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Systematic Review and Meta-analysis

Breaking Barriers in Functional Dyspepsia: A Systematic Review and Meta-analysis on Duodenal Tight Junction Protein Expression

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Background/Aims
Disruptions in tight junction (TJ) protein expression leading to duodenal epithelial barrier impairment may contribute to increased intestinal permeability, potentially playing a role in functional dyspepsia (FD) pathophysiology. Currently published studies evaluated the role of several TJ proteins in FD patients with inconsistent results. Therefore, we conducted this systematic review and meta-analysis to evaluate the duodenal mucosal expression of several TJ proteins in FD.

Methods
We performed a systematic electronic search on PubMed, EMBASE, and Scopus using predefined keywords. Diagnosis of FD by Rome III or Rome IV criteria was considered acceptable. Full articles satisfying our inclusion and exclusion criteria were included. The principal summary outcome was the mean difference of several TJ proteins in FD patients and control subjects.

Results
A total of 8 and 5 studies were included in our qualitative and quantitative synthesis, respectively, with a total population of 666 participants, out of which 420 were FD patients. No significant differences were observed between FD patients and controls in the expression of claudin-1 (–0.102 [95% CI, –0.303, 0.099]), claudin-2 (0.161 [95% CI, –0.134, 0.456)], claudin-3 (0.278 [95% CI, –0.280, 0.837]), claudin-4 (0.045 [95% CI, –0.264, 0.354]), ZO-1 (–0.221 [95% CI, –0.683, 0.241]), ZO-2 (–0.070 [95% CI, –0.147, 0.007]), ZO-3 (–0.129 [95% CI, –0.376, 0.118]), β-catenin (–0.135 [95% CI, –0.484, 0.214]), E-cadherin (–0.083 [95% CI, –0.229, 0.063]), and occludin (–0.158 [95% CI, –0.409, 0.093]).

Conclusions
The expressions of all evaluated proteins including claudin-1, claudin-2, claudin-3, claudin-4, ZO-1, ZO-2, ZO-3, β-catenin, E-cadherin, and occludin did not significantly differ between FD patients and controls. However, due to the limited number of included studies, results should be interpreted with caution.
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Key Words
Duodenal mucosal expression; Functional dyspepsia; Meta-analysis; Systematic review; Tight junction proteins

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Introduction

Functional dyspepsia (FD) represents a prevalent medical condition characterized by persistent or recurring discomfort or pain in the upper abdomen, devoid of any discernible structural abnormalities.[1-3] According to the Rome IV criteria, 2 subgroups of FD were proposed: postprandial distress syndrome (PDS) characterized by postprandial fullness or early satiation, and epigastric pain syndrome characterized by epigastric pain or burning sensation.[1-3] This condition is reported in approximately 7.1% of the general population.[4] Notably, up to 70% of patients with dyspepsia subjected to endoscopy exhibit unremarkable results and are consequently diagnosed with FD.[5] Although the precise causes of FD are not fully understood, it has been associated with a range of factors, including genetic influences, impaired gastric motility, heightened exposure of the duodenum to stomach acid, visceral hypersensitivity, and alterations in the interactions within the brain-gut duodenal axis.[6-15]

Recent research suggests that low-grade inflammation in the duodenum may be a contributing factor to the pathogenesis of FD.[16] Furthermore, the function of the duodenal barrier may be compromised due to changes in the expression of epithelial junctional proteins. Tight junction (TJ) proteins, integral to the mucosal barrier in the gastrointestinal tract, play a crucial role in this context.[17,18]

TJs, observed as the closest and thinnest electron-dense pentalaminar structures, consist of both transcellular proteins, such as claudins (claudin-1, claudin-2, claudin-3, claudin-4, claudin-5, etc), occludin, and junction adhesion molecule 1 (JAM-1), and bridging proteins like zonula occludens (ZO) including ZO-1, ZO-2, and ZO-3. These bridging proteins serve to connect the transcellular proteins with intracellular actin and myosin filaments. Among these components, claudin-2 is believed to be responsible for forming ion-selective pores, while claudin-3 and claudin-4 are tasked with sealing these pores. Occludin and JAM are primarily involved in maintaining cell polarity and regulating cell migration. ZO-1 is critical for the clustering of the transcellular fibrillary proteins.[19-31] The coordinated interactions among all these proteins are essential for the regulation and preservation of intestinal permeability.

Currently, published studies evaluated the role of several TJ proteins in FD patients with inconsistent results. Therefore, we conducted this systematic review and meta-analysis to evaluate the duodenal mucosal expression of several TJ proteins in FD, enhancing our knowledge about their potential role in FD.

Materials and Methods

In writing this systematic review and meta-analysis, the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020 statement was adhered to.[32]

Data Sources and Search Strategy

We looked for studies assessing TJ proteins expression in FD patients by searching 3 electronic databases: PubMed, Embase, and Scopus. Supplementary Material provides a description of the search keyword that was used (available in additional materials). We also manually looked for relevant publications that had been omitted from the references of the papers that were included. Two researchers carried out the literature search independently from inception till August 23, 2023. A consensus was reached through discussion in case of discrepancies. There was no language, country, duration restrictions, or filters applied during the search. The full texts of the studies that satisfied our inclusion and exclusion criteria were assessed after screening the abstracts and titles for eligibility. Two researchers extracted the data, and any discrepancies were resolved by referring to the original paper. Retrieved data included author names, publication year, country, sample size, mean age, sex distribution, body mass index, values of duodenal TJ protein expression in both FD and control groups, and Rome criteria used in diagnosing FD.

Eligibility Criteria

The following were the inclusion criteria for original papers that were included in our systematic review and meta-analysis: observational or interventional studies that evaluated duodenal mucosal mRNA expression levels of 1 or more of the following TJ proteins (claudin-1, claudin-2, claudin-3, claudin-4, ZO-1, ZO-2, ZO-3, and occludin) or adherens junction proteins (E-cadherin and β-catenin) were assessed by reverse transcription polymerase chain reaction.

We excluded studies that met the following criteria: (1) published abstracts without full articles and conference abstracts; (2) editorials, letters, brief surveys, and commentaries; (3) case reports; (4) review articles; and (5) practice guidelines.

Risk of Bias Assessment

The investigators assessed the internal validity and bias risk of the included studies using the Newcastle-Ottawa Scale.[33] Discussions were utilized to settle discrepancies between the obtained qual-

ity assessment results between the 2 investigators. Each reviewed study received a score based on the obtained number of stars. After the criteria for the selection, comparability, and outcome sections were confirmed, the study was given a star rating that ranged from 0 to 9. The stars in each study were summed to measure the quality of the included studies objectively. Studies with 7 stars or higher were found to be of good quality. The evaluation of methodological quality had no influence on the studies' eligibility.

Summary Measures and Synthesis of Results

The main outcome was the mean difference (MD) of duodenal TJ proteins expressions in FD patients and controls. As we assumed clinical variability between studies, we calculated the estimates of the random effects using restricted maximum likelihood to assess variance heterogeneity. We ran the meta-analysis data analyses in R with the Metafor tool (OpenMeta [Analyst]).[34,35] The 2-based Q-test and I^2 were used to assess between-study heterogeneity. In accordance with the recommendations of the Cochrane Handbook,[36] we evaluated I^2 values of 0% to 40% as not significant, 30% to 60% as moderate heterogeneity, 50-90% as substantial heterogeneity, and 75% to 100% as considerable heterogeneity in order to recognize and quantify heterogeneity.

We calculated the mean and standard deviation (SD) for the included studies that provided medians and interquartile ranges (IQR) in order to conduct statistical analyses of the collected data.[37] Standard error of mean (SEM) was converted to SD by multiplying the SEM with the square root of the sample size of the respective group. All evaluated data were shown as the estimated MD together with a 95% CI. Pooling of the data was conducted if 2 or more studies looked at comparable groups and produced the same result that was considered of interest, using mean ± SD or median (IQR).

Results

The PRISMA flow diagram outlines the search and selection strategy as shown in Figure 1. The initial search yielded a total of 174 publications (PubMed, n = 22 articles; EMBASE, n = 76; and Scopus, n = 76 articles). Thirty studies were excluded after being identified as duplicates. Subsequently, a preliminary screening was conducted on 144 articles to evaluate their eligibility. We excluded a total of 127 studies during the screening phase. We thoroughly assessed the 17 remaining publications to provide a more comprehensive eligibility assessment. Among the available 17 studies, we removed 8 conference abstracts and 1 conference poster.[38-46] Hence, a total of 8 articles were included in our qualitative synthesis[47-54] and 5 articles were included in our quantitative synthesis.[47-49,52,54] Studies that did not presented that data as mean ± SD, mean ± SEM or median (IQR) were excluded from the quantitative analysis, as were the studies where the data was not available.[50,51,53]

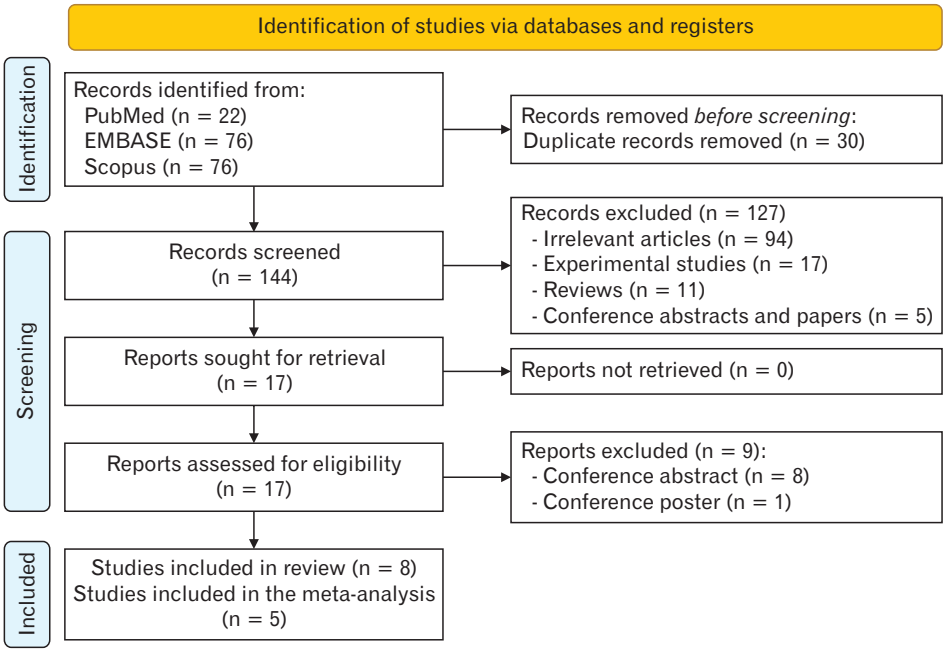


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-analyses flow diagram for the identification, screening, and inclusion of articles in our systematic review and meta-analysis.

Study Characteristics

The key characteristics of the studies that were included are summarized in Supplementary Table 1. This systematic review and meta-analysis comprised 666 subjects in total. Among those, 420 were diagnosed with FD, of which 142 were males. Patients with FD were diagnosed based on RomeE III criteria in 5 studies[47-50,54] and based on Rome IV in 3 studies.[51-53]

Claudins

Protein expression of claudin-1 was assessed in a total of 5 studies comparing FD patients to controls.[47-49,52,54] The pooled analysis revealed an overall MD of −0.102 (95% CI, −0.303 to 0.099). There was moderate heterogeneity with an I^2 of 59.51% (P-value of 0.051).

Protein expression of claudin-2 was examined in 4 studies comparing FD patients to controls.[47,48,52,54] The combined analysis indicated an overall MD of 0.161 (95% CI, −0.134 to 0.456). Non-significant heterogeneity was observed with an I^2 of 24.1% (P-value of 0.352).

Protein expression of claudin-3 was studied in 3 studies comparing FD patients to controls.[48,52,54] The pooled analysis demonstrated an overall MD of 0.288 (95% CI, −0.267 to 0.844). There was considerable heterogeneity with an I^2 of 82.89% (P-value of < 0.001).

Protein expression of claudin-4 was analyzed in 4 studies comparing FD patients to controls.[47,48,52,54] The pooled analysis indicated an overall MD of 0.026 (95% CI, −0.215 to 0.268). Non-

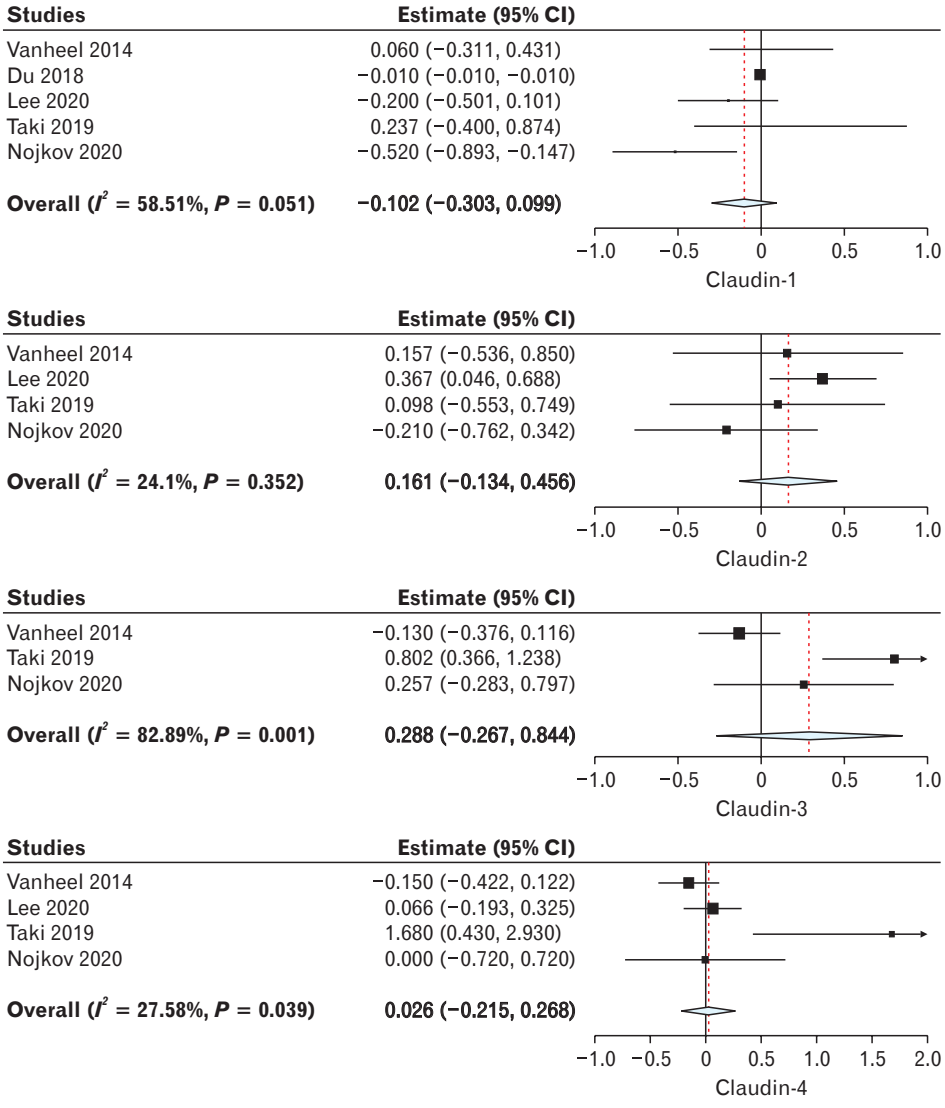


Figure 2. Claudin-1, Claudin-2, Claudin-3, and Claudin-4 levels in functional dyspepsia patients vs controls.

significant heterogeneity was observed with an I^2 of 27.58% (*P*-value of 0.039).

Figure 2 outlines the pooled analyses for claudin-1, 2, 3, and 4.

Zonula Occludens

ZO-1 protein expression was assessed in 4 studies comparing FD patients to controls.[47,48,52,54] The combined analysis revealed an overall MD of −0.229 (95% CI, −0.717 to 0.258). Considerable heterogeneity was reported with an I^2 of 82.63% (*P*-value of 0.010).

ZO-2 protein expression was investigated in 2 studies comparing FD patients to controls.[52,54] The combined analysis indicated an overall MD of -0.070 (95% CI, −0.301 to 0.161). There was no significant heterogeneity with an I^2 of 0% (*P*-value of 1.000).

ZO-3 protein expression was assessed in 2 studies comparing FD patients to controls.[52,54] The combined analysis indicated an overall MD of −0.129 (95% CI, −0.376 to 0.118). No significant heterogeneity was observed with an I^2 of 0% (*P*-value of 0.800).

Figure 3 outlines the pooled analyses for ZO-1, 2, and 3.

Beta-catenin

Beta-catenin protein expression was evaluated in 2 studies comparing FD patients to controls.[52,54] The collective analysis showed an overall MD of −0.126 (95% CI, −0.482 to 0.230). Moderate heterogeneity was observed with an I^2 of 54.12% (*P*-value of 0.140) (Fig. 4).

E-cadherin

E-cadherin protein expression was examined in 2 studies comparing FD patients to controls.[52,54] The collective analysis indicated an overall MD of −0.020 (95% CI, −0.272 to 0.231). No significant heterogeneity was observed with an I^2 of 0% (*P*-value of 0.381) (Fig. 5).

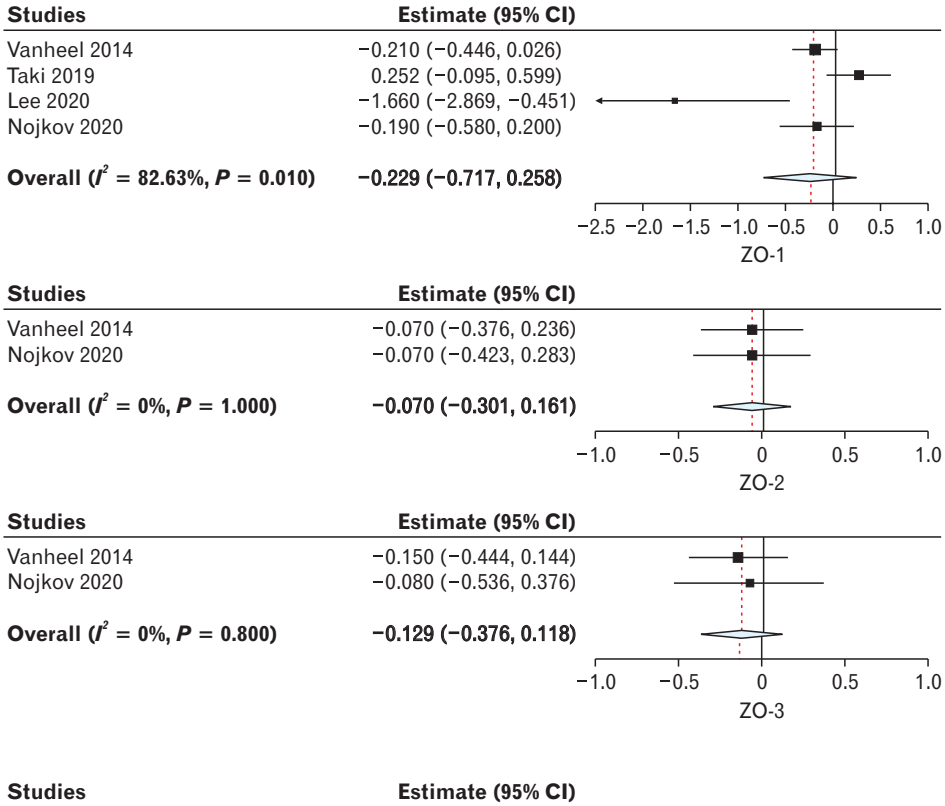


Figure 3. Zonula occludens (ZO)-1, ZO-2, and ZO-3 levels in functional dyspepsia patients vs controls.

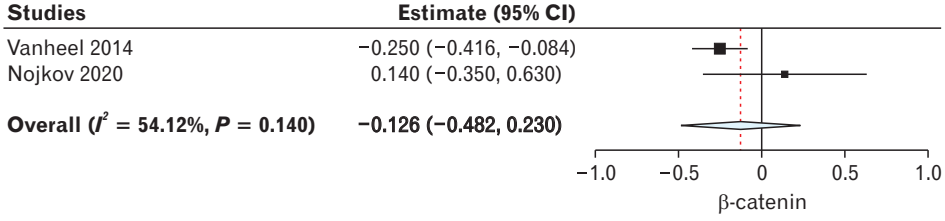


Figure 4. Beta-catenin levels in functional dyspepsia patients vs controls.

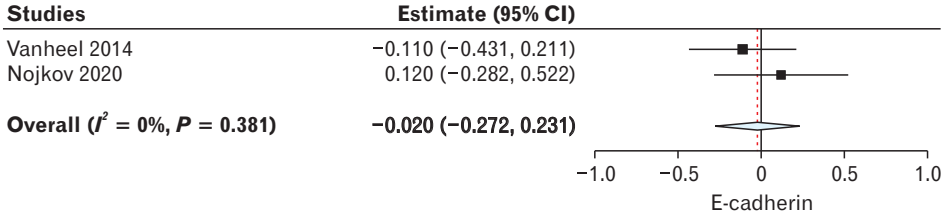


Figure 5. E-cadherin levels in functional dyspepsia patients vs controls.

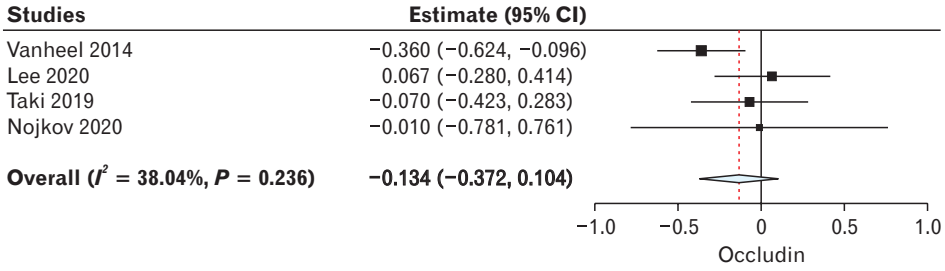


Figure 6. Occludin levels in functional dyspepsia patients vs controls.

Occludin

Occludin protein expression was studied in 4 investigations comparing FD patients to controls.[47,48,52,54] The pooled analysis demonstrated an overall MD of −0.134 (95% CI, −0.372 to 0.104). Moderate heterogeneity was reported with an I^2 of 38.04% (P-value of 0.236) (Fig. 6).

Quality Assessment

The Newcastle-Ottawa Scale for cross-sectional studies was used in a total of 8 studies, as outlined in Supplementary Table 2. Two studies scored 9 (Lee et al[47] and Vanheel et al[54]), 2 received a score of 7 (Du et al[49] and Komori et al[50]) and by this standard, the studies were considered "good quality." Three studies received a grade of 6 (Taki et al,[48] Wang et al,[51] and Narayanan et al,[53]) and by this standard were considered "moderate quality." Lastly, all evaluated studies had a clearly formulated research question and objective with a satisfactory sample size. All studies used clearly defined measures of exposure that are considered reliable and valid. All studies assessed the ascertainment of exposure in a satisfactory fashion.

Discussion

Our comprehensive analysis, incorporating data from 8 qualitative and 5 quantitative studies involving 532 participants, assessed the expression of TJ and adherens junction proteins in the duodenum of patients with FD compared to controls. We revealed the absence of statistically significant variations in the expression of several critical TJ proteins and adherens junction proteins in FD patients compared to control subjects. These proteins included claudin-1, claudin-2, claudin-3, claudin-4, ZO-1, ZO-2, ZO-3, and occludin. Nevertheless, the observed outcomes were accompanied by varying degrees of heterogeneity across the studies.

It is believed that there are multiple pathophysiological mechanisms involved in functional dyspepsia, considered a disorder of gut-brain interaction (DGBI), and that these mechanisms may contribute to any or all the subtypes of the condition. Gastrointestinal motor function abnormalities have historically been linked to FD, specifically PDS.[14,55] Eating meals significantly modulates symptoms and genetics may also play a part.[56,57] FD associated with *Helicobacter pylori* infection acknowledged as a distinct condition, while various gastrointestinal infections have been implicated as potential triggers for FD due to their adverse effects on gastric accommodation. Patients with FD commonly display sensorimotor disorders in the gastroduodenum, characterized by changes in motility and abnormal responses to mechanical and chemical stimuli.[58]

Even in the absence of observable macroscopic changes during examination, recent research suggests the presence of microscopic alterations in the duodenal mucosa of patients with FD. The duodenum has become increasingly recognized as an important factor in DGBI.[59] In functional dyspepsia, the role of the mucosal barrier and tight junctions is paramount in understanding the pathophysiology of the disorder.[58] Emerging evidence suggests that disruptions in the mucosal barrier integrity and alterations in TJ proteins contribute significantly to the development and progression of functional dyspepsia. These disruptions lead to increased mucosal permeability, allowing the passage of luminal antigens and patho-

gens into the underlying tissue, triggering inflammatory responses, and visceral hypersensitivity.[52,53] Additionally, impaired TJ function may exacerbate visceral hypersensitivity and dysmotility, key features of functional dyspepsia. Consequently, therapies aimed at restoring mucosal barrier function and preserving TJ integrity hold promise as potential therapeutic targets for managing FD and improving patient outcomes.[56,59]

In FD patients, intestinal permeability was assessed in several studies using urinary sugar excretion tests. In pediatric patients with FD, a sugar absorption test involving lactulose, mannitol, and sucrose, coupled with a 5-hour urine collection, yielded results similar to those of controls.[60] In adult patients with FD, the lactulose mannitol ratio between 1 hour and 2 hours was found to be higher compared to controls, even after adjusting for anxiety and depression, with no significant difference observed between PDS and epigastric pain syndrome.[53] In addition to molecular changes, structural alterations were identified, including a decreased proportion of junctions exhibiting perijunctional cytoskeleton condensation. Furthermore, there was a correlation observed between intercellular distance, the proportion of dilated junctions and the expression of TJ proteins, suggesting involvement of the leak pathway.[53,61] Ex vivo specimens can further characterize the epithelial barrier function at the molecular level by examining the expression of cell-to-cell adhesion proteins at both mRNA and protein levels.[61] It has been proposed that gastrointestinal inflammation can trigger an overlap of irritable bowel syndrome-FD.[62]

In the realm of clinical implications, these results offer critical insights into the role of junction proteins in the context of FD. While the absence of significant alterations in most of the studied junction proteins may suggest that tight junctions and adherens junctions do not play a pivotal role in FD pathophysiology within the populations under investigation. This underscores the need for additional research, especially on a larger scale, to validate and extend these findings. This avenue of inquiry holds promise for the development of novel insights and potential therapeutic strategies in the management of FD.

Our study has its limitations. Varying degrees of heterogeneity were observed across the analyzed proteins, which may limit the ability to draw definitive conclusions. Moreover, the analysis examined a limited selection of junction proteins, potentially excluding other relevant proteins. Due to the observation study design in the included analyses, causality cannot be confirmed nor negated. Furthermore, due to the limited number of published studies evaluating TJ proteins in FD, we were able to assess only a few studies, about 2 or 3 studies for each association. Hence, further future studies assessing TJ proteins in FD are considered necessary. Due to possible methodological flaws in included studies, results should be interpreted with caution.

Strengths of the study include the comprehensive analysis that incorporated data from numerous electronic databases, enhancing the robustness and generalizability of our findings. The use of a meta-analysis approach allowed for the quantitative synthesis of data from multiple studies, providing a more comprehensive overview of the subject matter. The study also offered a detailed examination of multiple TJ and adherens junction proteins in the duodenum, contributing to a thorough assessment.

In conclusion, FD patients lack significant differences in the expression of most TJ and adherens junction proteins compared to controls. These findings provide a platform for further exploration. Additional research is warranted to corroborate these findings, potentially paving the way for deeper insights and novel therapeutic approaches to address FD.

Supplementary Materials

Note: To access the supplementary material and tables mentioned in this article, visit the online version of *Journal of Neurogastroenterology and Motility* at http://www.jnmjournal.org/, and at https://doi.org/10.5056/jnm24013.

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