3.2. CCAT1-lncRNA. CCAT1-lncRNA, also known as colon cancer-associated transcript-1, was first found in colon cancer with a length of 2628 nucleotides and located on chromosome 8q24.2 [37]. CCAT1 is highly elevated in many types of cancers such as lung adenocarcinoma, gastric cancer, colorectal cancer, and esophageal squamous cell carcinoma and plays an important role in many biological processes, such as invasion, proliferation, drug resistance, migration, and survival [38–42]. In the last few years, some studies have suggested that CCAT1 can inhibit the function of the intestinal mucosal mechanical barrier through some mechanisms, leading to intestinal diseases. The mechanism of CCAT1-induced malignant transformation of IBD into CRC has also been discovered for the first time. Ma and other studies have found that CCAT1 can promote high levels of myosin light chain kinase (MLCK) expression by acting as a molecular sponge of miRNA and competitively binding to miR-185-3p, leading to an increase in intestinal barrier permeability and the weakening of intestinal mucosal

barrier function in patients with IBD, resulting in malignant diseases [43].

3.3. PlncRNA1. PlncRNA1, also called CBR3AS1, located in the antisense region of carbonyl reductase 3 (CBR3), was first found to be upregulated in prostate cancer [44]. It has been confirmed that it is also associated with other types of cancer, such as retinoblastoma, colorectal, and liver cancers [45–47]. A recent study suggested that PlncRNA1 can regulate the expression of downstream miR-34c by cooperating with the Myc gene, indirectly enhancing the expression of zinc finger protein (MAZ), ZO-1, and occludin in tight junction proteins and enhance the mechanical barrier of the intestinal mucosa. The authors confirmed that the overexpression of PlncRNA1 enhances the defense of the intestinal mucosal barrier against sodium sulfate glucose (DSS) injury. The authors concluded that PlncRNA1 could regulate the tight junction protein of the intestinal mucosal mechanical barrier and enhance the defense function of the intestinal barrier by regulating the content of downstream miR-34c [48].

3.4. lncRNA neat1. lncRNA neat1 is a nuclear-rich lncRNA located in accessory plaques [49], the integrity of accessory plaques [50]. Presently, studies have found that it is highly upregulated or downregulated in various tumor entities. Its main role is as a competitive endogenous RNA (Cerna) and competitive binding of tumor suppressor microRNA (miRNA). Sponge miRNA loses the ability to degrade, silence, or hinder its downstream, mainly carcinogenic-targeted transcript translation, and finally promotes cancer occurrence [51]. A recent study detected lncRNA NEAT1 in the intestinal mucosa of patients with IBD and found that its expression level was significantly upregulated compared to that in normal tissues. After further study, the authors found that specific knockout of NEAT1 in IBD mice could significantly reduce abnormally increased intestinal permeability, mediate macrophage polarization through the exocrine pathway, and weaken intestinal inflammation. Finally, the authors concluded that lncRNA NEAT1 could increase intestinal permeability abnormally and promote the inflammatory response in IBD by destroying the intestinal mucosal mechanical barrier [52].

3.5. lncRNA SPRY4-IT1. lncRNA SPRY4-IT1 is a 706 bp-length transcript found first in a sequence of adipose tissue cDNA [53]. It has been further confirmed to be widely expressed in various human tissues, including the intestinal mucosa [54]. SPRY4-IT1 is transcribed from the SPRY4 gene in the intron region, but SPRY4-IT1 is completely different in structure from SPRY4mRNA [55]. Previous studies have found that SPRY4-IT1 is highly expressed in various cancers, including melanoma [54], colorectal cancer [56], breast cancer [57], and systemic scleroderma [58]. Currently, some studies have found that the lncRNA SPRY4-IT can regulate the intestinal epithelial barrier. Xiao et al. [59] showed that lncRNA SPRY4-IT1 regulates intestinal epithelial barrier function by interacting with HUR and changing the expression of tight junction (TJ) proteins. In the in vitro experiment, the authors downregulated the expression of SPRY4-IT1. They found that the expression of TJ in the intestinal mucosal mechanical barrier was significantly inhibited, impairing the defense function of the intestinal barrier. On the contrary, increasing the expression level of SPRY4-IT1 in the intestinal mucosa not only prevented TJ inhibition induced by cecal ligation and perforation (CIP) but also protected the intestinal epithelial barrier from septic stress in vivo. The authors believe that SPRY4-IT1 is essential for maintaining the function of the intestinal mucosal mechanical barrier. Although it cannot increase the basic level of TJ proteins, it promotes the expression of tight junction proteins.

3.6. lncRNA uc.173. lncRNA (T-UCR), transcribed from UCR, originates from genomic elements located in many mammalian genomes, which is quite conservative in evolution; hence, it nickname, "dark matter" (DarkMaterial) in the human genome [60]. With the deepening of the study, researchers have found that it positively affects the intestinal mucosal mechanical barrier. Xiao et al. [61] proposed that uc.173 downregulates the expression of miRNA195 in intestinal epithelial cells by destroying the stability of pri-miR195. They found that miRNA195 can inhibit the expression of many proteins (CDK4, CDK6, CCND1, STIM1, and WEE1), which are important for cell migration and proliferation and significantly hinder the renewal of the intestinal mucosal barrier. In summary, the authors drew the following conclusions: in the analysis of intestinal epithelial cells and mice, uc.173 noncoding RNAs regulate the intestinal mucosal barrier and stimulate intestinal epithelial renewal by reducing the abundance of miRNA195. Wang et al. [62] found that uc.173 can act as a natural bait for miR-29b, specifically binding to RNA, reducing its inhibitory effect on CLDN1mRNA, and promoting the translation of the tight junction protein claudin-1 (CLDN1) and the repair of the intestinal mucosal mechanical barrier.

3.7. lncRNA Bmp1. Mouse Bmp1 is a full-length 4464 bp gene located on chromosome 14qD2. It was first found in bones and has been reported to play a variety of functions in bone formation [63]. Some studies have also found that this may be related to susceptibility to lung cancer [64]. Presently, some studies have found that the expression of its transcriptional product lncRNABmp1 is significantly increased in a burn mouse model, and the Bmp1 content is upregulated after intestinal mucosal injury. Through the experiment, the authors concluded that Bmp1 overexpression has a protective effect on the intestine of scalded mice, which can significantly improve the proliferation and migration of IEC-6 or HIEC-6 cells of intestinal crypt epithelial cells in rats through the Bmp1/miR-128-3p/PHF6/PI3K/AKT pathway and promote the repair of intestinal mucosal mechanical barrier [65]. This overexpression of lncRNA BMP1 can promote the repair of the intestinal mucosal barrier in burn patients.

3.8. lncRNA BC012900. In 2016, a study found for the first time that IBD is related to lncRNA, which is regulated by inflammatory stimulation and plays an important role in intestinal epithelial function. Wu et al. [66] screened

17,000 lncRNA genomes. By detecting lncRNA microarray and quantitative RT-PCR, the authors found that compared to normal tissues, 455 lncRNAs were significantly differentially expressed in the colon tissues of patients with UC in the active stage, among which lncRNA BC012900 was selected. This study found that overexpression of BC012900 downregulated the proliferation of HCT116 and HT29 intestinal epithelial cell lines and increased the susceptibility of intestinal epithelial cells to apoptosis, which was reversed by knocking out BC012900 expression by siRNA. Finally, the author concluded that overexpression of lncRNABC012900 in epithelial cells could significantly inhibit cell proliferation and increase cell sensitivity to apoptosis (Table 1).

4. lncRNA Participates in the Development of Intestinal Diseases by Regulating the Intestinal Mucosal Mechanical Barrier

4.1. lncRNA Participates in the Occurrence and Development of IBD by Regulating Intestinal Mucosal Mechanical Barrier

4.1.1. The Change of Intestinal Mucosal Permeability Is an Important Prodromal Symptom of IBD. Two new studies in 2020 have shown that changes in intestinal epithelial permeability in patients with IBD precede the onset of clinical symptoms, suggesting that specific interventions can be adopted to prevent IBD in the early stages of the disease [67]. Turpin et al. [68] reported a 7-year study on asymptomatic first-degree relatives of 1420 patients with Crohn's disease (CD) and quantified the permeability of the intestinal mucosal mechanical barrier by the ratio of lactulose to mannitol excretion fraction (LMR). It was found that intestinal permeability in patients with Crohn's disease was significantly higher during the follow-up period than in healthy people without Crohn's disease. The second study conducted by Torres et al. [69] examined serum samples from the Defense Department's Serum Bank (DoDSR), including Crohn's disease, ulcerative colitis (UC), and healthy individuals, and obtained a group of 51 protein biomarkers through data analysis. An IBD prediction model including hypothetical predictors (specific predictors cannot be determined) and protein biomarkers was established. When the AUROC is less than or equal to 0.76, the patient can predict the occurrence of Crohn's disease within 5 years; when the AUROC is less than or equal to 0.87, the patient can be diagnosed with Crohn's disease within one year at the earliest. These two studies suggest that the intestinal barrier function of patients with IBD has been disturbed a few years before clinical symptoms appeared, and the detection of intestinal permeability can alert us in advance of IBD occurrence.

4.1.2. The Injury of Intestinal Mucosal Mechanical Barrier Is a Typical Manifestation of IBD. By observing the lesion site of UC and CD patients, it was found that the tight junction structure of the lesion mucosa was most obviously destroyed [70], and the expression of occlusive tight junction protein was significantly downregulated, while the expression of pore-like tight junction protein was increased. These processes are often positively correlated with IBD symptoms [71]. IBD also induces intestinal barrier damage by inducing epithelial cell death or apoptosis. UC patients and colitis mouse models were accompanied by apparent death and destruction of colonic epithelial cells; through the anatomy of these organizations, there are many crypt-like microabscesses composed of inflammatory cells and dead cells in the intestinal tract of IBD mice, which increases the permeability of intestinal mucosal mechanical barrier in mice [72].

In the past few years, an increasing number of studies have shown that lncRNAs are closely involved in the pathogenesis of IBD [73]. lncRNAs are involved in many processes of IBD, such as the regulation of intestinal epithelial cell apoptosis and intercellular tight junction proteins related to lipid metabolism (Figure 2), thus regulating the permeability of the intestinal mucosal mechanical barrier [74]. Recent studies have reported that lncRNA CNN3-206 expression is increased in the intestinal tissue of CD patients. By acting as a molecular sponge to adsorb miR-212, activating the lncRNA CNN3-206-miR-212-Caspase10 regulatory network leads to increased apoptosis, migration, and invasion of intestinal epithelial cells [75]. Yang et al. [76] reported a new lncRNA named CRNDE, which can regulate the expression of downstream miR-495 and SOCS1, indirectly induce apoptosis of intestinal mucosal epithelial cells and aggravate the inflammatory response in IBD.

4.2. lncRNA Participates in Colorectal Cancer by Regulating Intestinal Mucosal Mechanical Barrier. Intestinal mucosal mechanical barrier deficiency can lead to direct contact between intestinal and luminal pathogens and toxins, thus promoting intestinal inflammation [77]. In addition, studies have shown that intestinal mucosal mechanical barrier injury can greatly increase IBD risk and colorectal cancer in mice, revealing the importance of the intestinal mucosal mechanical barrier in regulating inflammation and tumor process [78]. Current studies have found that the claudin family of intestinal mucosal mechanical barrier compact proteins is associated with different types of tumors, including breast cancer, prostate cancer, ovarian cancer, pancreatic cancer, gastric cancer, and colorectal cancer [79–81]. They may provide a signal pathway that connects the inside and outside of the cell and induces the proliferation and migration of cancer cells [82]. Mees et al. detected the content of tight junction proteins in the colon tissue of colorectal cancer patients with a history of UC and found that the expression of Claudin1,3,4 and β-catenin in patient tissue was significantly higher than that in healthy tissue [83]. Garcia-Hernandez et al. [84] found that when mucosal inflammation occurred, the expression of claudin-1, -2, and-18 in the intestinal epithelium increased, while the expression of claudin-3, -4, -5, -7, -8, and-12 decreased. The destruction of tight junction proteins in IBD tissue can often reflect the severity of inflammation and the prognosis of patients to a certain extent, and inflammation is also an important risk factor affecting the progression of inflammatory bowel disease and colorectal cancer. Many studies have found that lncRNAs can participate in the development of colorectal cancer by regulating claudin protein in the intestinal

Table 1: Regulatory effect and mechanism of different lncRNAs on intestinal mucosal mechanical barrier.

lncRNA	Impact on the barrier	Regulation methods	Action object	Functions
H19	↓	Direct	Paneth cell and goblet cell	Promote autophagy of small intestinal mucosa [25].
	↓	Indirect	miR-675	Inhibit the expression of TJ ZO-1 and E-cadherin, resulting in epithelial barrier dysfunction [27].
H19	↑	Indirect	miRNA LET-7G	Promote the repair of intestinal epithelial mucosa after burn [29].
	↑	Direct	AQP1, AQP3	Promote the expression of AQP and maintain the stability of intestinal mucosal mechanical barrier [32].
	↑	Indirect	P53, miRNA34a, let-7	Promote IECs proliferation and epithelial regeneration [33].
	↑	Indirect	miR-675-5p	Intestinal mucosal barrier damage caused by inhibition of VDR expression [36].
CCAT1	↓	Indirect	miR-185-3p	Increase the permeability of intestinal barrier and destroy the function of intestinal barrier [43].
PlncRNA1	↑	Direct	MAZ, ZO-1, occludin	Significantly enhance the protective function of intestinal barrier against sodium sulfate paste (DSS) injury [48].
neat1	↓	Direct	IEC macrophages	Participate in inflammatory response by regulating intestinal epithelial barrier and exocrine-mediated macrophage polarization [52].
SPRY4-IT1	↑	Direct	TJ	Change the expression of tight junction (TJ) protein to enhance the function of intestinal epithelial barrier [59].
uc.173	↑	Indirect	miRNA195 miR-29b	Promote the translation of TJ claudin-1 (CLDN1) and the repairment of intestinal mucosal mechanical barrier [61].
Bmp1	↑	Indirect	miR-128-3p	Increase the proliferation and migration of IEC-6 or HIEC-6 cells in rat intestinal crypt epithelial cells and promote the repair of intestinal mucosal mechanical barrier [65].
BC012900	↓	Direct	IECs	Inhibit the proliferation of intestinal epithelial cells and increase the sensitivity of cells to apoptosis [66].

mucosal mechanical barrier. The lncRNA CCAT-1 mentioned earlier in this paper is closely related to the occurrence and development of CRC. It has been found that in patients with colorectal cancer, CCAT1 can modulate MLCK in a miR-185-3p-dependent manner, regulate the role of tight junction proteins including claudin and ZO-1 in the distribution of MLCK, increase intestinal epithelial TJ permeability, and promote the malignant change of IBD [43]. lncRNA SPRY4-IT1 showed a similar effect. Some studies have suggested that it inhibits the translation of claudin-1, claudin-3, jam-1, and occludin in intestinal barrier tight junction proteins, reduce their stability, and lead to intestinal mechanical barrier dysfunction and promote the progression of colorectal cancer [59]. The lncRNA UC.173 plays the opposite role. lncRNA UC.173 can act as a molecular sponge of miR-29b that specifically binds to it, reducing its inhibitory effect on CLDN1 mRNA, promoting the expression of claudin-1, and indirectly repairing the damaged intestinal mucosal machinery barrier function to improve the symptoms of colorectal cancer patients [62].

4.3. lncRNA Participates in the Occurrence and Development of IBS-D by Regulating Intestinal Mucosal Mechanical Barrier. Irritable bowel syndrome with diarrhea (IBS-D) is the most common subtype of IBS. Patients often experience greater mental stress and psychological problems to a certain extent [85]. The intestinal permeability of IBS-D patients is often elevated, which is considered to be one of the causes of diarrhea [86]. In recent years, a growing number of studies have discovered that IBS-D occurrence is related to the intestinal mucosal mechanical barrier. An animal experiment confirmed that intestinal permeability was significantly increased in IBS-D mice [87]. Chao and Zhang's study also confirmed that IBS-D is caused by increased intestinal mucosal permeability, which could be related to the low expression of AQP 1, 3, 8 [88]. In particular, there is a significant correlation between the reduction in AQP3 and diarrhea symptoms [89]. Aquaporin exists mainly in human intestinal epithelial cells and plays an important role in maintaining the normal function of the intestinal tract [90]. We previously detected the expression levels of lncRNAH19, AQP1, and AQP3 in the colon of patients with IBS-D; we found that their expressions were significantly downregulated; we then demonstrated that their reduction is proportional through cell experiments. This suggests that the downregulation of lncRNA H19 affects the expression of AQP1 and AQP3, enhances the permeability of the intestinal mucosal mechanical barrier, and may be involved in IBS-D development [32].

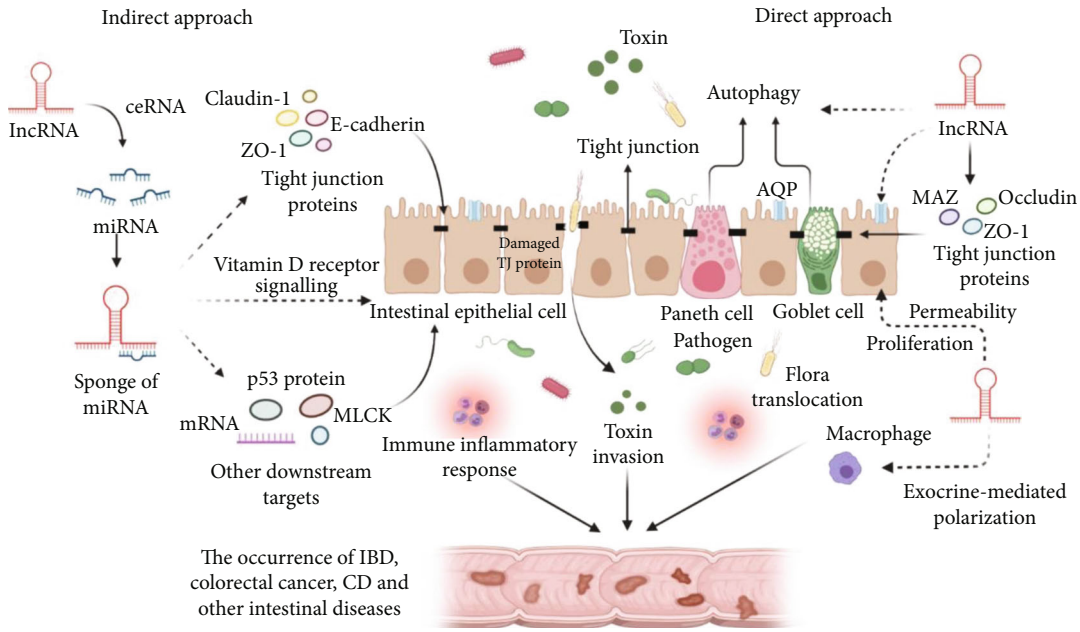

Figure 2: lncRNAs regulate the intestinal mucosal barrier directly (directly regulating TJ protein or AQPs) or indirectly (through miRNAs or other intermediates). The destruction of intestinal mucosal barrier participates in the progression of intestinal diseases such as IBD, colorectal cancer, and celiac disease through immune inflammatory reaction, toxin invasion, and flora translocation.

4.4. lncRNA Participates in the Progression of Celiac Disease by Regulating the Homeostasis of Intestinal Mucosal Mechanical Barrier. Celiac disease, also known as gluten allergy, is an autoimmune digestive tract disease typically characterized by intestinal inflammation and intestinal mucosal damage [91]. Castellanos-Rubio et al. [92] reported a lncRNA, lnc13, which contains a haplotype block associated with celiac disease and inhibits the expression of certain inflammatory genes under steady-state conditions. Lnc13 regulates gene expression by binding to hnRNPD, a member of the widely expressed heterogeneous ribonucleoprotein (HnRNP) family. The level of lnc13 decreased under stimulation, allowing the expression of inflammatory genes to increase. The authors believe that lnc13 plays an important role in maintaining intestinal mucosal barrier function, and the downregulation of lnc13 expression leads to the impairment of intestinal mucosal barrier function and an increase in intestinal barrier permeability. The level of LNC13 in small intestinal biopsies from patients with celiac disease was significantly decreased, suggesting that the downregulated expression of LNC13 may be one of the causes of inflammation in celiac disease. It has also been found that the noncoding regions of celiac disease-related SNPs can produce long noncoding RNAs (lncRNAs), many of which are regulators of gene expression. Many disease-related SNPs located in lncRNAs change their secondary structures or affect their expression levels, thus affecting their regulatory function, destroying the homeostasis of the intestinal mucosal barrier, thus promoting the development of the disease [93]. Recently, Santin et al. [94] reported a lncRNA with a new extraceliac risk variant named HCG14, which can regulate the expression of NOD1 in an allele-specific manner. NOD1, a member of the NOD-like receptor (NLR) family, is one of the most studied pathogen recognition receptors (PRRs). It acts as the first barrier against pathogens in several other tissues, including the intestinal tract. However, the mechanism underlying the increased risk of CD caused by HCG14 is still unknown and needs to be further explored.

5. lncRNA as a New Diagnostic and Therapeutic Marker of Inflammatory Bowel Disease

5.1. lncRNA as a New Diagnostic Marker of Inflammatory Bowel Disease. At present, IBD diagnosis lacks convincing gold standards. Routine diagnosis of IBD includes clinical symptom assessment combined with endoscopy, histology, serology, and radiology [95]. At the same time, IBD lacks specific biomarkers, often leading to misdiagnosis and delayed treatment. lncRNAs are a valuable diagnostic marker for various diseases. Its abundance in vivo is high and can be quickly detected by common molecular biological techniques, and has relative stability and tissue specificity [96]. Currently, many studies on IBD, colon biopsy, and lncRNA map data from blood samples suggest significant differences between the disease group and the healthy group [97]. By detecting lncRNAs, we can classify IBD subtypes and determine whether IBD is active. It is hoped that noninvasive lncRNAs based on humoral fluid can be used as biomarkers [98]. This utility improves our ability to diagnose IBD greatly and enables us to predict the occurrence of IBD before its clinical symptoms appear. In addition, because the pathological process of IBD is very complex, a single lncRNA corresponding to a certain stage is not sufficient to meet the needs of clinical diagnosis. Therefore, the combination of several candidate lncRNAs from different tissue sources and available biomarkers may be necessary to provide an accurate diagnosis. Overall, we can increase

Table 2: Summary and mechanism of lncRNA as a marker for diagnosis and treatment of various intestinal diseases.

Intestinal disease	Related lncRNA	Regulating mechanism	A potential role as a marker of diagnosis or treatment
IBD	CNN3-206	The lncRNA CNN3-206-miR-212-Caspase10 regulatory network	In intestinal lesions of patients with Crohn's disease, the expression of lncRNA CNN3-206 is significantly increased [74].
	CRNDE	miR-495 and SOCS1	Indirectly induce apoptosis of intestinal mucosal epithelial cells and aggravate the inflammatory response of IBD, can be used as a potential therapeutic target [75].
	NAIL	p38 and NFκB	Targeted knockout of NAIL can inhibit the expression of downstream inflammatory factors and greatly reduce the intestinal inflammatory response in patients with IBD [102].
Colorectal cancer	CCAT-1	miR-185-3p Claudin, ZO-1	In patients with colorectal cancer, the expression of CCAT is significantly increased, and the intestinal barrier function can be significantly improved by inhibiting its expression [42].
	SPRY4-IT1	Claudin-1, claudin-3, occludin, and jam-1	SPRY4-IT1 can destroy intestinal TJ and cause intestinal epithelial barrier dysfunction, which can be used as a potential therapeutic target [84].
	UC.173	miR-29b	Promoting the expression of uc.173 can advance the translation of TJ, claudin-1 (CLDN1), promotes the repair of intestinal mucosal mechanical barrier, and is beneficial to the improvement of symptoms in patients with colorectal cancer [61].
IBS-D	H19	AQP 1, 3, 8	Inhibiting H19 expression can significantly promote AQP1, AQP3, and AQP8 expression and significantly improve intestinal barrier function in IBS-D mice [87].
Celiac disease	Lnc13	hnRNPD	The expression of LNC13 in intestinal biopsies of patients with celiac disease was significantly decreased, suggesting that the downregulated expression of LNC13 may be one of the causes of inflammation of celiac disease [91].
	HCG14	NOD1	The content of HCG14 in intestinal tract of patients with celiac disease increased significantly, suggesting its potential value as a diagnostic index of celiac disease [93].

the likelihood of introducing reliable lncRNAs as IBD biomarkers through a larger cohort study of tissue biopsies and body fluids.

5.2. lncRNA as a Potential Therapeutic Target for Inflammatory Bowel Disease. lncRNAs are potential therapeutic targets for IBD. Downregulation of lncRNA by RNA interference or forced overexpression of lncRNA by appropriate vectors may affect the IBD process [99]. Although initial successes in treating intestinal diseases based on lncRNAs have been made in animal studies, these methods have not been proven feasible and safe in the clinic. Currently, immunosuppressant or hormone therapy is the main treatment for IBD [100]. Recent studies have shown that repairing the intestinal mucosal mechanical barrier can induce continuous clinical remission in patients with IBD, reduce the number of hospitalizations and operations, and improve the quality of life of patients [101]. Therefore, repairing the mechanical barrier of the damaged intestinal mucosa by artificially interfering with the expression of lncRNA has become an important research direction in the treatment of IBD. In recent years, there has been a new understanding of the role of lncRNAs in the inflammatory mechanism of IBD [102]. However, little is known about the key regulators that activate, fine-tune, or turn off NFκB activity under inflammatory conditions. Akıncılar et al. [103] designed the first genetic screening method to identify the specific lncRNA of NFκB and found a conservative lncRNA named NAIL. After a series of experiments, the authors found that NAIL can cooperate with another inflammatory factor, P38, to activate NF-κB and induce progenitor cells to differentiate into immature myeloid cells in the bone marrow, macrophages reassemble to the inflammatory region, and express inflammatory genes in colitis. Inactivated lncRNA NAIL can reduce the inflammatory response in colitis mice, suggesting that NAIL is an ideal target and biomarker for treating inflammatory bowel disease and other inflammation-related diseases.

5.3. lncRNA Can Be Used as a Marker for the Diagnosis and Treatment of Other Intestinal Diseases. In addition to IBD, lncRNAs can also be used as markers for diagnosing and treating other intestinal diseases such as colorectal cancer and celiac disease (Table 2). In the pathogenesis of colorectal cancer, many lncRNAs compete with specific mRNAs in binding to miRNAs. These lncRNA-miRNA-mRNA competitive endogenous RNA networks form a complex and highly regulated mechanism to control gene expression and cell function [104]. lncRNA members of this network are often involved in the advanced stage of colorectal cancer (such as CACS15, CYTOR, HOTAIR, MALAT1, TUG1, NEAT1, and MIR17HG) [105]. These lncRNAs may be

effective prognostic biomarkers. More importantly, the knockout or overexpression of these members in the colorectal cancer-related Cerna network significantly inhibits colorectal cancer progression, indicating their potential as therapeutic targets for colorectal cancer. In celiac disease, researchers propose that increasing the content of lnc13 can inhibit the expression of inflammatory genes associated with celiac disease, revealing the potential of lnc13 as a potential target for the diagnosis and treatment of celiac disease [91].

6. Summary and Outlook

The intestinal mucosal mechanical barrier is the most important barrier to prevent trillions of microorganisms, foreign antigens, and viruses from entering the intestinal environment. Its existence maintains the delicate dynamic balance between intestinal microorganisms and host epithelial cells, and plays a very important role in preventing intestinal mucosal damage. Damage to the intestinal mucosal mechanical barrier often leads to intestinal disease. As a rising star molecule in biology, lncRNAs have been shown to regulate various physiological and pathological processes. With the continuous progress of high-throughput sequencing technology [106], a growing number of lncRNAs have been annotated, but the functions of most lncRNAs remain unclear. Therefore, the study of lncRNAs is a broad unknown territory, which is of great research value and significance. In recent years, an increasing number of studies have revealed that lncRNAs have a regulatory effect on the intestinal mucosal mechanical barrier, and an increasing number of regulatory mechanisms are being found [107]. For example, various lncRNAs, including H19, regulate the function of intestinal epithelial cells and destroy the tight junctions between cells, resulting in an abnormal increase in the permeability of the intestinal mucosal mechanical barrier and affecting the normal function of the intestinal barrier. At the same time, injury to the intestinal mucosal mechanical barrier often leads to intestinal inflammatory diseases. A recent study found that the enhancement of intestinal mucosal mechanical barrier permeability is an important precursor of intestinal changes in patients with IBD and often occurs several years earlier. Presently, there is a lack of a gold standard for the diagnosis and treatment of IBD, which makes it urgent to find a specific marker for IBD diagnosis and treatment. Due to the gradual progress of high-throughput detection methods for lncRNA, the difficulty of detection and intervention of specific lncRNAs is significantly reduced, greatly improving the possibility of lncRNA becoming a new diagnostic and therapeutic target for IBD. Recently, there has been a breakthrough in studying the mechanism of inflammation caused by lncRNAs. Some studies [85] have found that lncRNAs play a very significant role in the process of activating intestinal inflammatory genes. We have every reason to believe that the diagnosis and treatment of intestinal diseases will have a broad and bright prospect in the near future through specific detection and monitoring of the corresponding lncRNA.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

S C, B H, and S Z were involved in the conception of the study. S C, C Z, and R H were involved in writing the article. S C, L X, and S Z critically revised the manuscript. All authors read and approved the final manuscript. S C, C Z, and B H were co-first authors and contributed equally to this work.

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